A holistic approach to bring sustainability and resilience via Plant and Microbe populations in the Marble Waste Polluted Ecosystem of Khyber Pakhtunkhwa, Pakistan

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A holistic approach to bring sustainability and resilience via Plant and Microbe populations in the Marble Waste Polluted Ecosystem of Khyber Pakhtunkhwa, Pakistan

A thesis submitted to the Quaid-i-Azam University in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

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(Plant Ecology and Conservation)

By

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This is to certify that research work presented in this thesis, entitled "**A holistic approach to bring sustainability and resilience via Plant and Microbe populations in the Marble Waste Polluted Ecosystem of Khyber Pakhtunkhwa, Pakistan**" was conducted by **Mr. Zeeshan Ahmad** under the supervision of **Dr. Shujaul Mulk Khan**. No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Department of Plant Sciences, Quaid-i-Azam University as a partial fulfillment of the requirement for the degree of Doctor of Philosophy in the field of **Plant Sciences/ Botany (Plant Ecology and Conservation)**, Department of Plant Sciences of Quaid-i-Azam University Islamabad, Pakistan.

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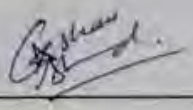

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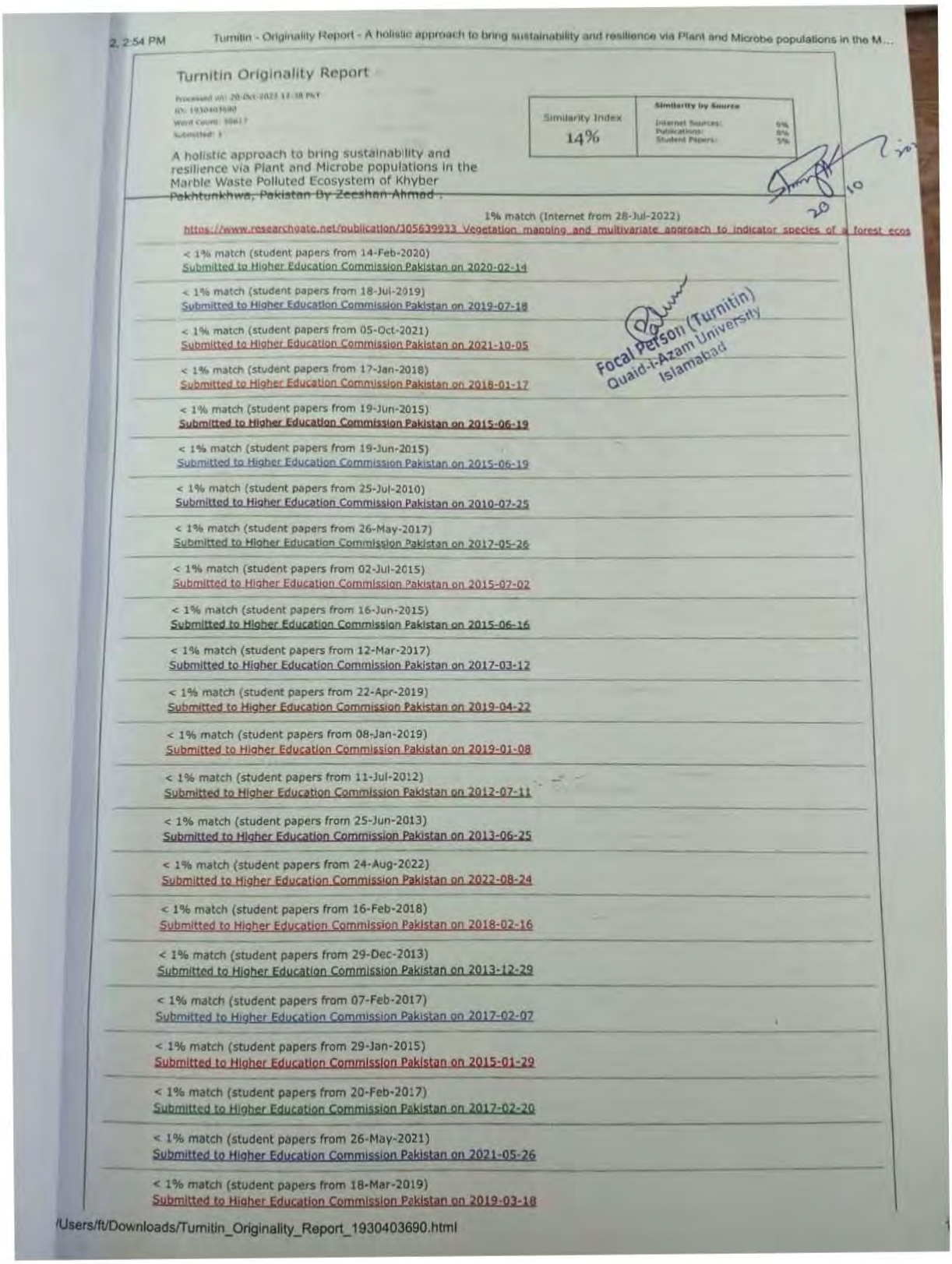

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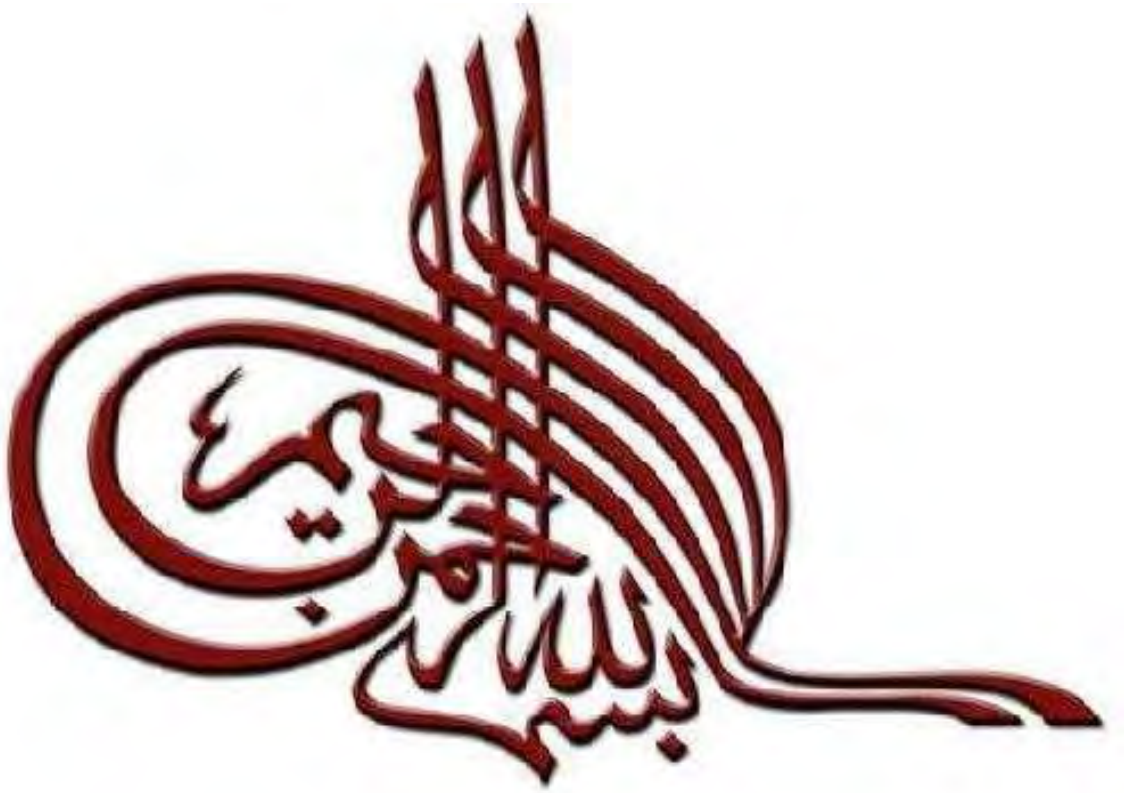

"In the name of ALLAH, most Gracious, most Compassionate"

AL QURAN

Assuredly the creation of the heavens ant the earth is a greater (matter) than the creation of men: yet most men understand not. Not equal are the blind and those who (clearly) see: not are (equal) those who believe and work deeds of righteousness, and those who de evil. Little do ye learn by admonition! The Hour will certainly come: therein is no doubt: yet most men believe not and your Lord says: Call on me; I will answer your (Prayer): but those who are too arrogant to serve me will surely find themselves in Hell in humiliation! It is Allah who has made the Night for you, that ye may rest therein, and the Day, as that which helps (you) to see. Verily Allah is full of Grace and Bounty to men: yet most men give not thanks. Such is Allah, your Lord, the Creator of all things. There is no God but He: then how ye are deluded away from the truth! Thus are deluded those who want to reject the Signs of Allah.

Al Mumin Verses 40:57 to 63.

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My loving **mother**, who is my inexhaustible source of comfort, love and optimism.

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Table of Contents

1 Chapter 1 3

1.1 Introduction 3

1.1.1 Pollution caused by the Marble Industry 4

1.1.2 Water used in the Marble Industry 5

1.1.3 Impact of Marble pollution on biodiversity and the concept 6

1.1.4 Role of Biodiversity in Pollution Reduction/ Bioremediation and Sustainable Environment 7

1.1.5 Spatial distribution or GIS mapping 8

1.1.6 Reclamation / Resilience in Marble waste polluted Ecosystem 9

1.1.7 Introduction to the Study area / Khyber Pakhtunkhwa Province 9

1.1.8 Research Rationale and Hypotheses 15

1.1.9 Aim of the study 16

1.1.10 Objectives of the study 16

1.1.11 Thesis Structure 17

2 Chapter 2 18

2.1 Introduction 18

2.1.1 Plant Biodiversity 18

2.1.2 Flora and vegetation 19

2.1.3 Pollution 20

2.1.4 Vegetation sampling through the Quadrat method 20

2.1.5 Gradients analysis 21

2.1.6 Isolation and Characterization of Microbes 22

2.1.7 Justification 23

2.1.8 Aims and objective 24

2.2 Materials and Methods 25

2.2.1 Vegetation sampling 25

2.2.2 Physiological attributes 28

2.2.3 Soil Data collection and Analysis 30

2.2.4 Climatic Data 31

2.2.5 Statistical data Analyses 31

2.2.6 Isolation of Micro Fungi 34

2.2.7 Morphological identification of fungus culture 34

2.2.8 Molecular analyses of fungi 35
2.3 Results 37
2.3.1 Plant species composition 37
2.3.2 Abundant and rare plants of the MWPE 44
2.3.3 Impact of environmental variables on abundant 48
2.3.4 Vegetation classification of Marble Waste Polluted Ecosystem 49
2.3.5 Characterization and Phylogenetic studies of Fungal Flora 88
2.3.6 Molecular and Phylogenetic analysis of the selected micro fungi 93
2.4 Discussion 95
2.5 Conclusion 100
3 Chapter 3 101
3.1 Introduction 101
3.1.1 Conceptual / Hypothesized Model 104
3.2 Materials and Methods 107
3.2.1 Diversity Indices, Species richness and evenness 108
3.2.2 Structural Equation Modeling 109
3.3 Results 111
3.3.1 Species area curves 111
3.3.2 Indicator of MWPE/ Vegetation classification of MWPE 112
3.3.3 Species richness and Diversity Indices 131
3.3.4 Direct ecological gradient through Canonical Correspondence 132
3.3.5 Structural Equation Modelling and reconfirmation of indicator 134
3.4 Discussion 152
3.5 Conclusion 156
4 Chapter 4 158
4.1 Introduction 158
4.1.1 Phytoremediation 158
4.1.2 Mechanisms of heavy metal uptake by plants 160
4.1.3 Factors effecting the accumulation of PTEs 161
4.1.4 Advantages of Phytoremediation 162
4.1.5 Limitations of Phytoremediation 163
4.1.6 Plants physiological response to pollution 163
4.1.7 Mycoremediation 165
4.1.8 Justification 166
4.2 Materials and Methods 167

4.2.1 Quantification of Potential Toxic Elements (PTEs)/Heavy Metals 167
4.2.2 Phytoremediation and plant selection 170
4.2.3 Determination of Photosynthetic pigments 171
4.2.4 Proline Quantification 172
4.2.5 Data Analyses 173
4.2.6 Bioremediation / Myco-remediation 174
4.3 Results 176
4.3.1 Phytoremediation ability of identified indicators 180
4.3.2 Pattern in chlorophyll contents of indicator plants 193
4.3.3 Proline accumulation as a survival mechanism in indicator plants 197
4.3.4 Mixed Effect Model 199
4.3.5 Myco-remediation / bioremediation ability of selected micro fungi 217
4.4 Discussion 225
4.5 Conclusion 229
5 Chapter 5 230
5.1 Introduction 230
5.1.1 Normalized Difference Vegetation Index (NDVI) 232
5.2 Materials and Methodology 234
5.2.1 Spatial distribution of heavy metals 234
5.2.2 Spatial autocorrelation 234
5.2.3 Determination of Normalized Difference Vegetation Index (NDVI) 234
5.2.4 Statistical Analyses 234
5.3 Results 235
5.3.1 Spatial Autocorrelation Moran‘s Index 247
5.3.2 Normalised Difference Vegetation Index (NDVI) 1986-2021 254
5.3.3 NDVI at a province level 256
5.3.4 NDVI at Factory Level 256
5.3.5 Impact of Marble pollution on NDVI through bivariate analysis 269
5.3.6 Impact of Marble pollution viz NDVI on Structural Equation 271
5.4 Discussion 274
5.5 Conclusion 278
6 Chapter 6 279
6.1 Discussion and Synthesis 279
6.1.1 Vegetation dynamics; abundance and rare plant species 280
6.1.2 Multivariate statistical approach Modelling 281

6.1.3 Concept of Indicator species in MWPE.....282
6.1.4 Physiological response as defense mechanism.....283
6.1.5 Bioremediation of indicator plants and Micro Fungi.....284
6.1.6 Spatial distribution pattern of PTE and temporal changes in NDVI ...285
6.1.7 Concept of Water tank for initial treatment of marble wastewater.....286
Conclusion……..288
Recommendations …… 290

List of Figures

Fig. 1.1 A view of a marble waste polluted ecosystem, Khyber Pakhtunkhwa.....5
Fig. 1.2 Elevation and slope map of the Khyber Pakhtunkhwa province, Pakistan 10
Fig. 2.1 Map of the study area showing the sampling points 26
Fig. 2.2 A glimpse of marble waste polluted ecosystem, KPK, Pakistan.....27
Fig. 2.3 The impact of Marble pollution on vegetation. 27
Fig. 2.4 Percentage distribution of herbs, shrubs and trees around marble factories. .37
Fig. 2.5 The topmost abundant tree species in the subtropical Marble waste. 44
Fig. 2.6 Rare plant species of the MWPE, KPK, Pakistan. 45
Fig. 2.7 The foremost abundant shrub species of the MWPE, KPK, Pakistan. 46
Fig. 2.8 The rare shrub species with minimum IVI in the subtropical MWPE. 46
Fig. 2.9 The top ten most abundant herb species reported from subtropical MWPE ..47
Fig. 2.10 Rare herb species with minimum IVI in the studied MWPE. 47
Fig. 2.11 The TWCA dendrogram comprehended the distribution of 220.....50
Fig. 2.12 TWCA dendrogram of 124 plant species and 66 stations/quadrats. 51
Fig. 2.13 The topmost abundant tree species in the Humid subtropical Marble. 52
Fig. 2.14 Rare tree species based on minimum IVI in the Humid subtropical. 53
Fig. 2.15 The most abundant shrub species with higher IVI in the Humid 53
Fig. 2.16 The rare shrub species with minimum IVI in the studied region. 54
Fig. 2.17 The topmost abundant herb species of Humid MWPE. 55
Fig. 2.18 The rare herb species of Humid MWPE.....55
Fig. 2.19 DCA showing the distribution of 124 plant species in the humid zone. 58
Fig. 2.20 DCA showing the distribution of 66 stations/quadrats in the humid zone...59
Fig. 2.21 CCA biplot representing the distribution of plant species in relation 61
Fig. 2.22 CCA biplot of all the station in conjunction with explanatory variables. 62
Fig. 2.23 TWCA dendrogram of 159 plant species and 138 stations/quadrats 63
Fig. 2.24 The most abundant tree species with higher IVI in the Semi-humid. 64
Fig. 2.25 Rare tree species with minimum IVI in the Semi-humid MWPE 65
Fig. 2.26 The foremost abundant shrub species of the Semi-humid MWPE.....65
Fig. 2.27 Topmost rare shrubs of the semi-humid MWPE 66
Fig. 2.28 The top ten abundant herb species based on IVI in the region. 67
Fig. 2.29 The top ten rare herb species in the semi humid MWPE. 67
Fig. 2.30 Ecological gradient through DCA biplot representing the distribution.....69
Fig. 2.31 Ecological gradient through DCA biplot representing the distribution 70
Fig. 2.32 Canonical correspondence Analysis biplot showing the distribution.....73
Fig. 2.33 Canonical correspondence Analysis biplot showing the distribution.....74
Fig. 2.34 TWCA dendrogram of 146 plant species and 123 stations/quadrats in. 75
Fig. 2.35 The topmost abundant tree species in the Dry subtropical MWPE, KPK....76
Fig. 2.36 The rare tree species in the Dry subtropical MWPE with minimum IVI. 77
Fig. 2.37 The topmost abundant shrubs with higher IVI in the subtropical MWPE ...77
Fig. 2.38 Rare shrubs of the Dry subtropical MWPE, KPK. 78
Fig. 2.39 The top ten abundant herb species recorded in the Dry subtropical. 79
Fig. 2.40 Topmost rare herb species in the Dry subtropical MWPE, KPK, Pakistan..79
Fig. 2.41 DCA demonstrating the distribution of plants in the dry subtropical.....82
Fig. 2.42 DCA indicating the distribution of stations in the dry subtropical MWPE..83
Fig. 2.43 Ecological gradient through CCA biplot showing the distribution 86

Fig. 2.44 Ecological gradient through CCA biplot showing the distribution of.....87
Fig. 2.45 Pure fungal isolates from marble waste polluted ecosystem.....88
Fig. 2.46 Morphological and microscopic characterization of fungal isolates.. 92
Fig. 2.47 Molecular phylogenetic relationship of micro fungus species. 94
Fig. 3.1 A theoretical model for testing the proposed hypotheses.....106
Fig. 3.2 The species-area curves of 220 plant species distributed among. 111
Fig. 3.3 The data attribute plots of *Ficus carica* (1st indicator) of the humid.....114
Fig. 3.4 The data attribute plots of *Catharanthus roseus* (2nd indicator).....115
Fig. 3.5 The data attribute plots of *Erigeron canadensis* (3rd indicator).....116
Fig. 3.6 Data attribute plot of *Morus nigra* (1st indicator).....119
Fig. 3.7 Data attribute plot of *Datura innoxia* (2nd indicator).....120
Fig. 3.8 Data attribute plot of *Persicaria glabra* (3rd indicator).....121
Fig. 3.9 Data attribute plot of *Dalbergia sissoo* (1st indicator).....124
Fig. 3.10 Data attribute plot of *Withania somnifera* (2nd indicator).....125
Fig. 3.11 Data attribute plot of *Saccharum bengalense* (3rd indicator).....126
Fig. 3.12 CCA biplot presenting the distribution of indicator species.....133
Fig. 3.13 Structural Equation Modeling showing the impact of marble.....137
Fig. 3.14 The direct and indirect effects (standardized coefficient) derived. 138
Fig. 3.15 SEM representing the influence of marble pollution, climate,.....141
Fig. 3.16 The direct and indirect effects (standardized coefficient) obtained. 142
Fig. 3.17 SEM representing the influence of marble pollution, climate,.....145
Fig. 3.18 The direct and indirect effects (standardized coefficient) acquired. 146
Fig. 3.19 Structural Equation Modeling of the indicator species in subtropical.....150
Fig. 3.20 The direct and indirect effects (standardized coefficient) obtained. 151
Fig. 4.1 Accumulation mechanism on phytoremediation technology. 160
Fig. 4.2 Samples preparation for potential toxic elements/ heavy metals 168
Fig. 4.3 Samples filtration after its digestion on hotplate. 168
Fig. 4.4 Quantification of PTEs using atomic absorption spectrophotometer. 169
Fig. 4.5 A picture with atomic absorption spectrophotometer.....169
Fig. 4.6 Samples prepared for photosynthetic determination. 171
Fig. 4.7 Proline produced by plant species as response to marble waste pollution. ..173
Fig. 4.8 Samples preparation and processing for myco-remediation.....175
Fig. 4.9 Chlorophyll-a contents (mg/g) in the leaves of some identified. 194
Fig. 4.10 Chlorophyll-b contents (mg/g) in leaves of some identified indicator. 195
Fig. 4.11 Total carotenoids (mg/g) in leaves of identified indicator plants. 196
Fig. 4.12 Proline accumulation in the leaves of identified indicator plant species....198
Fig. 4.13 The relationship between chromium phytoremediation along. 208
Fig. 4.14 The impact of nickel phytoremediation on proline accumulation. 209
Fig. 4.15 The scattered plot for copper phytoremediation along with proline. 210
Fig. 4.16 The effect of cadmium phytoremediation leads to increase 211
Fig. 4.17 The scattered plot for zinc phytoremediation along with proline,.....212
Fig. 4.18 The influence of iron phytoremediation on proline, chlorophyll-a. 213
Fig. 4.19 The cobalt phytoremediation approach increases proline accumulation....214
Fig. 4.20 The scattered plot for calcium phytoremediation along with proline.....215
Fig. 4.21 The effect of sodium phytoremediation on proline, chlorophyll-a.....216
Fig. 4.22 Magnesium bioremediation by selected micro fungi over different.....217

Fig. 4.23 Calcium remediation shown by selected micro fungi isolated from. 218
Fig. 4.24 Detail of cadmium heavy metal remediation through micro fungi. 218
Fig. 4.25 Cobalt remediation by some of the selected micro fungi after specific 219
Fig. 4.26 Copper bioremediation by selected micro fungi over different time 220
Fig. 4.27 Iron heavy metal remediation through micro fungi after different 220
Fig. 4.28 Mercury mycoremediation ability of the studied species isolated 221
Fig. 4.29 Sodium bioremediation by micro fungal species. 222
Fig. 4.30 The nickel remediation ability of micro fungi isolated from MWPE.....222
Fig. 5.1 Spatial distribution pattern of calcium element in the marble. 236
Fig. 5.2 Spatial distribution of magnesium heavy metal secreted from.....237
Fig. 5.3 Mapping the distribution of iron heavy metal in the marble waste 238
Fig. 5.4 Spatial distribution of zinc heavy metal in the MWPE. 239
Fig. 5.5 Spatial distribution of manganese heavy metal in the MWPE. 240
Fig. 5.6 Spatial distribution of copper heavy metal in the studied region. 241
Fig. 5.7 Mapping of nickel concentration in the marble waste polluted ecosystem. .242
Fig. 5.8 Cadmium heavy metal in the MWPE Khyber Pakhtunkhwa Pakistan.....243
Fig. 5.9 Spatial distribution of chromium heavy metal in the studied region. 244
Fig. 5.10 Spatial distribution potassium element in the studied region. 245
Fig. 5.11 Spatial distribution of phosphorous element in the studied area. 246
Fig. 5.12 Spatial autocorrelation of calcium. 248
Fig. 5.13 Spatial autocorrelation of chromium. 248
Fig. 5.14 Spatial autocorrelation of potassium 249
Fig. 5.15 Spatial autocorrelation of magnesium 249
Fig. 5.16 Spatial autocorrelation of manganese 250
Fig. 5.17 Spatial autocorrelation of zinc 250
Fig. 5.18 Spatial autocorrelation of CaCO3 251
Fig. 5.19 Spatial autocorrelation of cadmium 251
Fig. 5.20 Spatial autocorrelation of iron 252
Fig. 5.21 Spatial autocorrelation of nickel 252
Fig. 5.22 Spatial autocorrelation of phosphorous 253
Fig. 5.23 Spatial autocorrelation of copper 253
Fig. 5.24 Variation in NDVI between marble polluted and non-polluted. 254
Fig. 5.25 Mean Annual NDVI maps of the Khyber Pakhtunkhwa, Pakistan 256
Fig. 5.26 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner 262
Fig. 5.27 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner. 263
Fig. 5.28 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner. 264
Fig. 5.29 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner. 265
Fig. 5.30 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble. 266
Fig. 5.31 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble. 267
Fig. 5.32 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble. 268
Fig. 5.33 Impact of marble pollution on NDVI in the contaminated and non-.....269
Fig. 5.34 Impact of precipitation on NDVI in the marble contaminated and non. 270
Fig. 5.35 The influence of temperature on NDVI in the MWPE.....270
Fig. 5.36 SEM representing the impact of marble pollution, precipitation. 271

List of Tables

Table 2.1 Braun Blanquet cover classes with their mid points for herb and 30
Table 2.2 Composition of Malt extract agar media (mass/volume).....34
Table 2.3 Detail of plant species along with their habit, family, life.....38
Table 2.4 Summary of Ordinary Least Square, logistic and probabilistic models. 48
Table 2.5 Summary of Ordinary Least Square, logistic and probabilistic.....56
Table 2.6 Summary of DCA of all plant species and stations representing 57
Table 2.7 Summary of ecological gradient through CCA along with Monte.....60
Table 2.8 The summary of OLS, logit and probit models of abundant and 68
Table 2.9 Summary of DCA of all plant species and stations in semi humid. 69
Table 2.10 The summary of CCA of all the plants and stations in relation to.....72
Table 2.11 Ordinary Least Square, Logistic and Probabilistic. 80
Table 2.12 Showing eigenvalues, gradient length and cumulative percentage. 81
Table 2.13 CCA summary of all the plants and stations in relation to. 85
Table 2.14 Morphological and anatomical characteristics of fungal colonies.....89
Table 3.1 The topmost indicator species in relation to significant measured. 113
Table 3.2 The topmost indicator species with respect to significant. 118
Table 3.3 The foremost three indicators species along with significant. 123
Table 3.4 Other indicator species of the three identified subtropical MWPE,. 127
Table 3.5 Diversity indices of all the three subtropical vegetation zones. 131
Table 3.6 Summary of direct ecological gradient through Canonical. 132
Table 3.7 The detail of Structural Equation Modeling (SEM) linking. 135
Table 3.8 Model Fit Indices and their respective values after SEM in order 135
Table 3.9 Summary of SEM representing the direct, indirect and combined.....136
Table 3.10 SEM representing the impact of explanatory on the indicator. 139
Table 3.11 Result of model fit indices showing the goodness of model fit for. 140
Table 3.12 Summary of SEM representing the direct, indirect and combined.....140
Table 3.13 The detail of SEM linking indicators, climate, soil, elevation. 143
Table 3.14 The precise determination of model fit indices for the. 144
Table 3.15 The direct, indirect and combined impact of marble pollution,.....144
Table 3.16 The detail of SEM of identified plant indicators in the subtropical.....148
Table 3.17 Summary of Goodness of Model Fit of SEM of indicators. 148
Table 3.18 Summary of SEM representing the direct, indirect and.....149
Table 4.1 The indicator species evaluated for their phytoremediation ability.....170
Table 4.2 Detailed summary statistics. 177
Table 4.3 The concentration of heavy metals recorded. 185
Table 4.4 Biological Concentration Factor (BCF), Translocation Factor (TF). 187
Table 4.5 The concentration of heavy metals recorded (mg/kg) in the root.....189
Table 4.6 BCF, TF and BAC for measured heavy metals of indicator.....191
Table 4.7 Detailed description of Chlorophyll-a, b and total. 196
Table 4.8 Pattern of proline accumulation in the indicator plant in.....198
Table 4.9 The detailed description of mixed effect model. 202
Table 4.10 Mycoremediation ability of selected micro fungi after. 223
Table 5.1 Global Moran‘s Index (MI) summary of the measured variables. 247
Table 5.2 Detailed average NDVI in the marble polluted and non-polluted. 254
Table 5.3 NDVI at polluted (contaminated) and non-polluted plots 257

Table 5.4 Summary of SEM represents the impact of marble pollution,. 272
Table 5.5 The measured variables' direct and indirect effect in marble. 272
Table 5.6 Model Fit values after the SEM. 273

List of Appendix

Appendix table 1 Plant families along with species number and percentage. 313
Appendix table 2 List of plant species reported from Marble waste. 314
Appendix table 3 Plant families along with species number. 318
Appendix table 4 Summary of Species-area Curves of all the. 321
Appendix table 5 Indicator species Analysis indicating the topmost indicator. 325
Appendix table 6 Soil characteristics at zone wise 336
Appendix table 7 NDVI at factory /quadrat level 349

Publications arising to date from this Dissertation

The following papers have been published based on some results presented in the thesis:

1. **Ahmad, Z.,** S. M. Khan, and S. Page, 2021. Politics of the natural vegetation to balance the hazardous level of elements in marble polluted ecosystem through phytoremediation and physiological responses*, Journal of Hazardous Materials*, 414: 125451. **(Impact factor: 14.224)**

2. **Ahmad, Z.,** S. M. Khan, S. Page, S. Alamri and M. Hashem, 2021. Plants predict the mineral mines–A methodological approach to use indicator plant species for the discovery of mining sites. *Journal of Advanced Research*. 39: 119-133. **(Impact factor: 12.822)**

3. **Ahmad, Z.,** S. M. Khan, M. I Ali, N. Fatima and S. Ali, 2019. Pollution indicandum and Marble Waste Polluted Ecosystem; Role of selected indicator plants in phytoremediation and determination of pollution zones, *Journal of Cleaner Production*, 236, 117709. **(Impact factor: 11.072)**

4. **Ahmad, Z.** and Khan, S.M., Microbial Flora of Marble Waste-Polluted Environment in the Phylogenetic Perspectives. In Climate Change and Ecosystems (pp. 1-30). CRC Press. ***(Book Chapter)***

5. **Z. Ahmad,** S.M. Khan, S. Page, H. Balzter, Abdullah, S. Ali, S. Jehangir and U. Ejaz 2021. Environmental sustainability and resilience; Phytoremediation of heavy metals and plant physiological response in the Marble waste polluted ecosystem, *Journal of Cleaner Production* (Under Review / JCLEPRO-D-22-20848)

6. **Z. Ahmad** and S.M. Khan. 2022. Angiosperms Distribution in the Marble Waste Polluted Ecosystem and Driving Microclimatic Factors, *Journal of Hazardous Materials Advances* (Under Review / HAZADV-D-22-00358)

7. **Ahmad, Z.,** S. M. Khan and S. Ali, 2018. *Zonation of the Marble waste polluted ecosystem and assessment of their respective indicator species in District Buner, Pakistan.* 2nd International Conference on ***"Conservation of Medicinal and Aromatic plants for improving the livelihood of Mountain Communities through Industrial linkage***" organized by Center for Plant Sciences & Biodiversity (CPS & B), University of Swat in collaboration with Higher Education Commission (HEC), (September 03-05, 2018). ***(Abstract Published***)

8. **Ahmad, Z.,** S. M. Khan, M. I. Ali, N. Fatima and M. Iqbal, 2018. *Fungal diversity of the Marble Waste Polluted ecosystem in the Phylogenetic perspectives.* 7th International and 16th National Conference on ***"Plant resources: Current Trends, Challenges and Solutions"*** at Islamia College Peshawar and University of Peshawar, Pakistan, (March 23-26, 2018). ***(Abstract Published***)

Abstract

Marble industry is one of the major waste generating industries. Marble mining and processing result in environmental deterioration in the form of water, soil and air pollution. This dissertation aims to find out the physio-chemical properties of sampled vegetation and soil of the marble waste polluted ecosystems with emphasis on the role of specific indicators (plants and Fungi) in remediation of potential toxic elements (heavy metals) and NDVI changes in the marble polluted region of the Khyber Pakhtunkhwa province, Pakistan.

Quadrat quantitative ecological techniques were adapted for sampling of vegetation. All the collected data of plant species and environmental variables were analyzed using different statistical techniques including Two-way Cluster Analysis, Indicator Species Analysis, Ordinary Least Square, Logistic and Probabilistic Models, Species Area Curves, Bivariate Analysis, Detrended Correspondence Analysis, Canonical Correspondence Analysis and Structural Equation Modeling. Indicator species were determined using Indicator Species Analysis that exhibit more fortitude and contest against marble waste polluted ecosystem for each polluted zone. The identified plant indicators, its prestigious dignity concerning various environmental variables was then examined through direct gradient analysis i.e., Canonical Correspondence Analysis (CCA) and Structural Equation Modelling (SEM) for most suitable indicator species. Some of the selected indicators were further assessed for their phytoremediation ability and physiological response (i.e., proline osmolytes, chlorophyll-a, chlorophyll-b & total carotenoid contents) growing naturally in the subtropical Marble Waste Polluted Ecosystem (MWPE). Micro fungi were isolated, characterized and identified from the MWPE. Malt extract agar media was used for fungus growth and isolation of pure colonies. All the fungal isolates were characterized via Lacto-phenol cotton blue stain for their anatomical examination. The hyphae length and width of fungi were measured in micrometer (µm) using Piximeter software. Nuclear ribosomal Internal Transcribed Spacer (ITS) was amplified using ITS1-F and ITS4-R universal primers. ITS sequences were compared via BLAST network service of National Center for Biotechnology Information (NCBI) to affirm identification of micro fungus species. While, the spatial distribution of heavy metals (Potential toxic elements) was constructed using ArcGIS 10.8. Google Earth Engine (GEE) was used for the calculation/ extraction of NDVI from 1986 to 2021.

A total of 220 plant species belong to 164 genera and 65 different plant families were recorded from Subtropical vegetation zones of Khyber Pakhtunkhwa, Pakistan. TWCA classified all the stations and plants into three primary vegetation zones i.e., Humid, Semi-humid and Dry subtropical, based on Sorenson distance and Ward‘s linkage methods. The topmost indicators recorded for these vegetation zones were *Ficus carica*, *Catharanthus roseus* and *Erigeron canadensis* (Humid), *Morus nigra, Datura innoxia* and *Persicaria glabra* (Semi humid), *Dalbergia sissoo*,

Withania somnifera and *Saccharum bengalense* (Dry subtropical indicators) based on Indicator Values in the region. Out of 220 plant species, 19 indicator plants, i.e., *Adiantum capillus-veneris, Ailanthus altissima, Albizia lebbeck, Calotropis procera, Cynodon dactylon, Datura innoxia, Debregeasia salicifolia, Desmostachya bipinnata, Dodonaea viscosa, Erigeron bonariensis, Ficus carica, Morus alba, Morus nigra, Parthenium hysterophorus, Persicaria glabra, Ricinus communis, Setaria viridis, Tamarix aphylla* and *Withania somnifera* were the significant phytoextractor and phyto-stabilizer of potential toxic elements (Cr, Ni, Cu, Mn, Zn, Fe, Co, Cd, Ca and Mg) based on bioaccumulation coefficient, translocation and biological concentration factors. These indicators increase the accumulation of proline osmolyte and decrease chlorophyll-a, chlorophyll-b and total carotenoids as a defense or survival mechanism against marble waste polluted ecosystems. These results were reconfirmed through mixed effect modeling and bivariate regression. Preliminary results identified a total of six pure micro fungal isolates. Their molecular identification and phylogeny resulted *Aspergillus brasilensi*, *Aspergillus sydowii*, *Aureobasidium leucosper, Fusarium petroliphilum, Curvularia aeria* and *alternaria alternata* fungal species from marble wastewater polluted ecosystem. Morphologically most of these strains comprehended aseptate hyphae and black, brown, green, white to dark green colors. Whereas, anatomically these strains range from cylindrical to round, hyaline in lacto-phenol blue, thick to thin walled, smooth to ornamented surface with sharp scale and fusoid to ellipsoid in shape. Among the identified micro fungi *Aspergillus sydowii, Aspergillus brasilensi, Curvularia aeria* and *Alternaria alternata* showed a significant mycoremediation ability against marble pollution. Furthermore, a significant NDVI difference was found in the marble polluted and non-polluted regions. The non-polluted areas have higher NDVI than the marble polluted regions. The overall average NDVI in the marble polluted and non-polluted regions were 0.263 and 0.382, respectively.

It is concluded that the studied plant indicators and micro fungi of marble waste have a significant role in the remediation of Marble Waste Polluted Systems (MWPS). Increasing proline accumulation and decreasing chlorophyll contents with an increase in pollution in the studied plant show resistance of the biome/biosphere in response to the external abiotic Lithospheric toxicities. It is recommended that these plant species could be grown to remediate the Marble Waste Polluted Systems (MWPS) in the marble processing industries and its catchments.

1.1 Introduction

Marble is a metamorphic rock that is usually composed of recrystallized carbonate minerals, generally dolomite or calcite (Török et al. 2011). These rocks are formed during metamorphism when limestone is exposed to high heat and pressure. Most marbles are formed at boundaries of convergent plates where the maximum area of the Earth‘s crust is exposed to regional metamorphism. Some marbles are formed via contact metamorphism when hot magma heats neighboring limestone or dolomite. When formed from limestone, this marble will be white in color, with very rare impurities. Marbles that contain clay minerals, bituminous or iron oxide impurities can be gray, black, bluish, yellow, pink or green in color. These marbles contain the chemical compounds MgO, CaO, SiO_2, Fe_2O_3, TiO_2, P_2O_5, Na_2O and Al_2O_3 (Knoche et al. 1995). Marbles usually occur in large deposits that are geographically extensive and hundreds of meters thick. This property allows them to be mined on a broader scale, with some quarries and mines generating millions of tons of marble annually. Usually, marble is cut into various dimensions or made into crushed stone. The dimension stone is produced through sawing marble into different size pieces. These are used in buildings, paving, monuments, sculptures and other projects. While crushed stone is utilized as an aggregate in railroad beds, highways, building foundations and other constructions (Kore and Vyas 2016).

Marble rocks have many unique properties which are utilized for sculptures and as architectural building materials for beautification. The chemical properties of marble are also exploited in pharmaceuticals and in agriculture to decrease acidity of the soil. Marble products are also used in paint, papers and cosmetics due to their optical properties and low cost (André et al. 2014; Karaşahin and Terzi 2007). A high purity marble with a bright white color is very useful. It is mined, crushed, and processed to remove impurities. This powdered marble is used as a coloring agent, in whitewash, cosmetics, papers, plastics, putty, grout, filler in paint and other manufactured goods. Marble is also one of the most effective acid neutralization agents due to its high content of calcium carbonate. In a crushed form it is used for acid neutralization in lakes, streams, soils and in the chemical industry. Antacid medicines are helpful to people who suffer from acid indigestion and reflux. These medicines contain calcium

carbonate which is sometimes made from powdered marble. The solubility and low hardness of marble allows it to be used as a calcium additive in animal feeds especially for egg producing chickens and dairy cows. The marble has three Mohs hardness scale. Hence, it is easy to carve and useful for making sculptures, floor tiles, facing stones, architectural panels, windowsills and other ornamental objects.

1.1.1 Pollution caused by the Marble Industry

Where marble outcrops are being exploited, the marble industry plays an important role in the socioeconomic conditions of people and provides employment to hundreds of people within local communities. Nevertheless, the marble industry is also a source of environmental pollution. The primary causes of pollution are dust, noise, vibration and oil produced by or used at quarry sites (Aukour and Al-Qinna 2008a; Celik and Sabah 2008; Miliša et al. 2010). The marble industry in Pakistan wastes approximately 70 % of precious mineral resources during polishing, processing and mining processes (Ahmad et al. 2019; Gazi et al. 2012). Worldwide, about 40 % of marble wastes are generated in quarrying operation processes in the form of rock fragments. Such wastes are dumped into empty pits and river beds, and on roads and agriculture fields or pasture lands, resulting in wide ranging environmental pollution (Akbulut and Gürer 2007; Aukour and Al-Qinna 2008b; Mendoza et al. 2014). Marble pollution decreases topsoil permeability or porosity and hence limits the infiltration of water. It enhances soil alkalinity and decreases soil fertility. Mining and the processing of marbles result in environmental deterioration in the form of soil, air and water pollution. The dry marble powder or slurry has a high pH value causing lung and eye infections (El Haggar 2010).

When discarded into water reservoirs and bodies, marble waste affects surface water reserves (Vijayalakshmi et al. 2003) by adding potential toxic elements or heavy metals to the ecosystem. These can be removed by primary treatments and detoxification procedures (El-Maghraby et al. 2013). Marble waste dumped onto the land significantly impacts the local ecology and has a negative effect on the aesthetic of the natural environment. It directly or indirectly affects the composition of the flora and fauna of an ecosystem as well as causing other chemical and physical alterations of the environment (Yu et al. 2005) (Fig. 1.1).

Fig. 1.1 A view of a marble waste polluted ecosystem, Khyber Pakhtunkhwa, Pakistan.

1.1.2 Water used in the Marble Industry

Water is one of the most important natural resources consumed in the processing of marble stone. During marble processing i.e., cutting, cleaning, washing, cooling of saws and polishing, a large quantity of water is consumed, resulting in an equally large quantity of wastewater. The processing of one ton of marble stone results in one ton of slurry comprising 300 kg (30%) sawing dust and 700 kg (70%) water (Dhanapandian et al. 2009). Between 50 and 150 m^3 of water per day is required for an average size factory. The majority of marble industries are situated near to river sides, as a result of which waste from these units goes directly into the river where it causes pollution. Wastewater pollutants affect not only the aquatic flora and fauna but also downstream agricultural fields and crop production as well, because the river water is used for crop irrigation.

1.1.3 Impact of Marble pollution on biodiversity and the concept of indicator species

Marble industries and associated pollution cause a rapid change in the structure and function of the surrounding natural ecosystems. Plant and animal species experience various environmental issues in such polluted ecosystems. Some of these problems are slow to manifest, whereas others have an immediate effect (Bryson 1974). These changes may be serious for certain organisms which cannot adjust themselves to the changing conditions, especially for species relatively low genetic diversity and narrow ecological amplitude (Heath et al. 1993). The identification of indicator species is gaining more and more importance in the evaluation of environmental health. As most indicator species tolerate a limited range of environmental pollution, they can be used to assess the natural ecosystem health. In contrast, rare species with a narrow ecological tolerance are too sensitive to pollution and therefore only infrequently reflect the pollution response. Similarly, ubiquitous species possess a very broad tolerance and hence are likely to be less sensitive to pollution. Examples of numerous indicator species can be found in different environments; for example, lichens and bryophytes are commonly used to assess the air pollution (Dymytrova 2009; Larsen et al. 2007; Nash III and Wirth 1988). Use of indicator species differs from the classic chemical and physical measurements of environmental quality. The indicators add a temporal component corresponding to life span / residence time of an organism in a specific habitat. In addition, pollutants can occur in very low concentrations. Tedious analyses with high associated costs are required to detect such low levels of pollution. In contrast, the tolerance levels of indicator species can often provide a clear, low cost picture of a polluted environment. Furthermore, the indicator species rely upon the complex intricacies of ecosystems and can be used to convey a representative overview of a dynamic environment. Within this framework, indicator species monitoring in the natural environment can provide information on physical and chemical changes, ecological processes and biodiversity.

1.1.4 Role of Biodiversity in Pollution Reduction/ Bioremediation and Sustainable Environment

The degradation or removal of contamination has been achieved through various methods including chemical extraction, electrolytic techniques, ion exchange methods, precipitation, polymer micro encapsulation excavation and land filling (Antunes et al. 1998; Waltner-Toews 2001). Solidification, stabilization, flushing, soil washing, incineration, thermal desorption and extraction approaches have also been used to degrade or remove various types of pollutants (Gomez 2014; Oruru 2014). However, such types of approaches are relatively expensive especially for developing countries to apply on a larger scale (Moscoso et al. 2012).

In contrast, bioremediation or biological treatments are the best alternatives for the restoration or cleaning of polluted sites which are relatively achievable and low in cost (Autry and Ellis 1992; Cunningham et al. 1996; Vidali 2001). Such types of environmental friendly approaches have significant potential and can be used to degrade or remove contaminants from the polluted environment (Elliot et al. 2010). For example, the removal of petroleum from contaminated soil via the bioremediation procedure can save approximately 2.8 US$ per square meter. The cost benefits can increase up to 48 million US$ by applying enhanced bioremediation procedures for the removal of multiple types of pollutants (Romero et al. 2006; Verta et al. 1989).

Bioremediation is one of the most rapidly advancing fields of environmental science. It uses microorganisms in order to decrease and detoxify the harmful effects of various pollutants (Mani and Kumar 2014; Singh et al. 2011). It can also help to preserve the biodiversity of an ecological ecosystem and sustain life beyond the microbial flora (Srivastava and Vellend 2005). The microbial flora helps in the removal of pollutants from the environment via breakdown of complex molecules into simpler substances. Metabolic products in all such processes are then used by higher plants (Ali 2010; Shukla et al. 2010). Ecofriendly management procedures to utilize these pollutants via microorganisms can be identified and implemented. Phytoremediation is one of the strategies to restore polluted ecosystems at a low cost using plant species (Nascimento and Xing 2006; Wei et al. 2020; Yan et al. 2020). Certain plant species retain the inherent ability of bioaccumulation, translocation and degradation of different types of pollutants (Sepehri et al. 2020). They play a role as a

sink for biologically hazardous materials (Schwitzguébel 2017). Phytoremediation is a low cost technology driven by natural sunlight energy which takes place in situ, where the plants accumulate pollutants from the environment (Salt et al. 1998). Plants accumulate these pollutants (both organic and inorganic contaminants) in their tissues and extract out nutrients for their rapid growth (Anderson and Coats 1995).

1.1.5 Spatial distribution or GIS mapping

Mining and processing of marbles result in environmental deterioration in the form of soil, air and water pollution. Pollution adds certain types of heavy metals to the natural ecosystem, especially Fe, Mg, Ca, Na, Cu, Cr, Pb and Cd. Therefore, its spatial distribution mapping using GIS technology is a useful tool for accurate assessment of the status of heavy metal pollution. Spatial distribution or mapping is an arrangement of a phenomena on the Earth‘s surface. It is an important approach in geographical and environmental statistics. It can summarize the raw data directly or may reflect the outcome in more sophisticated forms. Different aspects of the data can be represented in a single graphical display using a suitable choice of various colors for differentiation. Such spatial data can help scientists in defining the regions or sites where pollution risk is high, thereby assisting decision makers in identifying the locations where efforts should be focused. The main benefit of a geostatistical approach is an unbiased estimation of the value of variables in unsampled regions through the application of interpolation (Goovaerts 1999; Webster and Oliver 2007). Complementary methods include the use of other measures of vegetation health. The normalized difference vegetation index (NDVI) provides a quantitative estimation from the surface reflectance of vegetation cover and biomass (Arabameri and Pourghasemi 2019). It monitors vegetation of an area from space in the visible and infrared portion of the spectrum (Bannari et al. 1995; Baret and Guyot 1991; Justice et al. 1986; Tucker et al. 1991; Tucker et al. 1985). NDVI has been recognized as a good indicator of vegetation productivity and therefore can also be used to understand the impact of pollution on vegetation (Wang et al. 2001). Hence, the combined use of a GIS, which provides resources to store, analyze and visualize spatial data, combined with the results from remote sensing and related innovations in computational specialist tools can provide a powerful method for land-based pollution assessment (Austin 2007; García et al. 2007).

1.1.6 Reclamation / Resilience in Marble waste polluted Ecosystem

Marble pollution has a destructive impact on the natural ecosystem. The absence of fertile topsoil is the main concern or issue in the marble waste polluted ecosystem. Therefore, the progress of natural vegetation process is very slow in the polluted regions. To prevent the consequences of marble pollution some remedial measures are essential for the reclamation or resilience of the degraded habitat in order to restore its natural condition and productivity. But the reclamation of marble polluted regions is often difficult due to their physical, chemical and biological characteristics. Selective planting of native, abundant and indicator species is desired in most cases along with the introduction of some local wild microorganisms. The restoration of these polluted sites by restoration plantings along with bioremediation and phytoremediation are essential for the natural environment resilience or restoration (Bini et al. 2017; Fiorentino et al. 2018).

1.1.7 Introduction to the Study area / Khyber Pakhtunkhwa Province

Pakistan as a custodian of the Hindu-Himalayan belt is of great geological importance. It is the home of the world‘s purest and finest grade marbles and granites. Globally, Pakistan is the 6th largest extractor of marble minerals and granite. According to Pakistan Stone Development Company (PASDEC), reservoirs containing 297 billion tons of marble and granite are present in Pakistan. While, according to Pakistan Federal Boards of Revenue Directorate of Training and Research, there are 160.2 million tons of the marble reserves in the country. Out of these, 158 million tons (98%) are present in the province of Khyber Pakhtunkhwa.

1.1.7.1 Location

The Khyber Pakhtunkhwa province is located in the northwestern part of the country at 31°49′–35°50′N latitude and 70°55′–71°47′E longitude. It covers a total area of 39,282 square miles (101,741 km^2) (Jan et al 2019) (Fig. 1.2). It is further divided into 35 districts.

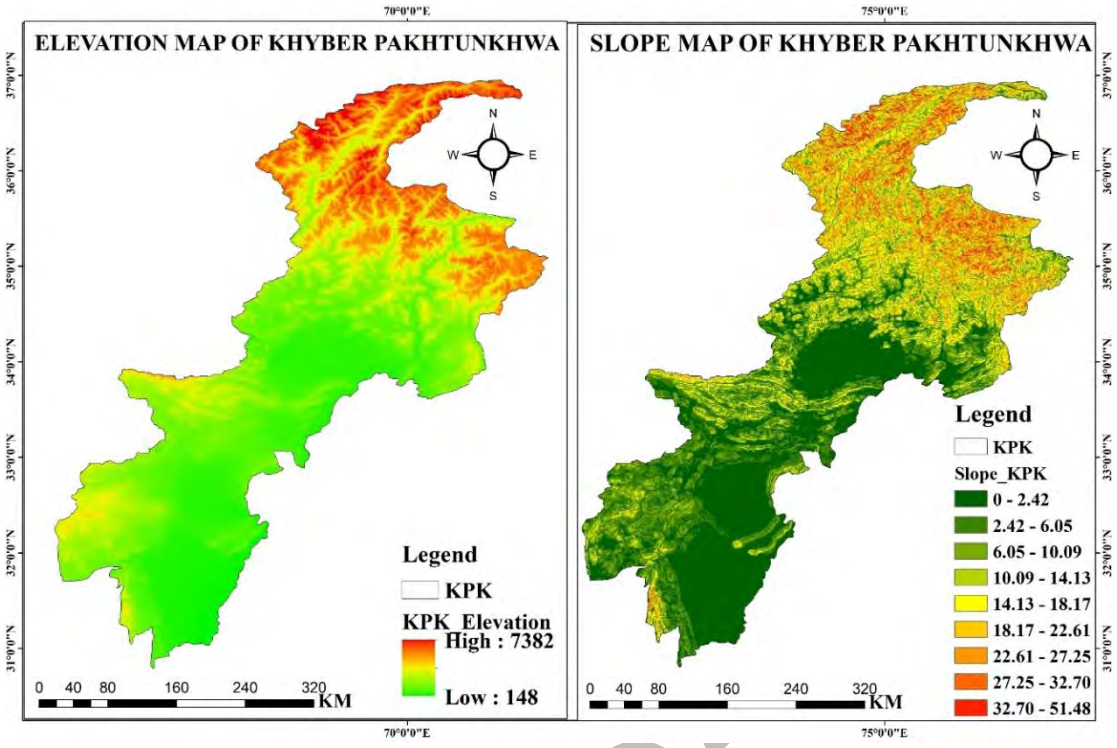


Fig. 1.2 Elevation and slope map of the Khyber Pakhtunkhwa province, Pakistan

1.1.7.2 Geographical boundaries

Geographically this province has a west and north boundary with Afghanistan. Azad Kashmir and Pakistani administrated areas of Kashmir (northern areas) are situated to the east and northeast sides, while Punjab and Baluchistan provinces are located to the southeast and southwest of the Khyber Pakhtunkhwa province. The Hindu Kush mountains lie on the north side of the province.

1.1.7.3 Terrain

The topography of the Khyber Pakhtunkhwa province is a mixture of rugged mountainous ranges, submontane areas of an undulating nature and plain areas surrounded by hills. The province has four topographical regions; the north-western mountainous zone extending to the Malakand region (where the Himalayan and Hindukush ranges meet), the north-eastern region of Hazara (extending to the Himalayan and Karakorum ranges), the central zone, and the southern zone (Gouleta 2015).

1.1.7.4 Geo-climate

Cold and snowy winters can be experienced in northern regions with heavy rainfall and short, pleasant summers. There is a very hot season during summer and moderate rainfall during winter. Extreme summer can be seen in the arid southern zone with relatively cold winters. The diverse landscapes of the province reflect varied amounts of precipitation. The average precipitation of the province is 406.4 mm (Rahman and Dawood 2018). Larger parts of the region experience a typically dry climate, but other parts (e.g. on the eastern periphery) receive monsoon rainfall from mid-June to mid-September, making these regions the wettest areas of Pakistan.

1.1.7.5 Brief history

The Khyber Pakhtunkhwa province is the product of a series of sacrifices of the inhabitants, who have been subject to the invasions of greater warriors over history. More than four thousand years ago, Aryans entered the region through the northern mountainous region. The Darius I (Persian) captured the Gandahara around 518 BC. The area then remained a hunting spot for various invaders including Greeks, Mauryans, the Bactrian Greeks, Scythians, the Kushanas, the White Huns, and the Guptas. By the tenth century the first Muslim king of Ghazni, Subuktagin, invaded Kabul which was then ruled by Hindu Shahiya kings. Subuktagin drove the Shahis down the country. Later this Frontier experienced a major transformation. Here, Turk descendants of earlier invaders and local Pashtun ethnic groups emerged as the dominant groups and replaced the former Hindu Shahi kings. During the Medieval period until 1818 the province remained part of the Muslim empire of India. The civil war started between various Pashtun tribes making them vulnerable to external attacks. The Sikh ruler of the Punjab, Ranjeet Singh, took full advantage of this opportunity and seized the areas of trans-Indus region, Dera Ismail Khan and Bannu, in 1818. Later, Sikhs conquered Peshawar in 1834, defeating the Pashtun at Nowshera. Fifteen years later, in 1849, the Sikhs were defeated, and the North-West Frontier districts became part of the British East India Company. The area remained part of Punjab until 1901. The viceroy of India, Lord Curzon, separated the five settled districts of, Bannu, Dera Ismail Khan, Hazara, Kohat, and Peshawar, and joined them to the five agencies of Kurram, Khyber, Malakand, North Waziristan, and South Waziristan, thus declared as a separate province: the Northwest Frontier

Province of India (Shah and Amjad 2011). After the division of the Indian sub-continent in 1947, NWFP became part of the newly made state of Pakistan. The name of the province, North West Frontier Province, remained until 15 April, 2010, when it was renamed as Khyber Pakhtunkhwa in 18th Amendment of the Constitution owing to the majority of Pashtuns in the region.

1.1.7.6 Education

The literacy rate of Khyber Pakhtunkhwa was 15.5% and 35.4% in 1972 and 1998 and it was 60%, 52%, 53% and 50% in the year 2012, 2013, 2014, 2015. According to the Pakistan Social and Living Standards Measurement level Survey 2019-20, the literacy rate of this province was 53%.

1.1.7.7 Population size

The province has a combination of both dense (urban centers) and less densely populated areas (mountains and countryside). During 1998, the estimated population of Khyber Pakhtunkhwa province was about 17,740 million. Majority of these population (14,740 million) lived in rural and minimum (3 million) lived in urban areas (Government of Khyber Pakhtunkhwa, 2015). The population was recorded as 24,700 million during 2011 (Gouleta 2015). The population of the province is 35 million with 52% males and 48% females, comprising of 11.9% of Pakistan's total population during 2021.

1.1.7.8 Economy

The Khyber Pakhtunkhwa has the 3rd largest economy of all provinces in Pakistan. It contributes 13% to gross domestic product (GDP) and 20% in mining output. Industries that support the economy of the province are mainly canning, preservation of fruits and vegetables, sugar refining, manufacturing, cotton textiles, furniture, cement, and tobacco processing. Other contributing industries include the mineral products including marble, limestone, gypsum and rock salt. Trade is another component helping the economy. The province dominates the economy in forestry and agriculture by generating heavy revenue. The economic growth of the province has been recorded with an annual growth rate of 4.5 %. Overseas remittances contribute 5% of GDP (Muhammad and Umar 2019).

1.1.7.9 Agriculture

Agriculture is the main important cash crop of the province. Different crops grown include wheat, maize, tobacco, rice, sugar beets, vegetables and fruits. In Khyber Pakhtunkhwa, the agriculture sector provides livelihoods to 85 percent of the population. Agriculture accounts for 14 percent of the provincial GDP and employs 37 percent of the labor force. The total cultivated area in Khyber Pakhtunkhwa is 1.6 million ha (7 percent of the country‘s total), half of which is rainfed. It produces about 75 percent of the country‘s tobacco, 17 percent of maize, 16 percent of barley, and 8 percent of sugarcane. However, the province is a net importer of agricultural produce and depends heavily on production from other provinces – especially from Punjab – for important food commodities such as wheat (64 percent import share), rice (74 percent), citrus (75 percent) and vegetables (90 percent). Promoting agricultural development and creating a vibrant rural economy is thus crucial for Khyber Pakhtunkhwa economic and social progress.

1.1.7.10 Ethnic diversity

Both cultural and linguistic diversity can be found in Khyber Pakhtunkhwa province. Diverse ethnic cultures of the province include Pashtuns, Hazarewal, Chitrali, Kalash, and Gujjars. Pashtuns are the dominant tribe of the province (more than 75%). Major Pashtun tribes include Afridi, Bangesh, Bannuchi, Bhittani, Daavi, Gandapur, Ghargasht, Khattak, Mohmand, Mahsud, Marwat, Orakzai, Qazi Khel, Wazir, and Yusufzai. While the non-Pashtun tribes living in the province are Abbasi, Syeds, Mughal, Jhut, Turks, and Rajputs. People mostly follow the jirga system to settle any kind of disputes. The inhabitants of the province are well known for their courtesy, hospitability, loyalty and bravery.

1.1.7.11 Languages

A majority of the residents speak the Pashto language which belongs to the Irani branch of the Aryan family of language. Other languages are spoken in the province including Hindko, Gojri, Persian, Khowar, Kalasha and Seraiki. Urdu, a national language, is used for communication purposes in academic institutions, while the English language is usually used for official correspondence.

1.1.7.12 Flora of Khyber Pakhtunkhwa

The diverse climate and unique landscape of Khyber Pakhtunkhwa regions has enriched the area with diverse flora. Even the desert plain of Dera Ismail Khan possesses a natural fertility which upon receiving heavy precipitation produces abundant crop and grasses (Shah and Amjad 2011). A variety of herbs, shrubs and trees are present in the region. Major tree species include *Pinus gerardiana, Cedrus deodara, Betula utilis, Thuja orientalis, Picea smithiana, Quercus sp.* and *Juglan regia* etc. Fruit trees include *Prunus malus, Prunus persica, Morus alba, Citrus sp.* etc.

1.1.7.13 Fauna of Khyber Pakhtunkhwa

The dense forests and rugged mountains provide shelter to diverse fauna in the area. Asiatic black bear, Eurasian lynx, Marmot, Indian leopard, Snow Leopard, Snow cock, snow partridge, and weasels (Sethi et al. 2020; Ullah et al. 2019) are present in different national parks of the province. Also, Markhor (*Capra falconeri*), national animal of Pakistan can be found profoundly in Chitral Gol National Park. A variety of fishes have also been reported from Khyber Pakhtunkhwa, including *Barilius vagra, B. Pakistanicus, Cyprinus Carpio, Labeo rohita, catla catla, Puntius ticto, P.sarana, P.sophore, Channa puntata,* and *C.striata* (Hasan et al. 2016).

1.1.7.14 Economic mineral resources of Khyber Pakhtunkhwa

Khyber Pakhtunkhwa Province hosts various metallogenic domains. The Hindu Kush Karakoram have vast mineral deposit including gold, arsenic, antimony, radioactive minerals, graphite, dolomite/limestone/ marble, polymetallic sulphides, coal, and gemstones. The Kohistan batholith which lies in the central part of arc consists of diorite, gabbro and granodiorite. The northern Indus suture represents obduction of the lower part of thick pile of thrust slice of ophiolitic rocks. The Kohistan terrain contains volcanic rocks throughout the Karakoram Suture along with traces of gold, antimony, zinc, copper and lead in its northern parts. Northern Indus Suture is characterizing the deposits of iron, serpentine, asbestos, peridot, chromite, magnesite, talc, soapstone, platinum and group of minerals associated with the gold. While Western Indus Suture contains deposits and showing of copper, lead-zinc, asbestos, chromite, fluorite, magnesite, iron, manganese, soapstone, talc, platinum, serpentine

and nickel. Khyber-Hazara that makes high metamorphic Zone contains beryl, fluorite, feldspar, galena, graphite, garnet, magnesite, magnetite, marble, quartz, scheelite, talc and gemstones. Gemstones present in this metamorphic zone include aquamarine, garnet, moonstone, peridot, pinktopaz, ruby, spessartine, and tourmaline. Whereas Khyber-Hazara low metamorphic Zone contains coal, phosphate, gypsum, iron, quartz, manganese, marble, and soapstone etc. Khyber Pakhtunkhwa and Ex-FATA include the Sulaiman Basin, Kohat Sub-Basin and interfingering of Sulaiman Basin with Kohat Sub-Basin in its southern part. The deposits include bentonite, bauxite/laterite, coal, clays, fuller‘s earth, fire clay, gypsum, glass sand, iron ore, lead-zinc ores, limestone/dolomite, potash salts, phosphate, manganese, radioactive minerals, rock salt sandstone, sulphur, oil shale, oil and gas (Malkani et al. 2017).

There has been extensive mining of both marble and granite in the province. Marble mining and processing industries are responsible for a heavy pollution loading on the natural environment. There is, therefore, a need to understand the impact of this pollution, particularly on the natural vegetation, and to identify plant and microbial indicators of pollution as a contribution to low-cost pollution assessment. With this in mind, the research hypotheses for this thesis are:

1.1.8 Research Rationale and Hypotheses

It is hypothesized that:

1. Marble waste polluted ecosystems host the Natural vegetation zones/associations with specific plant and micro fungus indicator species that can grow, survive and tolerate more successfully than others and can be utilized in managing such systems.
2. Indicator plants increase proline osmolyte and decrease chlorophyll contents as a defense response or survival mechanism against marble pollution.
3. An increase in marble waste pollution significantly decreases vegetation cover and plant health (green-ness) as measured by NDVI.
4. Proper land management and pollution reduction strategies may enhance the sustainability and remediation of the regions surrounding marble industries.

1.1.9 Aim of the study

The aim of this study is to find out the physio-chemical properties of sampled vegetation and soil of the marble waste polluted ecosystems with emphasis on the role of specific indicators (plants and Fungi) in remediation of potential toxic elements (heavy metals) and NDVI changes in the marble polluted region of the Khyber Pakhtunkhwa province, Pakistan.

1.1.10 Objectives of the study

1. To quantify phyto-sociological attributes through quantitative ecological techniques in the Marble Waste Polluted Ecosystem (MWPE) Khyber Pakhtunkhwa, Pakistan.
2. To explore the impact of Marble wastewater on plant species composition, distribution pattern, abundance and their indicators in various vegetation zones using ecological modelling approaches.
3. To isolate, characterize and identify micro fungi of the marble waste polluted ecosystem via biochemical tests, DNA based sequencing techniques and their phylogenetic relationship for possible future use.
4. To evaluate role of the plant & fungi indicators species in degradation of heavy metals in the marble wastes.
5. To determine physiological responses of indicators in the plant in terms of proline accumulation and chlorophyll contents in the vicinities of the marble industries.
6. To map heavy metal (potential toxic elements) concentrations present in marble waste polluted ecosystem of the Khyber Pakhtunkhwa province, using geo-informatics techniques.
7. To estimate temporal changes in the NDVI for the last forty years due to marble pollution in the studied area.
8. To recommend most fit indicator species for abatement of water pollution and bringing resilience in the ecosystem.

1.1.11 Thesis Structure

Keeping thesis aims and objectives in mind, the thesis consists of six chapters. Brief particulars of each chapter are as follows. Chapter 1 comprises a general introduction that provides an overview of marble, marble pollution, indicator species concept, reclamation of polluted sites, research hypotheses, aims and objectives. Chapters 2-5 have been written in the style and format of journal articles, each having an introduction, methods, data analyses, results, discussion and conclusion. Chapter 2 covers vegetation structure, composition, distribution pattern, dynamics and identification of micro fungi associated with the marble wastewater polluted ecosystem. Chapter 3 describes the detailed statistical procedure/methods for identifying indicator plants at the subtropical MWPE level in terms of marble pollution, climate, elevation and edaphic factors using indicator species analysis and structural equation modelling. Chapter 4 determines the phytoremediation ability of the naturally occurring indicator plant species *(identified in chapter 3)* and their physiological responses in terms of proline accumulation and reduction in chlorophyll contents in the vicinities of the marble waste polluted ecosystem. It also assesses the bioremediation ability of identified micro fungi for potential toxic elements remediation. Chapter 5 evaluates spatial distribution of heavy metals concentration present in MWPE and assessment of temporal changes in the NDVI for the last forty years in the region. Chapter 6 provides a discussion and synthesis with special reference to the main findings of the study and their comparison, conclusion and recommendations.

Chapter 2

Assessment of Vegetation and Micro Fungi in the Subtropical Marble Waste Polluted Ecosystem, Khyber Pakhtunkhwa, Pakistan

2.1 Introduction

Assessment of Vegetation and Micro Fungi was carried out to establish a baseline data for identification and further evaluation of indicators and best remediators of the pollutants.

2.1.1 Plant Biodiversity

Biodiversity is the term used for all forms of living organisms in an ecosystem. The term biodiversity refers to almost every aspect of the living world, applying across a wide range of spatial and temporal scales. It encompasses variability within individuals, communities, ecosystems and even at the trait and genetic levels (Mace et al. 2012; Öztürk et al. 2021). It focuses on flora of any ecological area, across numerous biogeographic regions (Gaston 2000). So, it is the priceless gift of nature that should be utilized wisely in order to conserve it for future generations.

Biodiversity is not only defined as the species number in an ecological region, but it is also the study of species richness with their relative abundance in a specific region (Hussain et al. 2022; Pielou 1977). It is the property of an area which refers to the differences among living organisms and their assemblage, the biotic processes acting on ecological communities, and the amount and specific structure of each. It can be restrained by scale, ranging from a specific microclimate habitat to the entire biosphere. Various types of geographic regions can be separated on the basis of biodiversity. Most of the time, edaphic and climatic ingredients have the strongest influence on plant species diversity (Hussain et al. 2022). Changes in biotic and abiotic factors can also result in changes in other associated components. Abiotic factors include elevation and soil texture (Laughlin and Abella 2007) as well as anthropogenic factors, including industrialization and other forms of human disturbance such as loss of forest cover (Namgail et al. 2012). Protecting biodiversity not only helps to conserve living species for future generations but may also lead to reductions in the rate of plant endangerment and extinction (Hoekstra et al. 2005).

2.1.2 Flora and vegetation

All plant species collectively make up the flora of a region (Ali 2008). The inventory of floras by plant researchers has been undertaken in many regions across the globe. Through such practices, sufficient data can be gathered and used as a reference for future studies. Vegetation differs from Flora. Vegetation is an assembly of plants growing together in a specific area. It is the name for the combined cover of plants of a particular locality (Jennings et al. 2004). Flora is the study of many species, while vegetation is the species distribution and abundance (Ali 2008; Badshah et al. 2013). Vegetation is made up of discrete and distinct plant communities as defined by Braun-Blanquet 1932; Clements 1916; Khan et al. 2013a).

Floral diversity defines all the plant species (including wild and cultivated) in a particular region. Floristic inventories assist in understanding different features of plants, soil and climate. They are a basic necessity for descriptive research in the field of ecology and can be used for displaying patterns of species diversity or understanding species distributions in relation to numerous factors (Khan et al. 2017a). Such studies are imperative for the evaluation of vegetation classification relative to its environments. This discipline has provided various methods to grasp vegetation taxonomy, which is helpful for the natural ecosystem conservation, quantification of ecosystem services and vegetation mapping (Ewald 2003; Khan 2012). Floristic studies come with numerous inspection tools and methods which are helpful at all levels (species, community and habitat level) (Huai and Pei 2004). Understanding the plant communities is a first necessity for the ecosystem ecology, natural resource management and conservation. Such information is mainly essential for studying rare or infrequent species and their management. These techniques make it easy for naturalists to determine species richness, abundance and diversity in an ecosystem. Such studies play their role in species conservation and also help in finding the indicators of a specific habitat/region. They can also give a clear picture of vegetation heterogeneity of a particular geographic area (Da Cunha and De Albuquerque 2006; Ullah et al. 2015). Furthermore, constancy, fidelity and frequency analyses specify the most threatened species in need of habitat protection (Baillie et al. 2004; Hester and Brooker 2007).

Research in the environmental sciences has increased in recent few decades, and has been important in describing the effects of rapid changes in the environment, e.g. as a result of human disturbances or climate change. Such variations can have serious consequences for those plants with low genetic assortment and limited ecological amplitude to cope with these varying conditions (Critchfield 1985; Davis et al. 2000). Some species adjust themselves against changing conditions through different strategies such as growth forms, their development, and changes in life cycle, which eventually can result in changes in structure and formation of plant communities. The ecologist must determine how environmental variation effects plant species, their composition and the structure of plant communities (Guisan and Zimmermann 2000; Økland and Eilertsen 1993).

2.1.3 Pollution

Industrial pollution directly and indirectly influences natural vegetation (Marini et al. 2007) and can bring about a rapid change in the ecosystem, especially in its structure, function and composition (Vitousek et al. 1997). Hence, there is an increasing demand for the proper development of various environmental principles and pollution controls, especially at the international level. The effect and degree of pollution in a wide range of environments has increased since the 1960s (Likens and Bormann 1974) and is now a main driver of environmental disturbance (Freedman and Hutchinson 1980). The effect of various pollutants on the environment is going to increase. It exerts pressure on the natural ecosystem that remains a serious problem (Cheevaporn and Menasveta 2003). The degradation of the forest community is a solid example of the adverse effects of pollution on the terrestrial ecosystems (Appannagari 2017).

2.1.4 Vegetation sampling through the Quadrat method

The quadrat method is widely employed by ecologists for vegetation sampling (Cox 1990). Usually, a Quadrat demarcates an area of a specific size, where one can count the number of plant species and their cover is estimated or recorded for each species. The establishment of a quadrat can be regular, random, or subjective in the study area. If the growth of plants is usually in clumps, the narrow and long sample plot is helpful. It can cover more plant species than round or square shaped quadrats of equal size (Barbour et al. 1987; Khan et al. 2011). Suitable quadrat size is one of the exigent

things for a sample plot that depends on certain factors. For example, the size bears no value if the cover is targeted for measurement purposes. But if the number of plant species is targeted, then the sample plot or quadrat size does matter, which can be selected through the minimal area method. All the phytosociological attributes can be determined using quadrat methods. Sampling plots with their plants having roots outside the quadrat's boundary will be included during measurements of cover when they have their canopy inside the sampling plot (Barbour et al. 1987). There are many more sampling techniques in the field of vegetation ecology. Based on unbiased sampling, there are three types of sampling mechanisms i.e., Simple, Stratified and Systematic.

2.1.5 Gradients analysis

There are two approaches to gradient analysis. The first approach is direct gradient analysis (Whittaker 1967) or ecological ordination (Austin 1968) whereby the vegetation data is investigated mathematically/ graphically in relation to environmental gradients. (Whittaker 1978; Whittaker and Niering 1965) were the first ecologists to use direct gradient analysis to analyze vegetation patterns along moisture and elevation gradients. This technique comprises various graphs for the proper interpretation of species distribution along ecological gradients.

The indirect gradient analysis is another approach used in vegetation ecology (Austin 1968; Whittaker 1978). Here, different mathematical methods are used to summarize the collected data. There are usually no a priori suppositions regarding the most essential ecological gradients that influence the vegetation. The results reveal which samples are at extremes and which are at the intermediate position in the environmental gradients. After this stage, different statistical tests are carried out, to highlight any significant correlations (Ahmad et al. 2019; Rasheed et al. 2021).

Ordination techniques are also of frequent use in vegetation science. The ordination approach is used to find out the pattern or tendencies in a multivariate data matrix. The main target is to reduce or turn the intricate data matrix into a few significant dimensions (Austin 1976; Whittaker 1978). The ordination method has been considered an essential tool or technique especially devised to analyze relationships between environmental gradients and vegetation. Local Nonmetric Multidimensional Scaling (LNMDS) and Detrended Correspondence Analysis (DCA) are the two

ordination methods used most frequently in the literature. The primary function of the ordination is to summarize the data regarding distinct units such as abundance data of species by generating a low dimension space of ordination. Similar samples and species are plotted so that they come close together while the dissimilar samples and species are placed far apart from each other. In general terms, ordination techniques are frequently used for a description of relationships between the ecological gradients and pattern of species composition that affect these patterns. Ordination can determine the species that are frequently found in association with each other and can further clarify how the composition of species in a distinct unit change with environmental gradient. The interpretation of patterns in the data sets is complicated, but the ordination helps the researcher to pinpoint this intricate pattern that is otherwise difficult or impossible to understand.

2.1.6 Isolation and Characterization of Microbes

Kingdom fungi represents a diverse group of eukaryotic organisms comprised of mushrooms and microbes like mold and yeast (Blackwell 2011; Taylor et al. 2006). Fungi have worldwide distribution and grow in a wide range of ecological environments. Approximately, there are 2.2-3.8 million fungal species of which only 5% have been described/ classified (Hawksworth and Lücking 2017). According to an early taxonomical studies, fungi were broadly classified based on their morphological i.e. spores color/microscopic and physiological characteristics (Petersen et al. 2015; Zhou et al. 2010). Nowadays, advances in molecular genetics has opened the way for modern taxonomic classification that challenged the historical grouping based on morphology and various other characters (da Cruz et al. 2018; Shimono et al. 2018; Takamatsu et al. 2018; Wheeler 2004). The phylogenetic studies based on molecular studies placed fungi into a separate kingdom that was sub divided into one subkingdom, seven phyla and ten sub phyla (Hawksworth and Iturriaga 2006; Okane and Ono 2018; Ruggiero et al. 2015; Shimono et al. 2018). It was kept apart from the reset of kingdoms due to chitin (a polymer of N-acetylglucosamine) in their cell wall, distinctive morphology, physiology, biochemical nature and solitarily molecular characteristics (Gilmore et al. 2013; Moore 2002). Fungi are crucial decomposers in any ecological ecosystem (Osono et al. 2011). They accomplish their indispensable role in decomposition of organic matter and have a pivotal part of the nutrient recycling and exchange with the environment (Shoji et al. 2006).

The isolation and identification of microbes is one of the most important steps in studying microbes. There are numerous practical applications for the identification of unknown microorganisms. The method used in diagnostic laboratories is mostly based on the phenotypic characteristics of microbes. Preliminary procedures include simple tests i.e., isolation in pure forms, staining reactions, morphological features, culture characteristics, metabolism, biochemical tests and growth on different type of culture media etc.

Molecular techniques are the major reliable tools for identification of microorganisms. They are one of the most effective and fastest technologies for the identification of microbes in various types of habitats. Organisms are identified from some unique part of their RNA or DNA providing definite information regarding their diversity. Molecular techniques have many advantages as compared to traditional methods which are not so appropriate for identification of microbial diversity. Fungi and bacteria are generally identified through 18S rRNA and 16S rRNA sequencing techniques. The rRNA (rDNA) is the most conserved region in all types of microbe cells due to hyper variable region. It is extensively used to determine phylogeny (evolutionary relationships), and taxonomy that estimates the rate of divergence in species. Primers are designed to bind with the conserved region to amplify the variable region. These types of sequences are extensively used for the identification of large numbers of species. The sequences from tens of thousands of isolates are available on the NCBI (National Center for Biotechnology Information) web link. The NCBI also provides the search algorithms to compare new sequences with those already available on the data base.

2.1.7 Justification

Rapid rates of industrialization and increasing human populations are increasing the demands for the protection of the natural environment from over exploitation and contamination. In some parts of northern Pakistan, a lack of adequate measures to treat marble pollutants has strongly affected the surrounding ecosystems in the form of physical and chemical changes that in turn harm the natural ecosystems as well as local human inhabitants who depend on those systems. Pollution affects people, animals and plants directly in the form of diseases and indirectly in the form of food safety and or habitat destruction. Marble industries have a deleterious effect on the

environment. In such situations there is a strong incentive for researchers to study the environmental issues arising from marble pollution, their impact on local flora, and to identify the different microbes associated with pollution.

2.1.8 Aims and objective

This chapter aims to find out the whole vegetation structure, composition, distribution pattern, dynamics and identification of micro fungi associated with the marble wastewater polluted ecosystem (MWPE) in the Khyber Pakhtunkhwa, Pakistan. The detailed research objectives are as follows:

1. To quantify phyto-sociological attributes through quantitative ecological techniques in the Marble Waste Polluted Ecosystem (MWPE) Khyber Pakhtunkhwa, Pakistan.
2. To explore the impact of Marble wastewater on plant species composition, distribution pattern, abundance and their indicators in various vegetation zones using ecological modelling approaches.
3. To isolate, characterize and identify micro fungi of the marble waste polluted ecosystem via biochemical tests, DNA based sequencing techniques and their phylogenetic relationship for possible future use.

2.2 Materials and Methods

Quadrat quantitative ecological techniques were used for the sampling of vegetation of Marble waste polluted ecosystem in the subtropical geographic zone, Khyber Pakhtunkhwa, Pakistan. The detailed description of materials and methods are as follows:

2.2.1 Vegetation sampling

The research work was carried out around the areas affected by pollution produced by the Marble industries located in the Khyber Pakhtunkhwa province, Pakistan (Fig. 2.1; Fig. 2.2;

Fig. 2.3). A total of 327 stations/marble factories were randomly selected during 2019-2020. Quadrat quantitative ecological techniques were implemented for the sampling of vegetation. A total of 981 quadrats were taken for the sampling of vegetation. At each marble factory different sizes of quadrats i.e., 100 m, 25 m and 1 m were taken for trees, shrubs and herbs vegetation, respectively. Phytosociological attributes i.e., cover, frequency, density, relative cover, relative frequency, relative density and importance value index, were measured for every plant species at each station. The cover and its relative values for tree species were calculated at the basal area of a stem through Diameter at Breast Height techniques. Basal area was calculated using formula = πr^2 (where r=radius) (Khan et al. 2014; Khan et al. 2013c; Khan et al. 2017b).

All the reported plant species were collected, appropriately tagged, placed in a newspaper and pressed in a plant presser (Ali and Nasir 1990; Ali and Qaiser 1995; Khan et al. 2013b; Khan et al. 2016). The Mercuric chloride and Ethyl alcohol solutions were utilized to poison specimens and, after that mounted on standard herbarium sheets. At last, all the plant specimens were identified with the help of Flora of Pakistan and other expert taxonomists (Nasir et al. 1972).

The geographical coordinates (longitude, latitude and elevation) for each subtropical station were recorded using GPS (Garmin etrex). A Geographical Information System (GIS) generated map was prepared for the sampling points using ArcGIS 10.5 software (Fu et al. 2013; Khan et al. 2016).

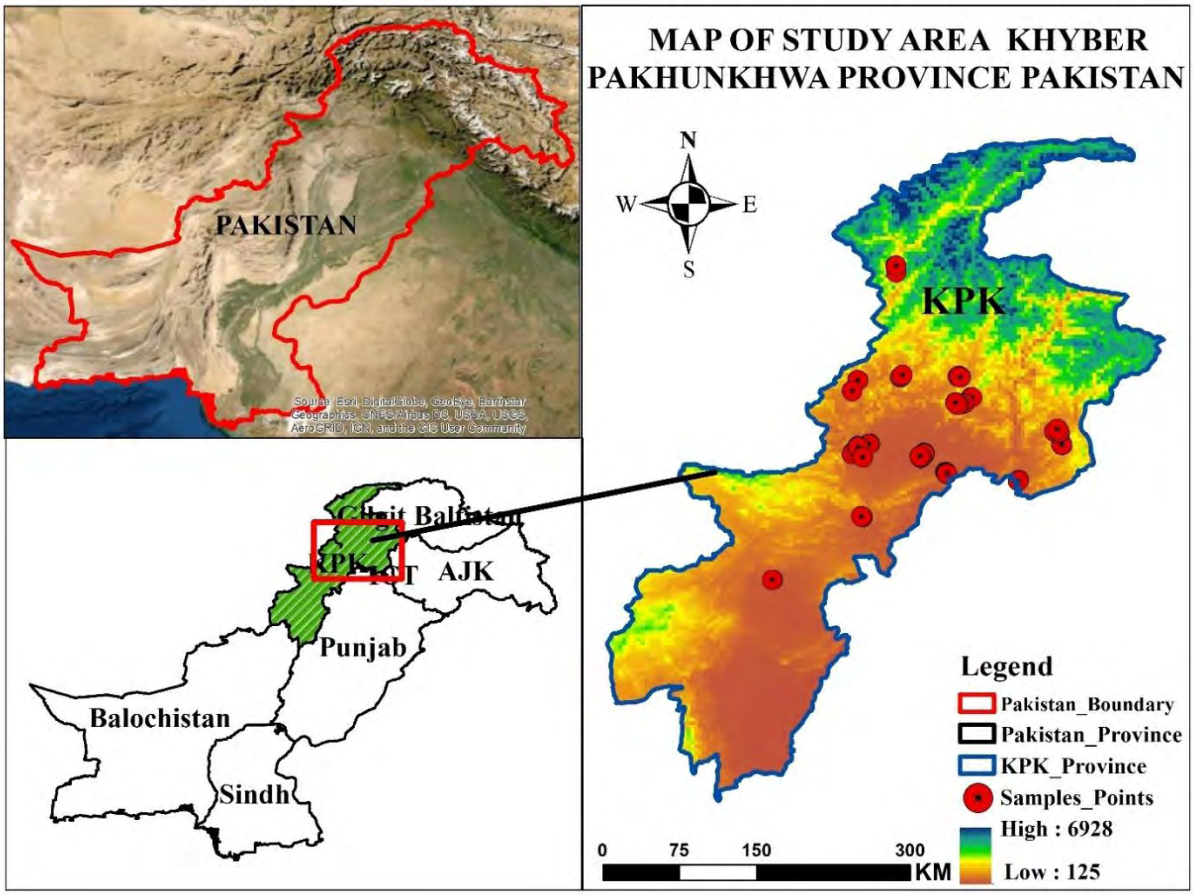


Fig. 2.1 Map of the study area showing the sampling points in subtropical zone of MWPE, Khyber Pakhtunkhwa, Pakistan.

Fig. 2.2 A glimpse of marble waste polluted ecosystem, KPK, Pakistan.

Fig. 2.3 The impact of Marble pollution on vegetation.

2.2.2 Physiological attributes

Vegetation data i.e. frequency, relative frequency, density, relative density, cover, relative cover and importance value index of each plant species in each quadrat were measured by using the following formulas:

2.2.2.1 Density

Density is the total numbers of individuals of a plant species in a sampled region. It was calculated according to (Khan 2012; Oosting 1956).

$$\text{Density (D)} = \frac{Total\ no.of\ individuals\ of\ a\ species\ found\ in\ a\ quadrat}{Total\ sampled/quadrat\ area} \ldots\ldots\ldots\ldots\ldots\ldots (i)$$

2.2.2.2 Relative density

Relative density is the % age distribution of an individual species in a sampled area.

$$\text{Relative Density (RD)} = \frac{Density\ of\ individuals\ plant\ species\ in\ a\ quadrat\ \times 100}{Total\ density\ of\ all\ plant\ species} \ldots\ldots\ldots (ii)$$

2.2.2.3 Frequency

The percentage of number of a quadrats/sampled stations in which a plant species is present is termed as frequency. It was calculated using the formula of (Cheevaporn and Menasveta 2003).

$$\text{Frequency (F)} = \frac{No.of\ quadrats\ in\ which\ a\ plant\ species\ present\ \times 100}{Total\ no.of\ quadrats\ taken} \ldots\ldots\ldots (iii)$$

2.2.2.4 Relative frequency

Relative frequency is the percentage frequency an individual species of the total frequencies.

$$\text{Relative Frequency (RF)} = \frac{Frequency\ of\ individual\ plant\ species\ \times 100}{Total\ frequency\ of\ all\ plant\ species} \ldots\ldots\ldots (iv)$$

2.2.2.5 Cover

It is the basal area occupied by herb, shrub or tree.

$$\text{Relative Frequency (RF)} = \frac{Total\ basal\ area\ of\ all\ individuals\ of\ a\ species}{Total\ quadrat/sampled\ area\ size} \ldots\ldots\ldots (v)$$

2.2.2.6 Relative cover

The percentage value obtained through division of total cover of an individual's plant species divided by total cover of all plant species is termed as relative cover.

$$\text{Relative Cover (RC)} = \frac{Cover\ of\ individual\ plant\ species\ \times 100}{Total\ cover\ of\ all\ plant\ species} \ldots\ldots\ldots (vi)$$

2.2.2.7 Basal area

The diameter of tree species was measured at Breast Height (in inches) using measuring tape at height of 4.5 feet above the ground.

$$CBH(cm) = CBH\ (inch) \times 2.54$$

According to the formula, values are required in meter to measure the basal area. The below conversion has been followed.

$$CBH(m) = \frac{CBH(cm)}{100}$$

To find the radius, the value of ‗r' was obtained by using the formula of circumference given below.

$$C = 2\pi r$$

This formula was rearranged as:

$$r = \frac{CBH(m)}{2\pi}$$

For the calculation of the basal area radius is required in square (r^2). The derivation of the r^2 is given as.

$$r^2 = r \times r$$

All the above-mentioned derivations was requisite for the below given formula of the basal area.

$$Basalarea\ (BA) = \pi r^2$$

2.2.2.8 Cover classes for herb and shrub species

The percentages of cover for herbs and shrubs were estimated and noted down in the field (Braun-Blanquet 1932). Percentage cover range and cover classes are given in below Table 2.1 along with their mid points.

Table 2.1 Braun Blanquet cover classes with their mid points for herb and shrub species.

Cover range (%)	Mid-point	Class
> 1%	0.5	Class ∞
1-5 %	3.0	Class 1
6-25 %	15.5	Class 2
26-50 %	38.0	Class 3
51-75 %	63.0	Class 4
75-100 %	88.0	Class 5

2.2.2.9 Importance value index (IVI)

The IVI of each plant species at each station were calculated according to (Khan 2012). The relative values of density, frequency and cover were added and then divided by 3. Mathematically it is given as:

$$\text{Importance Value Index (IVI)} = \frac{RD + RF + RC}{3} \ldots\ldots\ldots (vii)$$

2.2.3 Soil Data collection and Analysis

Soil samples were collected from all stations (in replicates) at a depth of 0.3m with a soil sampling instrument, put in polythene bags, labelled and dried at room temperature. The collected soil samples were analyzed for different physicochemical properties, including Electrical Conductivity (EC), pH, Total Dissolved Solids (TDS), Organic matter, $CaCO_3$, Potassium (K), Phosphorus (P), Manganese (Mn), Nickle (Ni), Cadmium (Cd), Chromium (Cr), Copper (Cu), Zinc (Zn), Iron (Fe), Magnesium (Mg) and Calcium (Ca). Soil EC, pH and TDS were determined following McLean methods (McLean 1982). Ten grams of well sieved and air-dried soil were homogenized in 50mL distilled water through a magnetic stirrer for sixty minutes (1h.). The solution was filtered using filter paper. The EC, pH and TDS were

determined using EC (Adwa AD3000), pH (Russel RL060P) and TDS meters. Organic matter concentration was analyzed using the method of (Tfaily et al. 2017). Whereas, $CaCO_3$ was determined in soil through the protocol (Chaney et al. 1982). The concentration of the elements K, P, Mn, Ni, Cu, Cd, Fe, Cr, Zn, Mg and Ca was analyzed using Atomic Absorption Spectrophotometry (Ahmad et al. 2019). One gram of sieved and dried sample was taken in a 250 mL conical flask. Ten mL of Per-chloric ($HCLO_4$) and Nitric acid (HNO_3) solution in a 1:3 ratio was added and placed for 24 hours. Soil samples were digested by placing on a hot plate at an initial temperature of 150 °C for 1 hour and a final 235 °C until the red fumes of nitric acid disappear and white fumes appear. The solution was filtered after cooling through filtered paper (Whatman No. 42) and 40 mL distilled water was added to raise its volume. The blank reagents were also prepared. The atomic absorption spectrophotometer VARIAN, AA240FS, was used for the aforementioned elemental analyses. The final elements concentration was obtained using the formula below:

$$\text{Element concentration(mg/kg)} = \text{AA reading} - \text{Blank reagents/sample weight (kg)} \times \text{volume raised} \times \text{df}$$

Where, AA= atomic absorption reading, df= dilution factor.

2.2.4 Climatic Data

The climatic data i.e., precipitation and temperature were obtained from the Metrological Department Government of Pakistan.

2.2.5 Statistical data Analyses

All the collected plant species, stations and environmental data of the marble waste polluted ecosystem were analyzed using different multivariate statistic software i.e., PC-ORD v.5, Canoco v 4.5, SPSS v.20, STATA v.14, and R v.4.0.2. Plant data along with environmental variables were arranged in Microsoft EXCEL work sheet for

further analyses. The detailed description of the subsequent analyses is provided below:

2.2.5.1 Species-area curves

PC-ORD version 5 was used to draw species area curves. This was performed to establish whether the sample sizes were adequate or not. Species area curves are mostly utilized in science of vegetation ecology to realize species composition in relation to sample size. Plant abundance data with Sorensen distance values were used to create species area curves at each subtropical zone of the marble waste polluted ecosystem.

2.2.5.2 Two-way Cluster Analysis

The Two-way Cluster Analysis of PCORD V5 was used to identify significant subtropical vegetation zones and distribution of plant species at individual level in each quadrat. This was based on pattern similarity index through Sorenson distance measurement and Wards Linkage Method (Ahmad et al. 2016b; Greig-Smith 1983; Khan et al. 2016).

2.2.5.3 Dominant and rare identification

The dominant and rare plant species of each habitat i.e., herb, shrub and tree species were identified based on higher and lower importance values index in the studied region as a whole and at zone level as well. Dominant and rare graphs or figures were drawn using R software.

2.2.5.4 Logit and Probit Model

The logit and probit model is also called the logistic and probabilistic model. It is used for the analysis of binary outcome variables. In this model we used non normal distribution of the probability in order to check why some of the plant species are abundant and some of them are rare in the MWEP. Hence, the binary outcome variables are abundant or rare. The explanatory variables included soil Electrical Conductivity (EC), pH, Total Dissolved Solids (TDS), Organic matter, $CaCO_3$, Potassium (K), Phosphorus (P), Manganese (Mn), Nickle (Ni), Cadmium (Cd), Chromium (Cr), Copper (Cu), Zinc (Zn), Iron (Fe), Magnesium (Mg), Calcium (Ca), temperature, precipitation and elevation. First, we hypothesized that there are overall factors influencing plant abundance of MWPE, KPK, Pakistan. The whole of the region was classified into three zones and each examined for the effect of explanatory

variables on plant species abundance and rarity. Binary dependent variable i.e., abundant/dominant and rare was taken as 1,0. The simple linear regression in our case did not give an appropriate result. Therefore, we adopted a specification that was designed to handle the specific requirement of the binary dependent variable. The probability of the observed model is:

$$\text{Probability } (DR = 1|xi, \beta) = 1 - Y(-xi\,\beta)$$

Where, Y is a continuous variable and β is the coefficient of the explanatory variables. The DR = 1 represent the abundance of plants, while DR = 0 showed rarity of plants. For rare plants we used the following probability function:

$$\text{Probability } (DR = 0|xi, \beta) = Y(-xi\,\beta)$$

To estimate the parameters of the model using the method of the probit and logit model we used the equation below:

$$l(\beta) = DR \log(1 - Y(-xi\,\beta) + (1 - DR) \log(Y(-xi\,\beta))$$

The following generalized model was used to establish the impact of soil (micro and macro elements), elevation and climate on plant abundance.

$$DR_i^* = xi\,\beta + \mu i$$

Where DR dependent variable (dominant and rare plant) xi indicate the explanatory variables of the model β showed the coefficient of the explanatory variables and μi is the unobserved error term of the model.

$$DRi = \{1 \text{if } DR > 0 \text{ and } 0 \text{ if } DR < 0$$

2.2.5.5 Detrended correspondence analysis (DCA) and Canonical correspondence analysis (CCA)

The indirect and direct gradient analysis i.e., DCA CCA was performed using CANOCO software to examine the significant relationships among plant species, stations/quadrats and environmental variables at each sort of subtropical MWPE. CCA analyzes plant relationships by multiple linear regression with environmental gradients and gives us an interpretable graphical presentation of species responses to environmental variables (Dufrêne and Legendre 1997; Ter Braak and Prentice 1988).

2.2.6 Isolation of Micro Fungi

Fungal populations in soil and wastewater of marble was isolated following the Harley and Prescott methods (Prescott et al. 1993). One hundred microliters sample solution was taken directly for fungal growth rather than making serial dilutions. Malt Extract Agar Media was prepared, autoclaved at 121˚C and cooled to around 50 ˚C (Table 2.2) (Ottow and Glathe 1968). In a Laminar Flow Hood, 20 ml of the agar media was poured into labelled petri dishes to prepare agar plates for fungal growth. 100 µl solutions were taken from each sample through a pipette and smeared on the surface of the agar plates. The petri plates were wrapped in clingfilm (Wiegand et al. 2008) and were kept at 28 ˚C for 48 -72 hours. Fungal colonies were formed after the incubation process. Isolation of pure fungal strains was done using hyphal tip isolation techniques.

Table 2.2 Composition of Malt extract agar media (mass/volume) for fungal growth.

S.NO.	Ingredients of Malt extract agar (for Fungi)	
1	Peptone	6gm
2	Malt extract	20gm
3	Agar	15gm
4	Dextrose	20gm
5	pH	5.5

2.2.7 Morphological identification of fungus culture

Shape, structure and type of each colony in terms of colony appearance and spore color were analyzed through observation. Lactophenol Cotton Blue staining procedure was carried out for further morphological identification. In the center of a clean microscopic glass slide a drop of Lactophenol Cotton Blue was stained and subsequently 2- 3 mm of fungal colony was taken via an inoculating loop, placed in the center of a slide and covered with a clean cover slip. All the 15 prepared slides were observed under 10X, 40X and 100X microscope lenses. Immersion oil was used for clear observation of microscopic characteristics under 100X lens. Color reactions of fungi tissues were noted with the help of Melzer‘s reagents, lactic acid and 5- 10 %

(w/v) KOH. The hyphae length and width of fungi were measured in micrometers (µm) using Piximeter software.

2.2.8 Molecular analyses of fungi

2.2.8.1 DNA Extraction

DNA extraction is an essential requirement of all molecular analyses and recombinant DNA manipulation. Fungal fresh cultures were grown for DNA extraction. DNA of selected fungal samples was extracted using the following standard protocol.

1. Three to 5 mg sample of each fungal strain was taken in Eppendorf tube, vortexed and centrifuged at 10000 rpm for 2 min at 4 ˚C.
2. The samples were ground in chilled condition in the solution of 2 to 3 ml of CTAB buffer.
3. Five to six beads of glass were added in Eppendorf tube and then vortexed.
4. Then 300 µl 10% (w/v) SDS was added into each Eppendorf tube and incubated for 45 minutes at 65 ˚C in a water bath.
5. All the samples were centrifuged for 15 to 20 min at 10000 rpm at 4 ˚C.
6. The supernatant was taken out and 500 µl (3ml) of sodium acetate was added to it and incubated at -20 ˚C for 20 min.
7. All the samples were again centrifuged for 5-6 min at 10000 rpm at 4˚C.
8. The supernatant was taken again into a fresh eppendorf tube, added to 500 µl of Isopropanol and incubated at room temperature for 5 minutes.
9. Moreover, samples were centrifuged for 10 minutes at 10000 rpm and supernatants were transferred into a new fresh Eppendorf tube.
10. The pellets obtained were washed with 70 % (v/v) ethanol for 2 minutes at 10000 rpm at 4 ˚C.
11. Ethanol layer was removed and 50 µl T.E. buffer was added and the sample was stored at -20 ˚C in the freezer.
12. DNA was run on one percent (w/v) agarose gel for confirmation through Gel electrophoresis.

2.2.8.2 DNA Sequencing

Determination of precise nucleotides sequences in a sample of DNA is termed as DNA sequencing. Nuclear ribosomal Internal Transcribed Spacer (ITS) was amplified

using ITS1-F and ITS4-R universal primers (Dentinger et al. 2010; White et al. 1990). ExoSAP-IT® was used to purify PCR products. BigDye® Terminator V 3.1 Cycle Sequencing Kit combined with primers in 10 µL reactions to performed dye-terminated unidirectional sequencing. Ethanol precipitation was used to clean sequencing reaction following the manufacturer‘s instructions, again suspended in 30 µL distilled water and run on ABI 3730 DNA sequencer in the Bejing Genomics Institute (BGI) Shenzen, China (Dentinger et al. 2010).

2.2.8.3 Computational editing of sequences & BLAST Analysis of ITS sequences

Basic Local Alignment Search Tool (BLAST) is a web-based program. It aligns the search of thousands of different types of sequences in the database to show homology with top match sequences. ITS sequences were matched/ compared via BLAST network service of National Center for Biotechnology Information (NCBI) to confirm identification. BioEdit software was used to edit and prepare the sequence where required and this was aligned through other sequences present in GenBnak via Muscle Alignment Tool. All the characters were simiarly weighted and gap positions were treated as missing data in aligned sequences.

2.2.8.4 Phylogenetic Analysis

Phylogenetic trees of each fungal species sequences were made separately. The Maximum Likelihood (ML) analysis was carried out via Molecular Evolutionary Genetic Analysis (MEGA 7) through default setting program like Jukes-Cantor Model and for ML Hauristic Nearest Neighbor Interchange (NNI) method (Tamura et al. 2011). One hundred bootstrapping replicates were performed for the analysis. The phylogenetic position of some selected fungi species were confirmed by making Maximum Parsimony Trees with bootstrapping.

2.3 Results

A total of 220 plant species belonging to 164 genera and 65 different plant families were recorded around marble factories of the Khyber Pakhtunkhwa, Pakistan. The detailed description and zone wise composition and distribution pattern are discussed step wise below:

2.3.1 Plant species composition

Habitat wise, the recorded plant species comprised of 145 herbs (65.9% of the total vegetation), 24 shrubs (10.9%) and 51 trees (23.18%) (

Fig. 2.4; Table 2.3). Family Poaceae was the leading family covering 12.7 % of the total vegetation followed by Asteraceae (7.27 %), Fabaceae (5 %), Amaranthaceae, Polygonaceae and Rosaceae (each with 4.55 % share), Solanaceae (4.09 %), Moraceae (3.64 %) and Brassicaceae (3.18 %) share. Euphorbiaceae has 2.73 % share, accompanied by Cucurbitaceae, Myrtaceae, Nyctaginaceae, Pteridaceae, Rutaceae and Verbenaceae each with 2.27 % share. The remaining plant families shared less than 2 % each of the total vegetation around Marble factories (Appendix Table 1).

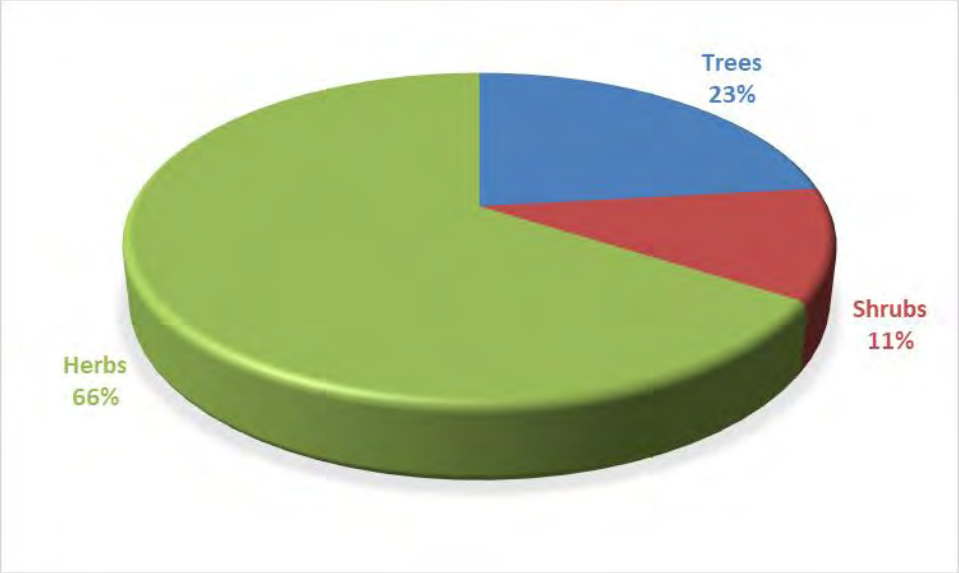


Fig. 2.4 Percentage distribution of herbs, shrubs and trees around marble factories of the Khyber Pakhtunkhwa, Pakistan.

Table 2.3 Detail of plant species along with their habit, family, life and leaf forms reported from the Subtropical zones around marble factories of the Khyber Pakhtunkhwa, Pakistan.

S. No.	Botanical Names	Habit	Family	Life Form	Leaf Form
1	*Acacia modesta* Wall	Tree	Fabaceae	Megaphanerophyte	Leptophyll
2	*Acacia nilotica* (L.) Delile	Tree	Fabaceae	Megaphanerophyte	Leptophyll
3	*Ailanthus altissima* (Mill.) Swingle	Tree	Simaroubaceae	Mesophanerophytes	Leptophyll
4	*Albizia lebbeck* (L.) Benth.	Tree	Fabaceae	Megaphanerophyte	Nanophyll
5	*Araucaria heterophylla* (Salisb.) Franco	Tree	Araucariaceae	Megaphanerophyte	Nanophyll
6	*Azadirachta indica* A.Juss.	Tree	Meliaceae	Megaphanerophyte	Microphyll
7	*Bombax ceiba* L.	Tree	Malvaceae	Megaphanerophyte	Mesophyll
8	*Broussonetia papyrifera* (L.) L'Hér. ex Vent	Tree	Moraceae	Megaphanerophyte	Megaphylls
9	*Callistemon lanceolatus* (Sm.) Sweet	Tree	Myrtaceae	Mesophanerophyte	Microphyll
10	*Celtis australis* L.	Tree	Cannabaceae	Megaphanerophyte	Microphyll
11	*Citrus aurantium* L.	Tree	Rutaceae	Microphanerophyte	Mesophyll
12	*Citrus limon* (L.) Osbeck	Tree	Rutaceae	Microphanerophyte	Mesophyll
13	*Citrus medica* L.	Tree	Rutaceae	Microphanerophyte	Mesophyll
14	*Citrus reticulata* Blanco	Tree	Rutaceae	Microphanerophyte	Mesophyll
15	*Citrus sinensis* (L.) Osbeck	Tree	Rutaceae	Microphanerophyte	Mesophyll
16	*Cupressus sempervirens* L.	Tree	Cupressaceae	Mesophanerophyte	Leptophyll
17	*Dalbergia sissoo* DC.	Tree	Fabaceae	Megaphanerophyte	Microphyll
18	*Diospyros lotus* L.	Tree	Ebenaceae	Microphanerophyte	Mesophyll
19	*Eriobotrya japonica* (Thunb.) Lindl.	Tree	Rosaceae	Mesophanerophyte	Mesophyll
20	*Eucalyptus camaldulensis* Dehnh.	Tree	Myrtaceae	Megaphanerophyte	Mesophyll
21	*Eucalyptus globulus* Labill.	Tree	Myrtaceae	Megaphanerophyte	Mesophyll
22	*Ficus benjamina* L.	Tree	Moraceae	Mesophanerophyte	Mesophyll
23	*Ficus carica* L.	Tree	Moraceae	Mesophanerophyte	Mesophyll
24	*Ficus macrophylla* Desf. ex Pers.	Tree	Moraceae	Mesophanerophyte	Mesophyll
25	*Ficus palmata* Forssk	Tree	Moraceae	Mesophanerophyte	Mesophyll
26	*Grevillea robusta* A.Cunn. ex R.Br.	Tree	Proteaceae	Mesophanerophyte	Microphyll
27	*Juglans regia* L.	Tree	Juglandaceae	Megaphanerophyte	Mesophyll
28	*Litchi chinensis* Sonn.	Tree	Sapindaceae	Mesophanerophyte	Mesophyll
29	*Mangifera indica* L.	Tree	Anacardiaceae	Megaphanerophyte	Mesophyll
30	*Morus alba* L.	Tree	Moraceae	Megaphanerophyte	Mesophyll
31	*Morus macroura* Miq.	Tree	Moraceae	Megaphanerophyte	Mesophyll
32	*Morus nigra* L.	Tree	Moraceae	Megaphanerophyte	Mesophyll

33	*Phoenix dactylifera* L.	Tree	Arecaceae	Mesophanerophyte	Mesophyll
34	*Pinus wallichiana* A.B.Jacks.	Tree	Pinaceae	Megaphanerophyte	Mesophyll
35	*Platanus orientalis* L.	Tree	Platanaceae	Megaphanerophyte	Mesophyll
36	*Populus alba* L.	Tree	Salicaceae	Megaphanerophyte	Mesophyll
37	*Populus ciliata* Wall. ex Royle	Tree	Salicaceae	Megaphanerophyte	Mesophyll
38	*Prosopis juliflora* (Sw.) DC.	Tree	Fabaceae	Microphanerophyte	Nanophyll
39	*Prunus armeniaca* L.	Tree	Rosaceae	Mesophanerophyte	Mesophyll
40	*Prunus domestica* L.	Tree	Rosaceae	Mesophanerophyte	Microphyll
41	*Prunus persica* (L.) Batsch	Tree	Rosaceae	Mesophanerophyte	Mesophyll
42	*Psidium guajava* L	Tree	Myrtaceae	Mesophanerophyte	Mesophyll
43	*Punica granatum* L.	Tree	Lythraceae	Microphanerophyte	Microphyll
44	*Pyrus communis* L.	Tree	Rosaceae	Mesophanerophyte	Mesophyll
45	*Robinia pseudoacacia* L.	Tree	Fabaceae	Mesophanerophyte	Mesophyll
46	*Salix tetrasperma* Roxb.	Tree	Salicaceae	Mesophanerophyte	Mesophyll
47	*Sapium sebiferum* (L.) Roxb.	Tree	Euphorbiaceae	Therophyte	Microphyll
48	*Syzygium cumini* (L.) Skeels	Tree	Myrtaceae	Megaphanerophyte	Mesophyll
49	*Tamarix aphylla* (L.) H.Karst.	Tree	Tamaricaceae	Mesophanerophyte	Laptophyll
50	*Tribulus pentandrus* Forssk.	Tree	Zygophyllaceae	Hemicryptophyte	Laptophyll
51	*Ziziphus jujuba* Mill.	Tree	Rhamnaceae	Microphanerophyte	Microphyll
52	*Bougainvillea spectabilis* Willd.	Shrub	Nyctaginaceae	Microphanerophyte	Microphyll
53	*Calotropis procera* (Aiton) Dryand	Shrub	Apocynaceae	Nanophanerophyte	Mesophyll
54	*Catharanthus roseus* (L.) G.Don	Shrub	Apocynaceae	Chamaephyte	Microphyll
55	*Cestrum nocturnum* L.	Shrub	Solanaceae	Nanophanerophyte	Mesophyll
56	*Combretum indicum* (L.) DeFilipps	Shrub	Combretaceae	Phanerophytes	Mesophyll
57	*Datura innoxia* Mill.	Shrub	Solanaceae	Therophyte	Mesophyll
58	*Datura metel* L.	Shrub	Solanaceae	Therophyte	Mesophyll
59	*Debregeasia saeneb* (Forssk.) Hepper & J.R.I.Wood	Shrub	Urticaceae	Microphanerophyte	Mesophyll
60	*Dodonaea viscosa* (L.) Jacq	Shrub	Sapindaceae	Nanophanerophyte	Microphyll
61	*Duranta stenostachya* Tod	Shrub	Verbenaceae	Nanophanerophyte	Microphyll
62	*Duranta erecta* L	Shrub	Verbenaceae	Nanophanerophyte	Microphyll
63	*Indigofera heterantha* Brandis	Shrub	Fabaceae	Nanophanerophyte	Nanophyll
64	*Lantana camara* L	Shrub	Verbenaceae	Nanophanerophyte	Microphyll
65	*Nannorrhops ritchieana* (Griff.) Aitch.	Shrub	Arecaceae	Microphanerophyte	Megaphylls
66	*Parthenocissus inserta* (A.Kern.) Fritsch	Shrub	Vitaceae	Nanophanerophyte	Microphyll
67	*Ricinus communis* L.	Shrub	Euphorbiaceae	Microphanerophyte	Microphyll
68	*Rosa indica* L.	Shrub	Rosaceae	Nanophanerophyte	Microphyll
69	*Rosa webbiana Wall. ex Royle*	Shrub	Rosaceae	Nanophanerophyte	Microphyll
70	*Rubus fruticosus L.*	Shrub	Rosaceae	Nanophanerophyte	Microphyll
71	*Rumex hastatus D. Don*	Shrub	Polygonaceae	Chamaephyte	Microphyll

72	*Senna occidentalis (L.) Link*	Shrub	Fabaceae	Therophyte	Microphyll
73	*Vitis vinifera L.*	Shrub	Vitaceae	Microphanerophyte	Mesophyll
74	*Withania somnifera (L.) Dunal*	Shrub	Solanaceae	Nanophanerophyte	Microphyll
75	*Ziziphus nummularia (Burm.f.) Wight & Arn.*	Shrub	Rhamnaceae	Nanophanerophyte	Microphyll
76	*Achyranthes aspera L*	Herb	Amaranthaceae	Therophyte	Mesophyll
77	*Acrachne racemosa (B.Heyne ex Roth) Ohwi*	Herb	Poaceae	Therophyte	Microphyll
78	*Adiantum capillus-veneris L*	Herb	Pteridaceae	Hemicryptophyte	Nanophyll
79	*Adiantum incisum Forssk.*	Herb	Pteridaceae	Hemicryptophyte	Nanophyll
80	*Adiantum venustum D. Don*	Herb	Pteridaceae	Hemicryptophyte	Nanophyll
81	*Aerva javanica (Burm.f.) Juss. ex Schult.*	Herb	Amaranthaceae	Chamaephyte	Microphyll
82	*Aloe vera (L.) Burm.f*	Herb	Xanthorrhoeaceae	Nanophanerophyte	Microphyll
83	*Amaranthus retroflexus L*	Herb	Amaranthaceae	Therophyte	Microphyll
84	*Amaranthus spinosus L*	Herb	Amaranthaceae	Therophyte	Microphyll
85	*Amaranthus viridis L*	Herb	Amaranthaceae	Therophyte	Microphyll
86	*Apluda mutica L*	Herb	Poaceae	Hemicryptophyte	Microphyll
87	*Aristida adscensionis L.*	Herb	Poaceae	Hemicryptophyte	Microphyll
88	*Artemisia vulgaris L.*	Herb	Asteraceae	Chamaephyte	Microphyll
89	*Artemisia persica Boiss*	Herb	Asteraceae	Chamaephyte	Microphyll
90	*Artemisia scoparia Waldst. & Kitam.*	Herb	Asteraceae	Chamaephyte	Microphyll
91	*Arundo donax L.*	Herb	Poaceae	Nanophanerophyte	Mesophyll
92	*Asparagus racemosus Willd.*	Herb	Asparagaceae	Nanophanerophyte	Laptophyll
93	*Bidens bipinnata L.*	Herb	Asteraceae	Therophyte	Microphyll
94	*Bidens pilosa L.*	Herb	Asteraceae	Therophyte	Microphyll
95	*Boerhavia diandra L.*	Herb	Nyctaginaceae	Hemicryptophyte	Nanophyll
96	*Boerhavia diffusa L.*	Herb	Nyctaginaceae	Hemicryptophyte	Nanophyll
97	*Boerhavia procumbens Banks ex Roxb*	Herb	Nyctaginaceae	Hemicryptophyte	Nanophyll
98	*Brachiaria ramosa (L.) Stapf*	Herb	Poaceae	Therophytes	Microphyll
99	*Brassica campestris*	Herb	Brassicaceae	Therophytes	Mesophyll
100	*Brassica nigra (L.) K.Koch*	Herb	Brassicaceae	Therophytes	Mesophyll
101	*Campsis radicans (L.) Seem.*	Herb	Bignoniaceae	Microphanerophyte	Microphyll
102	*Canna indica L.*	Herb	Cannaceae	Hemicryptophyte	Mesophyll
103	*Cannabis sativa L.*	Herb	Cannabaceae	Therophytes	Microphyll
104	*Capsella bursa-pastoris (L.) Medik*	Herb	Brassicaceae	Therophytes	Nanophyll
105	*Capsicum annuum L.*	Herb	Solanaceae	Therophytes	Microphyll
106	*Cheilanthes acrostica (Balb.) Tod.*	Herb	Pteridaceae	Hemicryptophyte	Nanophyll
107	*Chenopodium album L.*	Herb	Amaranthaceae	Therophytes	Microphyll
108	*Chenopodium murale L.*	Herb	Amaranthaceae	Therophytes	Microphyll

109	*Chrozophora tinctoria (L.) A.Juss.*	Herb	Euphorbiaceae	Therophytes	Microphyll
110	*Citrullus lanatus (Thunb.) Matsum. & Nakai*	Herb	Cucurbitaceae	Therophytes	Microphyll
111	*Cleome viscosa L*	Herb	Cleomaceae	Therophytes	Microphyll
112	*Colocasia esculenta (L.) Schott*	Herb	Araceae	Geophytes	Megaphylls
113	*Commelina albescens Hassk.*	Herb	Commelinaceae	Geophytes	Microphyll
114	*Commelina benghalensis L.*	Herb	Commelinaceae	Geophytes	Microphyll
115	*Convolvulus arvensis L*	Herb	Convolvulaceae	Therophyte	Microphyll
116	*Corchorus olitorius L.*	Herb	Malvaceae	Therophyte	Microphyll
117	*Cortaderia selloana (Schult. & Schult.f.) Asch. & Graebn.*	Herb	Poaceae	Hemicryptophyte	Microphyll
118	*Cucumis melo var agrestis*	Herb	Cucurbitaceae	Therophyte	Microphyll
119	*Cucurbita maxima Duchesne*	Herb	Cucurbitaceae	Therophyte	Megaphylls
120	*Cymbopogon citratus (DC.) Stapf*	Herb	Poaceae	Chamaephyte	Microphyll
121	*Cynodon dactylon (L.) Pers.*	Herb	Poaceae	Hemicryptophytes	Nanophyll
122	*Cynoglossum lanceolatum Forssk.*	Herb	Boraginaceae	Hemicryptophytes	Microphyll
123	*Cyperus difformis L.*	Herb	Cyperaceae	Geophytes	Microphyll
124	*Cyperus rotundus L.*	Herb	Cyperaceae	Geophytes	Microphyll
125	*Dactyloctenium aegyptium (L.) Willd*	Herb	Poaceae	Therophyte	Nanophyll
126	*Desmostachya bipinnata (L.) Stapf*	Herb	Poaceae	Hemicryptophyte	Microphyll
127	*Dichanthium annulatum (Forssk.) Stapf*	Herb	Poaceae	Hemicryptophyte	Nanophyll
128	*Dicliptera bupleuroides Nees*	Herb	Acanthaceae	Chamaephyte	Microphyll
129	*Digera muricata (L.) Mart.*	Herb	Amaranthaceae	Therophyte	Microphyll
130	*Digitaria ciliaris (Retz.) Koeler*	Herb	Poaceae	Therophyte	Microphyll
131	*Dryopteris stewartii Fraser-Jenk.*	Herb	Dryopteridaceae	Hemicryptophyte	Microphyll
132	*Dysphania ambrosioides (L.) Mosyakin & Clemants*	Herb	Amaranthaceae	Therophyte	Mesophyll
133	*Dysphania nepalensis (Link ex Colla) Mosyakin & Clemants*	Herb	Amaranthaceae	Therophyte	Mesophyll
134	*Echinochloa colona (L.) Link*	Herb	Poaceae	Therophyte	Microphyll
135	*Eleusine indica (L.) Gaertn.*	Herb	Poaceae	Therophyte	Microphyll
136	*Emex spinosa (L.) Campd*	Herb	Polygonaceae	Therophyte	Microphyll
137	*Epipremnum aureum (Linden & André) G.S.Bunting*	Herb	Araceae	Chamaeophyte	Microphyll
138	*Equisetum arvense L.*	Herb	Equisetaceae	Hemicryptophyte	Aphyllous
139	*Erigeron bonariensis L.*	Herb	Asteraceae	Therophyte	Nanophyll
140	*Erigeron canadensis L.*	Herb	Asteraceae	Chamaeophyte	Mesophyll
141	*Euphorbia helioscopia L.*	Herb	Euphorbiaceae	Therophyte	Nanophyll
142	*Euphorbia hirta L.*	Herb	Euphorbiaceae	Therophyte	Nanophyll
143	*Euphorbia prostrata Aiton*	Herb	Euphorbiaceae	Therophyte	Laptophyll

144	*Fragaria nubicola (Lindl. ex Hook.f.) Lacaita*	Herb	Rosaceae	Hemicryptophyte	Microphyll
145	*Fragaria vesca L.*	Herb	Rosaceae	Hemicryptophyte	Microphyll
146	*Helianthus annuus L.*	Herb	Asteraceae	Therophyte	Mesophyll
147	*Heliotropium europaeum L.*	Herb	Boraginaceae	Therophyte	Microphyll
148	*Heliotropium strigosum Willd*	Herb	Boraginaceae	Therophyte	Nanophyll
149	*Ipomoea purpurea (L.) Roth*	Herb	Convolvulaceae	Therophyte	Mesophyll
150	*Iris hookeriana Foster*	Herb	Iridaceae	Hemicryptophyte	Mesophyll
151	*Jasminum sambac (L.) Aiton*	Herb	Oleaceae	Nanophanerophyte	Mesophyll
152	*Juncus maritimus Lam.*	Herb	Juncaceae	Hemicryptophyte	Microphyll
153	*Lepidium didymum L.*	Herb	Brassicaceae	Therophyte	Nanophyll
154	*Lepidium sativum L.*	Herb	Brassicaceae	Therophyte	Nanophyll
155	*Luffa cylindrica (L.) M.Roem.*	Herb	Cucurbitaceae	Therophyte	Megaphylls
156	*Malva sylvestris L.*	Herb	Malvaceae	Therophyte	Mesophyll
157	*Malvastrum coromandelianum (L.) Garcke*	Herb	Malvaceae	Chamaeophyte	Microphyll
158	*Medicago polymorpha L.*	Herb	Fabaceae	Therophyte	Nanophyll
159	*Mentha arvensis L.*	Herb	Lamiaceae	Hemicryptophyte	Nanophyll
160	*Mentha longifolia (L.) L.*	Herb	Lamiaceae	Hemicryptophyte	Microphyll
161	*Mentha royleana Wall. ex Benth.*	Herb	Lamiaceae	Hemicryptophyte	Microphyll
162	*Mirabilis jalapa L.*	Herb	Nyctaginaceae	Geophytes	Mesophyll
163	*Momordica charantia L.*	Herb	Cucurbitaceae	Therophyte	Microphyll
164	*Musa paradisiaca L*	Herb	Musaceae	Cryptophyte	Megaphyll
165	*Nasturtium officinale R.Br.*	Herb	Brassicaceae	Therophyte	Microphyll
166	*Nepeta laevigata (D.Don) Hand.-Mazz.*	Herb	Lamiaceae	Hemicryptophyte	Microphyll
167	*Oenothera rosea L'Hér. ex Aiton*	Herb	Onagraceae	Therophyte	Nanophyll
168	*Opuntia dillenii (Ker Gawl.) Haw*	Herb	Cactaceae	Nanophanerophyte	Aphyllous
169	*Oxalis corniculata L*	Herb	Oxalidaceae	Hemicryptophyte	Nanophyll
170	*Parthenium hysterophorus L.*	Herb	Asteraceae	Therophyte	Microphyll
171	*Parthenocissus quinquefolia (L.) Planch.*	Herb	Vitaceae	Nanophanerophyte	Microphyll
172	*Paspalum distichum L.*	Herb	Poaceae	Hemicryptophyte	Microphyll
173	*Persicaria barbata (L.) H.Hara*	Herb	Polygonaceae	Hemicryptophyte	Microphyll
174	*Persicaria glabra (Willd.) M.Gómez*	Herb	Polygonaceae	Hemicryptophyte	Microphyll
175	*Persicaria hydropiper (L.) Delarbre*	Herb	Polygonaceae	Hemicryptophyte	Microphyll
176	*Persicaria maculosa Gray*	Herb	Polygonaceae	Hemicryptophyte	Microphyll
177	*Phalaris minor Retz.*	Herb	Poaceae	Therophyte	Microphyll
178	*Phyla nodiflora (L.) Greene*	Herb	Verbenaceae	Hemicryptophyte	Nanophyll
179	*Physalis divaricata D. Don*	Herb	Solanaceae	Therophyte	Microphyll
180	*Plantago lanceolata L.*	Herb	Plantaginaceae	Hemicryptophyte	Microphyll
181	*Plantago major L.*	Herb	Plantaginaceae	Hemicryptophyte	Mesophyll

182	*Poa annua L.*	Herb	Poaceae	Therophyte	Nanophyll
183	*Poa bulbosa L.*	Herb	Poaceae	Therophyte	Nanophyll
184	*Polygonum aviculare L.*	Herb	Polygonaceae	Hemicryptophyte	Nanophyll
185	*Polygonum plebeium R.Br.*	Herb	Polygonaceae	Hemicryptophyte	Laptophyll
186	*Polypogon monspeliensis (L.) Desf.*	Herb	Poaceae	Therophyte	Microphyll
187	*Portulaca grandifloraL.*	Herb	Portulacaceae	Therophyte	Nanophyll
188	*Portulaca oleracea L.*	Herb	Portulacaceae	Therophyte	Nanophyll
189	*Pteris cretica L.*	Herb	Pteridaceae	Hemicryptophyte	Microphyll
190	*Ruellia simplex C.Wright*	Herb	Acanthaceae	Chamaeophyte	Microphyll
191	*Rumex nepalensis Spreng.,*	Herb	Polygonaceae	Therophyte	Nanophyll
192	*Rumex dentatus L.*	Herb	Polygonaceae	Therophyte	Mesophyll
193	*Saccharum bengalense Retz.*	Herb	Poaceae	Nanophanerophyte	Mesophyll
194	*Saccharum spontaneum L.*	Herb	Poaceae	Nanophanerophyte	Mesophyll
195	*Sagittaria sagittifolia L.*	Herb	Alismataceae	Geophytes	Mesophyll
196	*Sesbania sesban (L.) Merr.*	Herb	Fabaceae	Therophyte	Nanophyll
197	*Setaria pumila* (Poir.) Roem.	Herb	Poaceae	Therophyte	Microphyll
198	*Setaria verticillata (L.) P.Beauv.*	Herb	Poaceae	Therophyte	Microphyll
199	*Setaria viridis (L.) P.Beauv.*	Herb	Poaceae	Therophyte	Microphyll
200	*Sisymbrium irio L.*	Herb	Brassicaceae	Therophyte	Microphyll
201	*Solanum americanum Mill.*	Herb	Solanaceae	Chamaeophyte	Microphyll
202	*Solanum lycopersicum L.*	Herb	Solanaceae	Therophyte	Microphyll
203	*Solanum surattense Burm. f.*	Herb	Solanaceae	Therophyte	Microphyll
204	*Sonchus asper (L.) Hill*	Herb	Asteraceae	Therophyte	Microphyll
205	*Sonchus oleraceus (L.) L.*	Herb	Asteraceae	Therophyte	Microphyll
206	*Sorghum bicolor (L.) Moench*	Herb	Poaceae	Therophyte	Mesophyll
207	*Sorghum halepense (L.) Pers.*	Herb	Poaceae	Hemicryptophyte	Microphyll
208	*Tagetes erecta L.*	Herb	Asteraceae	Therophyte	Microphyll
209	*Taraxacum officinale L.*	Herb	Asteraceae	Therophyte	Microphyll
210	*Tribulus terrestris L.*	Herb	Zygophyllaceae	Therophyte	Laptophyll
211	*Trifolium repens L.*	Herb	Fabaceae	Hemicryptophyte	Nanophyll
212	*Triticum aestivum L.*	Herb	Poaceae	Therophyte	Mesophyll
213	*Typha angustifolia L.*	Herb	Typhaceae	Geophyte	Mesophyll
214	*Verbascum thapsus L.*	Herb	Scrophulariaceae	Chamaeophyte	Mesophyll
215	*Verbena officinalis L.*	Herb	Verbenaceae	Hemicryptophyte	Microphyll
216	*Verbesina encelioides (Cav.) Benth. & Hook.f. ex A.Gray*	Herb	Asteraceae	Therophyte	Microphyll
217	*Xanthium strumarium L.*	Herb	Asteraceae	Therophyte	Microphyll
218	*Zea mays L.*	Herb	Poaceae	Therophyte	Mesophyll
219	*Tithonia diversifolia (Hemsl.) A.Gray*	Herb	Asteraceae	Therophyte	Mesophyll
220	*F9 (1) Ab*	Herb	-	-	-

2.3.2 Abundant and rare plants of the MWPE

The abundant and rare plant species were identified based on the importance value index. Their detailed descriptions are as follows:

2.3.2.1 Abundant and rare trees layer

The dominant or abundant tree species based on higher importance value index (IVI) were *Ficus carica*, *Morus alba* (2441 IVI), *Morus nigra* (1699 IVI), *Ailanthus altissima* (1655 IVI), *Populus alba* (1647 IVI), *Broussonetia papyrifera* (1624 IVI), *Eucalyptus globulus* (1308 IVI), *Dalbergia sissoo* (970 IVI), *Azadirachta indica* (898 IVI) and *Salix tetrasperma* (724 IVI) (Fig. 2.5; Appendix table 2). Whereas, the top ten rarest tree species were *Pyrus communis* (6.78 IVI) followed by *Cupressus sempervirens* and *Araucaria heterophylla* (8.46 IVI each), *Litchi chinensis* (12.22 IVI), *Juglans regia* (13.09 IVI), *Citrus limon* (16.67 IVI), *Sapium sebiferum* (17.42 IVI), *Ficus macrophylla* (17.56 IVI), *Ficus benjamina* (27.5 IVI) and *Citrus reticulata* (56 IVI) in the subtropical marble waste polluted ecosystem of Khyber Pakhtunkhwa, Pakistan (Fig. 2.6; Appendix table 2).

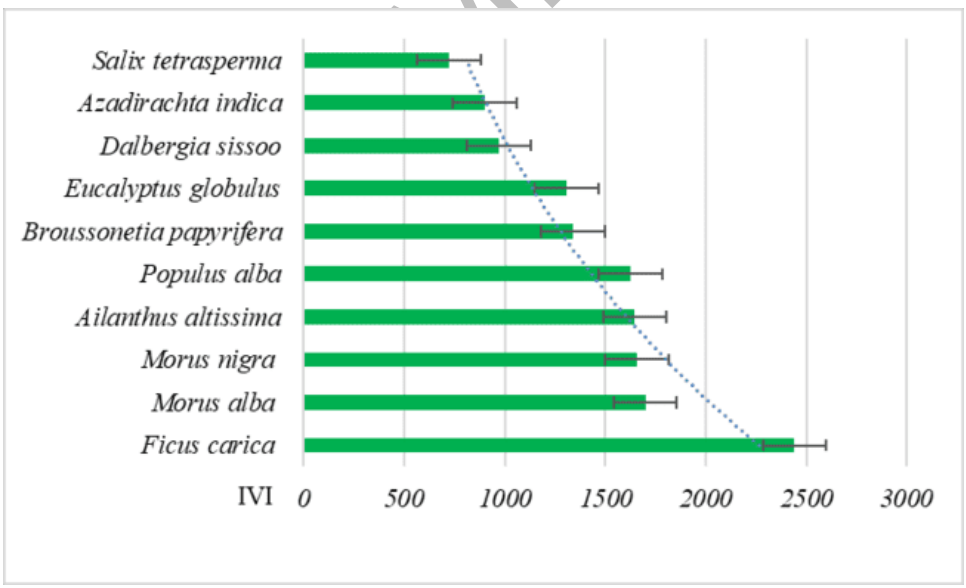


Fig. 2.5 The topmost abundant tree species in the subtropical Marble waste polluted ecosystem, KPK, Pakistan.

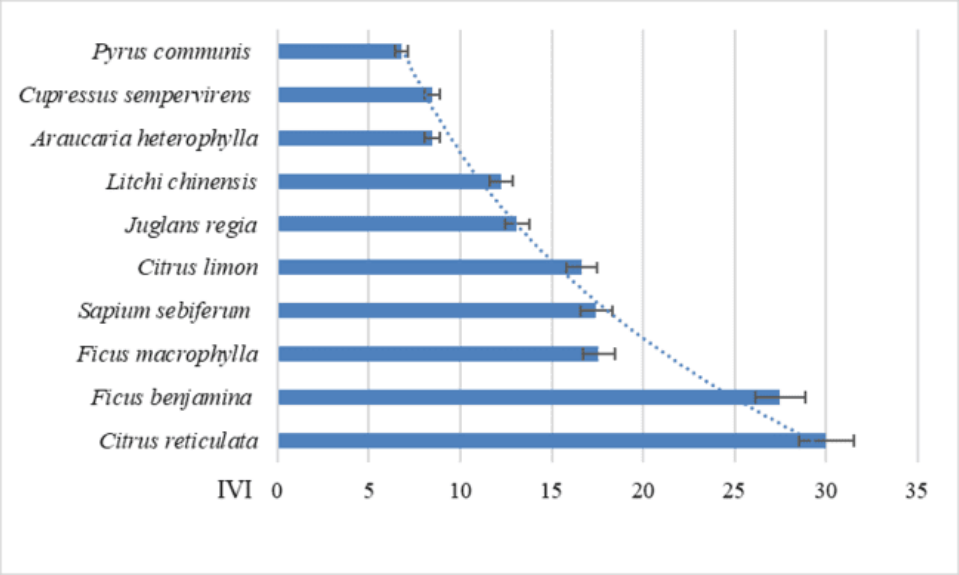


Fig. 2.6 Rare plant species of the MWPE, KPK, Pakistan.

2.3.2.2 Abundant and rare shrubs layer

The foremost abundant shrub species was *Calotropis procera* (1799 IVI), followed by *Datura innoxia* (1050 IVI), *Ricinus communis* (797 IVI), *Withania somnifera* (781 IVI), *Lantana camara* (444 IVI), *Ziziphus nummularia* (392 IVI), *Rosa indica* (382 IVI), *Senna occidentalis* (332 IVI), *Dodonaea viscosa* (320 IVI) and *Vitis vinifera* (317 IVI) in the subtropical MWPE, KPK, Pakistan (Fig. 2.7; Appendix table 2). At the same time, *Rumex hastatus*, *Combretum indicum* (13.88 IVI), *Bougainvillea spectabilis* (17.78 IVI), *Duranta stenostachya* (22.72 IVI), *Datura metel* (33.34), *Nannorrhops ritchieana*, *Indigofera heterantha*, *Duranta erecta*, *Debregeasia saeneb* (each with 66.67 IVI) and *Parthenocissus inserta* (120.74 IVI) were recorded as the rarist shrub species of the MWPE (Fig. 2.8; Appendix table 2).

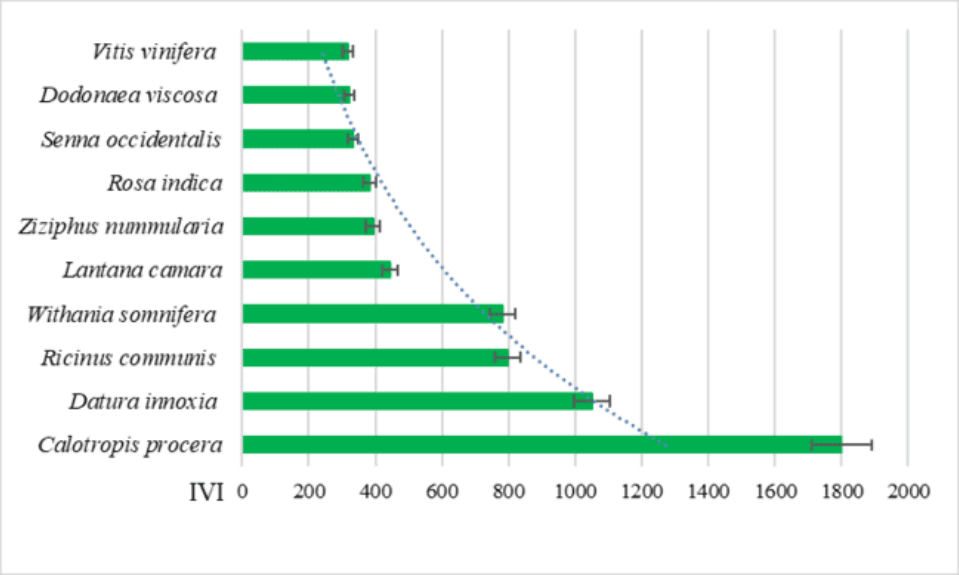


Fig. 2.7 The foremost abundant shrub species of the MWPE, KPK, Pakistan.

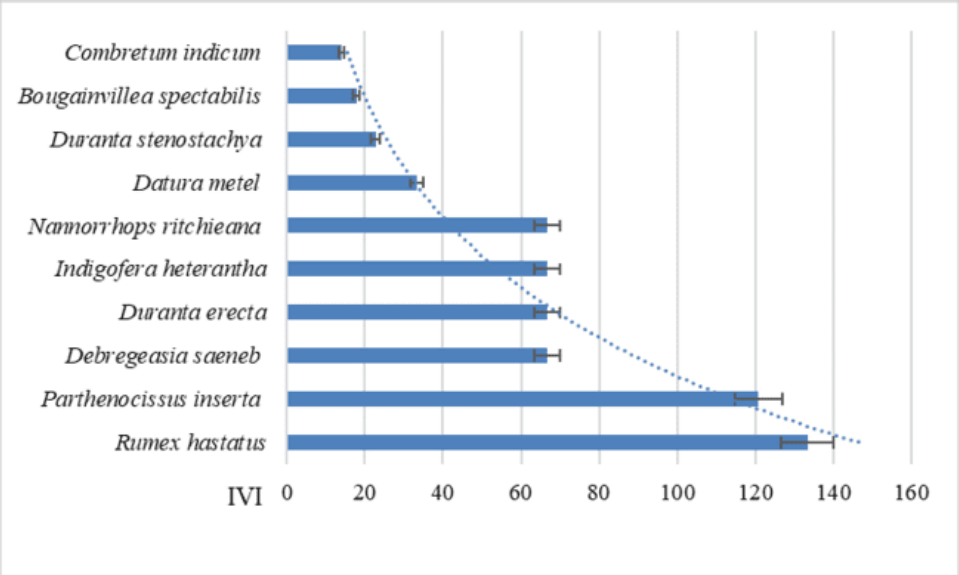


Fig. 2.8 The rare shrub species with minimum IVI in the subtropical MWPE.

2.3.2.3 Abundant and rare herbs layer

The top ten most abundant herb species were *Cynodon dactylon* (2982 IVI), *Parthenium hysterophorus* (1122 IVI), *Erigeron canadensis* (993 IVI), *Arundo donax* (795 IVI), *Adiantum capillus-veneris* (739 IVI), *Cannabis sativa* (664 IVI), *Xanthium strumarium* (633 IVI), *Taraxacum officinale* (627 IVI), *Amaranthus viridis* (621 IVI) and *Eleusine indica* (619 IVI) (Fig. 2.9; Appendix table 2). Though, *Bidens bipinnata* and *Pteris cretica* (3.67 IVI each) were the rare herb species accompanied by *Aloe vera* (4.18 IVI), *Dichanthium annulatum* (4.53 IVI), *Capsella*

bursa-pastoris (5.04), *Malva sylvestris* (5.09 IVI), *Aerva javanica* (5.27 IVI), *Brassica campestris* (5.45 IVI), *Artemisia scoparia* (5.78 IVI) and *Sisymbrium irio* (6 IVI) in the MWPE of the region (Fig. 2.10; Appendix table 2).

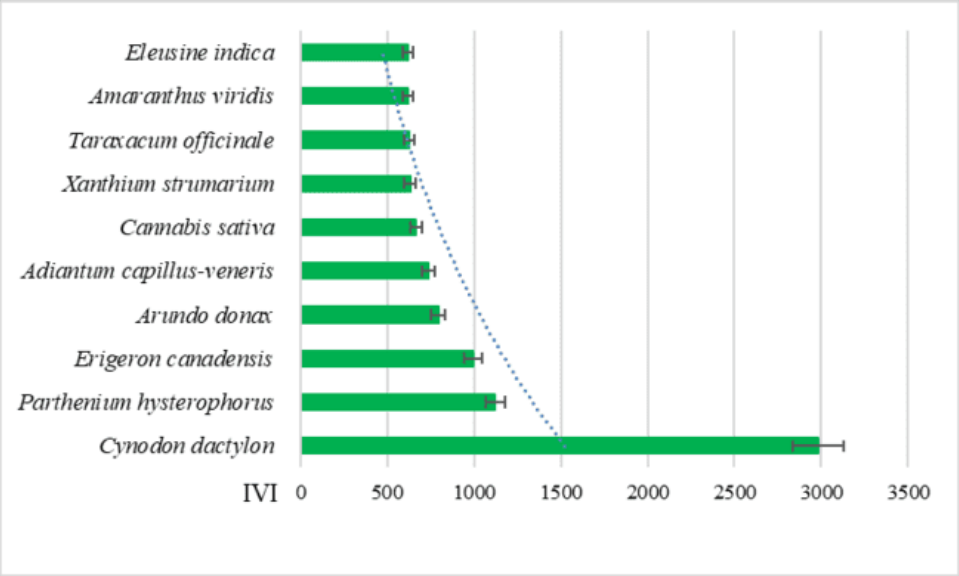


Fig. 2.9 The top ten most abundant herb species reported from subtropical MWPE

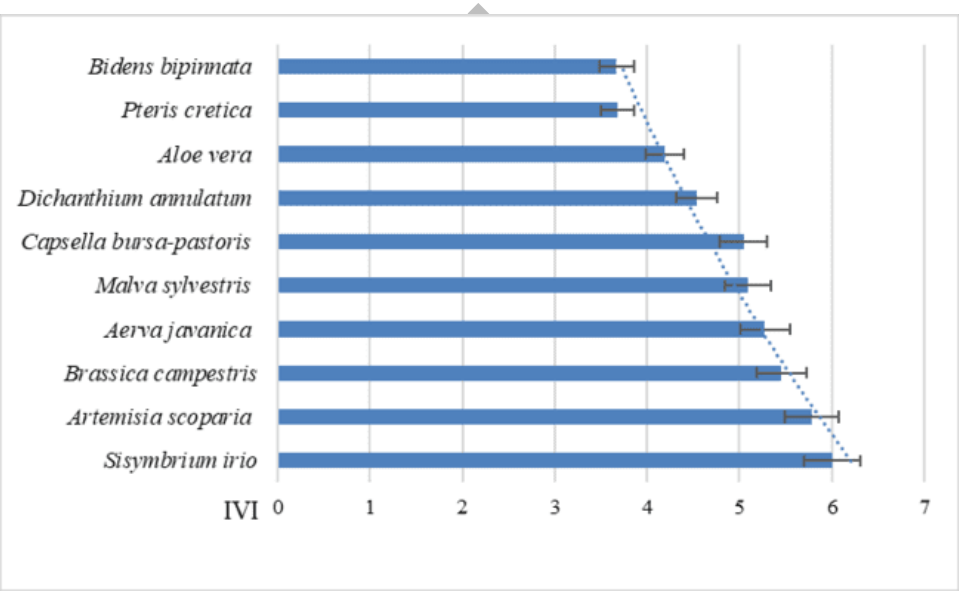


Fig. 2.10 Rare herb species with minimum IVI in the studied MWPE.

2.3.3 Impact of environmental variables on abundant and rare plants through Ordinary Least Square, Logistic and Probabilistic Models

The Ordinary Least Square (OLS), logistic and probabilistic models were used to determine the impact of environmental variables on binary/dependent variables. The coefficient of IVI has a positive and significant impact on plant abundance and rarity. The TDS (>0.002), Ni (>0.07), Cr (>0.02), P (>0.02) and precipitation (>0.001) had a positive and significant impact on the abundant and rare plant species of the MWPE. Whereas, the major composition of MWPE possesses $CaCO_3$, which has a negative significant effect (- >0.042) on plant abundance and rarity in the region. The detailed description of OLS, logistic and probabilistic models for each measured variables are given in table 2.3.4. Our model is best fit based on R^2 (0.67), Akaike Information Criteria (AIC) (510), Chi-square (20.29) and Probability values (0.0001) (Table 2.4).

Table 2.4 Summary of Ordinary Least Square, logistic and probabilistic models of the abundant and rare plant species of the MWPE, Khyber Pakhtunkhwa, Pakistan.

Variables	OLS	Logit	Probit	Variables	OLS	Logit	Probit
IVI	0.0001 (-0.00001)	0.022 (-0.002)	0.011 (-0.001)	Cd	0.001 (-0.001)	0.005 (-0.014)	0.001 (-0.008)
pH	-0.014 (-0.017)	-0.218 (-0.37)	-0.209 (-0.199)	Zn	-0.00003 (-0.0002)	0.002 (-0.004)	0.001 (-0.002)
EC	-0.00002 (-0.00002)	-0.001 (-0.001)	-0.0003 (-0.0004)	Fe	0.00001 (-0.0001)	-0.001 (-0.002)	-0.001 (-0.001)
TDS	0.0001 (-0.00002)	0.002 (-0.001)	0.001 (-0.0004)	K	0.00001 (-0.0001)	-0.002 (-0.001)	-0.001 (-0.001)
OM	0.035 (-0.071)	0.472 (-1.377)	-0.007 (-0.751)	P	0.012 (-0.006)	0.020 (-0.121)	0.029 (-0.065)
$CaCO_3$	-0.002 (-0.002)	-0.072 (-0.039)	-0.042 (-0.021)	Ca	0.00005 (-0.00004)	0.0001 (-0.001)	0.0002 (-0.001)
Ni	0.005 (-0.001)	0.071 (-0.025)	0.041 (-0.013)	Mg	0.00001 (-0.00005)	-0.001 (-0.001)	-0.001 (-0.001)

Cr	0.0002 (-0.001)	0.022 (-0.011)	0.012 (-0.006)	Temp	0.002 (-0.001)	0.027 (-0.028)	0.02 (-0.015)
Cu	-0.001 (-0.001)	-0.004 (-0.011)	-0.001 (-0.006)	Precipitation	0.001 (-0.0003)	0.003 (-0.005)	0.002 (-0.003)
Mn	0.0001 (-0.0003)	0.003 (-0.006)	0.001 (-0.003)	Constant	0.598 (-0.172)	-3.563 (-3.679)	-1.17 (-1.97)

R^2= 0.678, F-statistics/Chi-square= 20.429, AIC (510.569; 528.386)

2.3.4 Vegetation classification of Marble Waste Polluted Ecosystem

The hierarchical Two-way Cluster Analysis (TWCA) using Ward Method (based on minimizing increases in the squares' error sum) and Sorenson distance of PC-ORD software classified all the stations and plants into three major subtropical vegetation zones i.e., Humid, Semi Humid and Dry subtropical in the Marble waste polluted ecosystem. It further comprehended each plant species' distribution at a particular station and even quadrat level in different subtropical regions (Fig. 2.11).

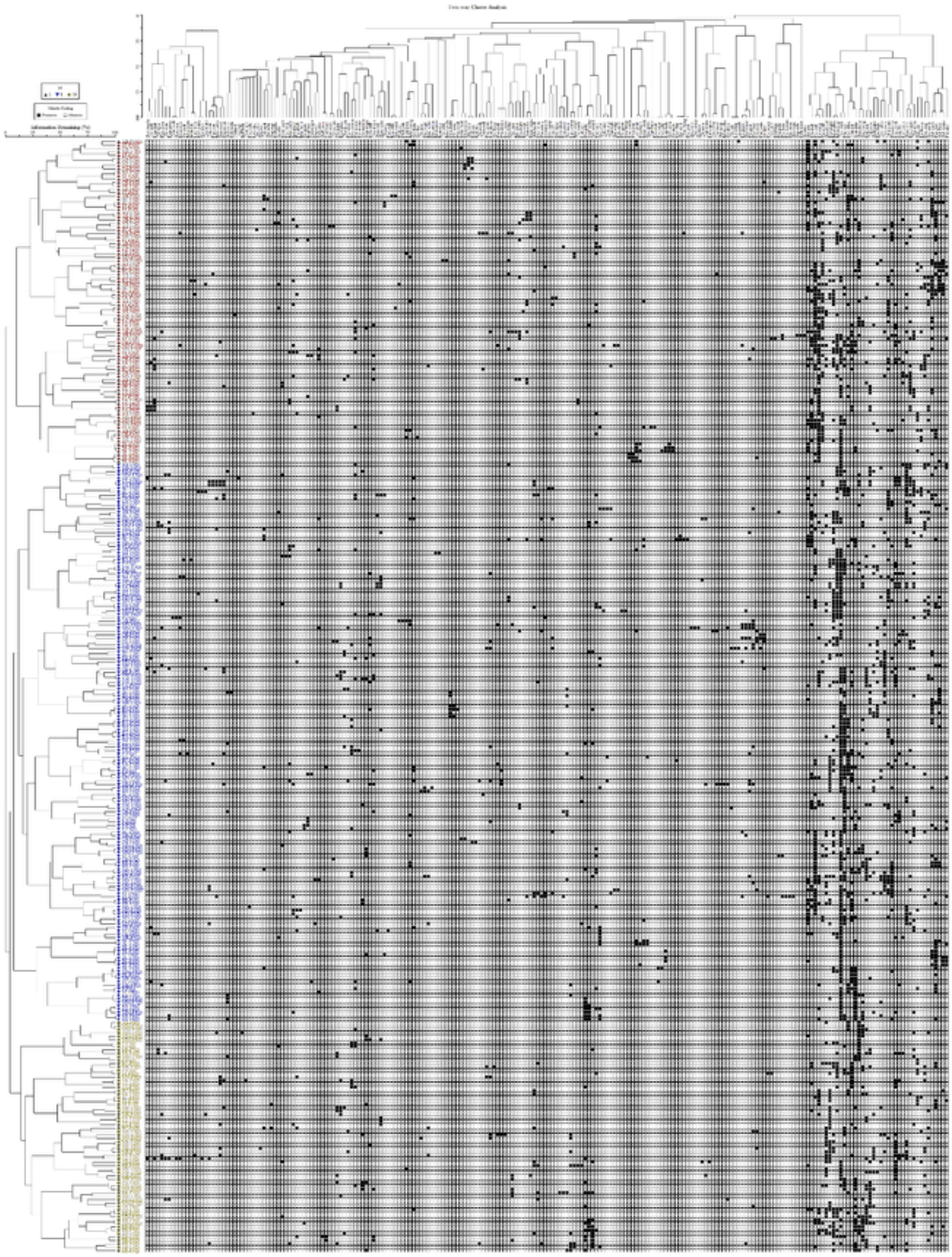


Fig. 2.11 The TWCA dendrogram comprehended the distribution of 220 plant species in the studied subtropical MWPE using Sorenson Distance Measurements with the Ward Linkage method (with narrow single-spaced width).

The detailed description of each subtropical vegetation zone of MWPE, their distinctive abundant/dominant and rare plant species are as follows:

2.3.4.1 Humid Subtropical Vegetation Zone of MWPE

This humid zone of MWPE is comprised of 66 stations encompassing 124 different plant species belonging to 47 families with an altitude range from 497.73-1213 m. Of which herb species were 80 (64.51% of the total humid vegetation), 13 shrubs (10.48%) and 31 tree species (25%). Family Poaceae was the leading family covering 11.47 % (14 species) of the total vegetation in the Humid subtropical marble waste polluted ecosystem. It was followed by Asteraceae (9 species; 7.37 %), Solanaceae (7 species; 5.73 %), Polygonaceae (6 species; 4.91 %), Amaranthaceae, Brassicaceae, Fabaceae and Rosaceae (each with 4 species & 4.09 % share). The Cucurbitaceae, Lamiaceae, Malvaceae, Moraceae and Myrtaceae each have 4 plant species (3.27 % each) followed by Euphorbiaceae and Pteridaceae each with 3 plant species (2.45 %). The remaining plant families shared less than 2 % each of the total vegetation, at this humid subtropical region around Marble factories (Appendix table 3). TWCA of humid subtropical MWPE were carried out using PC-ORD software. It further comprehends distribution of each plant species in the studied stations or quadrats (Fig. 2.12).

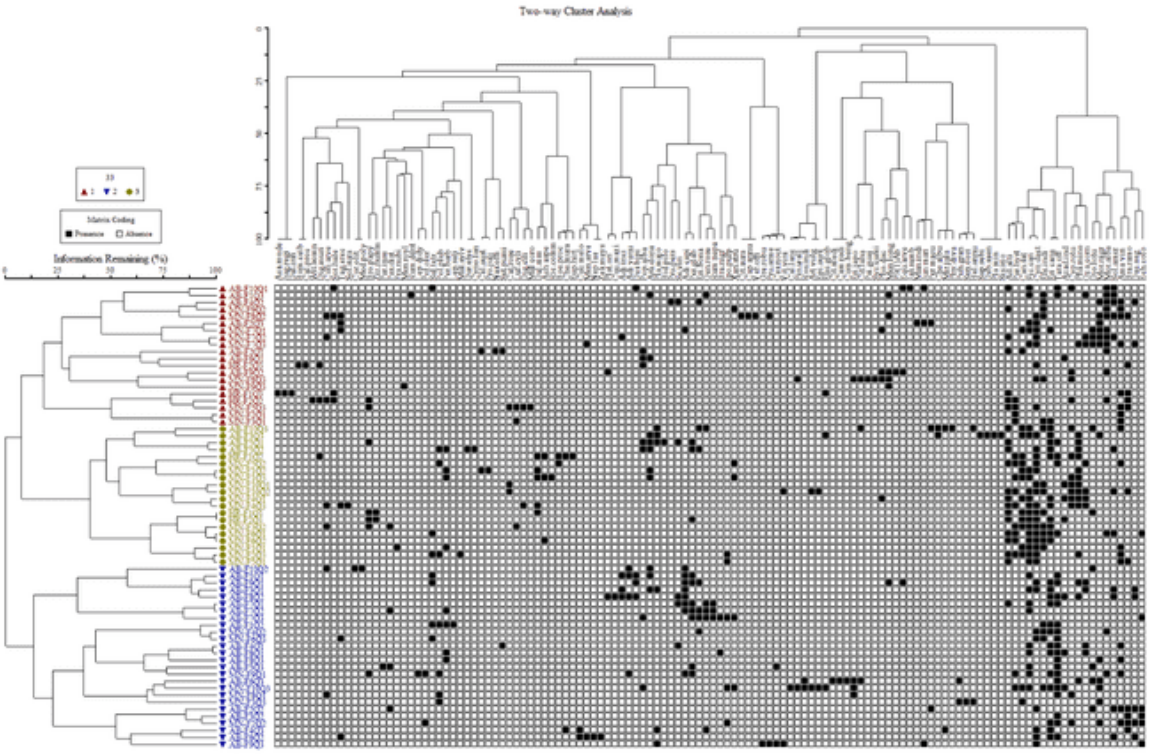


Fig. 2.12 TWCA dendrogram of 124 plant species and 66 stations/quadrats in the humid subtropical MWPE, KPK, Pakistan.

2.3.4.1.1 Abundant and rare plant species of the Humid subtropical MWPE

Tree layer:

The dominant or abundant tree species based on higher importance value index (IVI) in the humid subtropical MWPE was *Ficus carica* (840 IVI) followed by *Ailanthus altissima* (529 IVI), *Morus alba* (349 IVI), *Morus nigra* (305 IVI), *Salix tetrasperma* (280 IVI), *Populus alba* (263 IVI), *Diospyros lotus* (262 IVI), *Azadirachta indica* (202 IVI), *Dalbergia sissoo* (152 IVI) and *Broussonetia papyrifera* (238 IVI) (Fig. 2.13). Whereas, the top ten rare tree species were *Callistemon lanceolatus* (8.9 IVI) followed by *Psidium guajava* (11.42 IVI), *Juglans regia* (13.09 IVI), *Citrus medica* (13.33 IVI), *Sapium sebiferum* (17.42 IVI), *Acacia modesta* (17.85 IVI), *Pinus wallichiana* (17.97 IVI), *Syzygium cumini* (20.95 IVI), *Prunus persica* (22.23 IVI) and *Citrus aurantium* (22.61 IVI) in the humid MWPE (Fig. 2.14).

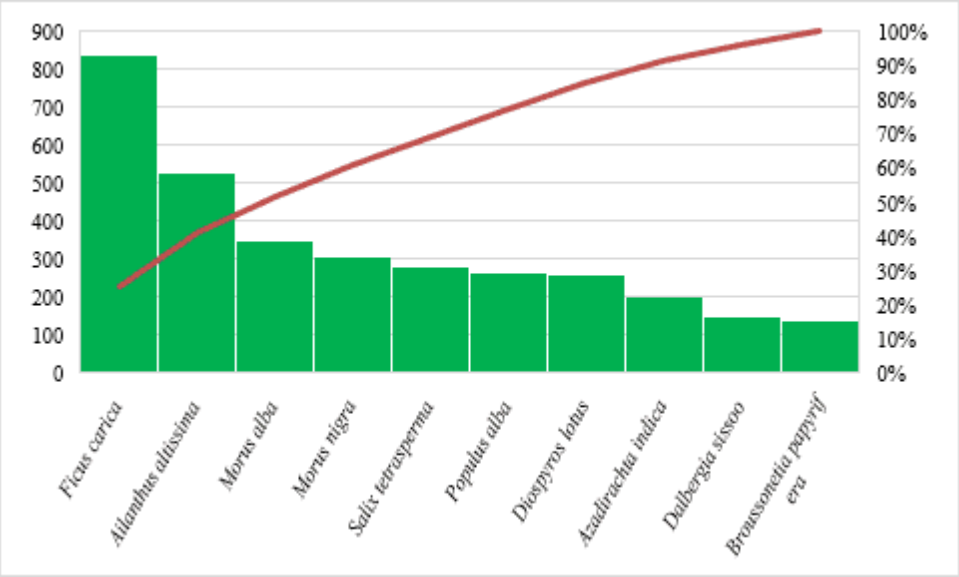


Fig. 2.13 The topmost abundant tree species in the Humid subtropical Marble waste polluted ecosystem, KPK, Pakistan.

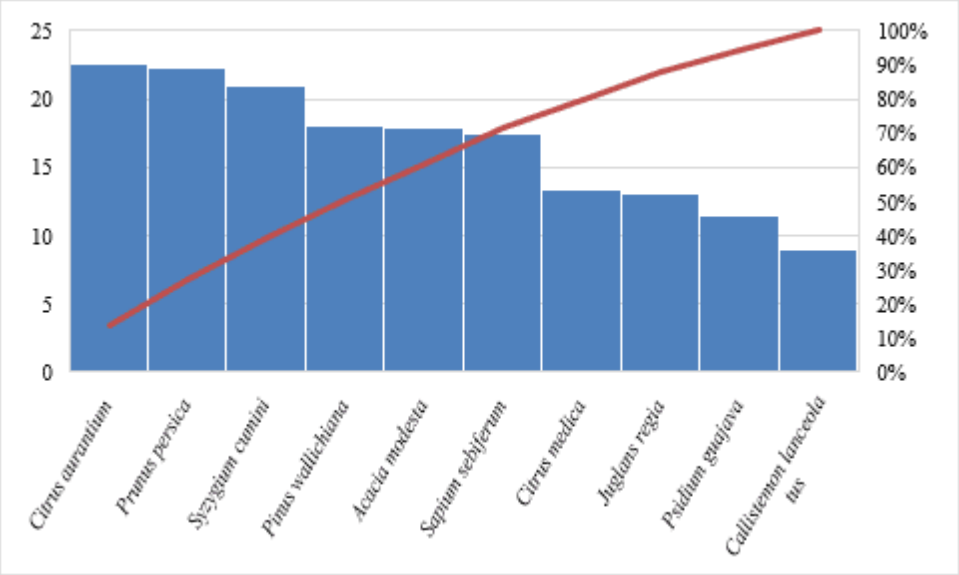


Fig. 2.14 Rare tree species based on minimum IVI in the Humid subtropical MWPE.

Shrub layer:

The foremost abundant shrub species were *Catharanthus roseus* (176 IVI), followed by *Rosa indica* and *Ziziphus nummularia* with 133 and 132 IVI in the region (Fig. 2.15). At the same time, *Parthenocissus inserta* (33.34 IVI), *Vitis vinifera* (56.67 IVI), *Withania somnifera, Senna occidentalis* and *Ricinus communis* (each with 66.67 IVI) were the rare shrub species of Humid MWPE (Fig. 2.16).

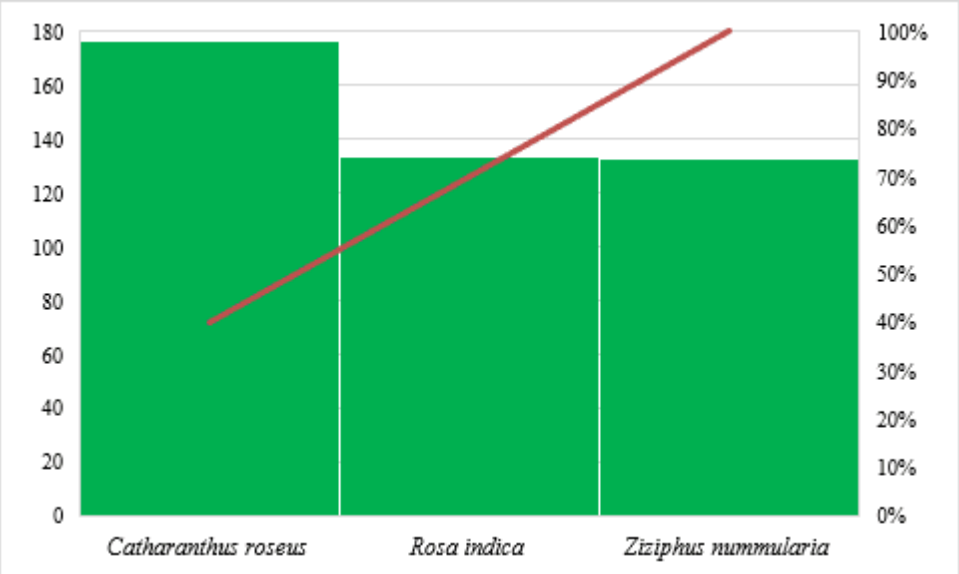


Fig. 2.15 The most abundant shrub species with higher IVI in the Humid subtropical MWPE.

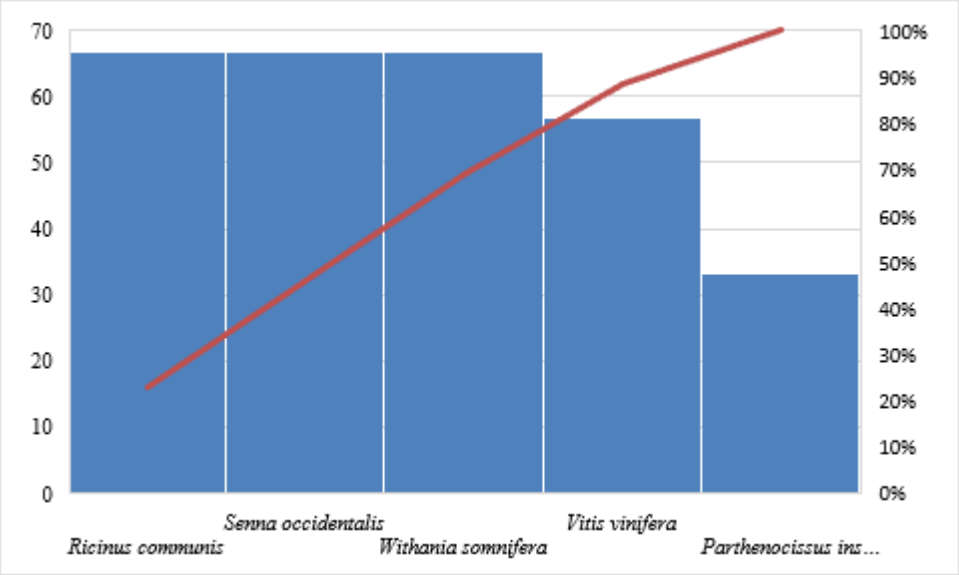


Fig. 2.16 The rare shrub species with minimum IVI in the studied region.

Herb layer:

The topmost abundant herb species were *Cynodon dactylon* (500 IVI), accompanied by *Erigeron canadensis* (271 IVI), *Taraxacum officinale* (240 IVI), *Amaranthus viridis* (237 IVI), *Eleusine indica* (223 IVI), *Cannabis sativa* (207 IVI), *Cyperus rotundus* (203 IVI), *Parthenium hysterophorus* (156 IVI), *Arundo donax* (144 IVI) and *Brachiaria ramosa* (142 IVI) in the humid subtropical MWPE (Fig. 2.17). While *Euphorbia hirta* (3.81 IVI), *Cucurbita maxima* (3.95), *Cynoglossum lanceolatum* (3.98 IVI), *Verbena officinalis & Capsicum annuum* (each with 4.14 IVI), *Solanum surattense* (4.24 IVI), *Zea mays* (4.52 IVI), *Physalis divaricata* (4.60 IVI), *Malva sylvestris* (5.09 IVI) and *Rumex dentatus* (5.31 IVI) were recorded as rare herb species of Humid subtropical MWPE (Fig. 2.18).

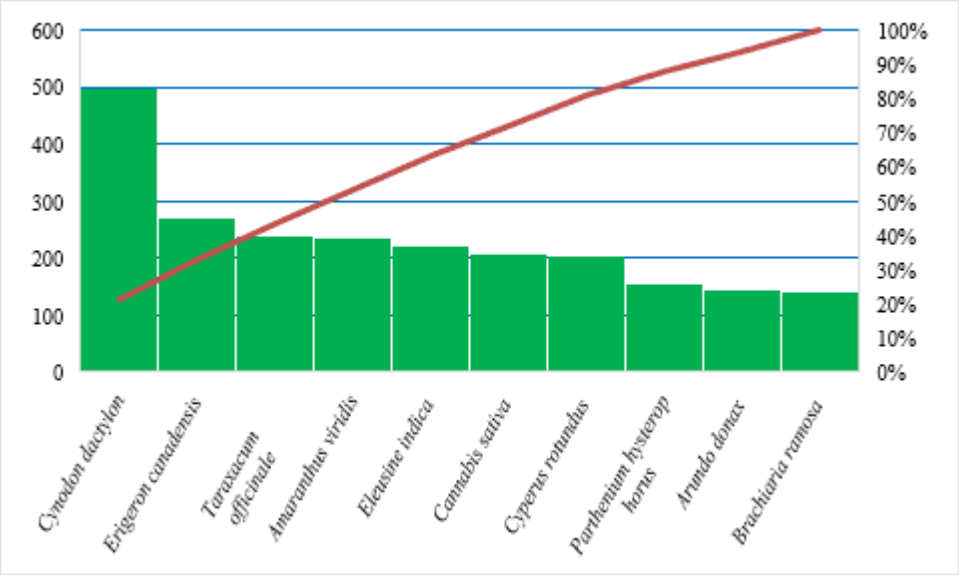


Fig. 2.17 The topmost abundant herb species of Humid MWPE.

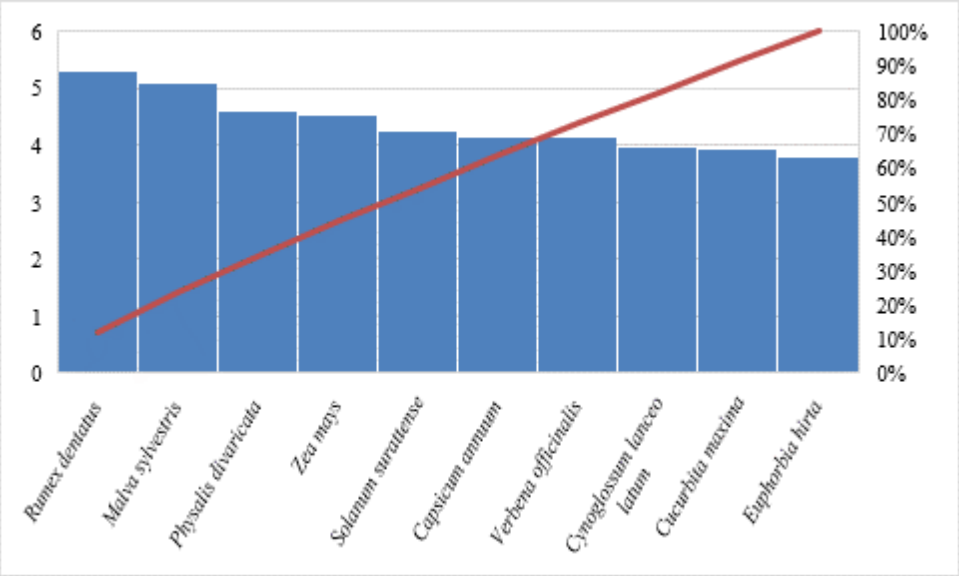


Fig. 2.18 The rare herb species of Humid MWPE.

2.3.4.1.2 Impact of measured variables on abundant and rare plants through OLS, Logistic and Probabilistic Models in Humid subtropical vegetation zone

The explanatory variables that showed significant positive effect on abundance and rare plant species of Humid subtropical MWPE were IVI (<0.039), EC (0.008), Cr (0.003), Zn (0.001), K (0.002) and precipitation (<0.24). While $CaCO_3$ (-0.240) and Ca (<-0.006) have negative and significant effect on plant species abundance and

rarity in the humid subtropical MWPE. Our models are perfect fits based on AIC (122), R^2 (0.59), Chi- square (5.84) and probability (0.001) values (Table 2.5).

Table 2.5 Summary of Ordinary Least Square, logistic and probabilistic models of the abundant and rare plant species of the Humid zone of MWPE.

Variables	**OLS**	**Logit**	**Probit**	**Variables**	**OLS**	**Logit**	**Probit**
IVI	0.0001 (-0.00002)	0.039 (-0.008)	0.018 (-0.003)	Cd	0.001 (-0.013)	-0.254 (-0.417)	-0.076 (-0.197)
pH	-0.058 (-0.045	-0.41 (-1.505)	-0.419 (-0.793)	Zn	0.001 (-0.001)	0.022 (-0.016)	0.009 (-0.008)
EC	0.0001 (-0.00003	0.008 (-0.005)	0.004 (-0.002)	Fe	0.0003 (-0.0005)	-0.017 (-0.017)	-0.007 (-0.008)
TDS	0.0002 (-0.0001	0.004 (-0.005)	0.002 (-0.003)	K	0.0002 (-0.0001)	0.006 (-0.005)	0.003 (-0.002)
OM	-0.23 (-0.177	-0.209 (-3.509)	0.5 (-1.932)	P	0.01 (-0.015)	-0.296 (-0.381)	-0.139 (-0.198)
$CaCO_3$	0.001 (-0.004	-0.240 (-0.139)	-0.104 (-0.065)	Ca	0.0002 (-0.0002)	-0.011 (-0.006)	-0.006 (-0.003)
Ni	0.013 (-0.005	0.311 (-0.198)	0.134 (-0.093)	Mg	0.0001 (-0.0002	0.007 (-0.006)	0.003 (-0.003)
Cr	0.003 (-0.002	0.032 (-0.054)	0.008 (-0.028)	Temp	-0.004 (-0.005)	0.002 (-0.122)	0.0002 (-0.064)
Cu	-0.0003 (-0.001	0.023 (-0.036)	0.015 (-0.017)	Precipitation	0.039 (-0.008)	0.008 (-0.001)	0.240 (-0.002)
Mn	-0.0001 (-0.001)	-0.026 (-0.022)	-0.011 (-0.01)	Constant	0.792 (-0.918	5.938 (-26.94)	2.465 (-13.414

R^2 = 0.590, F-statistics/Chi-square = 5.854, AIC= 122.303; 128.437

2.3.4.1.3 Ecological gradient through Detrended Correspondence Analysis (DCA) of Humid subtropical MWPE

Detrended correspondence analysis (DCA) was performed to examine the distribution pattern of 124 plants and 66 stations along the axes of Detrended gradient analysis (Fig. 2.19; Fig. 2.20). The maximum gradient length was recorded for axis 1 i.e., 8.43 with eigenvalue 0.787, followed by axes 2, 4 and 3 with eigenvalues 0.631, 0.490 and 0.438, respectively. The cumulative percentage variance of species data was observed maximum by axis 4 i.e., 15.5 accompanied by axes 3, 2 and 1 with 12.6, 9.3 and 5.2, correspondingly. While the sum of all eigenvalues or total inertia was recorded as 15.173 (Table 2.6).

Table 2.6 Summary of DCA of all plant species and stations representing eigenvalues along with different gradient lengths and cumulative percentage variance.

Axes	**1**	**2**	**3**	**4**	**Total inertia**
Eigenvalues	0.787	0.631	0.490	0.438	15.173
Lengths of gradient	8.437	5.927	4.089	4.437	
Cumulative percentage variance of species data	5.2	9.3	12.6	15.5	

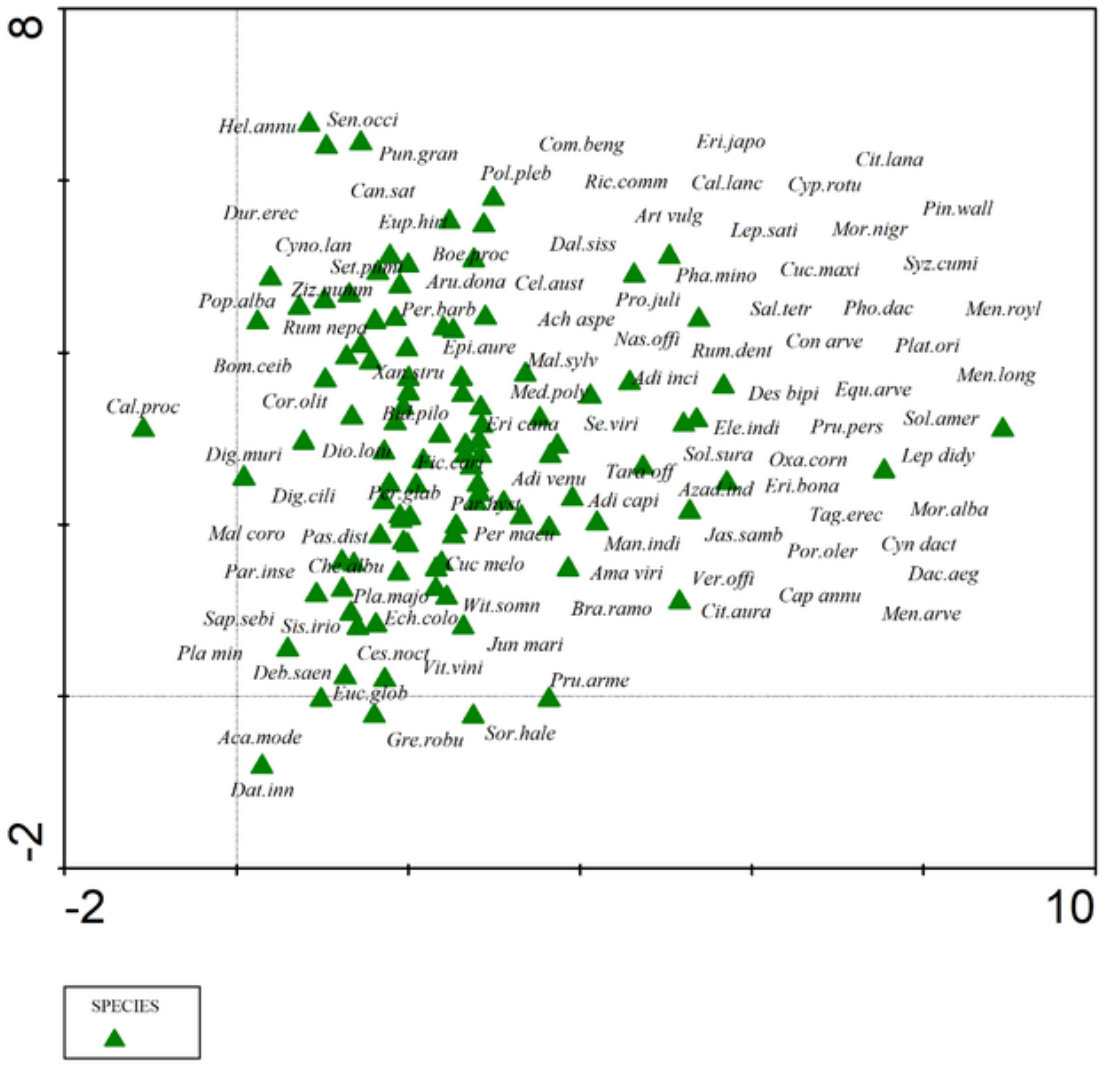


Fig. 2.19 DCA showing the distribution of 124 plant species in the humid zone of MWPE.

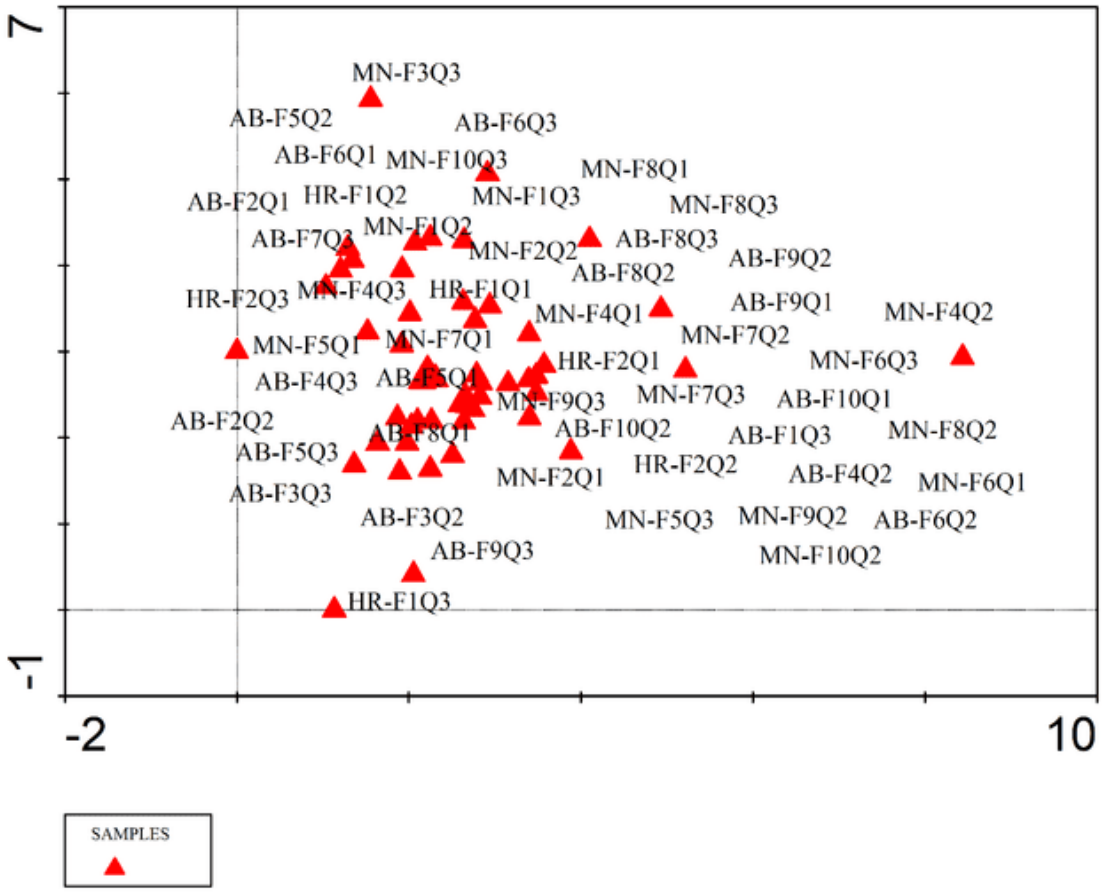


Fig. 2.20 DCA showing the distribution of 66 stations/quadrats in the humid zone of MWPE.

2.3.4.1.4 Ecological gradient through Canonical Correspondence Analysis (CCA) of Humid subtropical MWPE

The ordination of plant species through CCA biplot shows differential and similarity indexes in plant species and the distance between them. The results indicate that all the measured environmental variables i.e., elevation, soil pH, electrical conductivity (EC), total dissolved solids (TDS), organic matter (OM), calcium carbonate ($CaCO_3$), nickel (Ni), chromium (Cr), copper (CU), manganese (Mn), cadmium (Cd), zinc (Zn), iron (Fe), potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), temperature (Temp) and precipitation have a significant effect (probability=0.0015) on plant species composition and distribution in humid subtropical MWPE (Table 2.7). The 1st quadrant of CCA bi-plot clustered *Achyranthes aspera*, *Withania somnifera*, *Malva sylvestris*, *Punica granatum*, *Euphorbia hirta*, *Datura innoxia*, *Dalbergia sissoo*, *Acacia modesta*, *Calotropis procera*,

Solanum surattense etc. under the influence of higher temperature, $CaCO_3$ and lower precipitation, TDS, nickel, iron, magnesium, electrical conductivity, chromium, manganese, copper and potassium.

The 2nd quadrant comprehended the distribution of *Pinus wallichiana, Juncus maritimus*, *Taraxacum officinale*, *Medicago polymorpha*, *Rumex nepalensis*, *Adiantum capillus-veneris*, *Adiantum venustum*, *Parthenocissus inserta* and *Diospyros lotus* etc. under the consequence of higher elevation, cadmium, soil pH and less concentration of phosphorus, organic matter and zinc.

The 3rd quadrant indicated *Arundo donax*, *Equisetum arvense*, *Setaria viridis*, *Persicaria barbata*, *Morus alba*, *Epipremnum aureum, Brassica nigra*, *Artemisia vulgaris*, *Jasminum sambac* and *Rumex dentatus* under effect of higher precipitation, nickel, TDS, iron, magnesium, chromium, electrical conductivity, potassium, copper and lower amount of $CaCO_3$.

The 4th quadrant of CCA bi-plot distributed *Populus alba*, *Ailanthus altissima*, *Digitaria ciliaris*, *Parthenium hysterophorus*, *Amaranthus viridis*, *Malvastrum coromandelianum*, *Eleusine indica*, *Polygonum plebeium*, *Helianthus annuus* and *Senna occidentalis* under the influence of higher phosphorus, organic matter, zinc and lower soil pH and cadmium concentration (Fig. 2.21; Fig. 2.22 Table 2.7).

Table 2.7 Summary of ecological gradient through CCA along with Monte Carlo test in the Humid subtropical MWPE.

Axes	**1**	**2**	**3**	**4**	**Total Inertia**
Eigenvalues	0.508	0.444	0.417	0.373	15.173
Species-environment correlation	0.951	0.922	0.882	0.913	
Cumulative percentage variance of species data	3.3	6.3	9.0	11.5	
Cumulative percentage variance of species data	11.0	20.7	29.7	37.8	
Summary of Monte Carlo test					
Test of significance of 1st canonical axis	Eigenvalue	0.508	Test of significance of all canonical axes	Eigenvalue	4.606
	F-ratio	2.594		F-ratio	2.055
	P-value	0.0012		P-value	0.0015

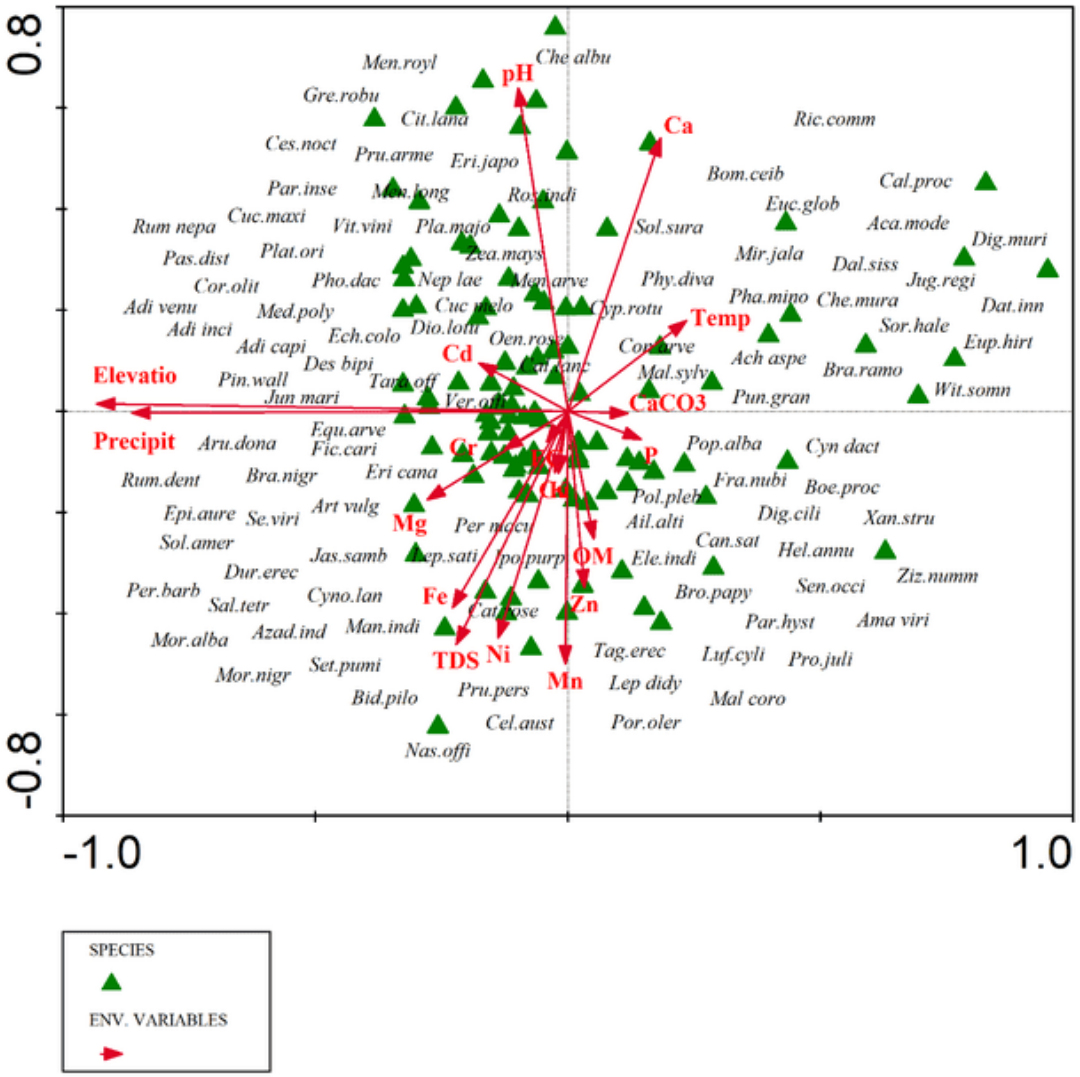


Fig. 2.21 CCA biplot representing the distribution of plant species in relation of measure environmental factors.

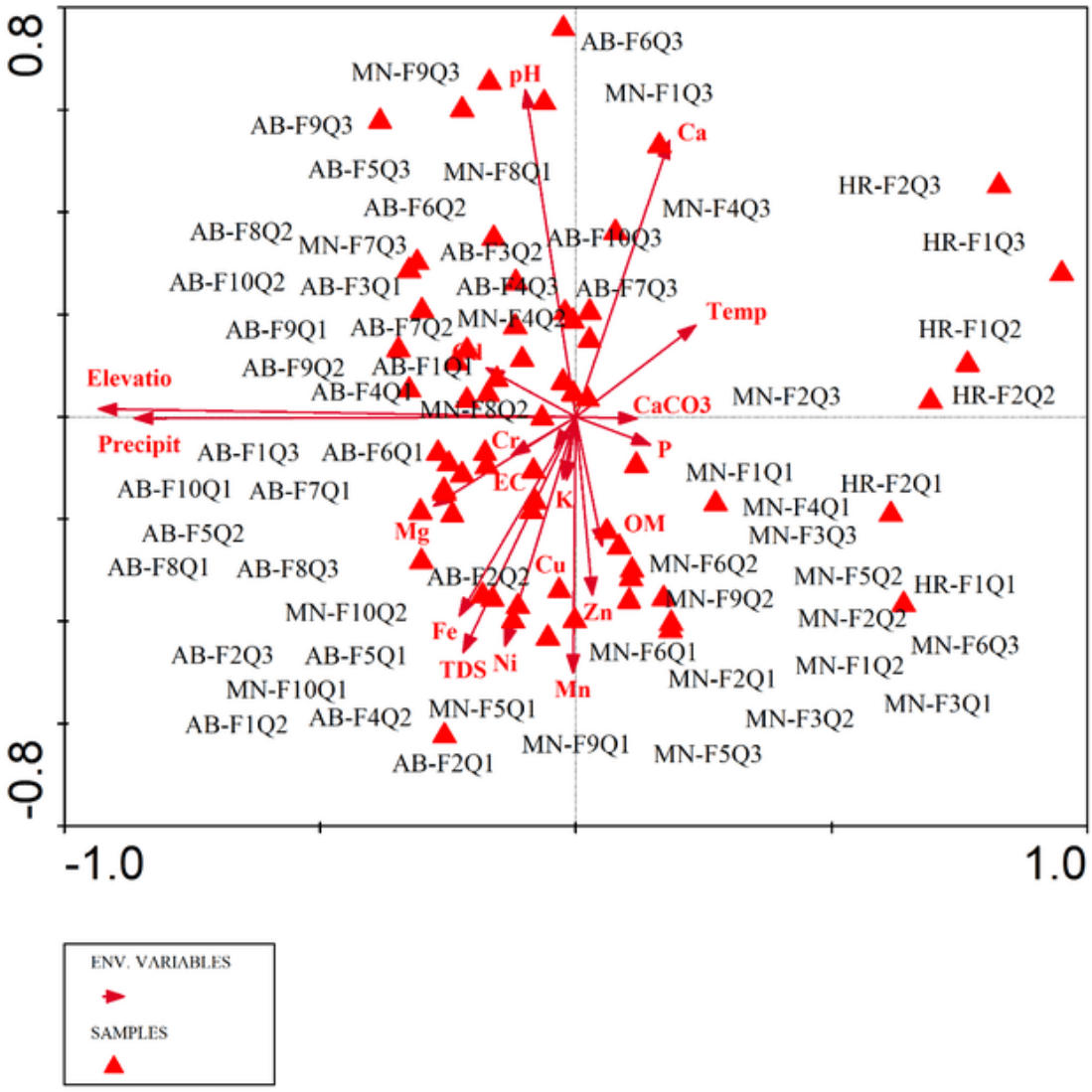


Fig. 2.22 CCA biplot of all the station in conjunction with explanatory variables in the humid subtropical MWPE.

2.3.4.2 Semi-Humid Subtropical Vegetation Zone (SHSVZ)

This semi-humid subtropical zone of MWPE encompassed 138 stations along with 159 different types of plant species belonging to 51 families. This zone lies between 275-920 m altitude. Herbs were the topmost habit with 107 species (67.29% of the total semi-humid subtropical vegetation) followed by 37 trees (23.27%) and 15 shrubs (9.43%). The family Poaceae was the leading with 24 plant species (15.09 %) followed by Asteraceae (11 species; 6.91 %), Fabaceae & Rosaceae (each with 9 species; 5.66 %), Amaranthaceae & Polygonaceae (each 8 species; 5.03 %), Solanaceae (7 species; 4.40%), Moraceae (6 species; 3.77 %), Euphorbiaceae,

Myrtaceae & Pteridaceae (each with 5 species; 3.14 %), Brassicaceae, Cucurbitaceae, Lamiaceae, Salicaceae, Verbenaceae and Vitaceae (3 species; 1.88 % each). Whilst the remaining plant families have less than 3 plant species and percentage (Appendix Table 3). The Two-way Cluster Analysis semi-humid subtropical MWPE further showed the distribution of 159 plant species in the 138 studied stations or quadrats (Fig. 2.23).

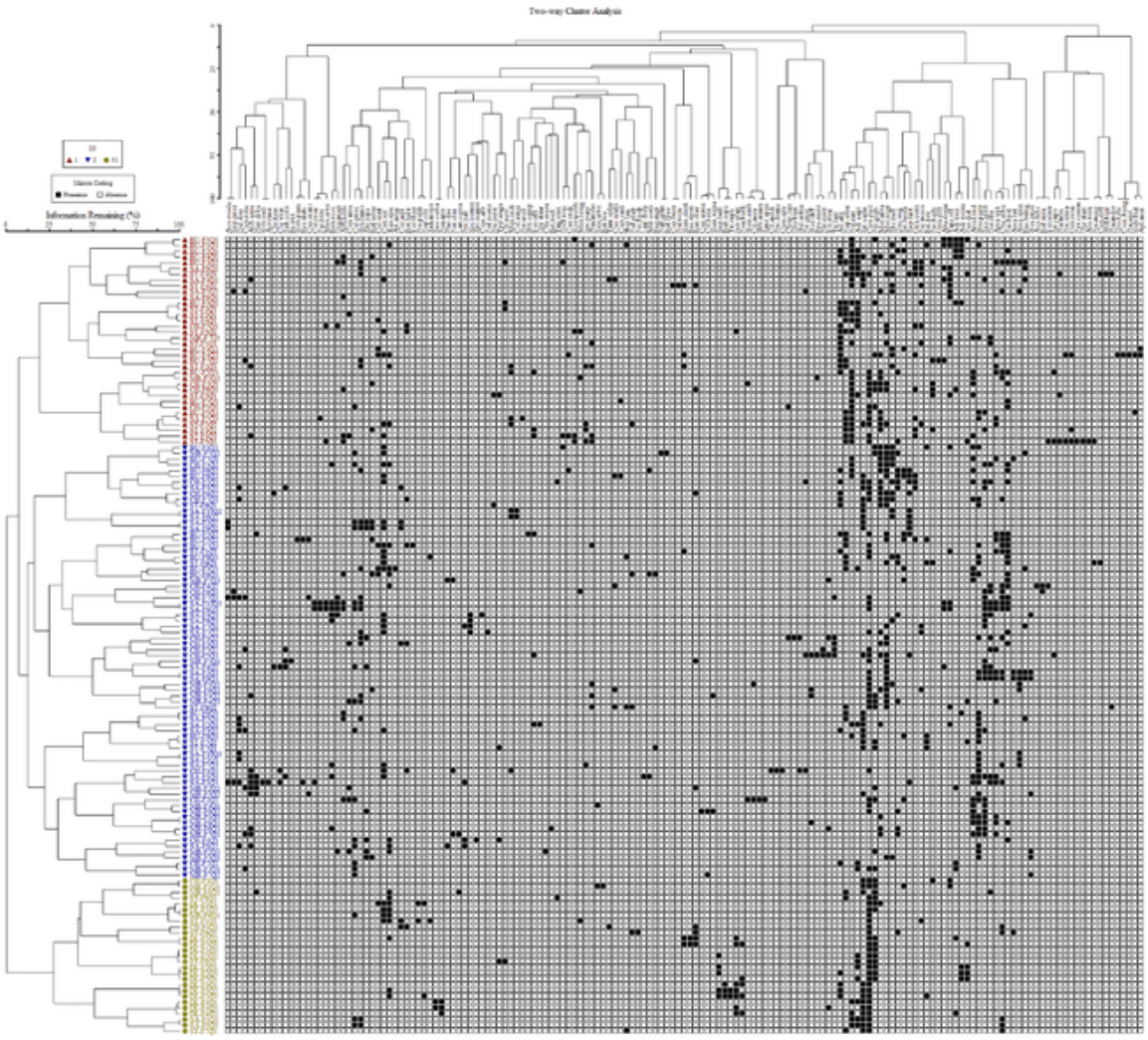


Fig. 2.23 TWCA dendrogram of 159 plant species and 138 stations/quadrats in the semi-humid subtropical MWPE, KPK, Pakistan.

2.3.4.2.1 Abundant and rare plant species of the Semi-humid subtropical MWPE Tree Layer:

Among the tree layer *Ficus carica* (1057 IVI) was the topmost dominant tree species based on IVI followed by *Ailanthus altissima* (831 IVI), *Eucalyptus globulus* (793 IVI), *Populus alba* (792 IVI), *Broussonetia papyrifera* (768 IVI), *Morus nigra* (768 IVI), *Morus alba* (545 IVI), *Azadirachta indica* (388 IVI), *Dalbergia sissoo* (261 IVI) and *Albizia lebbeck* (256 IVI) (Fig. 2.24). At the same time, *Pyrus communis* (6 IVI) was illustrated as rare tree species followed by *Pinus wallichiana* (8.33 IVI), *Diospyros lotus* (8.88 IVI), *Robinia pseudoacacia* (10.89 IVI), *Prunus domestica* (13.33), *Citrus medica* (13.33 IVI), *Ficus macrophylla* (17.56 IVI), *Punica granatum* (17.78 IVI), *Prunus armeniaca* (17.89 IVI) and *Syzygium cumini* (26 IVI) in the region (Fig. 2.25).

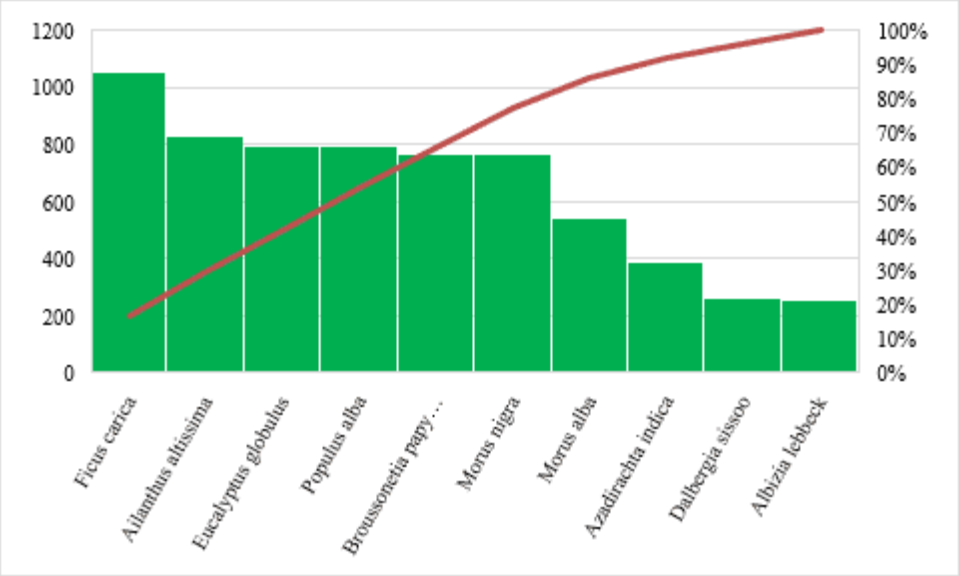


Fig. 2.24 The most abundant tree species with higher IVI in the Semi-humid subtropical MWPE.

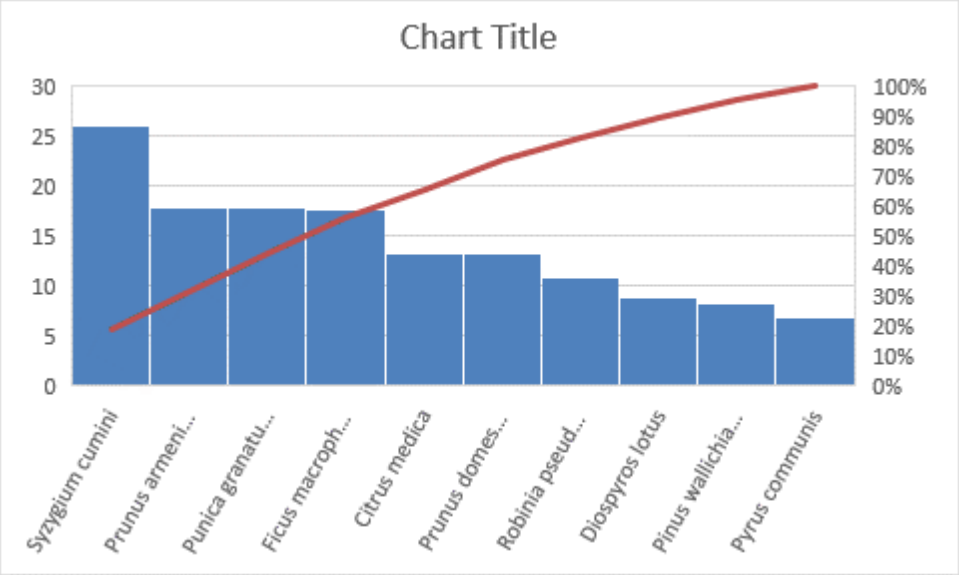


Fig. 2.25 Rare tree species with minimum IVI in the Semi-humid MWPE

Shrub Layer:

Calotropis procera (1005 IVI) was the foremost shrub species accompanied by *Datura innoxia* (750 IVI), *Senna occidentalis* (266 IVI), *Vitis vinifera* (261 IVI) and *Rosa webbiana* (193 IVI) (Fig. 2.26). However, *Rosa indica* (16.67 IVI), *Duranta stenostachya* (22.72 IVI), *Ziziphus nummularia* (33.33 IVI), *Withania somnifera* (38.89 IVI) and *Catharanthus roseus* (61.48 IVI) were the rare shrub species in semi humid subtropical MWPE (Fig. 2.27).

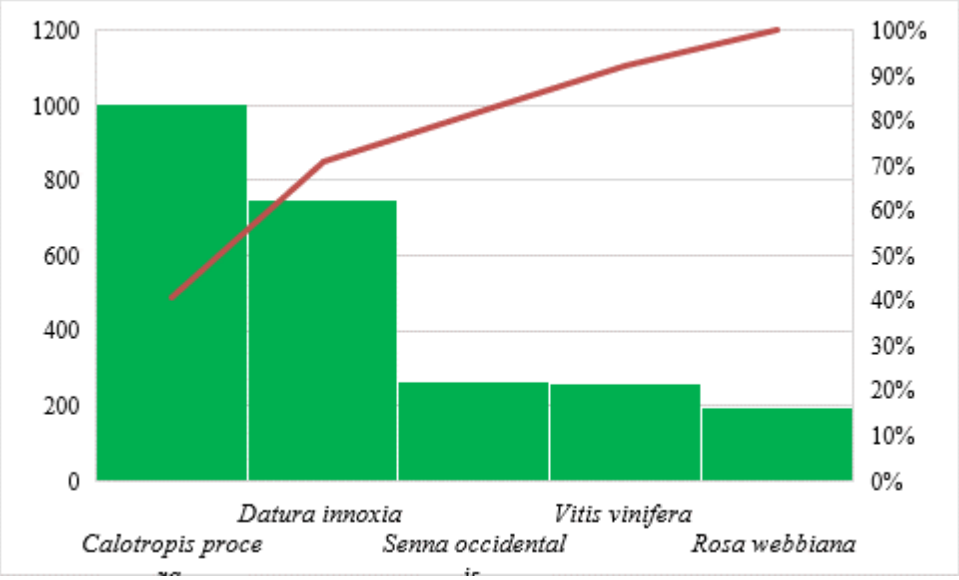


Fig. 2.26 The foremost abundant shrub species of the Semi-humid MWPE.

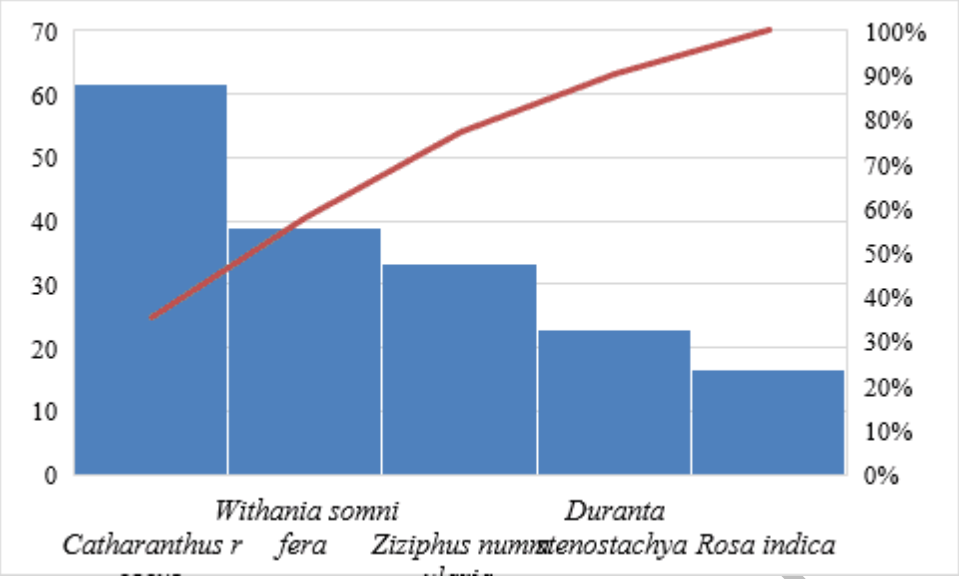


Fig. 2.27 Topmost rare shrubs of the semi-humid MWPE

Herb Layer:

The herbaceous layer was dominated by *Cynodon dactylon* (1129 IVI) followed by *Erigeron canadensis* (546 IVI), *Persicaria glabra* (417 IVI), *Adiantum capillus-veneris* (407 IVI), *Cannabis sativa* (357 IVI), *Eleusine indica* (341 IVI), *Parthenium hysterophorus* (336 IVI), *Amaranthus viridis* (283 IVI), *Taraxacum officinale* (279 IVI) and *Chenopodium album* (258 IVI) (Fig. 2.28). While *Commelina benghalensis* (3.22 IVI), *Bidens bipinnata* (3.67 IVI), *Pteris cretica* (3.67 IVI), *Aerva javanica* (4.18 IVI), *Capsella bursa-pastoris* (5.04 IVI), *Verbascum Thapsus* (5.44 IVI), *Brassica campestris* (5.45 IVI), *Sorghum bicolor* (6.09 IVI), *Aristida adscensionis* (6.09 IVI) and *Chenopodium murale* (6.53 IVI) were the top ten rare species in this region (Fig. 2.29).

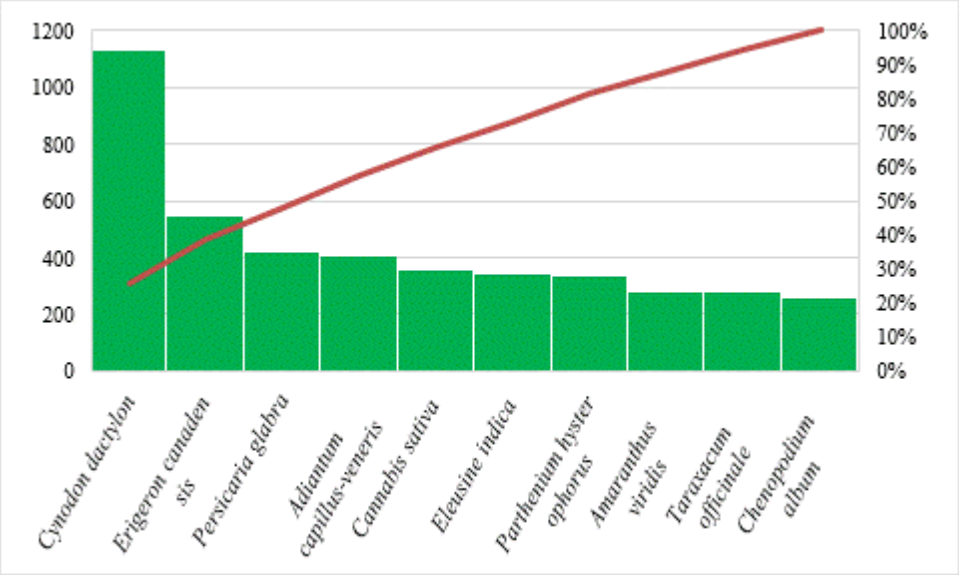


Fig. 2.28 The top ten abundant herb species based on IVI in the region.

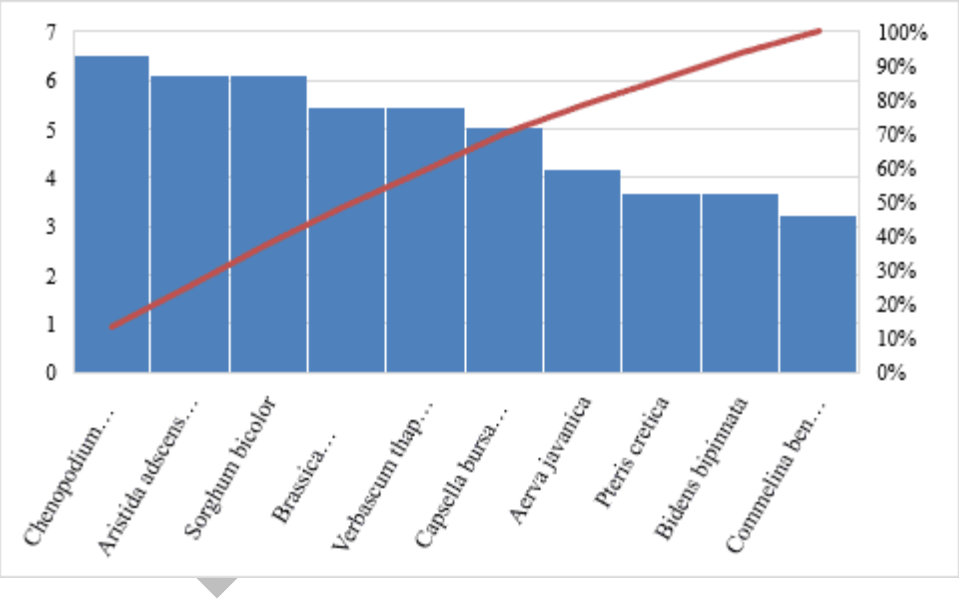


Fig. 2.29 The top ten rare herb species in the semi humid MWPE.

2.3.4.2.2 Results of Ordinary Least Square, Logistic and Probabilistic Models of Semi-Humid subtropical vegetation zone

Based on OLS, logistic and probabilistic models, the IVI (<0.023), EC (<0.0004), $CaCO_3$ (<0.045), Ni (<0.002), Cr (<0.41), Cu (<0.017), Mn (<0.004), Cd (<-0.012), Zn (<0.006), Fe (<0.0001), K (<0.0001), Ca (<0.00001), Mg (<-0.00001), temperature (<0.041) and precipitation (0.009) have significant effect on the occurrence of abundant and rare plants of semi humid subtropical vegetation. The AIC, R^2, Chi-

square and probability values were recorded as 253, 0.720, 9.261 and 0.001, respectively (Table 2.8).

Table 2.8 The summary of OLS, logit and probit models of abundant and rare plant species in the Semi humid MWPE.

Variables	OLS	Logit	Probit	Variables	OLS	Logit	Probit
IVI	**0.0001** (-0.00001)	**0.023** (-0.003)	**0.012** (-0.001)	Cd	-0.001 (-0.001)	-0.012 (-0.029)	-0.002 (-0.015)
pH	0.049 (-0.03)	-0.552 (-0.629)	-0.386 (-0.333)	Zn	-0.0002 (-0.0005)	0.006 (-0.009)	0.002 (-0.005)
EC	-0.0001 (-0.0001)	0.0004 (-0.001)	0.0001 (-0.001)	Fe	0.0001 (-0.0003)	-0.008 (-0.005)	**-0.005** (-0.003)
TDS	**0.0001** (-0.00004)	0.0004 (-0.001)	0.0004 (-0.0005)	K	0.0001 (-0.0001)	-0.005 (-0.003)	-0.002 (-0.002)
OM	-0.138 (-0.115)	-0.205 (-2.274)	-0.358 (-1.223)	P	0.007 (-0.01)	0.116 (-0.193)	0.088 (-0.105)
$CaCO_3$	-0.0004 (-0.003)	0.045 (-0.062)	0.025 (-0.033)	Ca	0.00001 (-0.0001)	-0.0005 (-0.002)	-0.0002 (-0.001)
Ni	0.002 (-0.003)	0.004 (-0.052)	0.017 (-0.027)	Mg	-0.00001 (-0.0001)	-0.001 (-0.002)	-0.001 (-0.001)
Cr	0.0003 (-0.001)	0.041 (-0.026)	**0.029** (-0.014)	Temp	-0.004 (-0.005)	0.037 (-0.091)	0.041 (-0.05)
Cu	-0.001 (-0.002)	0.017 (-0.029)	0.006 (-0.016)	Precipitation	0.002 (-0.001)	0.009 (-0.015)	0.002 (-0.008)
Mn	-0.0001 (-0.001)	0.0003 (-0.014)	0.004 (-0.007)	Constant	0.391 (-0.298)	1.093 (-5.819)	0.505 (-3.054)

R^2 = 0.720, F-statistics/Chi-square = 9.261, AIC=253.018

2.3.4.2.3 Ecological gradient through Detrended Correspondence Analysis (DCA) of Semi-Humid subtropical MWPE

Detrended correspondence analysis (DCA) was performed to examine the distribution pattern of 159 plants and 138 stations along the axes of Detrended gradient analysis (Fig. 2.30; Fig. 2.31). The maximum gradient length was recorded high for axis 2 i.e., 4.93 with eigenvalue 0.54, followed by axes 1, 3 and 4 with eigenvalues 0.60, 0.47 and 0.39, respectively. The cumulative percentage variance of species data was observed maximum by axis 4 i.e., 10.5 accompanied by axes 3, 2 and 1 with 8.4, 5.9

and 3.1 cumulative percentage variance. The sum of all eigenvalues or total inertia was recorded as 19.269 Table 2.9).

Table 2.9 Summary of DCA of all plant species and stations in semi humid MWPE representing eigenvalues along with different gradient length and cumulative percentage variance.

Axes	**1**	**2**	**3**	**4**	**Total inertia**
Eigenvalues	0.60	0.54	0.47	0.39	19.269
Lengths of gradient	4.86	4.93	4.85	4.20	
Cumulative percentage variance of species data	3.1	5.9	8.4	10.5	

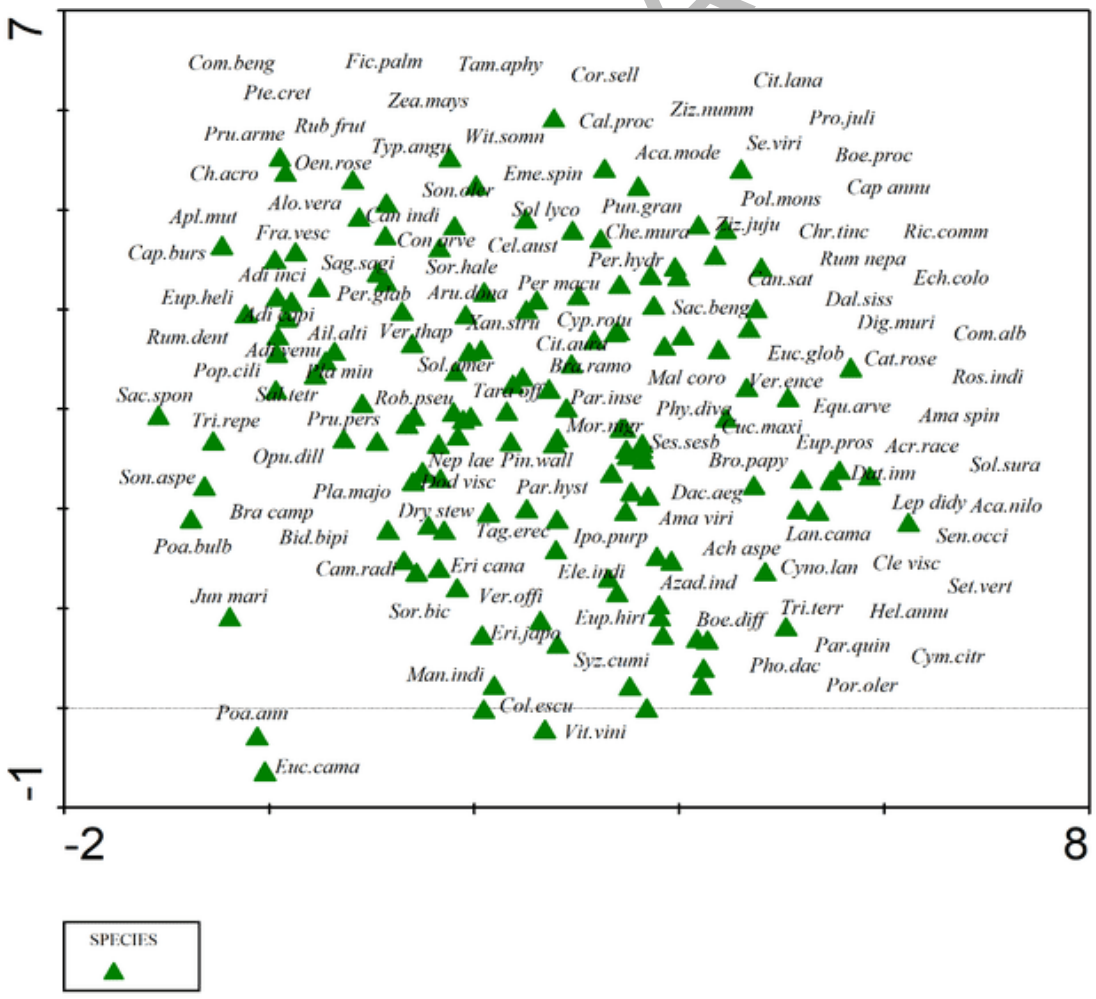


Fig. 2.30 Ecological gradient through DCA biplot representing the distribution of plant species in the semi humid subtropical MWPE.

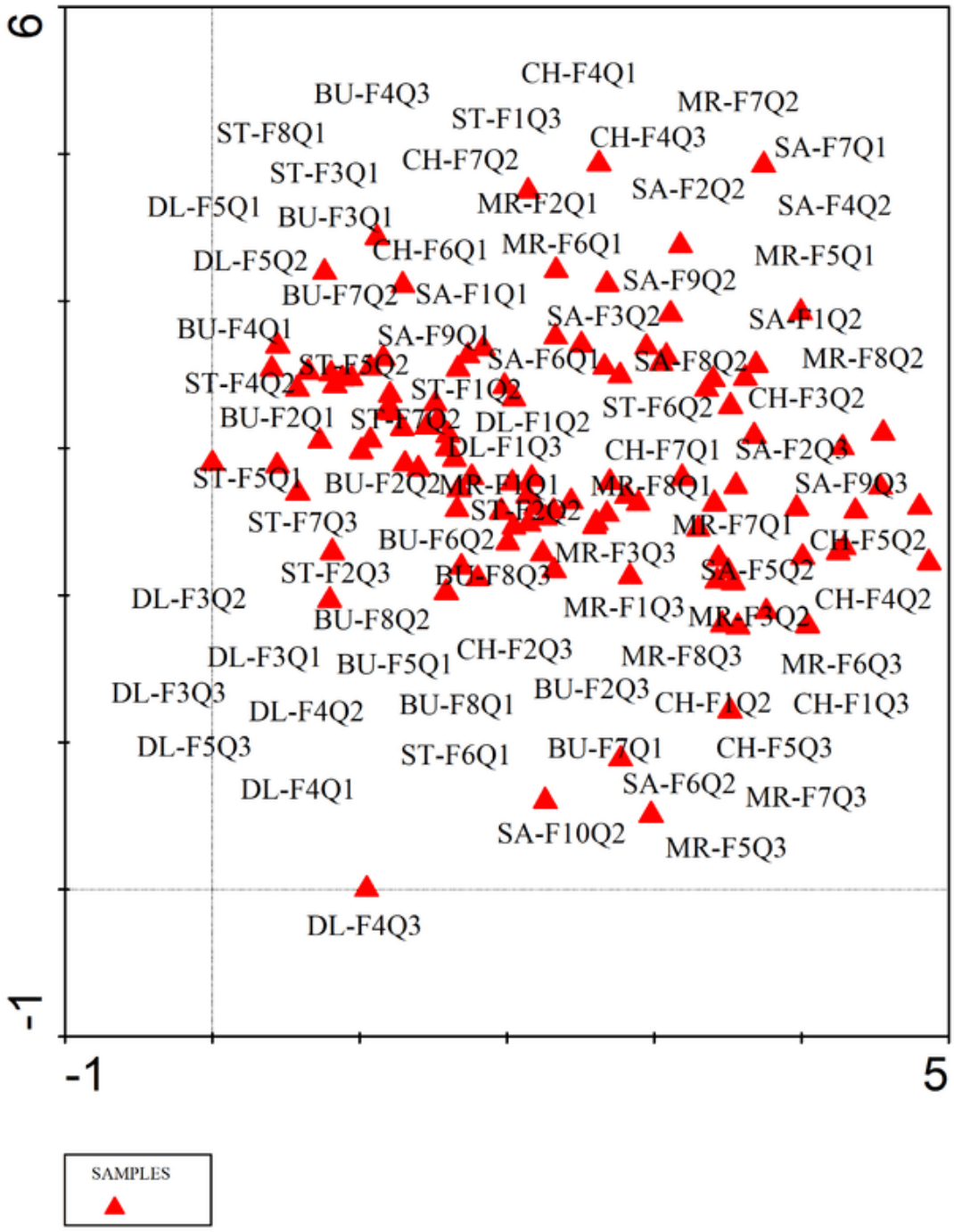


Fig. 2.31 Ecological gradient through DCA biplot representing the distribution of stations studied in the semi humid subtropical MWPE.

2.3.4.2.4 Ecological gradient through Canonical Correspondence Analysis (CCA) of Semi-Humid subtropical MWPE

The ordination of plant species through CCA biplot of Semi humid subtropical MWPE shows that the 1st quadrant of CCA bi-plot clustered *Acacia nilotica, Acacia modesta*, *Ziziphus jujuba*, *Cynoglossum lanceolatum*, *Calotropis procera*, *Lantana camara*, *Datura innoxia*, *Dalbergia sissoo*, *Euphorbia hirta* and *Solanum surattense* ., under the influence of higher values for electrical conductivity, manganese, total dissolved solids, iron, temperature and low elevation, soil pH and zinc concentration (Fig. 2.32).

The 2nd quadrant comprehended the distribution of *Poa annua*, *Sonchus asper*, *Trifolium repens*, *Erigeron canadensis*, *Adiantum incisum*, *Adiantum venustum*, *Cynodon dactylon*, *Ficus carica*, *Bombax ceiba* and *Paspalum distichum* ., under the consequence of higher precipitation, copper, nickel, chromium and less concentration of phosphorus, potassium, calcium carbonate, organic matter, magnesium, calcium and cadmium.

The 3rd quadrant indicated *Rosa webbiana*, *Prunus persica*, *Sagittaria sagittifolia, Campsis radicans*, *Diospyros lotus*, *Apluda mutica*, *Persicaria hydropiper*, *Oenothera rosea*, *Bidens bipinnata* and *Salix tetrasperma* , under effect of higher soil pH, zinc, elevation in conjunction with lower amount of electrical conductivity, manganese, total dissolved solids, iron, temperature.

The 4th quadrant of CCA biplot distributed *Dactyloctenium aegyptium*, *Morus alba*, *M. nigra*, *Euphorbia prostrata*, *Azadirachta indica*, *Chenopodium murale*, *Broussonetia papyrifera*, *Acrachne racemosa*, *Chenopodium album* and *Achyranthes aspera* , that were assembled under the influence of higher phosphorus, potassium, calcium carbonate, organic matter, magnesium, calcium, cadmium and lower precipitation, copper, nickel and chromium concentration (Fig. 2.33; Table 2.10).

Table 2.10 The summary of CCA of all the plants and stations in relation to measured environmental variables in the semi-humid subtropical MWPE.

Axes	**1**	**2**	**3**	**4**	**Total Inertia**
Eigenvalues	0.466	0.309	0.278	0.246	19.269
Species-environment correlation	0.913	0.680	0.822	0.793	
Cumulative percentage variance of species data	2.4	4.0	5.5	6.7	
Cumulative percentage variance of species data	14.2	23.6	32.1	39.5	
Summary of Monte Carlo test					
Test of significance of 1st canonical axis	Eigenvalue	0.466	Test of significance of all canonical axes	Eigenvalue	3.285
	F-ratio	2.922		F-ratio	1.276
	P-value	0.0002		P-value	0.0002

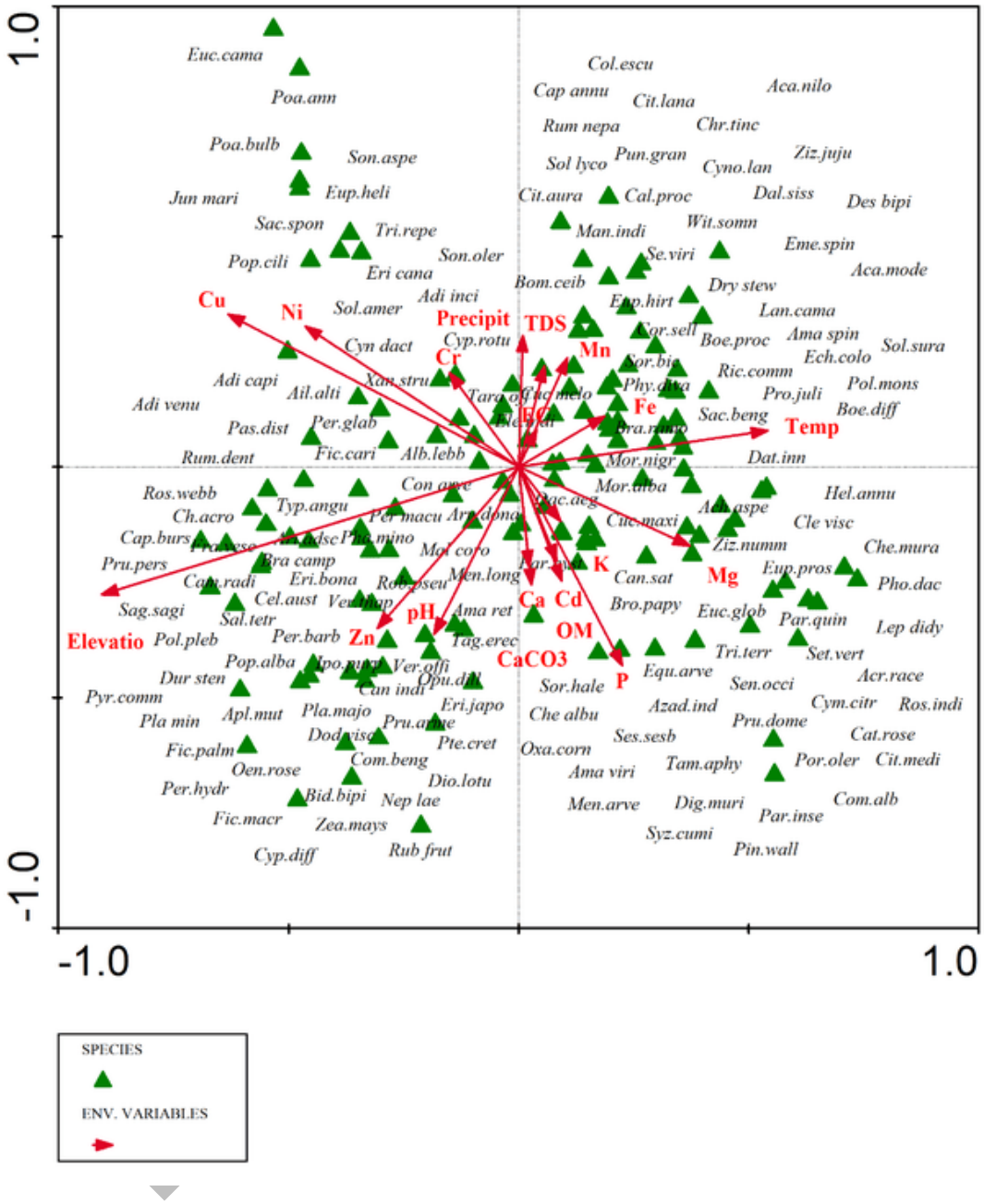


Fig. 2.32 Canonical correspondence Analysis biplot showing the distribution pattern of plant species under the influence of different variables.

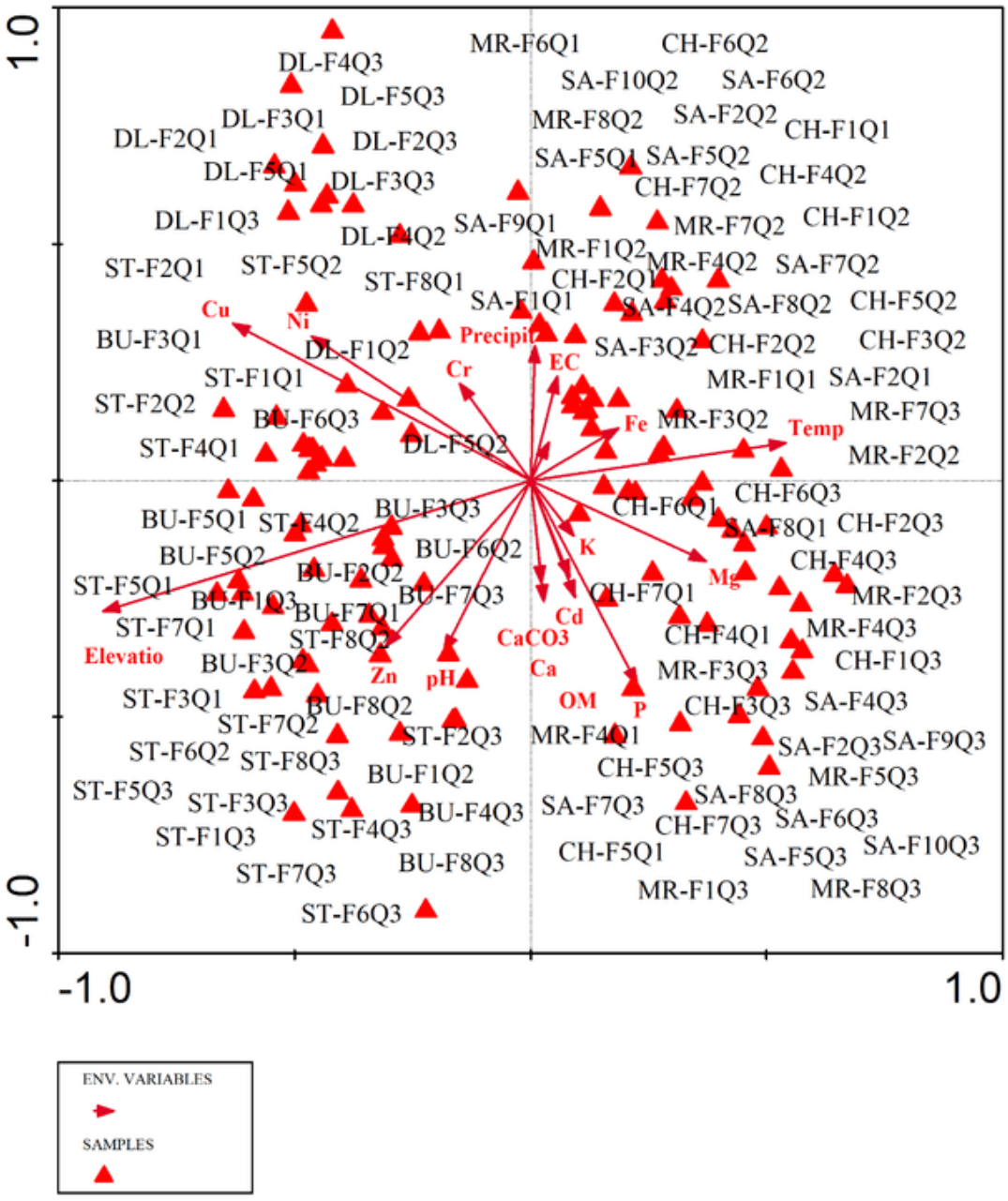


Fig. 2.33 Canonical correspondence Analysis biplot showing the distribution pattern of stations under the influence of measured environmental factors in semi-humid MWPE.

2.3.4.3 Dry Subtropical Vegetation Zone (DSVZ) of MWPE

This dry subtropical zone of MWPE comprises 123 stations and 146 various types of plant species after TWCA of the Sorenson similarity index. This zone is situated at an altitude range of 297-1437m. It comprised of 91 herb species (62.32%), 38 trees (26.02%) and 17 shrub species (11.64%). Family Poaceae with 18 number of plant species (12.33 %) was the most leading followed by Asteraceae 11 species (7.53 %), Amaranthaceae 9 species (6.16 %), Solanaceae 8 species (5.48 %), Fabaceae, Moraceae & Polygonaceae each wih 6 plants (4.11 %), Rodaceae 5 species (3.42 %), Cucurbitaceae, Lamiaceae, Myrtaceae, Nyctaginaceae & Rutaceae each with 4 plants (2.74 %), Euphorbiaceae, Pteridaceae and Verbenaceae with 3 plant species each (2.05 %) in this MWPE (Appendix table 3). The TWCA of dry subtropical MWPE further comprehends distribution of 146 plant species in the 123 studied stations or quadrats (Fig. 2.34).

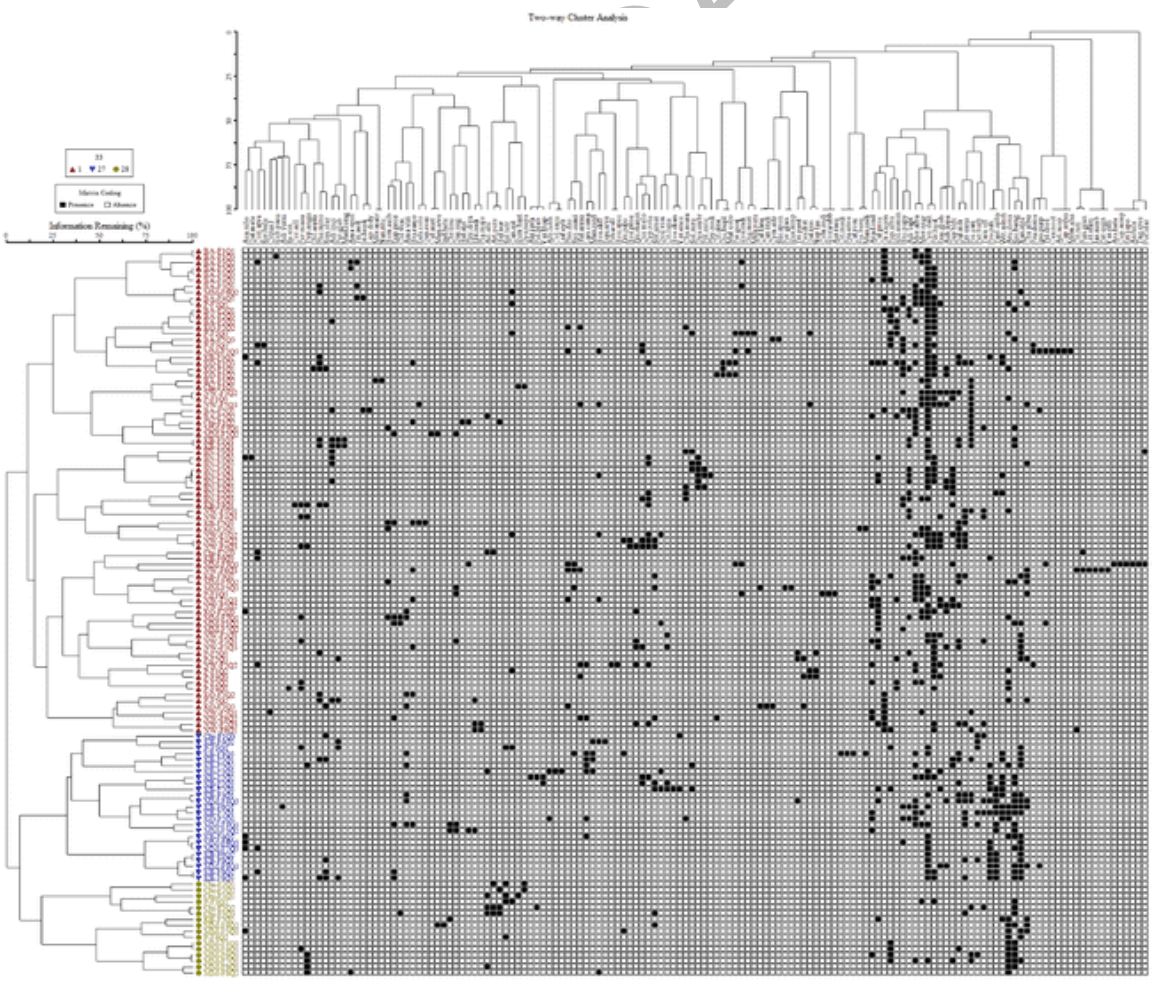


Fig. 2.34 TWCA dendrogram of 146 plant species and 123 stations/quadrats in the dry subtropical MWPE, KPK, Pakistan.

2.3.4.3.1 Abundant and rare plants of the Dry subtropical MWPE

Tree layer:

This layer was dominated by *Morus alba* (804 IVI), *Dalbergia sissoo* (590 IVI), *Morus nigra* (581 IVI), *Ficus carica* (576 IVI), *Populus alba* (567 IVI), *Eucalyptus globulus* (477 IVI), *Broussonetia papyrifera* (432 IVI), *Prosopis juliflora* (418 IVI), *Tamarix aphylla* (353 IVI) and *Azadirachta indica* (307 IVI) (Fig. 2.35). While, *Pinus wallichiana* (8.33 IVI), *Eriobotrya japonica* (8.47 IVI), *Cupressus sempervirens*, *Araucaria heterophylla* (8.47 IVI), *Prunus armeniaca* (8.93 IVI), *Citrus medica* (8.93 IVI), *Litchi chinensis* (12.22 IVI), *Citrus limon* (16.67 IVI), *Mangifera indica* (17.68 IVI), *Celtis australis* (22.22 IVI) and *Ficus benjamina* (27.50 IVI) were the rare tree species (Fig. 2.36).

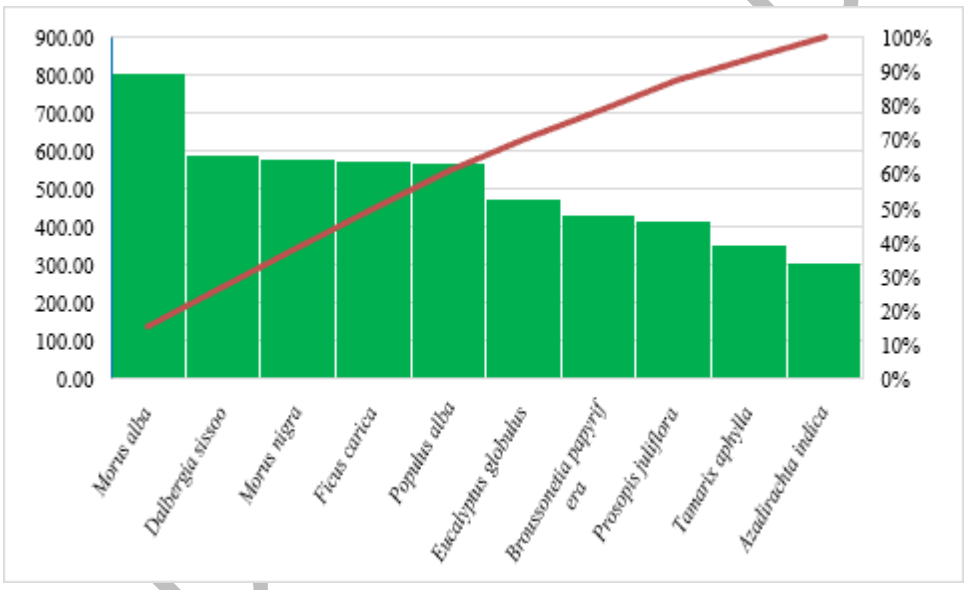


Fig. 2.35 The topmost abundant tree species in the Dry subtropical MWPE, KPK

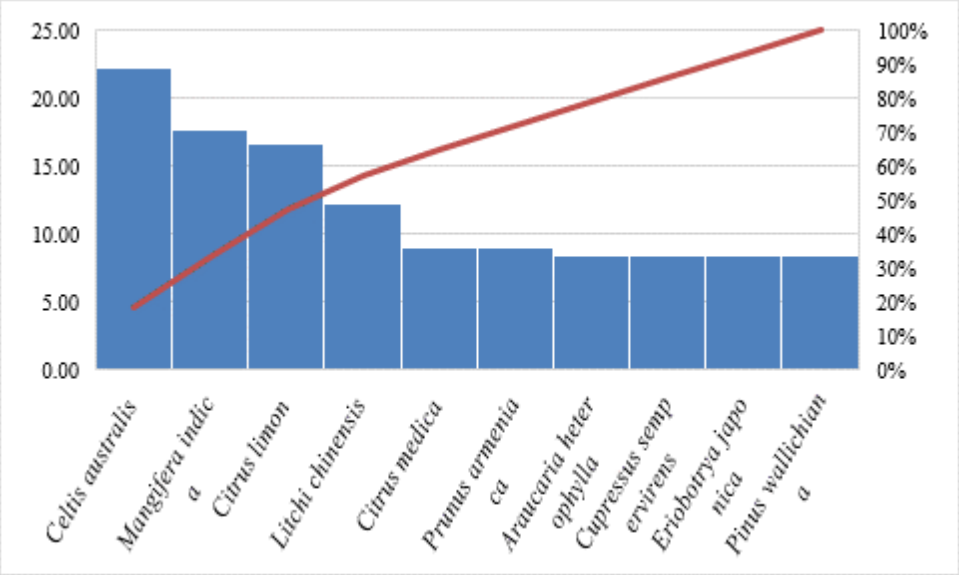


Fig. 2.36 The rare tree species in the Dry subtropical MWPE with minimum IVI.

Shrub Layer:

The *Calotropis procera* (727 IVI) was the topmost dominant shrub species followed by *Withania somnifera* (675 IVI), *Ricinus communis* (550), *Lantana camara* (344) and *Datura innoxia* (233 IVI) (Fig. 2.37). The rare shrub species recorded *Combretum indicum* (13.89 IVI), *Rosa webbiana* (16.67 IVI), *Bougainvillea spectabilis* (17.78 IVI), *Catharanthus roseus* (19.44 IVI) and *Datura metel* (33.33 IVI) from dry subtropical MWPE (Fig. 2.38).

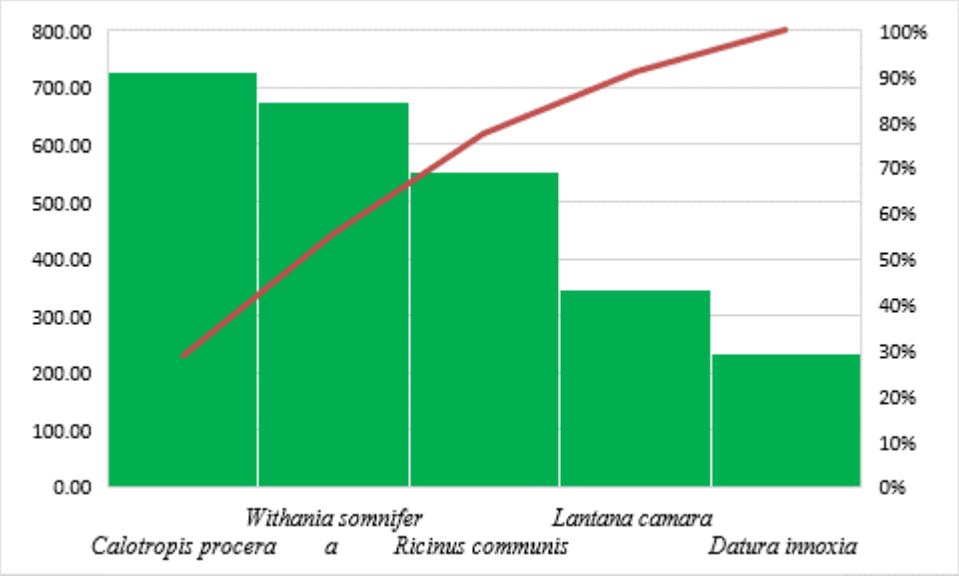


Fig. 2.37 The topmost abundant shrubs with higher IVI in the subtropical MWPE

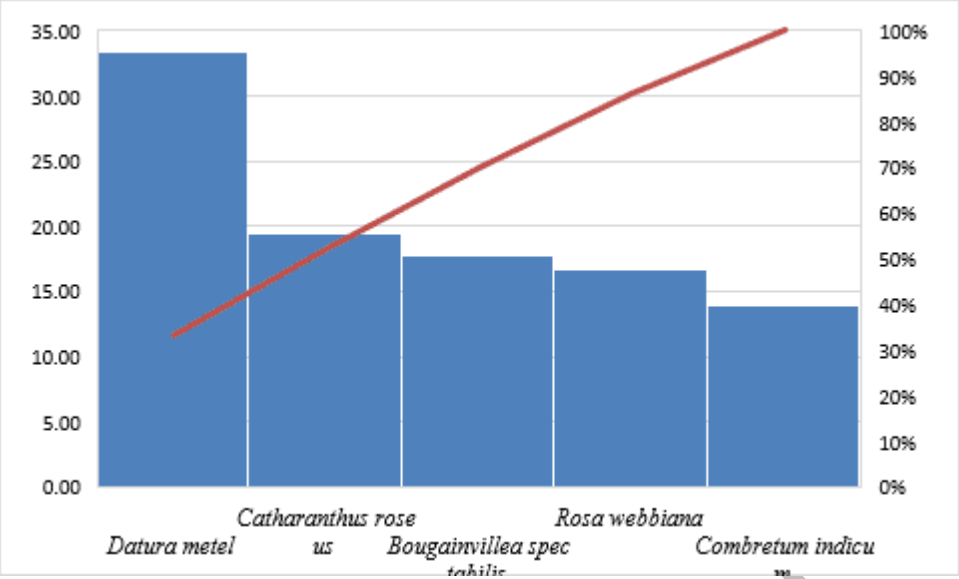


Fig. 2.38 Rare shrubs of the Dry subtropical MWPE, KPK.

Herb Layer:

This herbaceous layer was dominated by *Cynodon dactylon* (1362 IVI), *Parthenium hysterophorus* (630 IVI), *Arundo donax* (548 IVI), *Saccharum bengalense* (421 IVI), *Xanthium strumarium* (347), *Oxalis corniculata* (291 IVI), *Adiantum capillus-veneris* (261 IVI), *Desmostachya bipinnata* (254 IVI), *Chenopodium album* (204 IVI) and *Erigeron canadensis* (175 IVI) (Fig. 2.39). At the same time, *Dichanthium annulatum* (4.54 IVI), *Aerva javanica* (5.28 IVI), *Lepidium didymum* (5.71 IVI), *Capsicum annuum* (5.79 IVI), *Artemisia scoparia* (5.79 IVI), *Adiantum venustum* (6.98 IVI), *Parthenocissus quinquefolia* (7.52 IVI), *Momordica charantia* (7.87 IVI), *Asparagus racemosus* (9.26 IVI) and *Jasminum sambac* (9.48 IVI) were the rare plant species with minimum IVI in the region (Fig. 2.40).

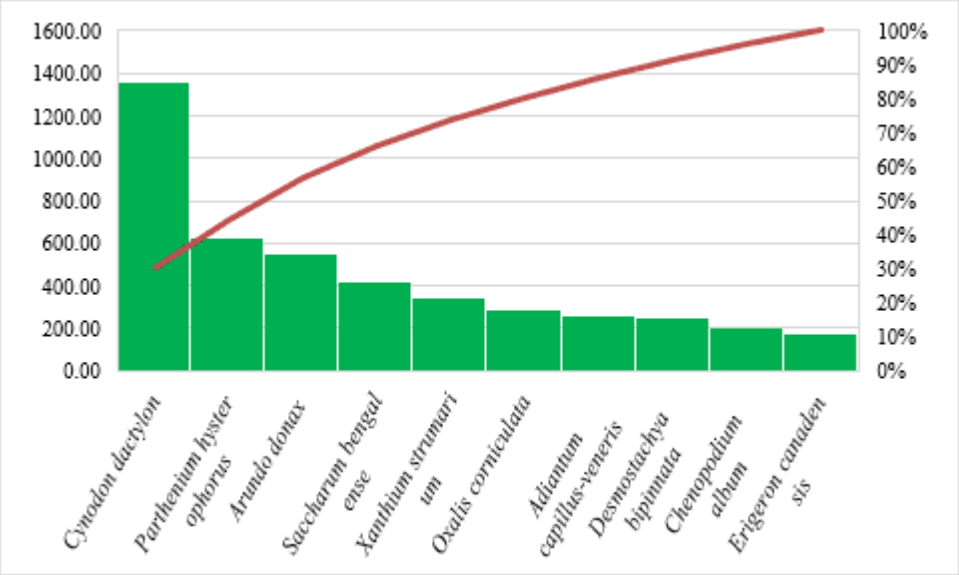


Fig. 2.39 The top ten abundant herb species recorded in the Dry subtropical MWPE.

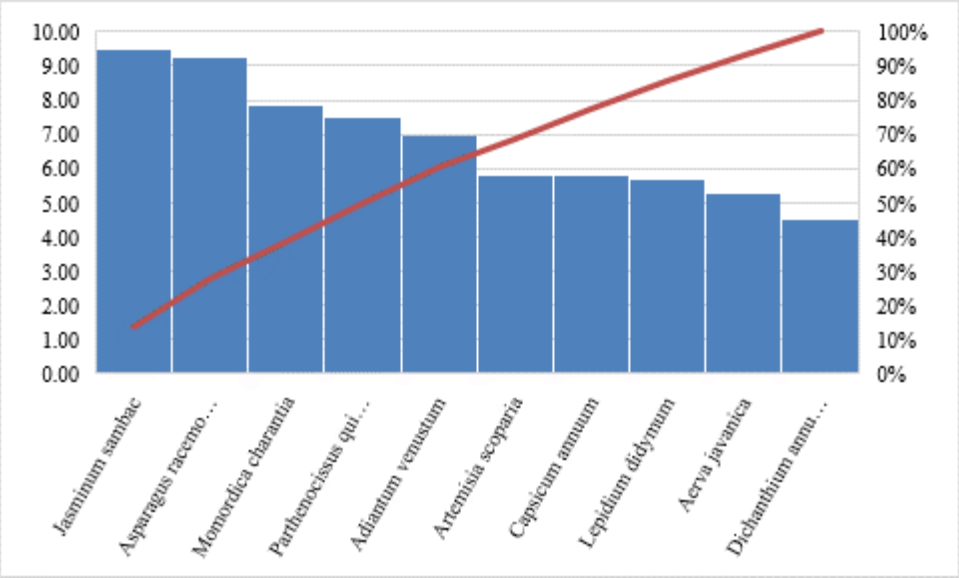


Fig. 2.40 Topmost rare herb species in the Dry subtropical MWPE, KPK, Pakistan

2.3.4.3.2 Results of Ordinary Least Square, Logistic and Probabilistic Models of Dry subtropical Zone

The Ordinary Least Square, Logistic and Probabilistic Models of Dry subtropical comprehended that the IVI (<0.024), TDS (<0.004), OM (0.370), Ni (<0.113), Cr (0.002), Cd (0.003), Zn (0.09), P (<0.696) and Precipitation (<231) have positive significant effect on the occurrence of abundant and rare plants. At the same, pH (-0.065), EC (-0.0001), CaCO3 (< - 0.361) and K (- 0.002) have negative significant

effect on the abundant and rare plant species of the Dry subtropical MWPE (Table 2.11). The AIC, R^2, Chi- square and probability values were recorded as 153, 0.550, 9.477 and 0.0001, respectively. Hence, it shows that our model is a perfect fit.

Table 2.11 Ordinary Least Square, Logistic and Probabilistic Models of abundant and rare plant species in the Dry subtropical MWPE, KPK , Pakistan.

Variables	**OLS**	**Logit**	**Probit**	**Variables**	**OLS**	**Logit**	**Probit**
IVI	0.0001 (-0.00001)	0.024 (-0.004)	0.012 (-0.002)	Cd	0.003 (-0.001)	0.017 (-0.04)	0.008 (-0.021)
pH	-0.065 (-0.032)	1.382 (-1.089)	0.568 (-0.57)	Zn	0.0002 (-0.0003)	0.015 (-0.009)	0.009 (-0.005)
EC	-0.0001 (-0.0001)	-0.001 (-0.002)	-0.001 (-0.001)	Fe	-0.0001 (-0.0002)	(-0.005 (-0.006)	-0.002 (-0.003)
TDS	0.0001 (-0.00004)	0.004 (-0.002)	0.002 (-0.001)	K	-0.0001 (-0.0001)	-0.004 (-0.003)	-0.002 (-0.001)
OM	0.370 (-0.125)	2.863 (-3.612)	0.93 (-1.869)	P	0.026 (-0.009)	0.696 (-0.295)	0.396 (-0.15)
CaCO3	-0.009 (-0.003)	-0.361 (-0.13)	-0.201 (-0.067)	Ca	0.00003 (-0.0001)	0.001 (-0.002)	0.001 (-0.001)
Ni	0.011 (-0.002)	0.113 (-0.065)	0.068 (-0.034)	Mg	-0.0001 (-0.0001)	-0.002 (-0.002)	-0.001 (-0.001)
Cr	0.002 (-0.001)	0.011 (-0.03)	0.009 (-0.016)	Temp	0.003 (-0.004)	0.084 (-0.134)	0.04 (-0.071)
Cu	0.001 (-0.001)	-0.011 (-0.03)	-0.005 (-0.016)	Precipitation	0.004 (-0.003)	0.231 (-0.083)	0.123 (-0.043)
Mn	-0.001 (-0.001)	0.004 (-0.015)	-0.0001 (-0.008)	Constant	0.469 (-0.353)	-30.580 (-12.191)	-14.475 (-6.215)

R^2= 0.550, F-statistics/Chi-square = 9.477, AIC=153.058; 156.678

2.3.4.3.3 Ecological gradient through Detrended correspondence Analysis of Dry-subtropical MWPE

DCA was carried out to illustrate the distribution pattern of dry subtropical vegetation via Detrended gradient analysis (Fig. 2.41; Fig. 2.42). The maximum gradient length was recorded high for axis 1 i.e., 8.36 with eigenvalue 0.72, followed by axes 2, 3 and 4 with eigenvalues 0.56, 0.52 and 0.44, respectively. The cumulative percentage variance of species data was observed maximum by axis 4 i.e., 11.7 accompanied by axes 3, 2 and 1 with 9.3, 6.7 and 3.8, correspondingly. While the sum of all eigenvalues or total inertia was recorded as 19.267 (Table 2.12).

Table 2.12 Showing eigenvalues, gradient length and cumulative percentage variance in the dry subtropical MWPE.

Axes	**1**	**2**	**3**	**4**	**Total inertia**
Eigenvalues	0.72	0.56	0.51	0.44	19.267
Lengths of gradient	8.36	6.59	4.19	4.36	
Cumulative percentage variance of species data	3.8	6.7	9.3	11.7	

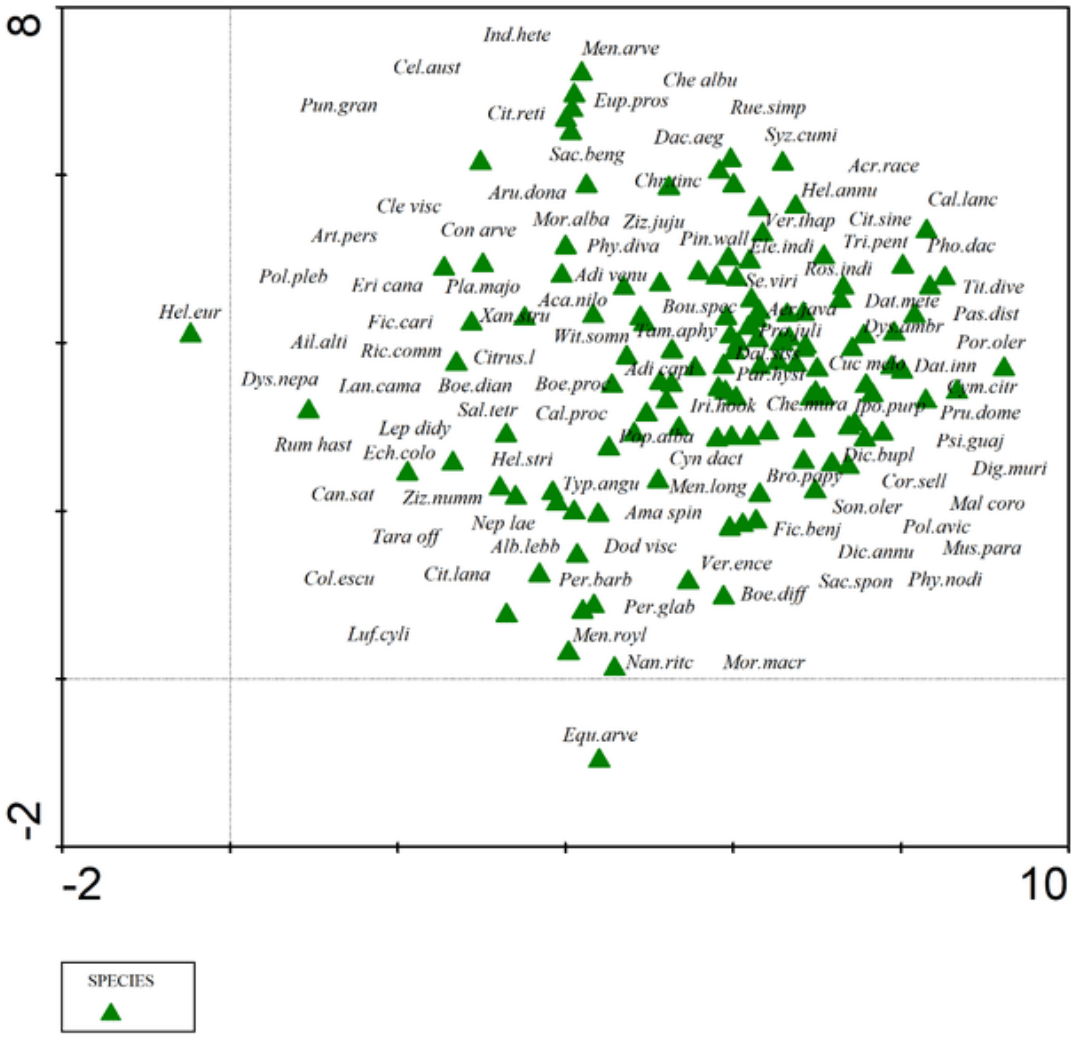


Fig. 2.41 DCA demonstrating the distribution of plants in the dry subtropical MWPE.

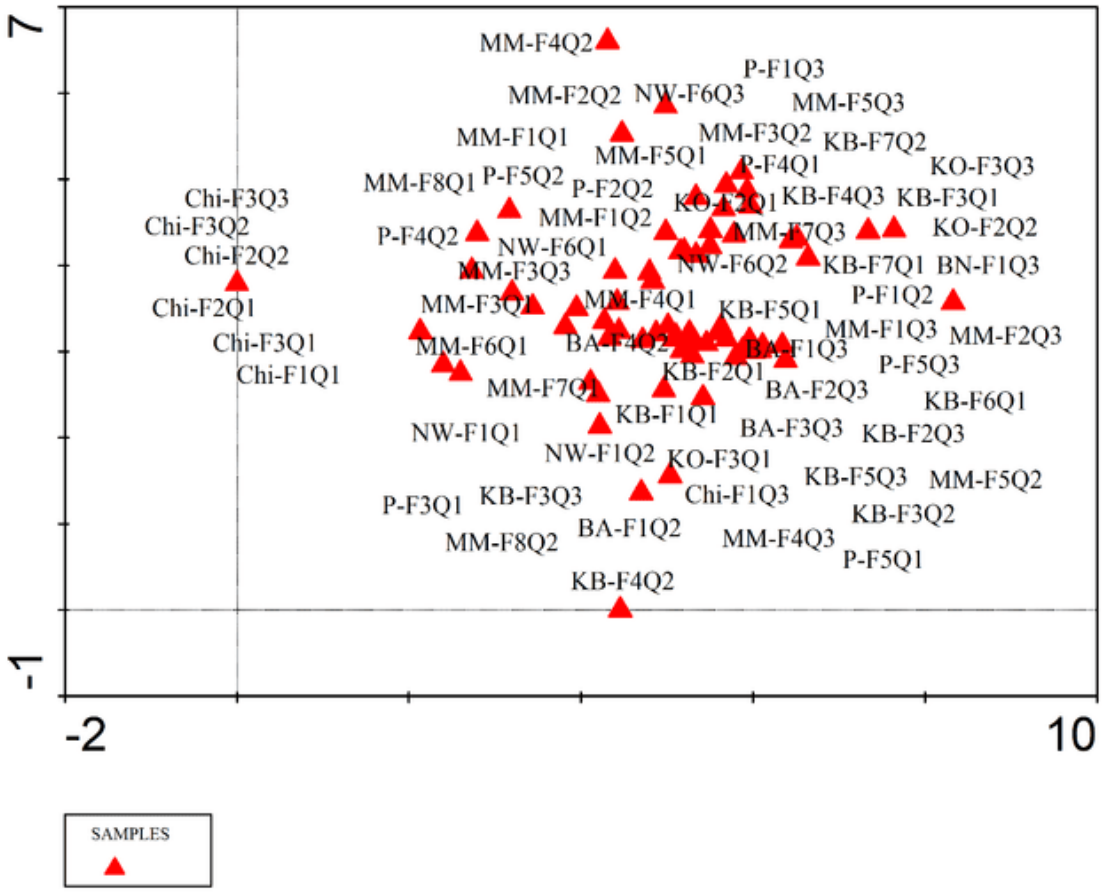


Fig. 2.42 DCA indicating the distribution of stations in the dry subtropical MWPE.

2.3.4.3.4 Ecological gradient through Canonical Correspondence Analysis (CCA) of Dry subtropical MWPE

CCA analysis resulted that all the measured environmental variables have significant effect (probability=0.0002) on plant species composition and distribution pattern in Dry subtropical MWPE (Table 2.13).

The 1st quadrant of CCA bi-plot distributed *Digera muricata*, *Sorghum halepense*, *Mentha longifolia*, *Ailanthus altissima*, *Cannabis sativa*, *Tribulus pentandrus*, *Heliotropium europaeum*, *Artemisia persica* and *Colocasia esculenta*, under the impact of higher concentration of cadmium, chromium and lower soil pH, manganese, iron, potassium and temperature (Fig. 2.43).

The 2nd quadrant comprehended the distribution of *Poa annua*, *Sonchus asper*, *Trifolium repens*, *Erigeron canadensis*, *Adiantum incisum*, *Adiantum venustum*, *Cynodon dactylon*, *Ficus carica*, *Bombax ceiba* and *Paspalum distichum*, under the consequence of higher precipitation, copper, nickel, chromium and less concentration of phosphorus, potassium, calcium carbonate, organic matter, magnesium, calcium and cadmium.

The 3rd quadrant clustered *Withania somnifera*, *Datura metel*, *Dalbergia sissoo*, *Xanthium strumarium*, *Acacia nilotica*, *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Chrozophora tinctoria*, *Tamarix aphylla* and *Phyla nodiflora*, under the effect of higher soil pH, manganese, iron, potassium, temperature and lower cadmium and chromium concentration in the region.

The 4th quadrant of CCA bi-plot distributed *Setaria viridis*, *Saccharum spontaneum*, *Amaranthus spinosus*, *Verbesina encelioides*, *Saccharum bengalense* and *Broussonetia papyrifera*, under the influence of higher nickel, copper, zinc, elevation and low organic matter, calcium carbonate, total dissolved solids, phosphorous, magnesium, electrical conductivity and precipitation (Fig. 2.44; Table 2.13).

Table 2.13 CCA summary of all the plants and stations in relation to measured environmental variables in the dry subtropical MWPE.

Axes	**1**	**2**	**3**	**4**	**Total Inertia**
Eigenvalues	0.577	0.349	0.330	0.293	19.267
Species-environment correlation	0.924	0.871	0.848	0.824	
Cumulative percentage variance of species data	3.0	4.8	6.5	8.0	
Cumulative percentage variance of species data	15.4	24.7	33.5	41.3	
Summary of Monte Carlo test					
Test of significance of 1st canonical axis	Eigenvalue	0.576	Test of significance of all canonical axes	Eigenvalue	3.748
	F-ratio	3.176		F-ratio	1.309
	P-value	0.0002		P-value	0.0002

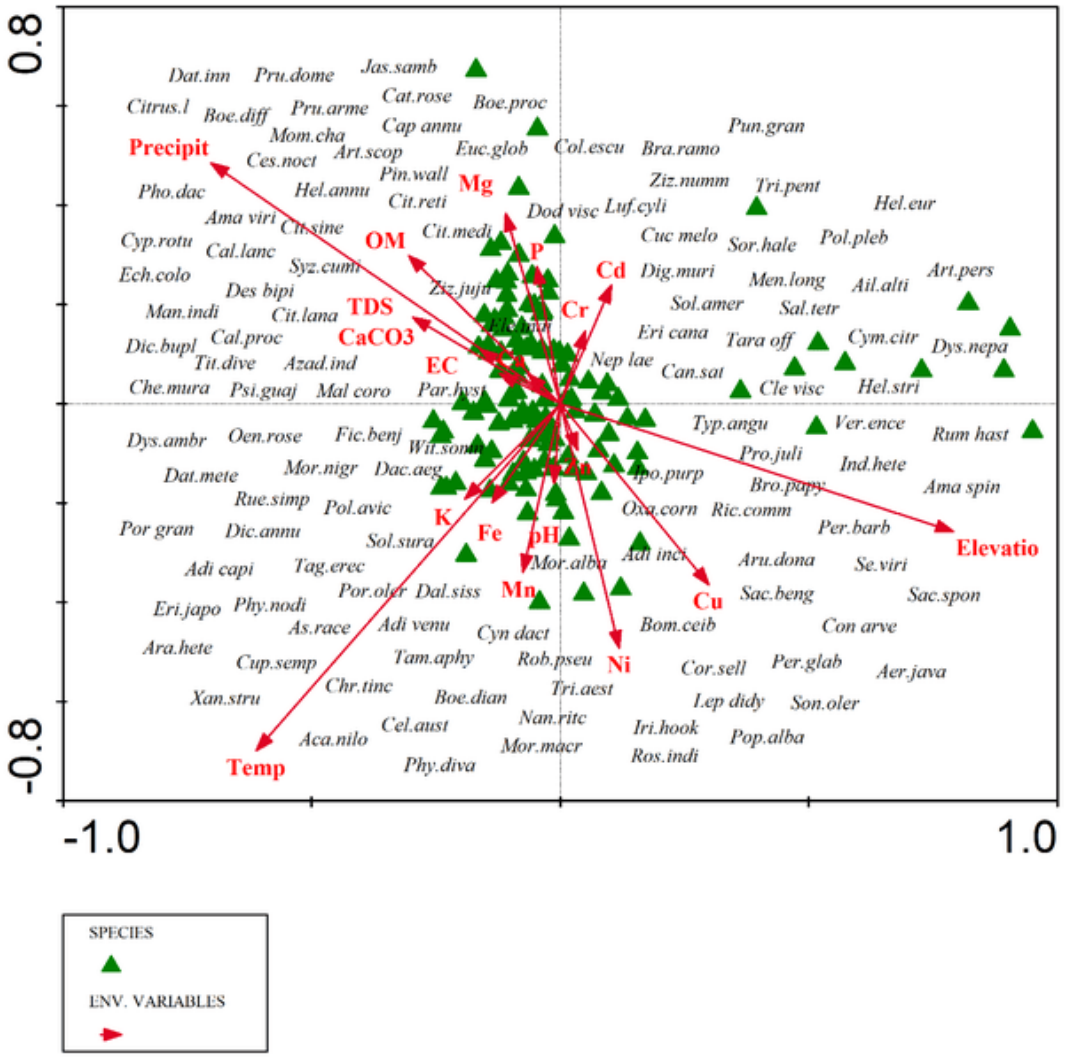


Fig. 2.43 Ecological gradient through CCA biplot showing the distribution of plant species under the impact of various environmental factors in dry subtropical MWPE

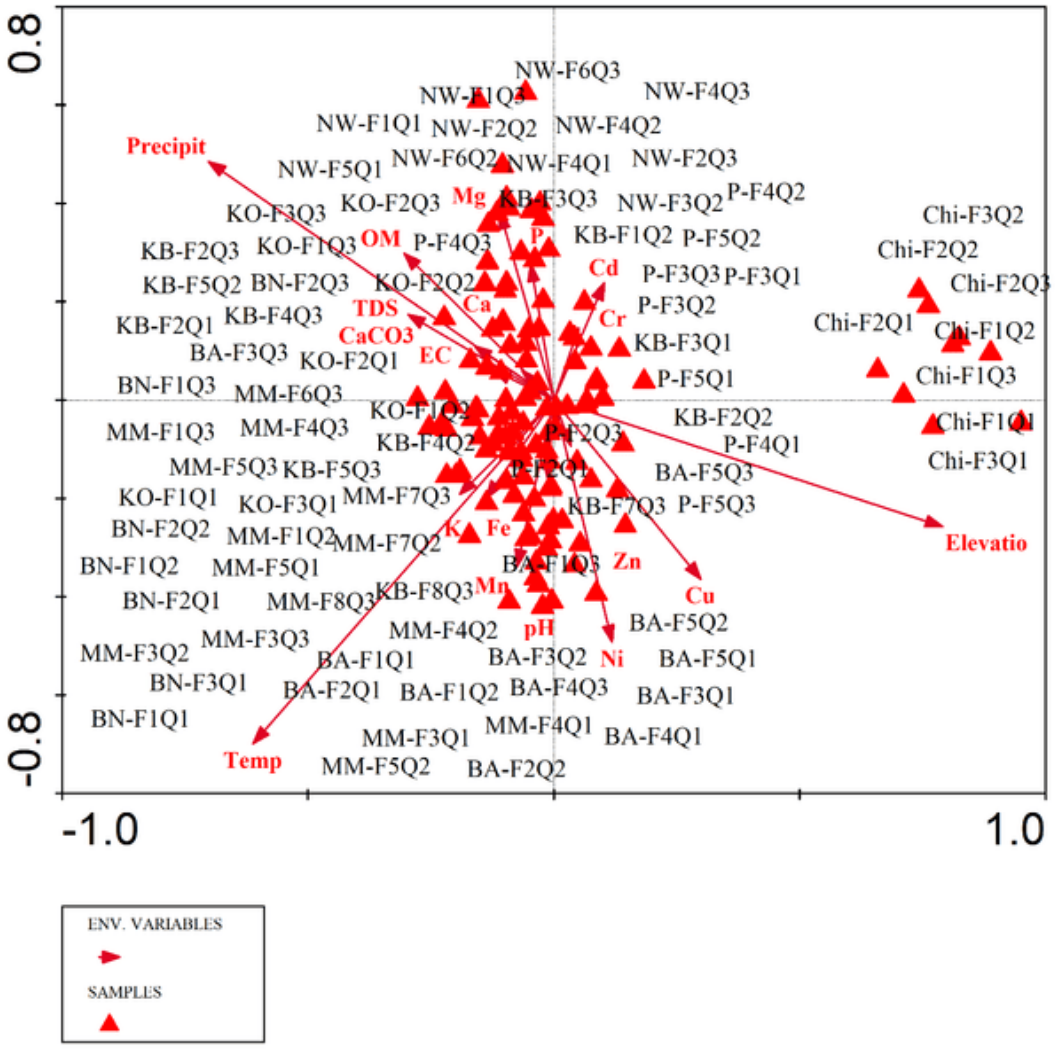


Fig. 2.44 Ecological gradient through CCA biplot showing the distribution of stations under the impact of various environmental factors in dry subtropical MWPE.

2.3.5 Characterization and Phylogenetic studies of Fungal Flora

A total of 15 fungal strains were isolated from Marble Waste polluted ecosystem of the studied region. Morphologically most of these strains comprised aseptate hyphae and black, brown, green, white to dark green colors (Fig. 2.45). Whereas, anatomically these strains range from cylindrical to round, hyaline in Lactophenol Blue, thick to thin walled, smooth to ornamented surface with sharp scale and fusoid to ellipsoid in shape (Fig. 2.46; Table 2.14). It showed a wide variety of variations among the fungi.

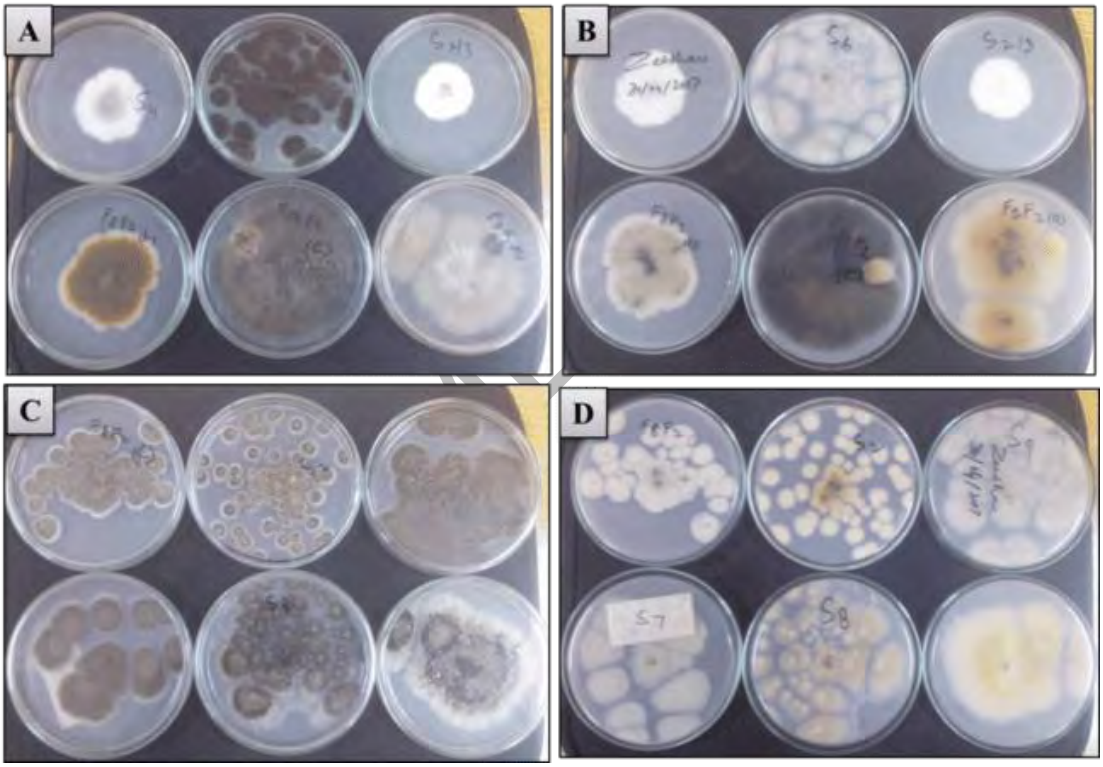
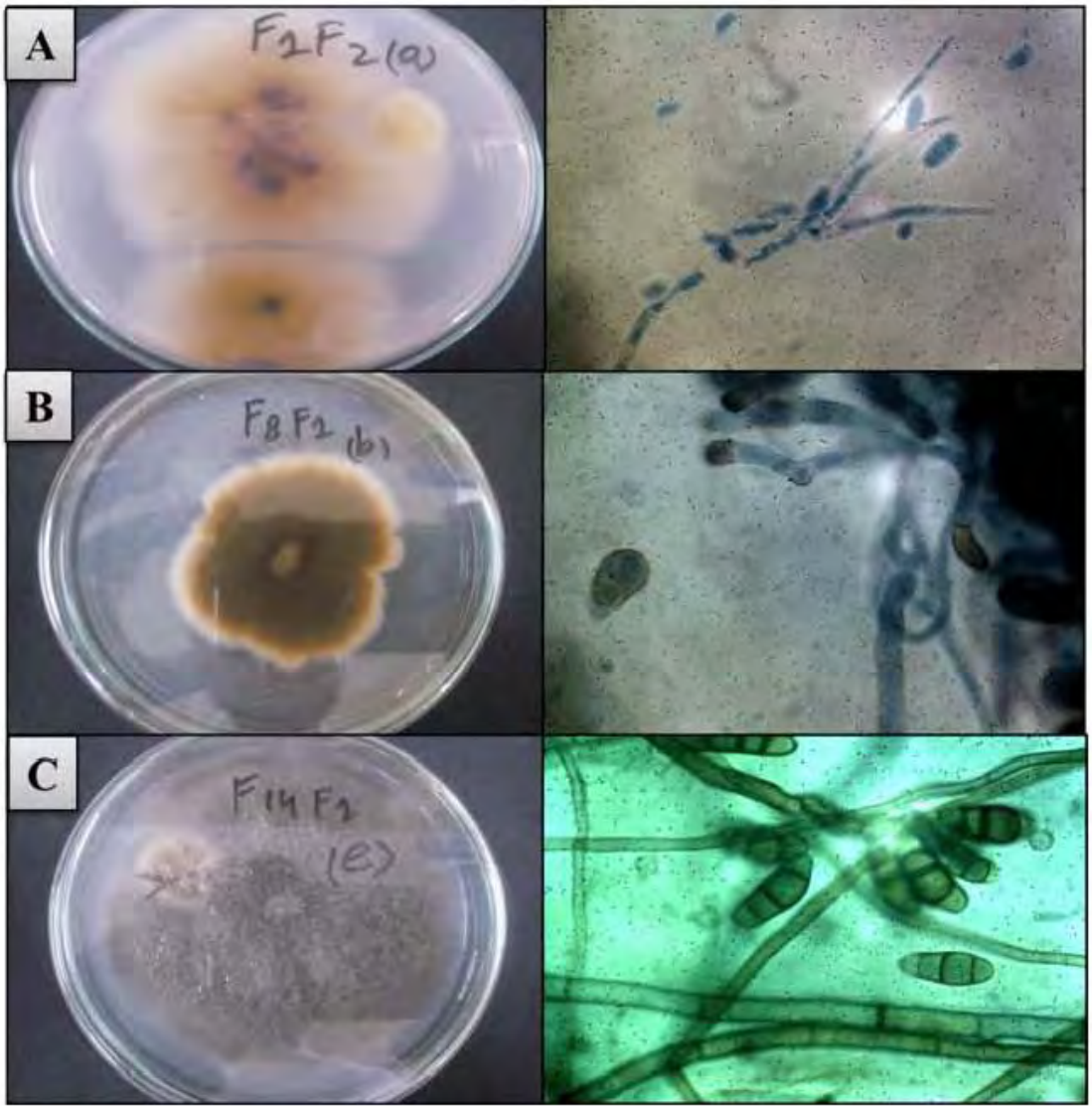


Fig. 2.45 Pure fungal isolates from marble waste polluted ecosystem

Table 2.14 Morphological and anatomical characteristics of fungal colonies grown on Malt extract agar plates and stained after 48 -72 hr. of incubation.

S. NO	Fungal strains	Spore color	Appearance of colonies	Anatomical characters/ Microscopic characters	Shape of hyphae
1	S1	White	Feather like	Cylindrical, hyaline in lactophenol and blue in staining, thick walled, 16 - 60 µm in length and 8 - 10 µm in width, smooth surface.	Septate
2	S2	Green	Foam like	Spherical, black in color, ornamented, thick walled, 6.4 - 7.6 µm in length and 6.1 - 7.3 µm in width.	Aseptate
3	S3	Brown	Point like	Clavate, hyaline in lactophenol and blue in staining, gattulated, thin walled, 16-20 µm in length and 6-8 µm in width, smooth surface.	Septate
4	S4	White	Foam like /spread	Cylindrical, hyaline in lactophenol, thick walled, 54 - 79 µm in length and 5.8 - 6 µm in width, smooth surface.	Septate
5	S5	Black	Foam like /spread	Rounded, black in color, surface ornamented, surface with sharp scales, 8 - 9 µm in length and 7 - 9 um in width.	Aseptate
6	S6	Black	Foam like	Spherical, hyaline, smooth surface, thick walled, 4.9 - 6.1 µm in length and 4.4 - 5.4 µm in width.	Aseptate
7	S7	Dark green	Conjugated hyphae	Hyaline in lactophenol and blue in staining, thin walled, retienlate in shape, 5.8 - 6.7 µm in length and 5.3 - 6.2 µm in width, smooth surface.	Aseptate
8	S8	Black & white Spores black	Foam & spread	Hyaline in lactophenol, thick walled, spherical and retienlate in shape, 6.5 - 8.3 µm in length and 6.3 - 6.9 µm in width, smooth surface.	Aseptate

9	S9	Dark brown spores green/brown	(pointed) spread	Hyaline in lactophenol, thin walled, spherical in shape, 5.1 - 7.6 µm in length and 4.6-5.3 µm in width.	Aseptate
10	F1 F2	Black	Pointed	Cylindrical, Hyaline in lactophenol, thin walled, 37 - 77 µm in length and 4-7 µm in width, smooth surface.	Aseptate
11	F8 FA	Black	Pointed	Clavate, sometime spherical, ellipsoid, brown color, thick walled, 21 - 39 µm in length and 11 - 20 µm in width, smooth surface, just like telio spores.	Septate
12	F8 f1 B	White/brown	Foam/ snow like	hyaline in lactophenol, thin walled, spherical in shape, surface ornamentation present, 6.1-4.6 µm in length and 4.6-5.3 µm in width.	Aseptate
13	F8f2 C	Green	Powdery	Fusoid to ellipsoid in shape, hyaline in lactophenol and blue in staining, thick walled, gattulated, sometime small apiculus are present, 12-21 µm in length and 9-11 µm in width, smooth surface.	Aseptate
14	F14 F1	Pure black	Feather like /spread	Olive brown color, thick walled, ellipsoid, clavate, 30-34 µm in length and 12-15 µm in width, smooth surface, just like telio spores.	Septate
15	F14 f4	White/ bacteria like	Shiny	Hyaline in color, thin walled, rod shape, equal, 17-32 in length and 2-3 µm in width, smooth surface.	Septate


A
F1F2(a)
B
F8F2 (b)
C
F14F2
(e)

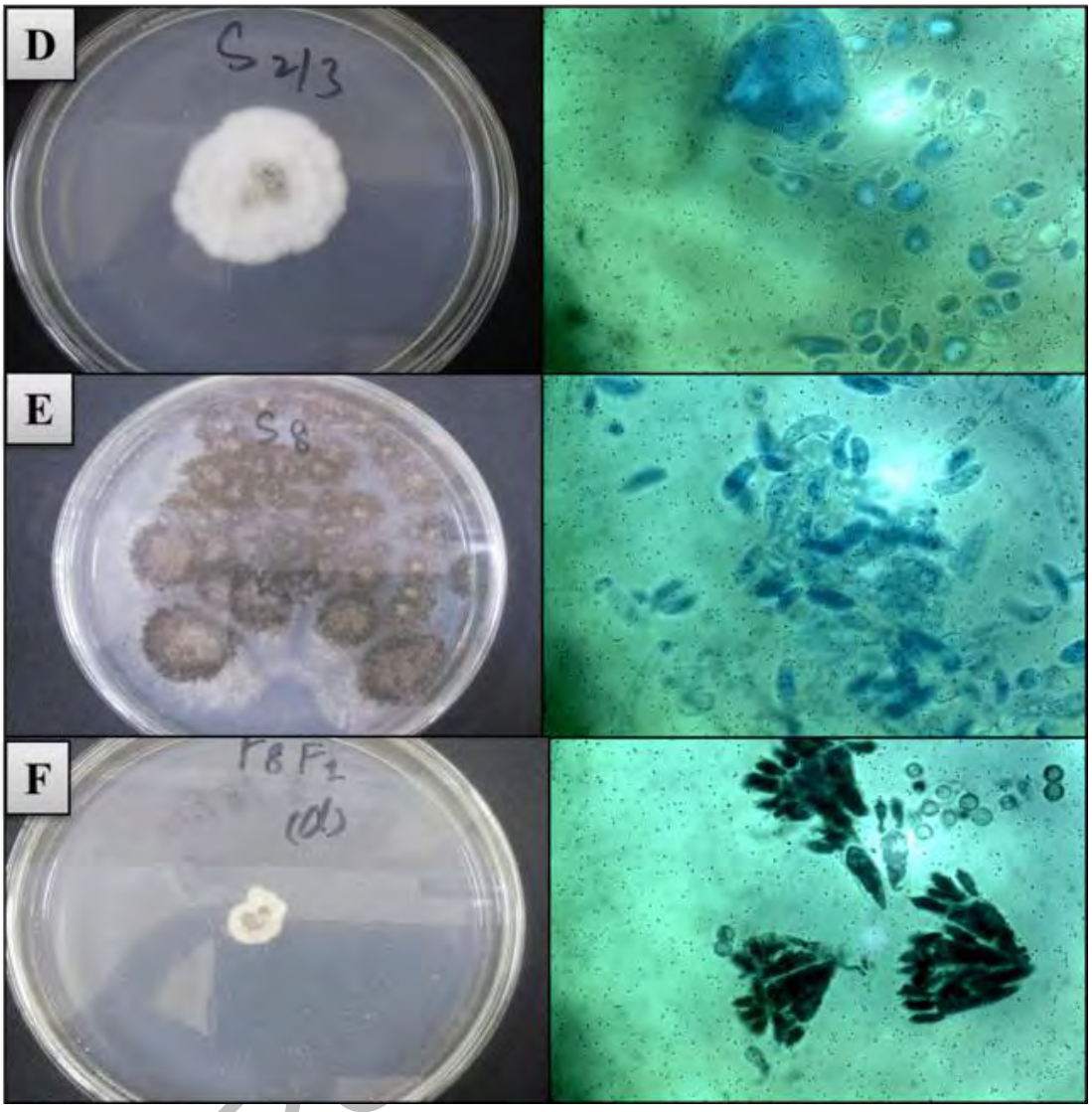


Fig. 2.46 Morphological and microscopic characterization of fungal isolates. A) fungal strain with black spore color and aseptate hyphae B) hyaline in lacto-phenol with aseptate hyphae C) pure black in color with septate hyphae, D) fungal colony with foam like appearance and aseptate hyphae E) spore black in color with aseptate hyphae F) white in color and aseptate hyphae.

2.3.6 Molecular and Phylogenetic analysis of the selected micro fungi

On the basis of aforementioned results i.e., isolation, morphological, anatomical/ microscopic characterization, some of the micro fungi were selected for further molecular analyses. The detailed descriptions are as follows:

Local Alignment Search Tool (BLAST) was used to find out the most similar sequence. All the consensus of ITS1F and ITS4 were analysed using a gene bank search. ZAF 04 and ZAF 05 strains showed 99% similarity with *Aspergillis brasiliensi* (KX011592), having 95% Query coverage. ZAF 02 and ZAF 03 resemble 99% to *Aspergillus sydowii* (KU687806) along with 97% Query Coverage. ZAF 07 strain showed 100% resemblance with *Aureobasidium leucosper* JN712487 having 99% Query coverage. ZAF 06 resemble 99% with *Fusarium petrophilium* (KP132225), acquiring 93% Query coverage. ZAF 08 resembled 99% in conjunction with 99% Query coverage to *Alternaria alternata* (KY609180). ZAF 09 comprehends 99% similarity with *Curvularia aeria* (KT933642.1) along 98% Query coverage. The final complete data set of 54 sequences resulted 1150 character including gaps, of these 486 characters was removed both on 5‘ and 5‘ end. The final data set consist of 664 characters, out of which 227 were conserved sequences, 395 variable, 297 parsimony informative sites and 98 singleton sites. The final phylogram is representing by 3 clades with *Lyomyces organensis* as root of the tree (out group). The clade one is further divided into three sub clades. The sub clade A with 100% ML bootstrap value contained ZAF 04 and ZAF 05 strains having close affinities with accessions KX011592, JQ316521, FJ195349, KC796389, KT378129, KM491891, FJ629321, FJ195348, KX098315, AM295181 and FJ717684 of *Aspergillis brasiliensi*. Where in sub clade B our strains ZAF 02 and ZAF 03 have close leaning with *Aspergillus sydowii* having accession number KU687806, LN898728, KJ413376 and KU687806 etc. at 93% ML bootstrap value. The consensus of these sequences comprehends polymorphism on one position which indicated minimum divergence. The sub clade C with 100% bootstrap value contained ZAF 07 along with *Aureobasidium leucosper* JN712487 and JN712488. The clade 2 representing ZAF 06 in conjunction with *Fusarium petroliphilum* having accession KP132225, KC254043, LC184213, LC184243 and LC184196 at 100% ML bootstrap value. Clade 3 was further divided into two sub clade i.e., sub clade A & B. Our strain ZAF 09 has close affinities with accession KT933642, KP131919, KT933641, KT933639, and

KU856631 etc. of *Curvularia aeria* at 100% ML bootstrap value. The sub clade B contained ZAF 08 strain with 74% bootstrap value with accession KY609180, KU182490, KX377683, KP739874, KP739875, KY075667, KX926578, KJ002064, AY154682 and KU179665 of *Alternaria alternata.* It shared 100 % genetic character with *Alternaria alternata* (KY609180) and presented minimum divergence (Fig. 2.47).

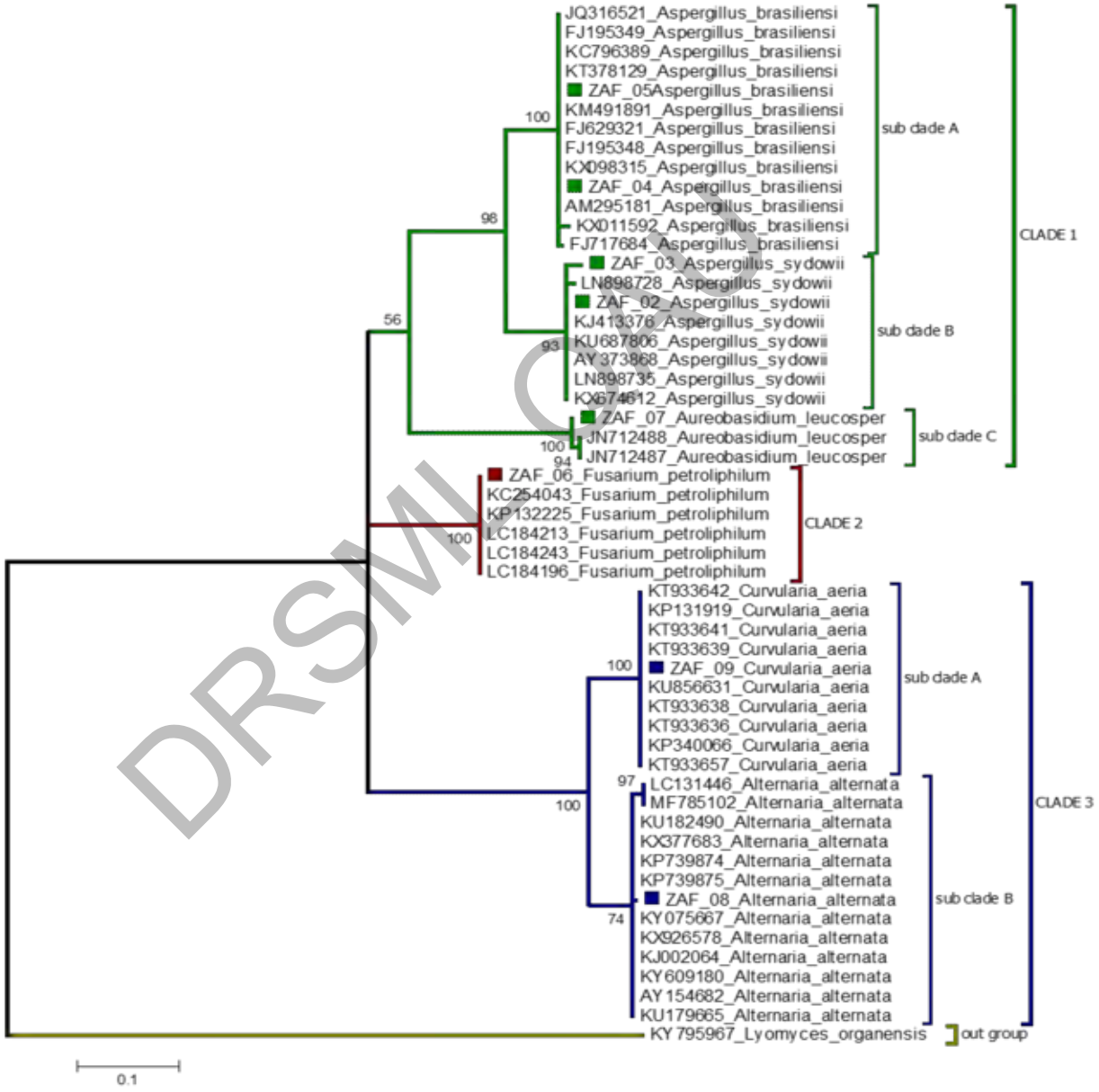


Fig. 2.47 Molecular phylogenetic relationship of micro fungus species with their allies inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model using nr ITS-rDNA data. The analysis involved 54 nucleotide sequences. There were a total of 298 positions in the final dataset. Species collected from the marble waste polluted ecosystem were labelled with (■) box.

2.4 Discussion

Every type of ecosystem has diverse flora and fauna which mark them as unique due to the particular niches that they occupy. A reasonable relationship exists between organisms within the environment, which are the main determinants of species abundance and diversity of fauna and flora of a particular region (Zhou et al. 2008). The current study acknowledges the flora of marble waste polluted ecosystem and their influence along with different environmental plus climatic factors on species composition and distribution pattern in the subtropical geographic region, Khyber Pakhtunkhwa, Pakistan. This study acknowledges a total of 220 plant species belonging to 164 genera and 65 plant families from the subtropical zones around marble factories of the Khyber Pakhtunkhwa province, Pakistan. Habit wise they comprise 145 herbs, 24 shrubs and 51 trees. Family Poaceae was the leading family followed by Asteraceae, Fabaceae, Amaranthaceae, Polygonaceae, Rosaceae, Solanaceae, Moraceae and Brassicaceae. Our findings are in close harmony with the work of (Anwar et al. 2022; Hussain 2009; Ilyas et al. 2013) who also reported family Poaceae were dominant in their respective studied regions. The reason beyond dominancy of Poaceae is its broad ecological amplitude, human disturbance and their perennating capabilities (Anwar et al. 2022).

The climatic condition of an area is described by the life form (Raunkiaer 1934). It is an important physiognomic attribute that has been widely used in vegetation studies. It indicates micro and macroclimate as well as human disturbance of a particular area. Different regions having similar biological spectrum indicate similar climatic condition and human disturbance. The life form of plant species reflects the adaptation of plants to the climate conditions. The current study indicated that the Therophytes were the leading life form class followed by Hemicryptophytes and Nanophanerophytes in the MWPE of Khyber Pakhtunkhwa, Pakistan. These dominances reveal the response to harsh environmental conditions and immense human disturbance i.e., marble pollution. The dominancy of therophytes followed by hemicryptophytes has also been observed (Badshah et al. 2013) in different types of polluted regions. Maximum numbers of therophytes are easily adapted to human disturbance (Shah 2013). In the current study microphylls was the dominant leaf form followed by Mesophyll, Nanophyll, Leptophylls, Megaphylls and Aphyllous in the region. The dominance of microphylls has also been reported (Haq et al. 2015) from

the adjacent areas. The abundance of microphylls leaf form are the result of arid climates and habitat disturbance/degradation (Samad et al. 2018; Zeb et al. 2020). The current study assessed dominant plant species including *Ficus carica*, *Morus alba*, *Morus nigra,* *Calotropis procera*, *Datura innoxia*, *Ricinus communis,* *Cynodon dactylon*, *Parthenium hysterophorus* and *Erigeron canadensis* from the MWPE. Similar to the current findings (Nazir et al. 2011) reported some of these plant species from the industrial polluted regions of adjacent cities i.e., Rawalpindi and Islamabad, Pakistan. While the (Noreen et al. 2019a) worked on the vegetation of Nashpa Oil and Gas pollution and reported *Calotropis procera*, *Datura innoxia*, *Ricinus communis,* *Cynodon dactylon*, *Parthenium hysterophorus* and *Justicia adhatoda* as dominant plant species of the polluted region.

In this chapter we have used the Species Area Curves and Two-way Cluster Analyses for the sampled size adequacy and distribution of each plant species at each quadrat/station. Similar techniques have also been used by a number of researchers to assess the sample size adequacy and plant species distribution in different regions (Ahmad et al. 2016a; Bano et al. 2018; Iqbal et al. 2018; Kamran et al. 2020; Khan et al. 2017a; Khan et al. 2016). The impact of measured variables in the marble waste polluted ecosystem were analyzed through Ordinary Least Square, Logistic and Probabilistic Models to determine why some of the plant species are abundant and some of them are rare in the MWPE. It showed that the higher calcium, magnesium, nickel, chromium, zinc, soil pH, TDS, $CaCO_3$ concentration influence plant species abundance and rarity. Like the current findings (Adewole and Adesina 2011) also reported that the particulate matter released from marble factories contained calcium and magnesium in maximum concentration. A major amount of these particulate matter accumulates on the water, soil and surrounding vegetation (Adewole and Adesina 2011; Ahmad et al. 2019). In such cases, plant growing in the vicinity of pollution absorb or assimilate the pollutants during nutrient uptake and cause significant physiological changes. Such changes may be serious for those plant species especially the ones which have relatively less genetic diversity and narrow ecological amplitude. As a result, some of the plant species cope or survive the environmental pollution stress while other may not (Brandt and Rhoades 1973; Joshi and Swami 2007; Prajapati and Tripathi 2008). In the current study Canonical Correspondence Analysis (CCA) and Detrended Correspondence Analysis (DCA) were used to evaluate plant species distribution pattern and composition along

measured environmental variables in the MWPE. Both the CCA and DCA are widely used ordination and classification techniques that are frequently used for the proper determination of significant relationships between environmental data and floristic composition (Dufrêne and Legendre 1997; Hill and Gauch 1980; Ter Braak and Prentice 1988). It showed that the $CaCO_3$, electrical conductivity, soil pH, calcium, magnesium, temperature, precipitation, elevation and organic matter have a significant role in the distribution pattern of all plant species in the region. An extensive review of the literature showed that there is no data on vegetation of marble waste polluted habitats for comparison as well as foundation purposes. However, (Kabir et al. 2010) reported eighty plants in a Cement polluted ecosystem from Karachi, Pakistan with higher concentration of Calcium carbonate, EC, pH, TDS, exchangeable Potassium, Sodium and low quantity of Organic Matter concentration. In addition, they documented the industrial soil was mostly of a porous type with considerable amount of water holding capacity. Further studies would be of great importance for better management of such regions and systems.

Furthermore, in the current study a molecular approach was carried out using consensuses of ITS1F and ITS4 primers for each species. The comparison of micro fungi 18S rRNA gene sequence has an emergent preferred genetic technique. Such techniques have been widely used as a molecular clock to estimate relationship among fungi (phylogeny). Recently, it has become important as a means to identify unknown fungi up to genus or species level (Shahid et al. 2014a). The use of 18S rRNA gene sequences to study fungi phylogeny is well documented (Smit et al. 1999). Taxonomy has been by far the most common housekeeping genetic marker used for several reasons. It includes i) presence in almost all fungi often existing as a multigene operon or family. ii) Function of 18S rRNA gene has not changed over time, indicating random sequence changes were more accurate measure of time (evolution). The rRNA molecular analysis is a key method in microbiology not only to explore diversity of microbes but also to identify new strains/ cultures. In the current study, molecular identification and phylogeny of isolated micro fungi revealed most of the species belong to genus *Aspergillus* i.e. *Aspergillus sydowii*, *Aspergillus brasilensi* and *Aspergillus phoenicis*. Other species included *Fusarium petrolilum*, *Auerobasidium leucosper*, *Alternaria alternata* and *Curvularia aeria*. The results are in close harmony with work of (Roussos et al. 1995), where they reported a total of

272 strains of filamentous fungi from soil, leaves of coffee plant and coffee cherries growing in a polluted region of Mexico. It included most of the isolated microorganisms belonging to *Aspergillus, Fusarium, Trichoderma, Pennicillium* and *Humicola* genera having the ability to degrade 100 % of the caffeine in liquid media. Whereas, (Maiti et al. 2013) also sequenced the ITS region of two fungi isolated from aerobic compost environment.

DNA extraction were carried out following enzymatic digestion and glass fiber filtration protocol (Dentinger et al. 2010). Nuclear ribosomal internal transcribed spacer (ITS) was amplified using universal primers ITS1-F and ITS4-R were used for fungi. ExoSAP-IT® was used to purify PCR products. BigDye® Terminator V 3.1 Cycle Sequencing Kit in 10 µL reactions with ITS4-R and ITS1-F primers were applied to perform dye-terminated unidirectional sequencing. Whereas, (Guo et al. 2003) conceded the molecular identification of endophytic fungi using the nuclear ribosomal DNA (nrDNA) sequence analysis. The 5.8S gene and flanking internal transcribed spacer (ITS1 & ITS2) regions of nrDNA were amplified and sequenced. Further identification were done by means of sequences similarity comparison and phylogenetic analysis of the ITS regions (Dentinger et al. 2010; White et al. 1990). Similarly (Manter and Vivanco 2007) used the ITS primers (ITS1F and ITS4) for the characterization of fungal abundance and diversity in mixed template samples through qPCR and length heterogeneity analysis. In addition, (Ranjard et al. 2001) analyzed GenBank database for length heterogeneity in fungi species through a various set of conserved rRNA primers (2234C and 3126T). The ITS1F and ITS4 primers showed a significant overlap between fungal taxonomic groups having size range of 390 to 1065 base pairs in 251 samples from 104 genera. Though, unlike ITS4 and ITS1F primers members of Oomycota, Chytridiomycota and Plasmodiophoromycota comprised conserved primer sequences and must be amplified through 2234C and 3126T primers (Ranjard et al. 2001). The (De Carolis et al. 2012) identified species of *Aspergillus, Mucorales* and *Fusarium* using direct surface analysis through matrix assisted laser desorption ionization time of flight mass spectrometry.

The DNA sequencing resulted in the identification of *Aspergillus sydowii* (ZAF 02), *Aspergillus brasilensi* (ZAF 03 & ZAF 05), *Fusarium petrolilum* (ZAF 06), *Auerobasidium leucosper* (ZAF 07), *Alternaria alternata* (ZAF 08) and *Curvularia aeria* (ZAF 09) respectively. The predominant *Aspergillus sydowii* was a saprophytic

fungus mostly found in soil occasionally pathogenic to human (causing aspergillosis, keratomycosis and onychomycosis) and also causes the contamination of food. It has been found in the sea water and shown to be the cause of aspergillosis in sea fans during 1990 (Rypien 2008). While, (Matkar et al. 2013) worked on the production of cellulose from *Aspergillus sydowii* a new isolated strain obtained from terrestrial region also being reported from marine habitats. They identified isolate using 18S rDNA sequencing. Sea fan crude extracts inhibited the growth of *A. sydowii* but were less effective at higher temperature (Alker et al. 2001). The *Aspergillus sydowii* was a causative agent of epidemics that effect gorgonian corals (sea fans) and has significantly affect their pollution in the Caribbean sea (Ein-Gil et al. 2009; Toledo-Hernández et al. 2008). Whereas Chiu et al., (2005) reported peritonitis caused through *Aspergillus sydowii* in a patient undergoing continuous ambulatory peritoneal dialysis. Current study also revealed the isolation, characterization and phylogeny of *Curvularia aeria* from marble wastewater. *Curvularia* spp. was the most common pathogenic fungi on Gramineae plants and was frequently found in other cellulosic substrates (Nakada et al. 1994). Furthermore, *Aureobasidium leucosper* was reported from marble waste water, mostly ubiquitous black in color found in different types of environments (water, soil, air and limestone) (Andrews et al. 2002). While, (Smithson et al. 2013) worked on halophilic fungi (*Aureobasidium leucosper*) that has a significant effect to clear-out slats formed on the surface of sand stone in Medamoud, Egypt. *Aureobasidium leucosper* has a vital importance in biotechnology in terms of production of various types of enzymes as well as used in biological control of plant disease (Ferreira-Pinto et al. 2006; Zhang et al. 2012). The (Di Francesco et al. 2015) worked on the production of volatile organic compounds from *Aureobasidium leucosper* and demonstrated that the *A. leucosper* has a significant role against five fruit postharvest pathogens (*Penicillium italicum, penicillium digitatum, penicillium expansum, Botrytis cinerea* and *Colletotrichum acutatum*). *Aureobasidium pullulans* decreased the incidence of blue and gray mold of apple by 67 and 89 % respectively (Ippolito et al. 2000).

Aspergillus brasilensis is one of the most common species of *Aspergillus* genus causing a black mold disease of certain vegetables and fruits. It was ubiquitous in nature (Varga et al. 2007). The *Aspergillus brasilensis* was extensively exploited by fermentation industries for the production of organic acids like citric acid and certain

types of enzymes (Pel et al. 2007). It has the ability to degraded xenobiotics through different oxidative, demethylation and hydroxylation reactions providing a potential role in bioremediations (Tudzynski et al. 2002). (Kapoor et al. 1999) worked on the removal of cadmium, copper, nickel and lead heavy metals using *Aspergillus brasilensis* and concluded a significant role of *Aspergillus* spp. in heavy metal removal. The (Wen et al. 2005) co-cultured *Aspergilus phoenicis* with *Trichoderma reesei* by means of dairy manure as a substrate produced a high level β-glucosidase.

2.5 Conclusion

It is concluded that the MWPE of Khyber Pakhtunkhwa province, Pakistan mostly contains 220 different plant species. Family Poaceae was the topmost dominant family of the polluted region. *Ficus carica, Calotropis prosera, Cynodon dactylon* were the dominant and *Pyrus communis, combretum indicum and Biden bippinta* were the rare plant species of MWPE. Based on OLS, logistic and probabilistic all the measured variables have significant role in the occurrence of dominant and rare plant species in the region. While CCA concluded that the $CaCO_3$, electrical conductivity, calcium, soil pH, magnesium, temperature, precipitation, elevation and organic matter have a significant role in the distribution pattern of plant species in the region. It is also concluded that marble wastewater mostly revealed six different types of micro fungal strains in Buner, Pakistan. Their molecular identification and phylogeny resulted in the identification of *Aspergillus sydowii* (ZAF 02 and ZAF 03), *Aspergillus brasilensi* (ZAF 04 and ZAF 05), *Fusarium petrophilium* (ZAF 06), *Aureobasidium leucosper* (ZAF 07), *Alternaria alternata* (ZAF 08) and *Curvularia aeria* (ZAF 09) species. Morphologically many of these strains exhibited aseptate hyphae and varying colors i.e., black, brown, green and white. Anatomically, these strains range from cylindrical to round, hyaline in lacto-phenol blue, thick to thin walled, smooth to ornamented surface with sharp scale and fusoid to ellipsoid in shape.

Chapter 3

Vegetation Indicators of the Marble Polluted Region

3.1 Introduction

As we have discussed in the previous chapter, the global distribution of plants is based on climatic patterns. On this basis, the world‘s natural regions are comprised of Equatorial and tropical (0-23.5°), Subtropical (23.6-34°), Warm temperate (34.1-45°), Cool temperate (45.1-58°), Subarctic (58.1-66.5°), Arctic (66.6-72°) and Polar (72.1-90°) regions on north and south latitudes. These natural regions are further divided into forests, grasslands and deserts based on physiognomic features (Loidi 2018). The subtropical geographic/climatic region is situated north and south of the tropical region. It is characterized by mild winter and hot summer seasons (Erlat and Türkeş 2013; Krüger et al. 2013). The subtropical geographic region is further divided into two i.e., dry summer/ Mediterranean climate (where seasonal rainfall occurs in the cooler months) and humid subtropical (where rainfall often occurs in the warmest months of the year). A significant portion of the world's deserts is located within the subtropics due to subtropical ridge development. The region bordering warm oceans is susceptible to locally heavy rainfall from tropical cyclones, contributing significantly to the annual rainfall. In comparison, the regions bordering cool oceans are prone to fog, aridity, and dry summers.

The natural vegetation of these geographic regions is, however, not only influenced by climate and other regional environmental factors such as geology and soil type, but also by anthropogenic factors. The latter include various forms of environmental pollution, notably from dumped waste, emissions, and industrial effluents. Pollution can bring about a rapid change in the natural ecosystem, especially in its structure, function and composition and is one of the main agents disturbing the environment. Improper disposal of industrial waste materials, such as those associated with mineral extraction, are known to cause a range of environmental impacts. For example, waste materials arising from the marble extraction industry may be dumped in empty pits, but also on river beds, roads, agriculture fields, or pasture lands, leading to wide ranging environmental pollution (Aukour and Al-Qinna 2008b; Mendoza et al. 2014). Without primary treatments and detoxification procedures, effluents from the marble industry can add potential toxic elements (heavy metals) to the ecosystem (El-Maghraby et al. 2013). Over time, levels of contamination in water and soil can

increase, with adverse effect on the local natural environment and for local inhabitants. The pollution also affects the composition of flora and fauna through chemical and physical alterations of the environment (Yu et al. 2005).

However, such types of pollution can give rise to diverse and unique vegetation types in the subtropical geographic region (Ganjurjav et al. 2020; Liu et al. 2020; Zhao et al. 2019). Various plant species absorb such toxic pollutants or potential toxic elements from the marble waste polluted ecosystem (Paz-Alberto and Sigua 2013; Treesubsuntorn and Thiravetyan 2018). They can act as sinks that absorb noxious waste concentrations (Prajapati and Tripathi 2008). This type of pollution abatement function is best performed by some pollutant indicators (Dyer et al. 2017; Haller et al. 2018). These organisms' presence in toxic substances/ heavy metals is termed an indicator, tolerant or hyperaccumulator of such habitat (Freeman et al. 2006).

Plant indicators can be defined as those species that consistently are found growing under distinct environmental factors and which do not occur elsewhere (Khan et al. 2016). Plant indicators are also known as biological, environmental or phyto-indicators. Based on their distribution, two types of plant environmental distributions can be described, i.e., eurytopic (that indicates a wide range of tolerance) and stenotopic (which indicates narrow limits of tolerance). Ideal indicators are those that are indicative of unique geographic conditions (Burgass et al. 2017; Wu et al. 2017). They can signal a change in an environment's biological condition and may be used as a proxy to analyze an ecological unit's health. The relationships between plant species and environmental variables can be utilized to indicate the ecosystem/environment. Therefore, indicators can be utilized as a priceless gift of nature and need preservation for future generations and research purposes compared to many other such species (Kwatra et al. 2016).

Several plant species are used as indicators of a particular habitat. These are used to define land resources' optimal use, e.g. for forest, agriculture and mining, etc. (Worrall et al. 2009). Edaphic indicators tell us which type of soil is suitable for a particular type of agricultural purpose. Certain climatic factors affect the growth rate of specific plants in addition to other parameters (Firn et al. 2019). If the growth is significant under a particular set of conditions, such an ecosystem or environment is considered suitable for agriculture and plantation (Ivanov et al. 2008). Usually, the growth of tall grasses indicates fertile soil conditions (Kelley 1922). Plant species and plant communities are also indicators of groundwater depth (Cannon 1971). In

addition, some plant species are the indicators of different forest types, where they grow abundantly. For instance, *Abies pindrow, Picea smithiana, Cedrus deodara, Pinus wallichiana, Pinus roxburghii, Pinus gerardiana* and *Juniperus macropoda* are the indicators of Coniferous forests of the temperate ecosystem. *Acacia modesta*, *Olea cuspidata* and *Dodonaea viscosa* are the indicators of subtropical dry forests zones. *Salvadora oleoides*, *Prosopis cineraria* and *Capparis aphylla* are the indicators of thorny tropical forests. Likewise, *Avicennia marina*, *Bamboo* spp., *Apluda* spp. and *Cenchrus* spp. are the mangrove wetland ecosystem indicators (Champion et al. 1965). Fungi, *Neottia*, *Strobilanthes* are indicators of humus that can prevent the regeneration of trees. *Ziziphus nummularia, Rhazya stricta* and *Datura metel* are the indicators of higher concentrations of Magnesium and Calcium in the soil. *Acacia modesta, Periploca aphylla* and *Cousinia prolifera* have been reported as indicators of higher electrical conductivity in soil (Noreen et al. 2019a). Besides these, many plants are the indicators of different mining types and minerals e.g., *Equisetum arvense*, *Papaver libonoticum* (Gold indicators), *Vallozia candida* (Diamond indicator), *Stellaria setacea* (Mercury indicator), *Astragalus* spp. (Uranium indicator), *Viscaria alpine*, *Gypsophila patrini* (Copper indicators), *Silene cobalticola* (Cobalt indicator), *Lycium juncus* (Lithium indicator), *Dacrydium caledonicum* (Iron indicator) and *Ulex aquifolium* are considered as Aluminum indicators. *Pteris aquilina* and *Pyronema confluens* grow well on burnt ground and hence are considered to be fire indicators. Some of the plant species are pollutant indicators that can grow in a region where particular kind of pollutants exist (Ahmad et al. 2019). Different plant species are also utilized to recognize climatic features of a particular area. For example, the presence of sclerophyllous vegetation indicates a long dry summer season compared to the luxuriant growth of bryophytes which indicates humid and cooler climatic conditions (Sampson 1939).

Researchers often utilize the approach of plant indicators without any conceptual background. There has been some occasional use of a recognized approach for the identification/selection of plant indicators using indicator analyses. Conventionally authors have mentioned the concept of dominant or characteristics species. Indicator Species Analysis (ISA) can be used to construct the performance of individual indicators across two or more groups of sampled units (Dufrêne and Legendre 1997) based on concepts of abundance and frequency (concentration of abundance in a

particular group and relative frequency within a group). It allows a natural companion to the multi-response permutation procedure of the data and provides a proficient means to minimize the complications inherent in natural vegetation. It detects significant environmental factors that elucidate these complications and give proper indicators (Iqbal et al. 2018; Khan et al. 2016). ISA distinguishes the main pattern in the relationships among species, environmental factors and assists in generating a hypothesis concerning the structure and peculiarity of indicator species in a specific ecosystem (Anderson et al. 2006; Beals 1984; Greig-Smith 1983). The structural equation modeling is a powerful multivariate analysis tool that has great potential in ecological research, as data accessibility continues to increase. However, it remains a challenge even though it was introduced to the ecological community decades ago. Regardless of its rapidly increased application in ecological research, well-established models remain rare. In fact, well-established models can serve as a prior model, as this has been extensively used in psychometrics, behavioral science, business, and marketing research. There is an overlooked yet valuable opportunity for ecologists to establish an structural equation modeling representing the complex network of any ecosystem.

3.1.1 Conceptual / Hypothesized Model

The Russian-German scientist Wladimir Koppen in 1900 proposed the most popular system of climatic classification (Köppen 1900). He suggested the different vegetation types based on diverse climatic regions while studying vegetation, precipitation and temperature (Köppen et al. 2011). These climatic regions are further divided into various climatic types i.e., tropical [wet (rainfall), monsoon, wet and dry (savanna)], dry (arid & semiarid), mild (Mediterranean, humid subtropical, marine), continental [warm summer, cool summer & subarctic(boreal)] and Polar (tundra & ice cap) climate. Whereas (Thornthwaite 1948) realized that precipitation alone is not a good indicator of moisture conditions in an environment. He explained the role of potential evapotranspiration derived from temperature and day length to estimate plants' water needs in a given environment.

The has previously been on plant species as indicators of climatic conditions with very little work done on the impact of pollution on vegetation and their respective

indicators. Therefore, keeping this research gap in mind, it was hypothesized that each type of subtropical vegetation zone of marble waste polluted ecosystem, such as Humid, Semi-humid and Dry subtropical, has definite vegetation structure and indicators that can survive, grow and manifest more tolerance to the polluted conditions as compared to other plants in the subtropical MWPE, KPK, Pakistan. The prediction is that (i) marble pollution has a direct positive significant effect on the indicators of each subtropical vegetation zone, (ii) Elevation and soil have a direct negative effect and climate may have a positive/negative significant effect, (iii) marble pollution harms climate and soil, (iv) while elevation and soil both have a positive relation with climate in the subtropical marble waste polluted ecosystem, Khyber Pakhtunkhwa, Pakistan (Fig. 3.1). For this purpose, the subtropical vegetation of MWPE was selected for further study. In this chapter, the main focus is the detailed statistical procedure/methods for identifying indicator plant species at the subtropical MWPE level in terms of marble pollution, climate, elevation and edaphic factors. Indicator species analysis (ISA) was used for the identification of indicator plants in the MWPE. At the same time, both structural equation modeling and canonical correspondence analysis were used to confirm identified indicators via statistical evaluation of the hypothesized multivariate models. The procedure adopted in this chapter could be followed to classify and identify indicator plants of any microhabitat type/ecosystem in any part of the world.

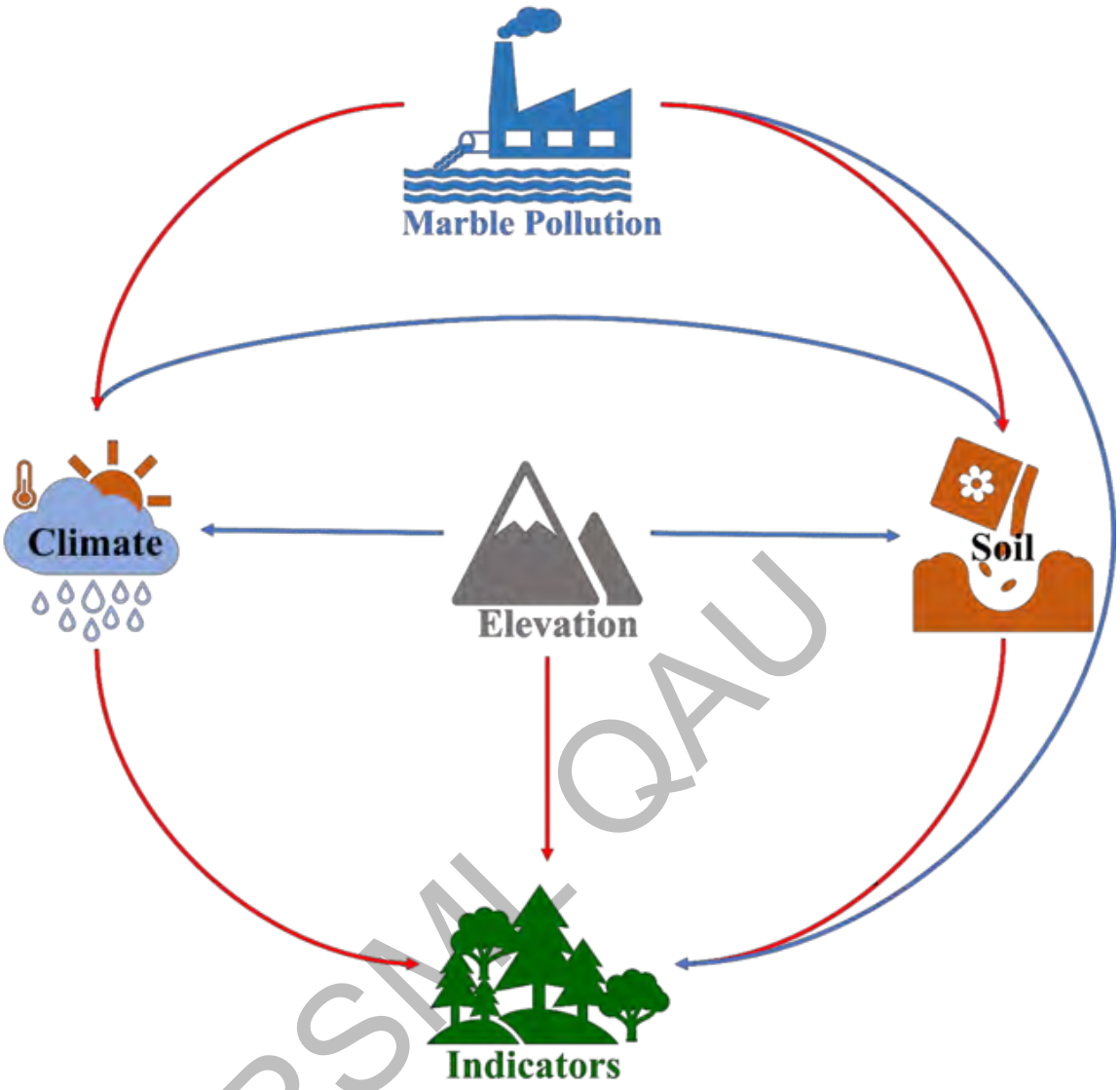


Fig. 3.1 A theoretical model for testing the proposed hypotheses of the current study for the subtropical vegetation of MWPE indicators. Blue and red arrows represent a positive and negative relationship, respectively.

3.2 Materials and Methods

All the collected data of plants and environmental factors from three subtropical vegetation zones of MWPE were analyzed for understanding the complex relation of indicator plants and MWPE through multivariate statistical packages (Lepš and Šmilauer 2003). According to the software's requirements, the absence and presence (0,1) data of all 327 stations and 220 plant species were arranged in the MS Excel sheet. The Two-way Cluster Analysis of PCORD v.5 was used to identify significant subtropical marble waste polluted zones based on pattern similarity index through Sorenson distance measurement and Ward's linkage method (Ahmad et al. 2016b; Greig-Smith 1983; Khan et al. 2016). The ISA was carried to find out indicators of each sort of subtropical MWPE (i.e., Humid, Semi humid and Dry subtropical). It provides knowledge about species fidelity with the particular habitat of specific subtropical vegetation zone of MWPE. The Monte Carlo Test was carried out for statistical significance after determining Indicator Values (%age of perfect indication established on combing values of relative abundance and frequency) of respective indicators using method initially adopted in a study at (Dufrêne and Legendre 1997). During ISA, a proportional abundance of a specific plant in a particular group, its relative abundance was calculated using the formula given below.

$$RA_{jk} = \frac{x_{kj}}{\sum_{k=1}^{g} x_{kj}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}$$

Where, RA*jk*=relative abundance, X*kj*= means an abundance of species *j* in group *k*, *g*=total number of groups.

Then, relative frequency of plants in each group was also calculated i.e., the proportion of sample units in each group that contains that plant species using the below formula. Percent/ faithfulness/ constancy of presence in a particular group is also expressed using these procedures.

$$RF_{kj} = \frac{\sum_{i=1}^{nk} b_{ijk}}{n_k} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(ii)}$$

Where, RF*kj* is the relative frequency of plant j in group *k*, b*ijk* is presence or absence of plant *j* in sample *i* of group *k, i* is sample unit.

At last, equations i & ii were gathered multiplication and the results were expressed as percentage yielding indicator value (IV*kj*) for each plant *j* in group *k.*

$$IV_{kj} = 100\ (RA_{kj} \times RF_{kj}) \ldots\ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

A threshold level of 25% indication and 95% significance ($p \leq 0.05$) was deliberated as a cut off value for determining the indicators. Once the significant indicators were identified, the direct gradient analysis i.e., CCA, was performed using CANOCO software to examine, reconfirm and draw the substantial and distinct indicators of each sort of subtropical vegetation zone of MWPE. CCA analyzes the indicator plants relation by multiple linear regression with environmental gradients and gives us an interpretable graphical presentation of species response environmental variables (Dufrêne and Legendre 1997; Ter Braak and Prentice 1988).

3.2.1 Diversity Indices, Species richness and evenness

Diversity indices provide a mathematical estimation of plant species diversity in a community. This gives us more information concerning community composition. The diversity indices i.e., Shannon Index (H'), Simpson Index (D), Simpson Index of diversity (1-D) and Pielou's Evenness (J) were calculated (Pielou 1966; Simpson 1949).

3.2.1.1 Shannon Index (H')

The Shannon index is usually used to know about plant species diversity in a particular community. It determines both the evenness and abundance of plant species present in a community. The Shannon index of diversity was calculated using below formula:

$$H' = -\sum_{i=1}^{s} Pi\ ln\ Pi \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(iv)}$$

Where, H'= Shannon Index, S= Total number of species in sample/community/zone (species richness), pi= Relative abundance of each species.

3.2.1.2 Simpson Index (D)

The Simpson index is also the measurement of plant species diversity. It was calculated using the below equation:

$$D = \frac{\Sigma n_i(n_i-1)}{N(N-1)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(v)}$$

Where, D=Simpson index, n= total number of any particular plant species, N= Total number of all species.

3.2.1.3 Simpson Index of diversity (1-D)

The Simpson index of diversity was determined using 1-D (D is Simpson index). Its value range between 0-1.

3.2.1.4 Pielou's Evenness (J) / Species Evenness

The Pielou's evenness index was used to determine the evenness of plant species using the below equation.

$$J = H' / H_{max} \text{..... (vi)}$$

Where, J= Pielou evenness, H'= Calculated Shannon Index, Hmax= ln(s) [species diversity under maximum equitability].

3.2.1.5 Species richness

The species richness was calculated using the below equation (Menhinick, 1964).

$$d = \frac{s}{\sqrt{N}} \text{..... (vii)}$$

Where, s=total number of species, N=total number of individuals and d=species richness

3.2.2 Structural Equation Modeling (SEM)

The SEM was designed to examine the research hypothesis via the structural relation among observed variables and different plant indicators in subtropical (Humid, Semi-humid and Dry) MWPE using R software. First, we have normalized and standardized the observed variables data as per the requirements of the structural equation model. The relationship among the explanatory variables was checked through the calculation of Variance Inflation Factor (VIF). The SEM with random effect was assessed for all three subtropical MWPE in order to avoid circulatory analyses in the model. First, we addressed the impact of marble pollution, climate, elevation and soil at three (Humid, Semi-humid and Dry) subtropical MWPE, separately and then combinedly through SEM using the hypothesized model (Fig. 3.1.1). We assessed Chi-square Statistics (X^2), Goodness of Model Fit Index (GFI), Adjusted Goodness of Model Fit Index (AGFI), Root Mean Square Error of Approximation (RMSEA), Standard Root Mean

Square Residual (SRMR), Normed Fit Index (NFI), Non-Normed Fit Index (NNFI), Comparative Fit Index (CFI) and Akaike Information Criterion (AIC) for the goodness of model fit for SEM.

Mathematical representations of the general and specific SEM are as follow:

$Y= \boldsymbol{\beta_{0+}}\ \boldsymbol{\beta_{1Z+}}\ \boldsymbol{\varepsilon_i}$……. (viii)

$\mathbf{Y= \beta_0 + \sum_{i=1}^{19} \beta i}\ (\mathrm{Xpollution} + \mathrm{X}climate + \mathrm{X}soil + \mathrm{X}elevation)\ +\ \varepsilon i$ …… (ix)

Equation (viii) shows the general structural equation model and equation (ix) specific model of our study. Where, **Y** represent indicator species, $\boldsymbol{\beta_0}$ denote the intercept of the equation, $\boldsymbol{\beta_1}$ disclose the coefficient of variable z, $\boldsymbol{\varepsilon_i}$ represent the unobserved variations in the model or error term in the equation, **βi** represents the coefficient of latent variables.

3.3 Results

3.3.1 Species area curves

PC-ORD version 5 was used to classify plant species into potential plant zones. The first test performed was a species-area curve. This was drawn to examine whether the sample size was adequate or not. It has mostly been utilized in the science of vegetation ecology to realize species composition with sample size. Plant abundance data with Sorensen distance values were used to create species-area curves for 327 quadrats/stations and 220 species. The first-order jack-knife estimate was observed at station number 274, followed by the second-order jack-knife at station number 303. A total of 54 plant species were recorded, with only one occurrence in the studied region. It showed that the maximum number of plant species appearing up to station number 274 (i.e., 210 within an average distance of 0.039). The average number of plant species occurring at station number 303 was 216 with an average distance of 0.024 and then the species curves become parallel with only four more species at the end. This proved that there had been adequate sampling in the targeted region (Appendix table 4; Fig. 3.2).

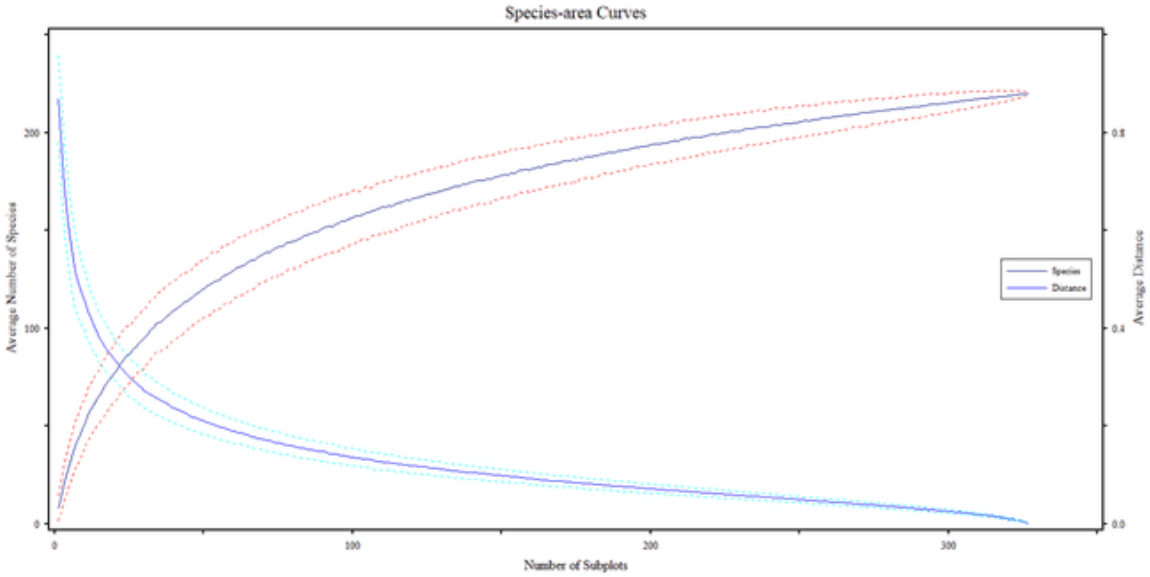


Fig. 3.2 The species-area curves of 220 plant species distributed among 327 stations in the MWPE, Khyber Pakhtunkhwa, Pakistan.

3.3.2 Indicator of MWPE/ Vegetation classification of MWPE

The detailed description of each subtropical vegetation zone of MWPE and their distinctive indicator plants are as follows:

3.3.2.1 Humid subtropical vegetation zone of MWPE

The indicator species were identified using indicator species analysis. The topmost indicators of this Humid subtropical MWPE were *Ficus carica* L., *Catharanthus roseus* (L.) G.Don and *Erigeron canadensis* L. along with indicator value ≥ 20% and Probability value ≤ 0.05 after the Indicator Species Analysis (ISA) (Fig. 3.3; Fig. 3.4; Fig. 3.5; Appendix table 5). These were the indicators of higher precipitation (138mm/ annum), copper, cadmium, lower calcium, phosphorous concentration and neutral soil pH in the humid subtropical zone of marble waste polluted ecosystem (Table 3.1). Other indicators of this humid subtropical zone were *Ailanthus altissima*, *Salix tetrasperma*, *Diospyros lotus*, *Punica granatum*, *Prunus persica*, *Pinus wallichiana*, *Amaranthus viridis*, *Eleusine indica*, *Brachiaria ramosa*, *Persicaria barbata*, *Solanum americanum*, *Echinochloa colona*, *Paspalum distichum*, *Tagetes erecta*, *Setaria viridis*, *Sorghum halepense*, *Oenothera rosea*, *Setaria pumila*, *Plantago major*, *Digera muricata*, *Rumex nepalensis, Arundo donax* and *Artemisia vulgaris* having IV≥20% and probability ≤ 0.05 (Table 3.4).

In more depth, all the Humid vegetation zone of MWPE indicators were influenced by different climatic, topographic and soil physicochemical conditions. It can be one of the foremost factors of distinct indicator species of this subtropical zone. Soil pH ranges from 7.38-9.3, EC deviated from 31-233.3 ppm, TDS varied from 33-356 ppm, OM ambit from 0.47-0.83%, $CaCO_3$ 0.48-17%, Ni 1.25-25.98 ppm, Cr 10.87-71.66 ppm, Cu 10.1-91.68 ppm, Mn 3.23-131.65 ppm, Cd orbit from 48.19-54.15 ppm, Zn 32.88-146.48 ppm, Fe ambit from 54.05-258.38 ppm, K 134.28-659.04 ppm, P 3.02-7.74 ppm, Mg array from 1.54-333.42 ppm, Ca 2.76-370.91 ppm, Mean Annual Temperature 20.88-29.46 °C and Precipitation 138.033 mm in the Humid subtropical region (Appendix table 6; Fig. S1).

Table 3.1 The topmost indicator species in relation to significant measured variables along with respective indicator value (IV), probability (p-value) and total importance value index in the humid MWPE.

S. No.	Indicator Species	Variable	Max Grp	IV	p-value	TIVI
1	*Ficus carica*	Ca	low	32	0.0334	840.64
		Cu	high	31.7	0.0246	
2	*Catharanthus roseus*	pH	neutral	20.5	0.0466	176.67
		Cd	high	37	0.0586	
3	*Erigeron canadensis*	P	low	44.3	0.0358	271.92

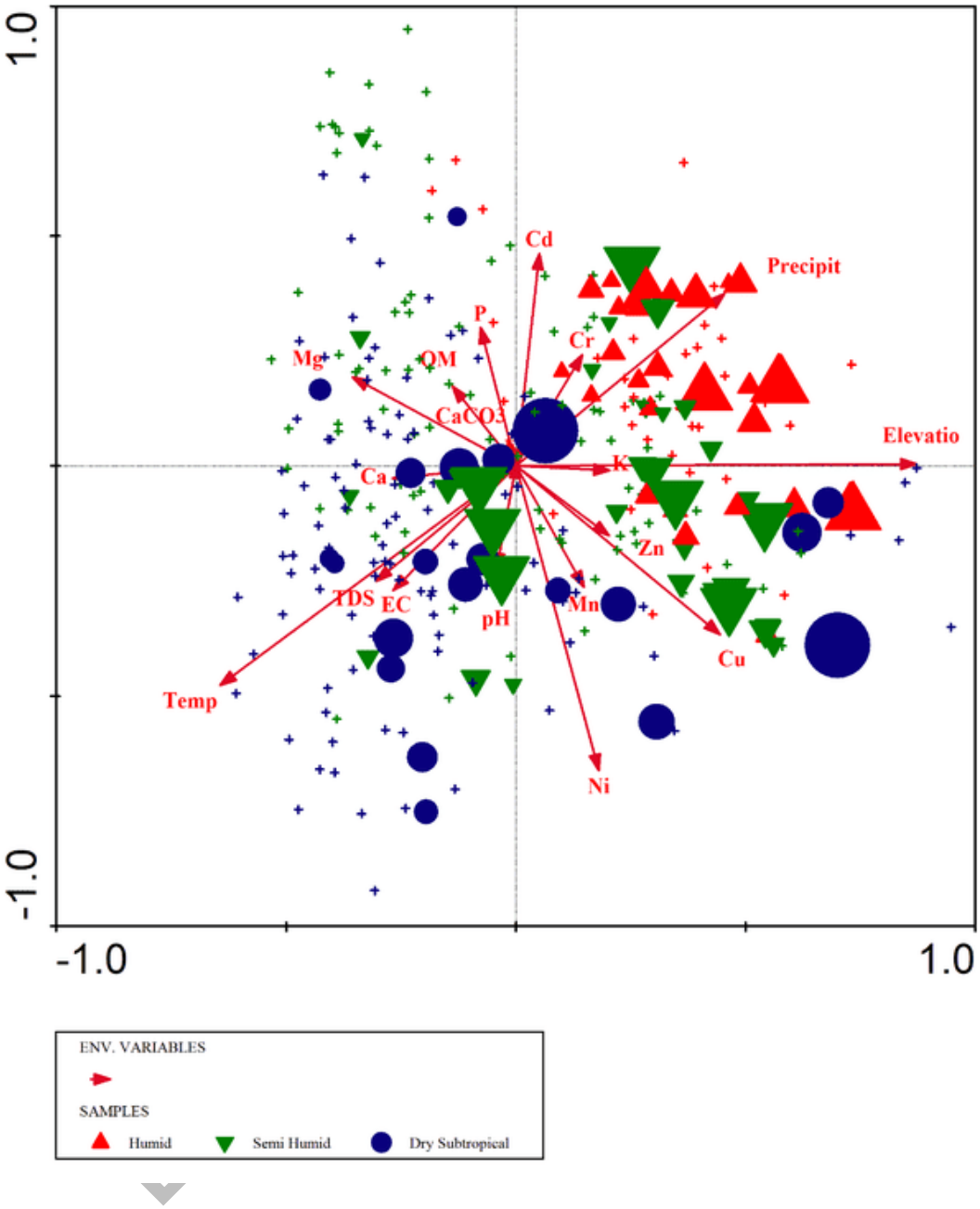


Fig. 3.3 The data attribute plots of *Ficus carica* (1st indicator) of the humid MWPE in relation to measured environmental factors after CCA of CANOCO software's reconfirming the identification of ISA graphically.

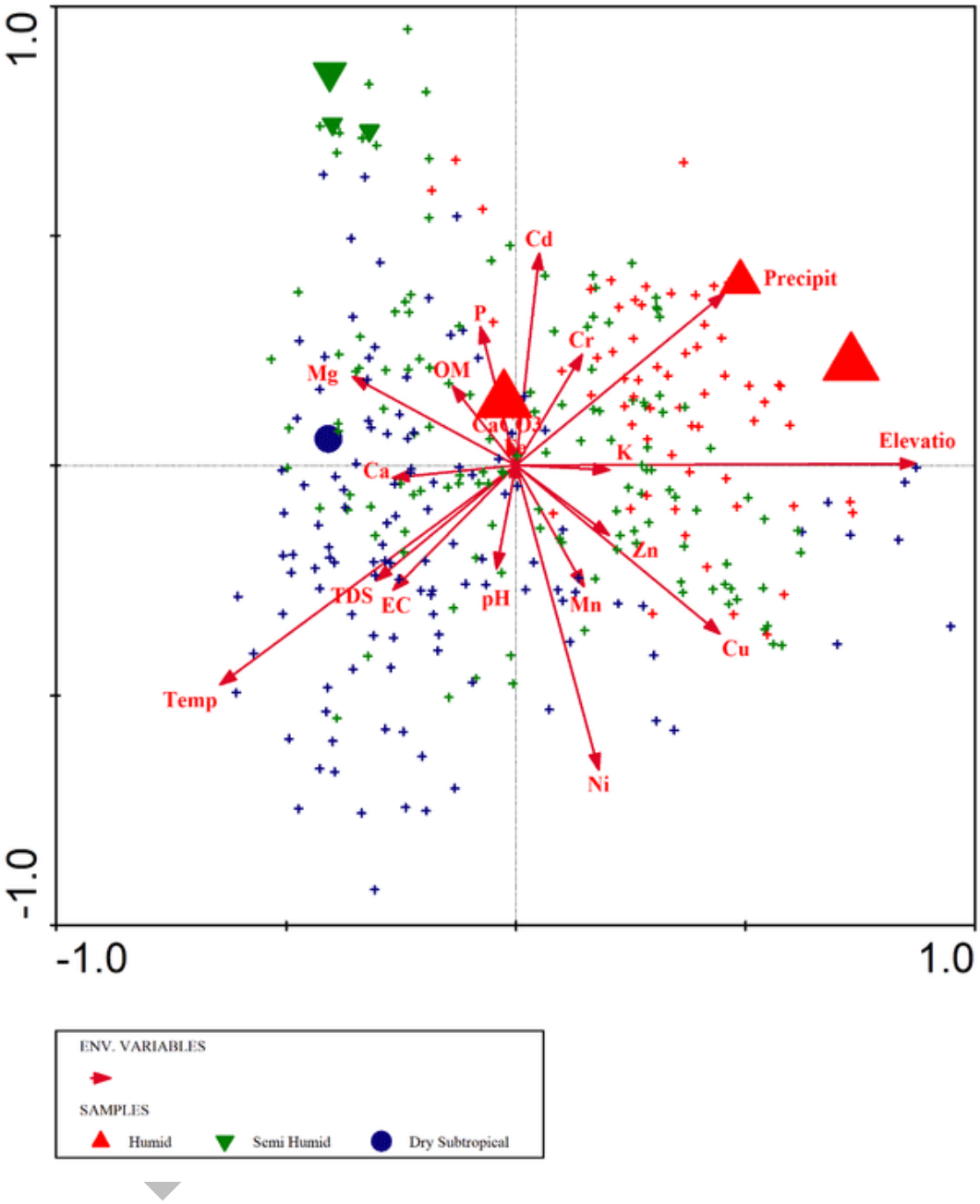


Fig. 3.4 The data attribute plots of *Catharanthus roseus* (2nd indicator) of humid MWPE in relation to different environmental variables after CCA of CANOCO software's.

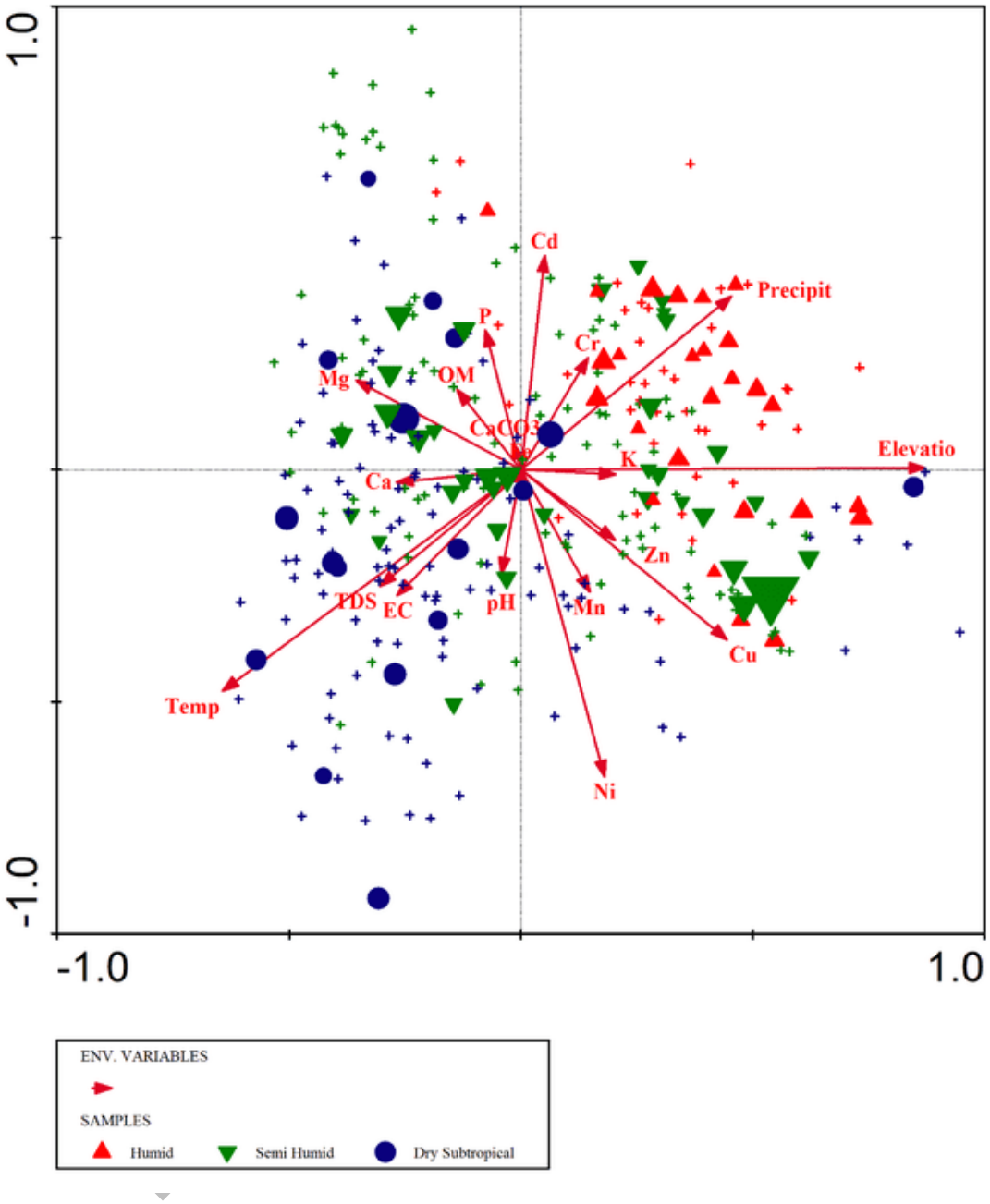


Fig. 3.5 The data attribute plots of *Erigeron canadensis* (3rd indicator) of humid MWPE with respect to various environmental factors.

3.3.2.2 Semi-Humid subtropical vegetation zone of MWPE

The topmost three indicator species of this semi-humid subtropical were *Morus nigra* L., *Datura innoxia* and *Persicaria glabra* (Willd.) M.Gómez one each from trees, shrubs and herbs, respectively (Fig. 3.6; Fig. 3.7; Fig. 3.8; Appendix table 5 & Fig. 3.3.6-8). These were the indicators of a moderate amount of precipitation (31mm), temperature, $CaCO_3$ and phosphorous, lower potassium, cadmium, manganese and neutral soil pH compared to other subtropical MWPE (Table 3.2). The other characteristic species of this vegetation zone were *Populus alba*, *Albizia lebbeck*, *Mangifera indica*, *Ziziphus jujuba*, *Celtis australis*, *Ficus palmata, Senna occidentalis, Adiantum capillus-veneris, Chenopodium album, Dactyloctenium aegyptium, Achyranthes aspera, Persicaria maculosa, Adiantum incisum, Euphorbia hirta, Juncus maritimus, Cortaderia selloana, Cheilanthes acrostica, Rumex dentatus, Poa annua, Typha angustifolia, Verbena officinalis, Saccharum spontaneum* and *Colocasia esculenta* (Table 3.4).

Furthermore, soil pH of this semi humid subtropical zone of MWPE varies from 7.8-9.64, EC fluctuates from 21.2-268 ppm, TDS ranges from 24-372 ppm, OM deviates from 0.47-0.83%, $CaCO_3$ stretch from 0.30-17.62 %, Ni ambit from 5.01-50.55 ppm, Cr 0.11-46.78 ppm, Cu extent from 3.51-51.48 ppm, Mn 0.67-120.67 ppm, Cd 0.59-56.93 ppm, Zn 0.7-147.19 ppm, Fe 14.02-225.28 ppm, K 3.44-346.69 ppm, P 3.18-7.96 ppm, Mg 23.114-702.47 ppm, Ca 2.4-643.85 ppm, temperature 19.75-37.46 ^oC and precipitation 31.66-64.13mm (Appendix table 6; Fig. S1).

Table 3.2 The topmost indicator species with respect to significant environmental factors, total importance value index, indicator and probability values of semi humid subtropical MWPE.

S. No.	Indicator Species	Variable	Max grp	IV	P	TIVI
1	*Morus nigra*	K	Low	30.6	0.0612	768.19
		pH	Neutral	36.9	0.0378	
2	*Datura innoxia*	$CaCO_3$	High	34.2	0.0578	1005.19
		Cd	Low	25.2	0.0652	
		Elevation	Moderate	25.3	0.0002	
		Precipitation	Moderate	15.4	0.0374	
3	*Persicaria glabra*	P	Moderate	23.7	0.0224	417.63
		Precipitation	High	24.7	0.009	
		Elevation	Moderate	20.5	0.0534	
		Mn	Low	38.5	0.0184	

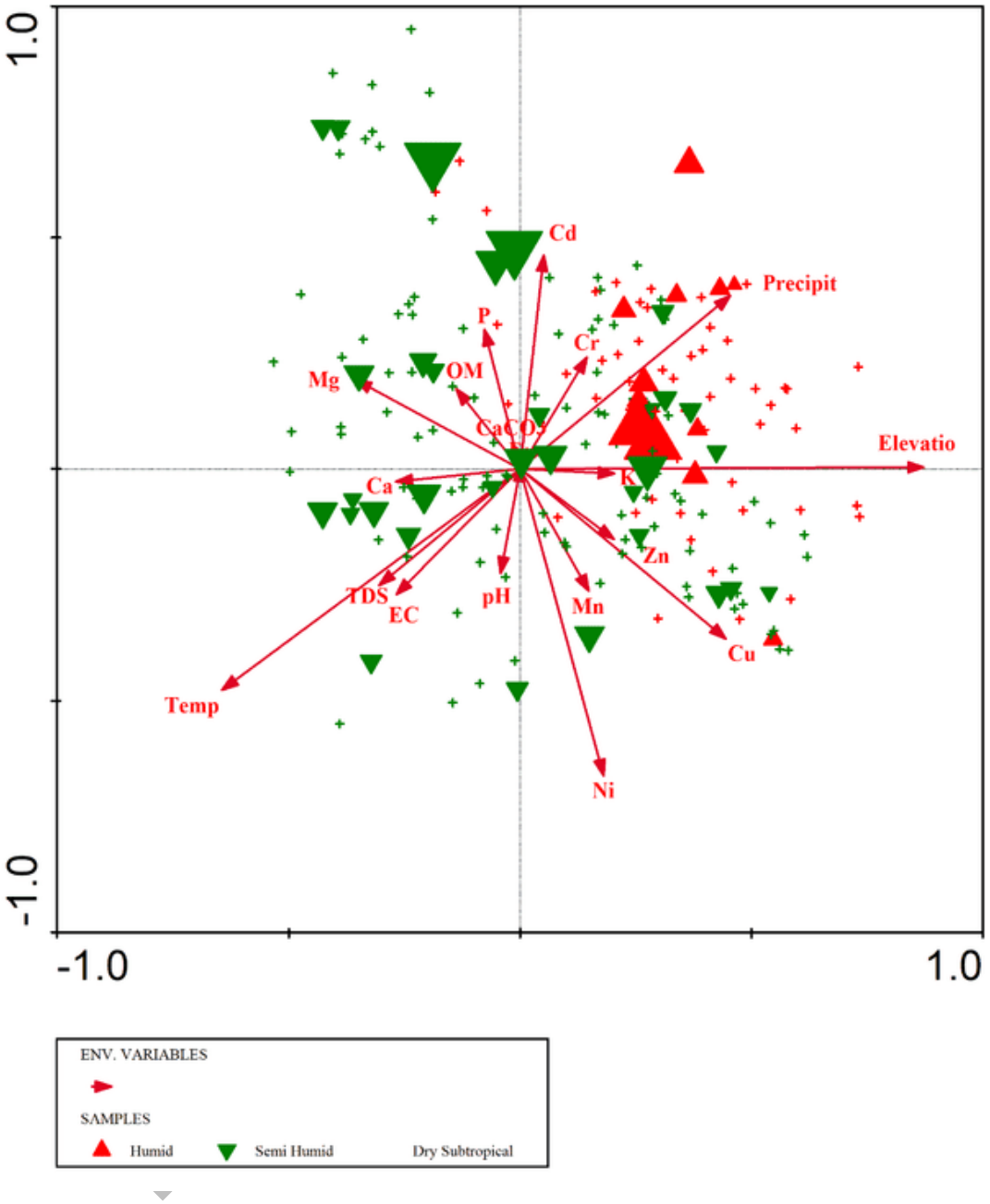


Fig. 3.6 Data attribute plot of *Morus nigra* (1st indicator) of Semi humid subtropical MWPE in conjunction with measured environmental factors.

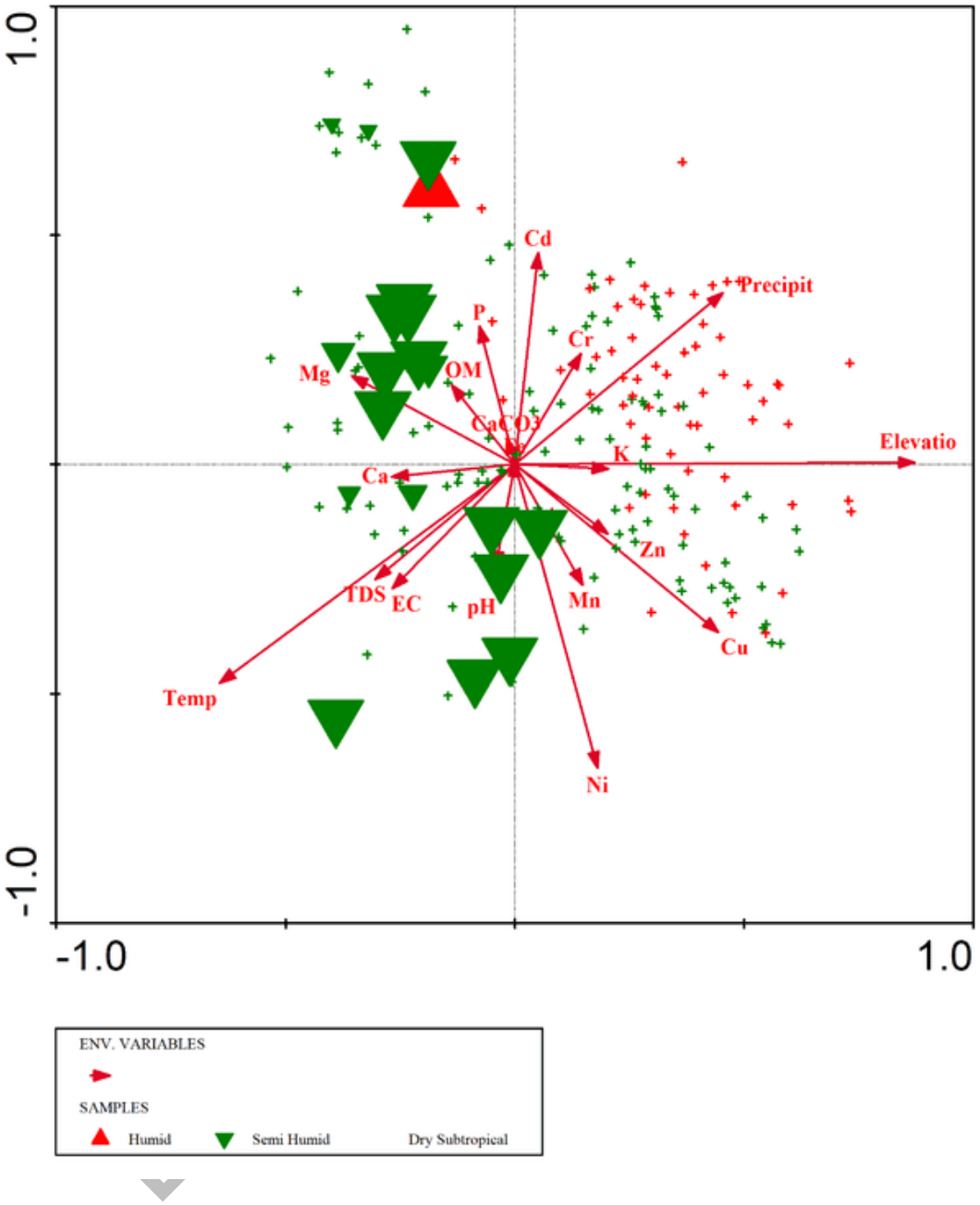


Fig. 3.7 Data attribute plot of *Datura innoxia* (2nd indicator) in relation with measured edaphic, climatic and topographic factors in Semi humid subtropical MWPE.

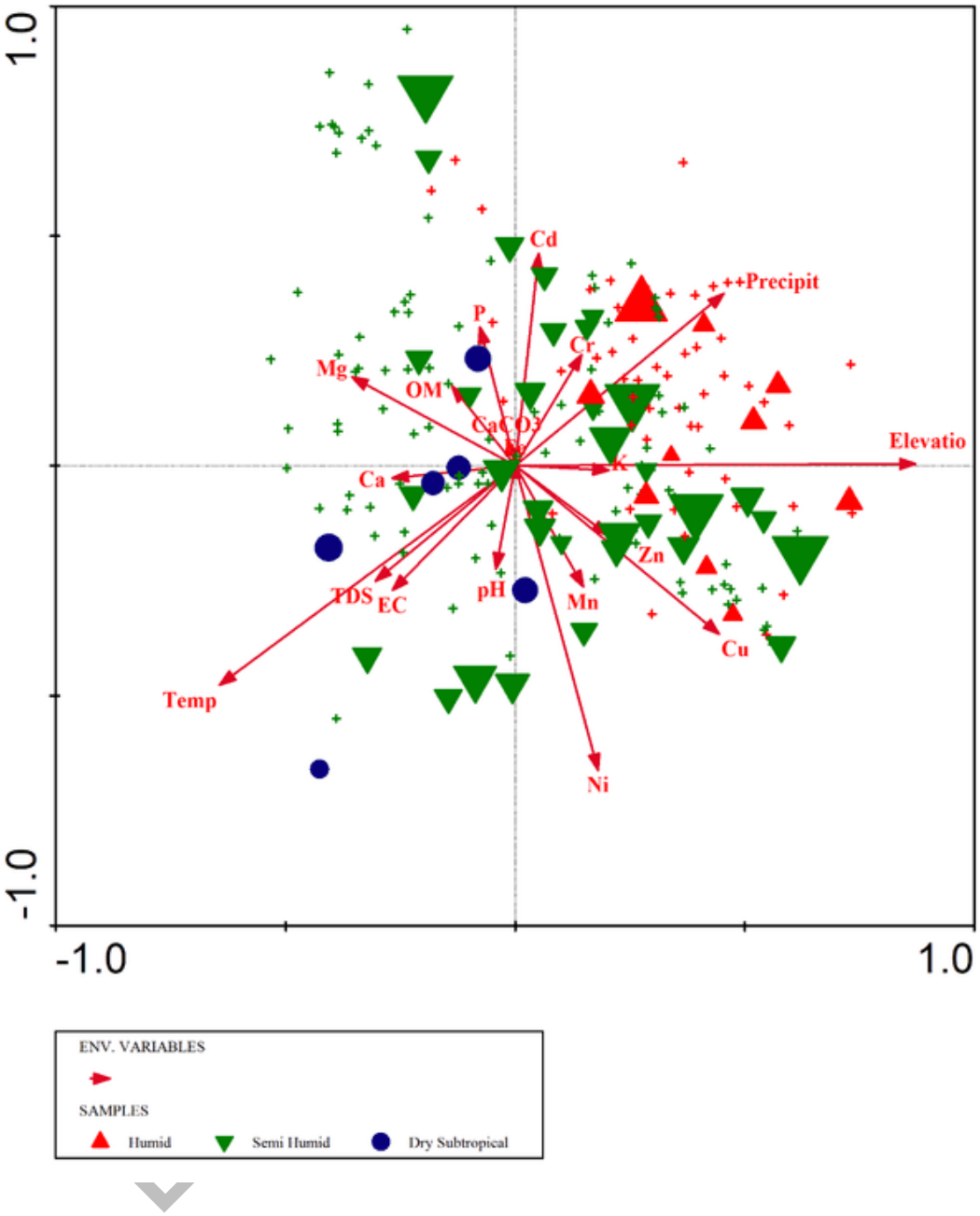


Fig. 3.8 Data attribute plot of *Persicaria glabra* (3rd indicator) in relation to measured environmental variables in the Semi humid MWPE, KPK, Pakistan.

3.3.2.3 Dry subtropical vegetation zone of MWPE

The topmost plant indicators of this dry subtropical MWPE were *Dalbergia sissoo* DC., *Withania somnifera* (L.) Dunal and *Saccharum bengalense* Retz. after the ISA (Fig. 3.9; Fig. 3.10; Fig. 3.11; Appendix table 5). These were the indicator species of higher temperature (38°C), electrical conductivity, moderate $CaCO_3$, low precipitation, pH, iron, copper, nickel and zinc concentration in the dry subtropical region (Table 3.3). Other characteristics species included *Prosopis juliflora* (Sw.) DC., *Tamarix aphylla*, *Tribulus pentandrus*, *Ricinus communis*, *Dodonaea viscosa*, *Rumex hastatus*, *Cynodon dactylon*, *Desmostachya bipinnata*, *Artemisia persica*, *Dysphania nepalensis*, *Euphorbia prostrata*, *Sonchus oleraceus*, *Chenopodium murale*, *Heliotropium europaeum*, *Boerhavia diffusa*, *Solanum surattense*, *Cucumis melo var agrestis* and *Dysphania ambrosioides* (Table 3.4).

When environmental factors change it sustains growth of various indicator species. The soil pH of this Dry subtropical zone deviate from 7.82-9.77, EC 1.84-371 ppm, TDS turn from 18-387 ppm, OM 0.48-0.83 %, $CaCO_3$ 0.35-17.49%, Ni ridge from 6.11-50.24 ppm, Cr 0.34-54.91 ppm, Cu 4.03-45.58 ppm, Mn varies from 0.35-93 ppm, Cd 0.86-55.27 ppm, Zn fluctuates from 2.61-148.55 ppm, Fe 0.38-276.05 ppm, K 6.75-557.94 ppm, P differ from 3.17-7.98 ppm, Mg 13.49-701.14 ppm, Ca range from 17.54-629.97 ppm, mean annual temperature 19.44-39.15 °C and 6.35-55.66 mm precipitation (Appendix table 6; Fig. S1).

Table 3.3 The foremost three indicators species along with significant environmental variables and their respective indicator & probability values and total importance value index in the Dry MWPE.

S. No.	Indicator Species	Variable	Max grp	IV	P	TIVI
1	*Dalbergia sissoo*	Fe	Low	30.9	0.0462	590.29
		Cu	Low	22.2	0.0646	
		Ni	Low	52.7	0.0136	
		Temp	High	24	0.048	
		Zn	Low	59.2	0.036	
2	*Withania somnifera*	Fe	Low	49.8	0.0086	675.64
		CaCO3	Moderate	14.4	0.0778	
		EC	High	39	0.013	
		pH	Low	85.6	0.0004	
3	*Saccharum bengalense*	Temp	High	21.2	0.0272	421.38
		Precipitation	Low	20	0.066	

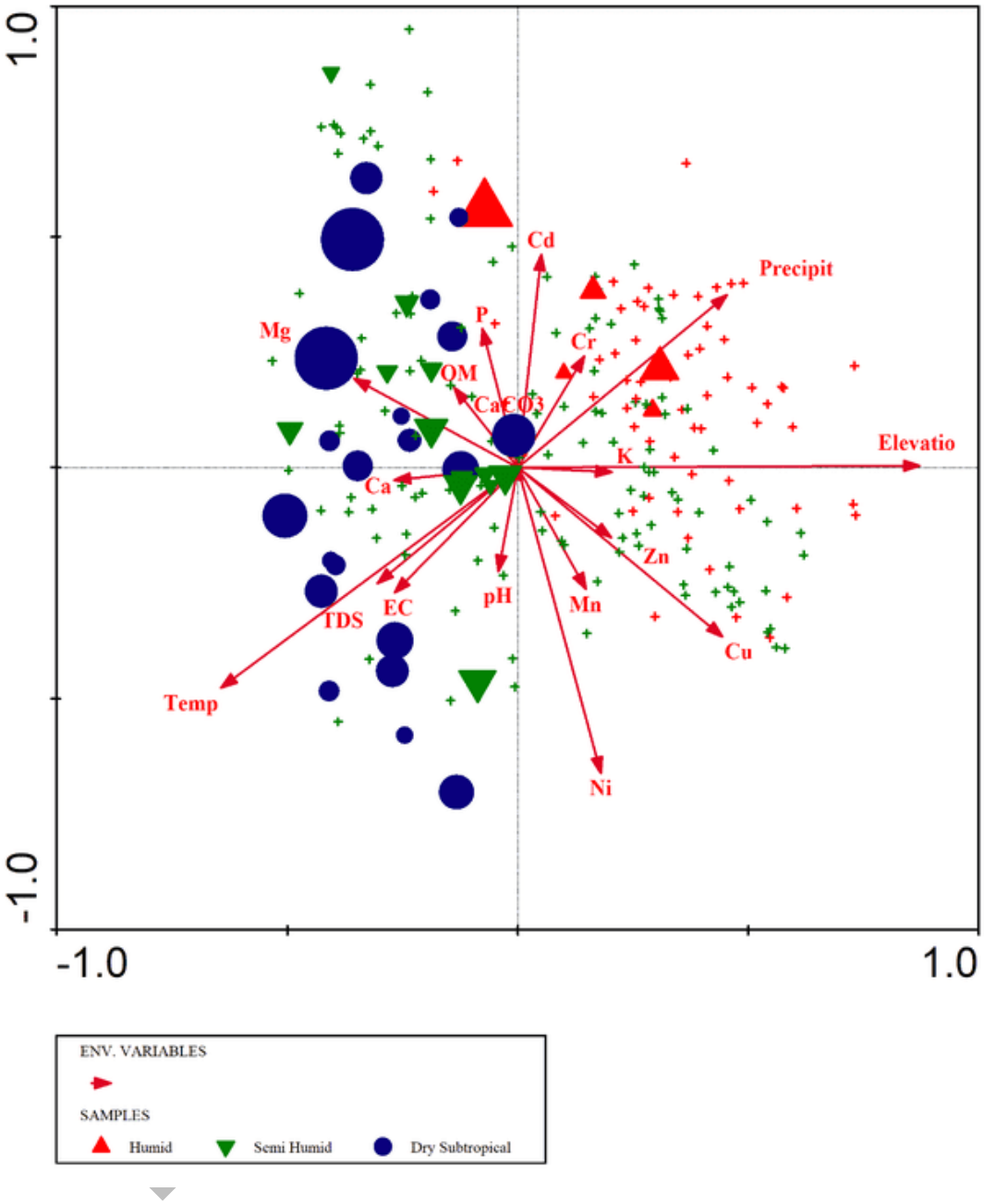


Fig. 3.9 Data attribute plot of *Dalbergia sissoo* (1st indicator) in relation to measured environmental variables in the Dry subtropical MWPE, KPK, Pakistan.

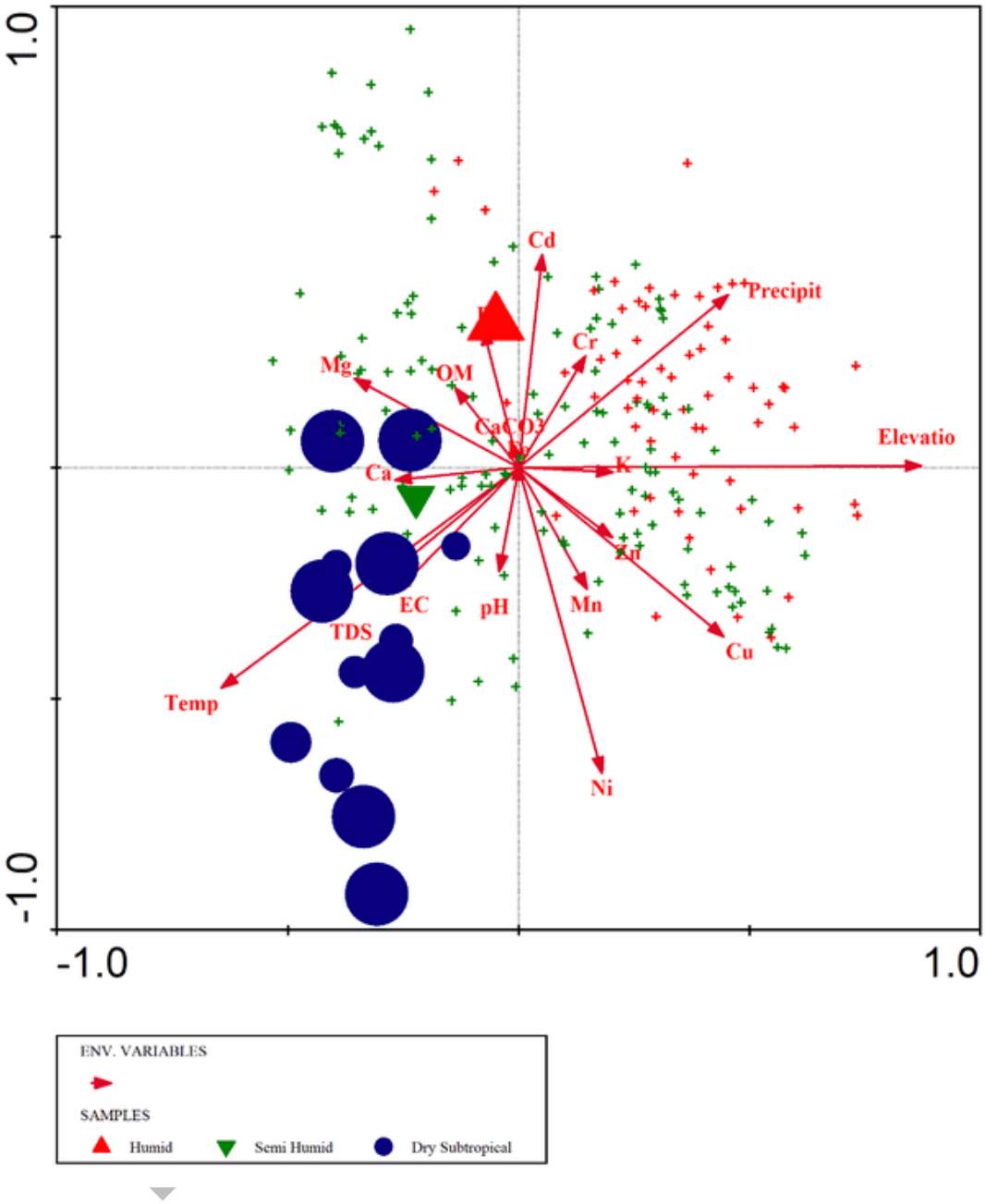


Fig. 3.10 Data attribute plot of *Withania somnifera* (2nd indicator) in relation with measured edaphic, climatic and topographic factors in Dry subtropical MWPE.

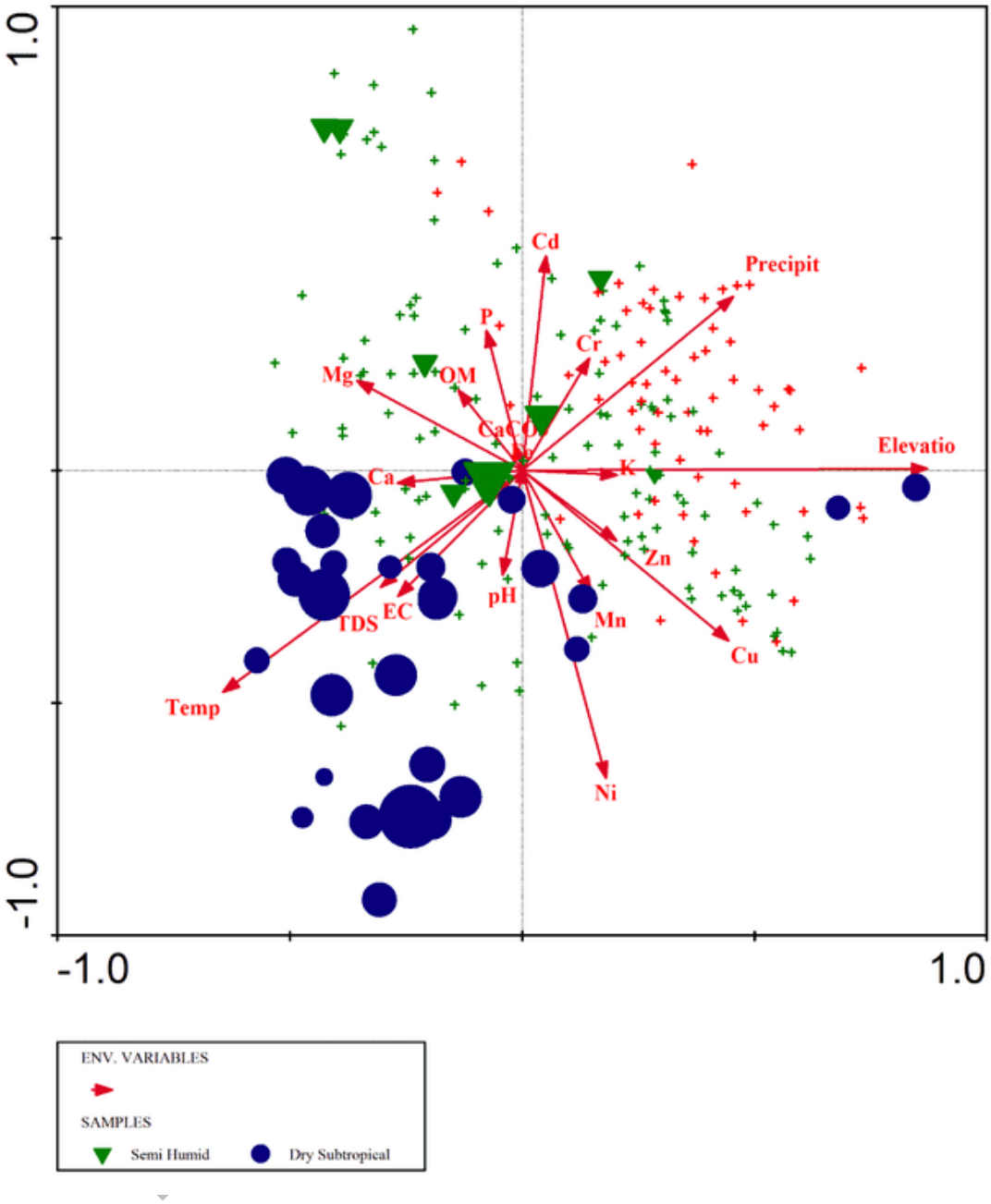


Fig. 3.11 Data attribute plot of *Saccharum bengalense* (3rd indicator) of Dry subtropical MWPE in conjunction with measured environmental factors.

Table 3.4 Other indicator species of the three identified subtropical MWPE, KPK, Pakistan.

Botanical Names	**Variables**	**IV**	**P**	**T-IVI**
Humid Subtropical MWPE				
Ailanthus altissima	Elevation	28.8	0.0444	1058.76
	pH	57.2	0.0006	
	Mn	41	0.023	
Platanus orientalis	CaCO3	40	0.0052	119.94
Senna occidentalis	Zn	15.4	0.0396	252.52
Taraxacum officinale	Ca	37.1	0.026	542.54
Amaranthus viridis	TDS	87.8	0.0016	521.98
Cannabis sativa	Elevation	31.7	0.0058	490.23
Echinochloa colona	Zn	18.5	0.0588	201.22
Persicaria barbata	Zn	24.1	0.0242	182.61
Paspalum distichum	Temperature	14.9	0.0392	134.89
Tagetes erecta	Mg	86.5	0.0032	130.46
	Ca	23.8	0.0526	
Polygonum plebeium	Zn	19.8	0.029	113.78
Oenothera rosea	pH	32.9	0.0116	98.98
	TDS	46.5	0.0092	
	EC	46.4	0.0212	
Setaria pumila	Mn	19	0.0542	96.64
Plantago major	CaCO3	41.7	0.015	95.77
Ipomoea purpurea	Temperature	21.5	0.0358	85.74
Digera muricata	Mn	19.6	0.0504	66.40
Luffa cylindrica	Cr	13.6	0.0306	57.98
	Ni	95.3	0.0014	
Rumex nepalensis	EC	31.8	0.0164	48.05
Nasturtium officinale	Mn	19	0.0542	35.13
Digitaria ciliaris	Mg	46.1	0.0428	23.76
	pH	25	0.0172	
	OM	16.7	0.0316	

Mirabilis jalapa	OM	16.7	0.0332	22.14
Bidens pilosa	CaCO3	25	0.0594	20.69
Semi-Humid Subtropical MWPE				
Populus alba	Cu	37.5	0.004	900.76
	Elevation	21.6	0.0112	
	Temperature	21.7	0.048	
Albizia lebbeck	pH	21.1	0.0472	278.98
Ziziphus jujuba	pH	18	0.0548	236.11
Mangifera indica	TDS	39.7	0.0322	210.66
Celtis australis	Elevation	8.3	0.039	115.61
Datura innoxia	Cu	19.8	0.0466	764.79
	Elevation	12.5	0.0456	
Parthenocissus inserta	Cu	18.7	0.0088	95.00
Adiantum capillus-veneris	Ca	54	0.0278	442.79
	Fe	23	0.0118	
	CaCO3	58	0.0112	
	Cu	39.8	0.002	
	EC	40.7	0.0464	
Amaranthus spinosus	Elevation	14.7	0.0006	216.19
Dactyloctenium aegyptium	Ni	42.6	0.0234	212.21
	K	46.7	0.0344	
Achyranthes aspera	Zn	32.6	0.008	154.98
Erigeron bonariensis	Ni	22.3	0.0526	154.07
	K	57.6	0.0054	
	Elevation	12.1	0.0258	
	Cu	25.6	0.013	
Adiantum venustum	Cr	25.2	0.0298	131.65
	K	44.9	0.0132	
	Temperature	15.2	0.0168	
	Elevation	10.7	0.0224	
Boerhavia procumbens	Elevation	13.3	0.0028	106.53
Rumex dentatus	TDS	39.9	0.0438	61.40

Verbena officinalis	Cd	21.7	0.0476	46.12
Physalis divaricata	Elevation	6.7	0.02	44.59
Tribulus terrestris	Fe	14.5	0.005	43.56
	Temperature	27.8	0.0002	
Euphorbia helioscopia	Cr	35.1	0.0058	33.50
	Temperature	26.7	0.0002	
Cyperus difformis	Mg	33	0.0186	16.85
Persicaria hydropiper	P	33.3	0.023	12.80
Dryopteris stewartii	Cd	25	0.0274	10.99
Fragaria vesca	Ca	25	0.0514	7.38
Sorghum bicolor	Cd	25	0.0276	6.62
Bidens bipinnata	K	33.3	0.0404	3.99
Dry Subtropical MWPE				
Tamarix aphylla	Ca	60.2	0.0142	431.67
Acacia nilotica	TDS	35	0.0554	298.02
Tribulus pentandrus	Fe	45.5	0.0232	178.41
Psidium guajava	P	43.1	0.0534	149.89
Bombax ceiba	CaCO3	31.5	0.0286	121.61
	P	43.6	0.0478	
Callistemon lanceolatus	Cr	16.3	0.0372	52.84
Ricinus communis	Fe	36.5	0.0434	623.08
Rumex hastatus	Precipitation	22.2	0.0054	162.6
Cynodon dactylon	Mg	37.8	0.0178	1657.18
	Temperature	43.5	0.0404	
Parthenium hysterophorus	EC	46.2	0.0156	771.50
	Cu	42.6	0.0486	
Xanthium strumarium	Temperature	28.2	0.028	422.33
Oxalis corniculata	Mn	16	0.0498	356.02
Desmostachya bipinnata	Elevation	45.2	0.019	299.97
	Cr	34.8	0.0044	
Convolvulus arvensis	Cr	34.8	0.0044	158.49
Artemisia persica	Elevation	44.4	0.0014	143.90

	Cr	22.7	0.027	
	Precipitation	44.4	0.0002	
Dysphania nepalensis	Precipitation	33.3	0.0002	127.37
	Temperature	30.9	0.0058	
	Temperature	33.3	0.0008	
	Elevation	33.3	0.002	
Helianthus annuus	OM	19.6	0.0046	117.51
	Elevation	64	0.0002	
Euphorbia prostrata	Zn	28.3	0.0216	115.08
Sonchus oleraceus	TDS	39.7	0.0246	102.21
Chenopodium murale	Zn	30.5	0.0144	89.05
	Temperature	33.3	0.0002	
	Cr	28.6	0.0088	
Mentha royleana	Zn	32.4	0.0074	87.27
	pH	48	0.0246	
Heliotropium europaeum	Precipitation	44.4	0.0002	87.07
	Temperature	49.2	0.0002	
	Temperature	44.4	0.0002	
	Elevation	44.4	0.0014	
Solanum surattense	Elevation	28.7	0.0284	71.28
Dysphania ambrosioides	Cr	30.6	0.0028	59.78
	Temperature	33.3	0.0002	
	Elevation	66.7	0.0002	
Portulaca oleracea	Fe	43.2	0.031	57.51
Triticum aestivum	K	31.4	0.036	48.94
	EC	46.2	0.0156	
Chrozophora tinctoria	P	47.2	0.0356	42.90
	TDS	27.4	0.0456	
	EC	39	0.0444	
Dicliptera bupleuroides	Temperature	16.7	0.0042	42.56
	pH	33.3	0.052	
Iris hookeriana	K	32.7	0.0222	40.33

	CaCO3	32.7	0.0222	
Polygonum aviculare	Cd	15.1	0.0172	36.64
	Cr	15.3	0.0444	
	Temperature	16.7	0.0036	
	Elevation	33.3	0.002	
Verbascum thapsus	OM	11.1	0.0222	21.34
Musa paradisiaca	P	50	0.0268	8.93

3.3.3 Species richness and Diversity Indices

The species richness values ambit from 124 to 159 plant species in these three major subtropical vegetation zones of MWPE. The highest Shannon Diversity Index was determined for Semi humid (H‘=4.112) followed by Humid (3.896) and Dry subtropical vegetation zone (3.852). The Simpson Index values, i.e., 0.964, 0.963 and 0.948, were determined for Humid, Semi humid, and Dry subtropical zones. At the same time, the Simpson Index of diversity was recorded between 0.036-0.052. The Semi humid subtropical vegetation zone has maximum Pielou‘s Evenness Index (0.811) followed by Humid (0.808) and Dry subtropical zones (0.773) (Table 3.5).

Table 3.5 Diversity indices of all the three subtropical vegetation zones.

S. No.	**Subtropical Vegetation Zones**	**Species Richness**	**Shannon Index (H’)**	**Simpson Index (D)**	**Simpson Index of diversity (1-D)**	**Pielou's Evenness H’/Hmax**
1	Humid	124	3.896	0.964	0.036	0.808
2	Semi Humid	159	4.112	0.963	0.037	0.811
3	Dry	146	3.852	0.948	0.052	0.773

3.3.4 Direct ecological gradient through Canonical Correspondence Analysis (CCA)

The plant indicators of different subtropical vegetation zone of MWPE were again confirmed by CCA. The direct ecological gradient analysis resulted that the precipitation, temperature, elevation, and soil (pH, EC, TDS, P, K, OM, $CaCO_3$, Ni, Cd, Cr, Mg, Ca, Mn, Cu, Zn) have a significant impact (p=0.0002) on indicator plants diversity of subtropical MWPE (Table 3.6). Like, indicator species of the Humid subtropical MWPE were clustered under the influence of higher precipitation, elevation, Cd, low temperature and alkaline soil pH. Whereas indicator species of Semi humid subtropical MWPE were under the impact of moderate amount of precipitation, temperature, nearly neutral soil pH, higher Mg and organic matter concentration compared to other subtropical zones. The indicators of Dry subtropical vegetation zones were under the consequence of low precipitation, elevation, higher temperature, EC and soil pH compared to other zones (Fig. 3.12).

Table 3.6 Summary of direct ecological gradient through Canonical Correspondence Analysis.

Axes	**1**	**2**	**3**	**4**	**Total Inertia**
Eigenvalues	0.337	0.196	0.137	0.109	15.837
Species-environment Correlation	0.798	0.640	0.614	0.551	
Cumulative %age variance of Species Data	2.1	3.4	4.2	4.9	
Test of significance of first canonical axis			Test of all canonical axes		
Eigenvalues	0.337		Trace	1.486	
F-ratio	6.565		F-ratio	1.646	
P-value	0.0002		P-value	0.0002	

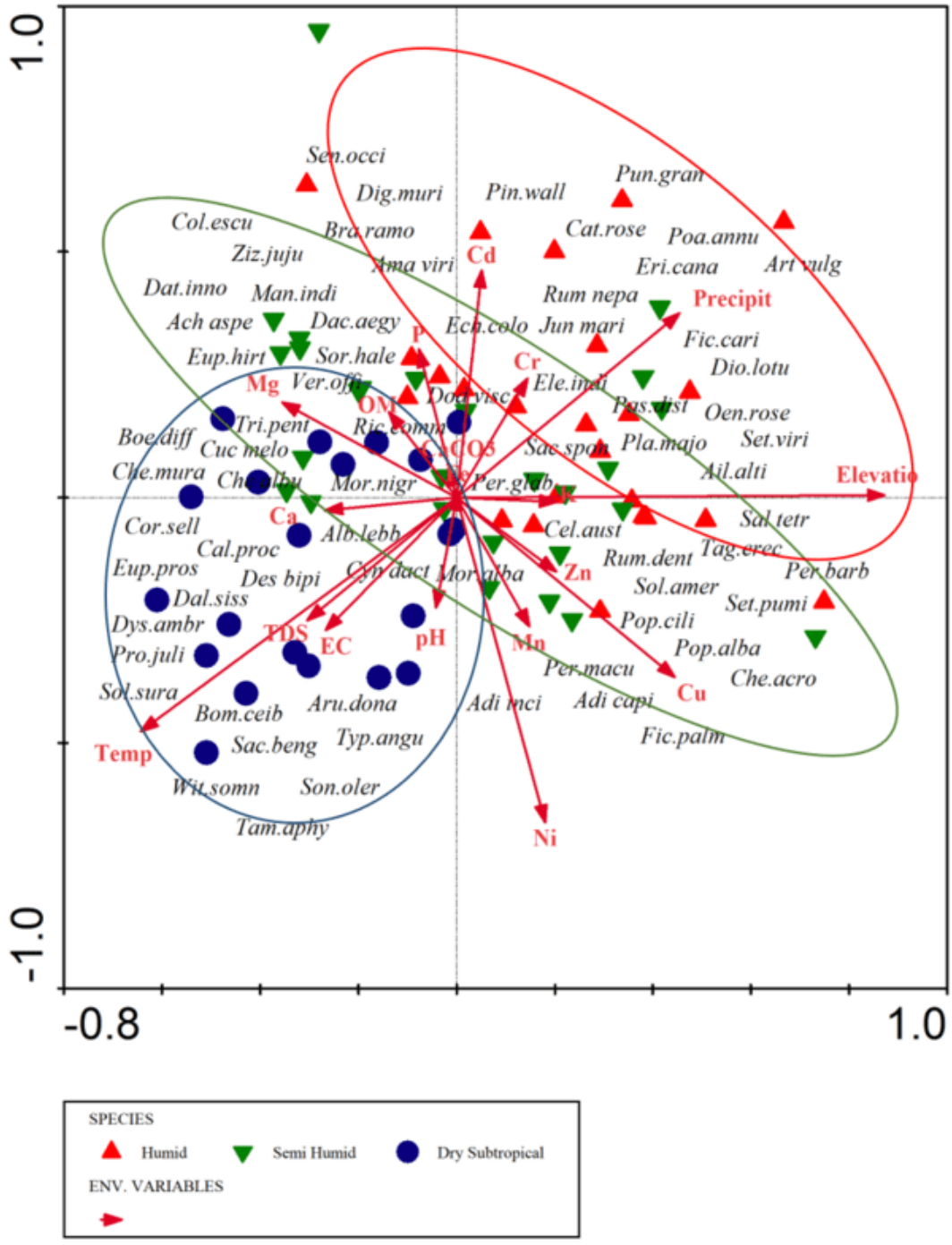


Fig. 3.12 CCA biplot presenting the distribution of indicator species in relation to measured environmental variables.

3.3.5 Structural Equation Modelling and reconfirmation of indicator species

SEM is mainly used for research that is designed to confirm a research study design. Therefore, we have used linear SEM to ensure indicator species that were identified through ISA, DCA and CCA. A detailed description of it is as follows:

3.3.5.1 SEM of Humid Subtropical MWPE

SEM again confirmed the identified indicators of humid subtropical MWPE. It revealed that marble pollution has a positive significant (β = 0.26) impact on the occurrence of identified indicator species. Whereas, elevation (β = -0.19), soil (β = -0.20) and climate (β = -0.07) have an insignificant negative impact on indicators. The impact amongst mediators such as elevation has a significant positive effect on climate (β = 0.32), while the climate has a significant and positive impact upon soil (β = 0.35) of the region (Table 3.7 & Fig. 3.13). Regarding the Goodness of Model Fit, our model is considered as a good fit because all the values, i.e., Chi-square (8.096), P-value (0.757), Goodness of Fit Statistic (GFI) (0.997), Adjusted Goodness of Fit Statistic (AGFI) (0.986), Comparative Fit Index (CFI) (0.925), Standardized Root Mean Square Residual (SRMR) (0.009), Root Mean Square Error of Approximation (RMSEA) (0.0001), Bentler-Bonett Index or Normed Fit Index (NFI) (0.996), Tucker Lewis Index or Non-normed Fit Index (NNFI) (0.947) and Akaike Information Criterion (AIC) (554.97) determined at a significant level (Table 3.8). We have also calculated the direct, indirect and combined effect of marble pollution on indicator species. It showed that the direct impact of predictor was higher than the indirect effect (Table 3.3.9). The marble pollution has both direct and indirect positive effects on indicator plants. While soil, elevation and climate have direct negative and indirect positive effects except for climate (i.e., negative) (Table 3.9; Fig. 3.14).

Table 3.7 The detail of Structural Equation Modeling (SEM) linking indicators, climate, soil, elevation and marble pollution in the humid subtropical MWPE.

S. No.	Response	Predictor	Standardized co-efficient (β)	S.E.	t-vale	p-value
1	Indicators	Pollution	0.2595	0.1196	2.1687	**0.034***
2	Indicators	Elevation	-0.1582	0.1263	-1.2523	0.2152
3	Indicators	Soil	-0.1978	0.1284	-1.5402	0.1287
4	Indicators	Climate	-0.0712	0.134	-0.5314	0.5971
5	Soil	Elevation	0.0375	0.1248	0.3003	0.765
6	Soil	Climate	0.3517	0.1248	2.8187	**0.0065****
7	Soil	Pollution	-0.0328	0.1182	-0.2774	0.7824
8	Climate	Elevation	0.3217	0.1193	2.6966	**0.009****
9	Climate	Pollution	-0.0344	0.1193	-0.2884	0.774

Significant codes: 0 =***, 0.001=**, 0.01=*

Table 3.8 Model Fit Indices and their respective values after SEM in order to check the goodness of model fit.

S. No.	Model Fit Indices	Value
1	Chi-sq	8.096
2	P-value	0.757
3	Goodness of Fit Statistic (GFI)	0.997
4	Adjusted Goodness of Fit Statistic (AGFI)	0.986
5	Comparative Fit Index (CFI)	0.925
6	Standardized root mean square residual (SRMR)	0.009
7	Root Mean Square Error of Approximation (RMSEA)	0.0001
8	Bentler-Bonett Index or Normed Fit Index (NFI)	0.996
9	Tucker Lewis Index or Non-normed Fit Index (NNFI)	0.947
10	Akaike Information Criterion (AIC)	554.971

Table 3.9 Summary of SEM representing the direct, indirect and combined impact of marble pollution, soil, climate and elevation on the indicators of humid MWPE, KPK, Pakistan.

S. No.	Response	Mediator	Predictor	Path label	Effect	est	S.E.	t-vale	p-value
1	Indicators	--	Pollution	a	Direct	0.259	0.115	2.256	**0.024**
2	Indicators	--	Soil	b	Direct	-0.198	0.123	-1.603	0.109
3	Indicators	--	Elevation	c	Direct	-0.158	0.121	-1.304	0.192
4	Indicators	--	Climate	d	Direct	-0.071	0.129	-0.551	0.582
5	Soil	--	Pollution	e	Direct	-0.032	0.115	-0.275	0.783
6	Climate	--	Pollution	f	Direct	-0.034	0.117	-0.295	0.768
7	Climate	--	Elevation	h	Direct	0.322	0.117	2.76	**0.006**
8	Soil	--	Climate	i	Direct	0.364	0.115	3.174	**0.002**
15	Indicators	Soil	Pollution	e*b	Indirect (single path)	0.006	0.023	0.271	0.786
16	Indicators	Climate	Pollution	f*d	Indirect (single path)	-0.002	0.009	-0.26	0.795
17	Indicators	Climate	Elevation	h*d	Indirect (single path)	0.023	0.042	0.54	0.589
18	Indicators	Climate, Soil	Pollution	f+(i*b)	Indirect (multiple paths)	-0.106	0.127	-0.838	0.402
19	Indicators	Climate, Soil	Elevation	h+(i*b)	Indirect (multiple paths)	0.25	0.127	1.967	**0.049**
20	Indicators	Direct + Indirect	Pollution	a+(e*b) +(f*d) +(f+(i*b))	Total	0.157	0.179	0.877	0.38
21	Indicators	Direct + Indirect	Elevation	c+(h*d)+ (h+(i*b))	Total	0.114	0.174	0.658	0.51

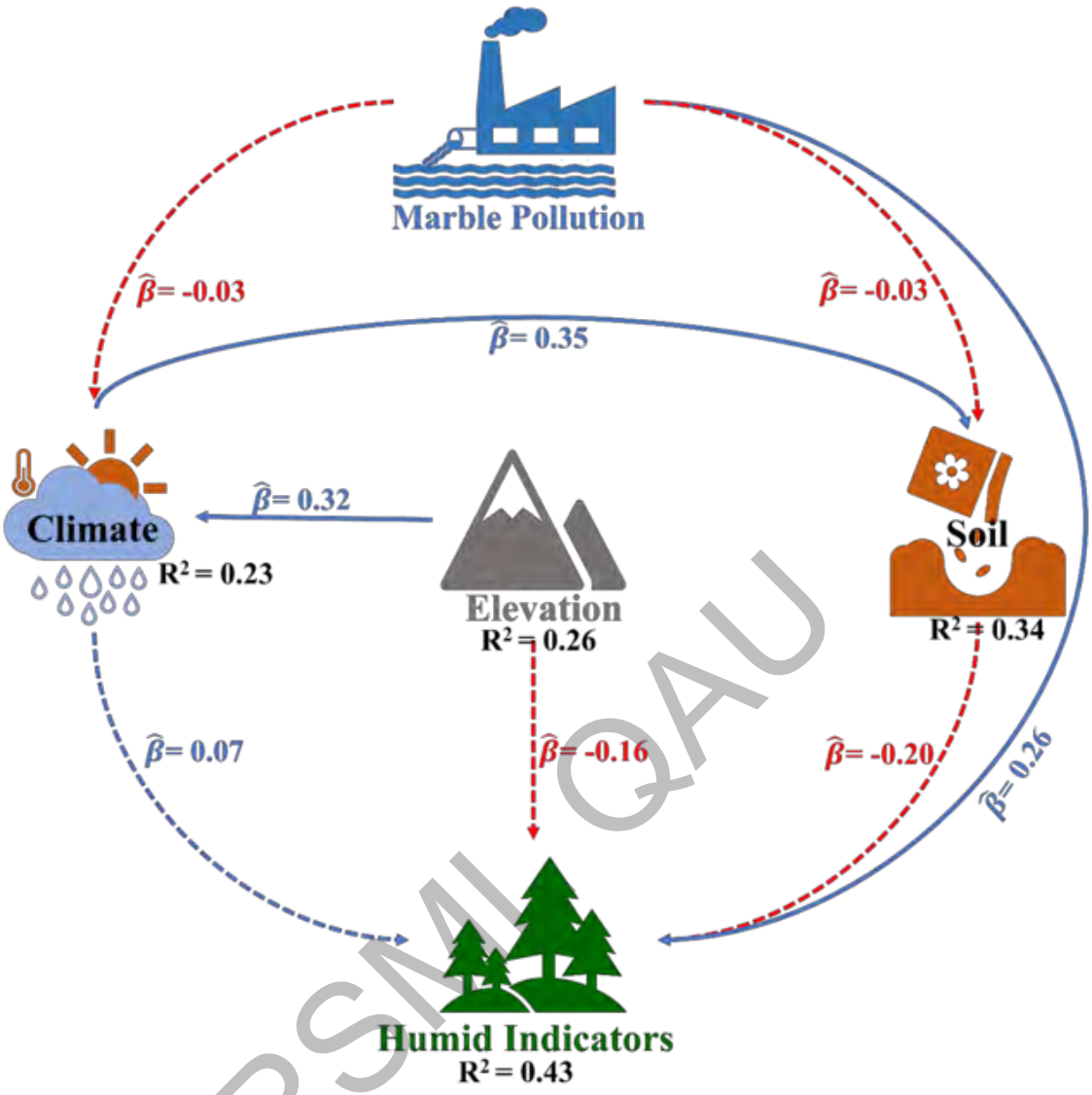


Fig. 3.13 Structural Equation Modeling showing the impact of marble pollution, climate, soil and elevation on indicator species of humid subtropical MWPE. Blue and red arrows represent the significant positive and negative paths, while dashed arrows comprehended the non-significant paths/effects. The standardized coefficient (β) has been shown.

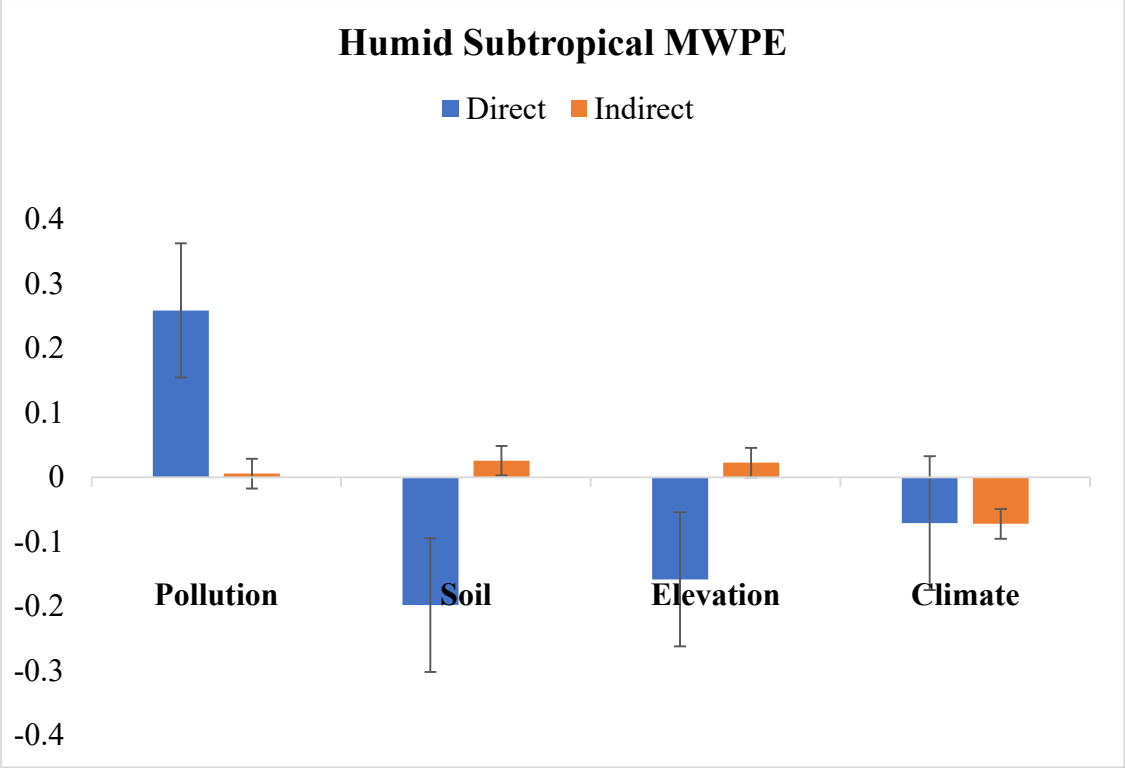


Fig. 3.14 The direct and indirect effects (standardized coefficient) derived from SEM of marble pollution, soil, elevation and climate in the humid MWPE.

3.3.5.2 SEM of Semi Humid Subtropical MWPE

SEM again confirmed the identified indicators of the semi-humid subtropical MWPE. It revealed that the occurrence of these indicator species decreases with an increase in marble pollution (β =-0.25). This decrease may be due to a large number of marble factories in the region. At the same time, the elevation (β = -0.30) and soil (β = -0.21) have a significant negative effect on indicator species of the region. The elevation and marble pollution have a significant negative impact on soil (β = -0.23) and climate (β = -0.24), respectively (Table 3.10; Fig. 3.15). The recorded Chi-square (7.443), p-value (0.506), GFI (0.998), AGFI (0.969), CFI (0.971), SRMR (0.014), RMSEA (0.0001), NFI (0.991), NNFI (0.967) and AIC (1143.44) showed that our model is the best fit. The total/combined effect of marble pollution is higher than the indirect and direct effect on the identified indicator species of the region. The direct and indirect effects of all the predicted variables followed the same pattern (i.e., negative) except marble pollution (i.e., negative for direct and positive for indirect) on the occurrence of semi-humid indicators (Table 3.12; 3.16).

Table 3.10 SEM representing the impact of explanatory on the indicator species of semi-humid subtropical MWPE. The significant effects are shown in bold and stared.

S. No.	Response	Predictor	Standardized co-efficient (β)	S.E. of β	t-value	p-value
1	Indicator	Pollution	-0.2479	0.0873	-2.8384	**0.0052****
2	Indicator	Elevation	-0.2974	0.0867	-3.432	**0.0008*****
3	Indicator	Soil	-0.2137	0.0818	-2.6113	**0.0101***
4	Indicator	Climate	-0.0623	0.0805	-0.7738	0.4404
5	Soil	Elevation	-0.2258	0.0894	-2.527	**0.0127***
6	Soil	Climate	0.0557	0.0848	0.6562	0.5128
7	Soil	Pollution	-0.1127	0.0917	-1.2298	0.2209
8	Climate	Elevation	-0.0007	0.0907	-0.0079	0.9937
9	Climate	Pollution	-0.241	0.0907	-2.658	**0.0088****

Significant codes: 0 =***, 0.001=**, 0.01=*

Table 3.11 Result of model fit indices showing the goodness of model fit for semi-humid SEM.

S. No.	Model Fit Indices	Value
1	Chi-sq	7.443
2	P-value	0.506
3	Goodness of Fit Statistic (GFI)	0.998
4	Adjusted Goodness of Fit Statistic (AGFI)	0.969
5	Comparative Fit Index (CFI)	0.971
6	Standardized root mean square residual (SRMR)	0.014
7	Root Mean Square Error of Approximation (RMSEA)	0.0001
8	Bentler-Bonett Index or Normed Fit Index (NFI)	0.991
9	Tucker Lewis Index or Non-normed Fit Index (NNFI)	0.967
10	Akaike Information Criterion (AIC)	1143.44

Table 3.12 Summary of SEM representing the direct, indirect and combined impact of marble pollution, soil, climate and elevation on the indicators of semi-humid MWPE. The significant effects are highlighted in bold.

S. No.	Response	Mediator	Predictor	Path label	Effect	β	S.E.	t-vale	p-value
1	Indicators	--	Pollution	a	Direct	-0.248	0.086	-2.887	**0.004**
2	Indicators	--	Soil	b	Direct	-0.214	0.08	-2.664	**0.008**
3	Indicators	--	Elevation	c	Direct	-0.297	0.085	-3.496	**0.0001**
4	Indicators	--	Climate	d	Direct	-0.062	0.079	-0.79	0.43
5	Soil	--	Pollution	e	Direct	-0.126	0.088	-1.43	0.153
6	Climate	--	Pollution	f	Direct	-0.241	0.09	-2.687	**0.007**
7	Soil	--	Elevation	g	Direct	-0.226	0.088	-2.561	**0.01**
8	Climate	--	Elevation	h	Direct	-0.001	0.09	-0.008	0.994
9	Indicators	Soil	Pollution	e*b	Indirect	0.027	0.021	1.26	0.208
10	Indicators	Climate	Pollution	f*d	Indirect	0.015	0.02	0.758	0.449
11	Indicators	Climate	Elevation	h*d	Indirect	0.001	0.006	0.008	0.994
12	Indicators	Soil	Elevation	g*b	Indirect	0.048	0.026	1.846	**0.065**
13	Indicators	Direct + Indirect	Pollution	a+(e*b) +(f*d)	Total	-0.737	0.156	-4.719	**0.0001**
14	Indicators	Direct + Indirect	Elevation	c+(g*b) +(h*d)	Total	-0.574	0.136	-4.183	**0.0001**

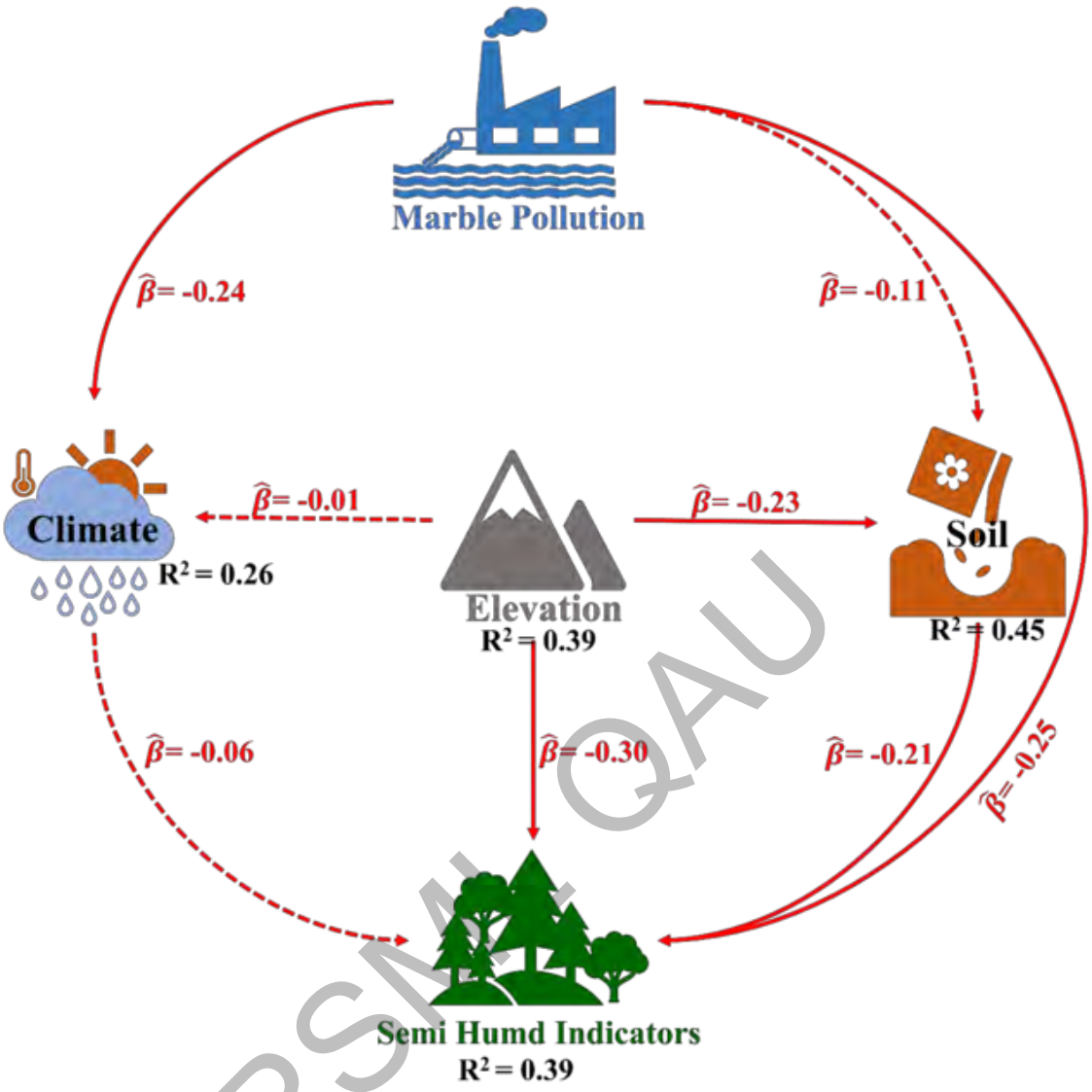


Fig. 3.15 SEM representing the influence of marble pollution, climate, soil and elevation on indicator species of semi-humid subtropical MWPE. Blue and red arrows represent the significant positive and negative paths, while dashed arrows comprehended the non-significant paths/effects. The standardized coefficient (β) has been shown.

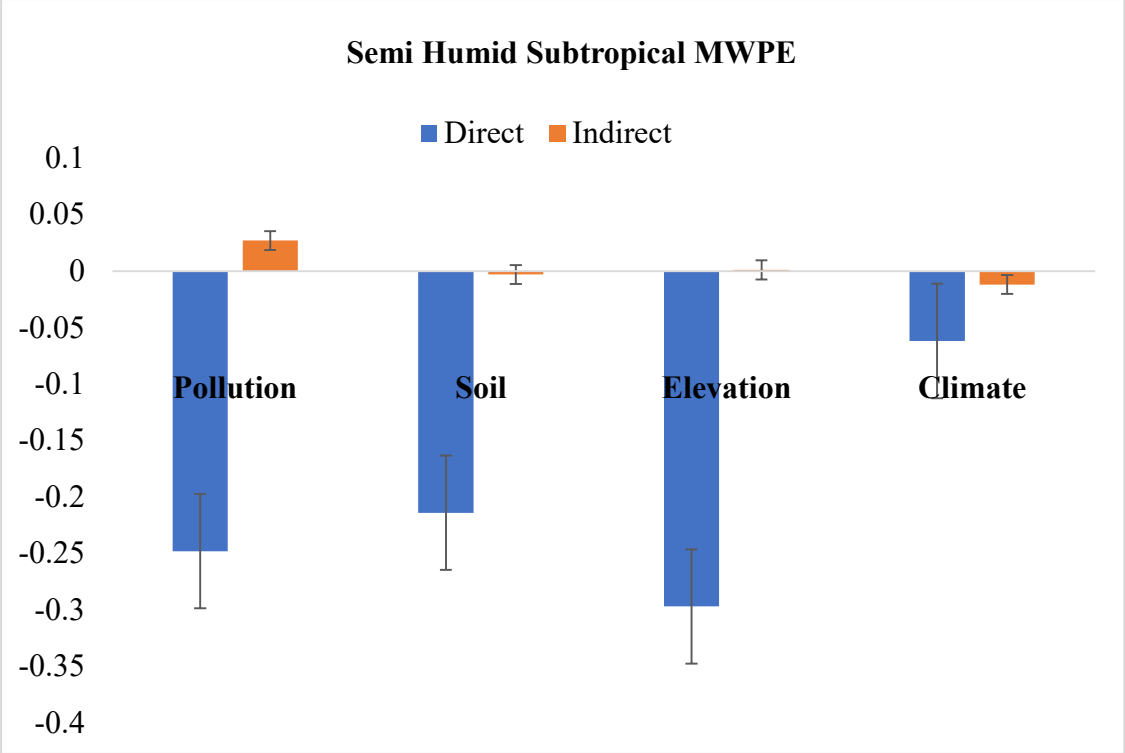


Fig. 3.16 The direct and indirect effects (standardized coefficient) obtained from SEM of marble pollution, soil, elevation and climate in the semi-humid MWPE, KPK, Pakistan.

3.3.5.3 SEM of Dry Subtropical MWPE

The SEM comprehended that the marble pollution (β = 0.18) has a significantly positive and climate (β = -0.24) has a significantly negative effect on the plant indicators of this MWPE. The soil (β = 0.19) has a positive nearly significant impact and elevation (β = -0.08) has an insignificant negative influence on the identified indicator species. While the climate (β = 0.28) has positive and pollution (β = -0.20) has a significant negative effect on the soil of this region (Table 3.13; Fig. 3.17). The Goodness of Model Fit was recorded as Chi-square (2.767), p-value (0.096), GFI (0.988), AGFI (0.916), CFI (0.982), SRMR (0.027), RMSEA (0.012), NFI (0.974), NNFI (0.936) and AIC (962.96) through the most popular fit statistics (Table 3.14). Furthermore, the elevation has a positive significant indirect effect via climate on the indicator species of dry subtropical MWPE. The indirect impact of marble pollution through soil and climate mediator was recorded higher (β = 0.58) than direct (β = 0.18) and combined effect (β = 0.51) on indicator species (Table 3.15). Both direct and indirect effect of marble pollution has a positive impact on indicator species of the region. While soil has positive direct & negative indirect effects, elevation and climate have a negative direct and positive indirect impact on the occurrence of indicator species of dry subtropical MWPE (Fig. 3.18).

Table 3.13 The detail of SEM linking indicators, climate, soil, elevation and marble pollution in the dry subtropical MWPE of Khyber Pakhtunkhwa, Pakistan.

S. No	Response	Predictor	Standardized co-efficient (β)	S.E. of β	t-value	p-value
1	Indicators	Pollution	0.1826	0.0915	1.9969	**0.0481***
2	Indicators	Climate	-0.2447	0.1205	-2.03	**0.0446***
3	Indicators	Soil	0.1936	0.0993	1.9505	0.0535
4	Indicators	Elevation	-0.0888	0.1193	-0.7449	0.4578
5	Soil	Elevation	-0.1792	0.1089	-1.6454	0.1025
6	Soil	Climate	0.2829	0.1082	2.6138	**0.0101***
7	Soil	Pollution	-0.1962	0.0825	-2.3773	**0.019***
8	Climate	Elevation	-0.6573	0.0695	-9.4512	**0.0001*****
9	Climate	Pollution	-0.0303	0.0695	-0.4359	0.6637

Significant codes: 0 =***, 0.001=**, 0.01=*

Table 3.14 The precise determination of model fit indices for the goodness of model fit for the identified indicators of dry subtropical MWPE.

S. No.	Model Fit Indices	Value
1	Chi-sq	2.767
2	P-value	0.096
3	Goodness of Fit Statistic (GFI)	0.988
4	Adjusted Goodness of Fit Statistic (AGFI)	0.916
5	Comparative Fit Index (CFI)	0.982
6	Standardized root mean square residual (SRMR)	0.027
7	Root Mean Square Error of Approximation (RMSEA)	0.012
8	Bentler-Bonett Index or Normed Fit Index (NFI)	0.974
9	Tucker Lewis Index or Non-normed Fit Index (NNFI)	0.936
10	Akaike Information Criterion (AIC)	962.968

Table 3.15 The direct, indirect and combined impact of marble pollution, soil, climate and elevation after SEM on the indicators of dry subtropical MWPE, KPK, Pakistan.

S. No.	Response	Mediator	Predictor	Path label	Effect	β	S.E.	t-vale	p-value
1	IVI	--	Pollution	a	Direct	0.183	0.089	2.047	**0.041**
2	IVI	--	Soil	b	Direct	0.194	0.096	2.014	**0.044**
3	IVI	--	Elevation	c	Direct	-0.089	0.116	-0.769	0.442
4	IVI	--	Climate	d	Direct	-0.245	0.121	-2.021	**0.043**
5	Soil	--	Pollution	e	Direct	-0.18	0.082	-2.211	**0.027**
6	Climate	--	Pollution	f	Direct	-0.03	0.069	-0.441	0.659
7	Soil	--	Climate	i	Direct	0.399	0.082	4.898	**0.001**
8	Climate	--	Elevation	h	Direct	-0.657	0.069	-9.569	**0.001**
9	IVI	Soil	Pollution	eb	Indirect (single path)	0.035	0.023	1.489	0.137
10	IVI	Climate	Pollution	fd	Indirect (single path)	-0.007	0.017	-0.431	0.666
11	IVI	Climate	Elevation	hd	Indirect (single path)	0.161	0.081	1.978	**0.048**
12	IVI	Climate, Soil	Pollution	f+(i*b)	Indirect (Multiple paths)	0.58	0.08	7.227	**0.001**

13	IVI	Climate, Soil	Elevation	h+(i*b)	Indirect (Multiple paths)	0.108	0.08	1.341	0.18
14	IVI	Direct+ Indirect	Elevation	c+(h*d)+ (h+(i*b))	Total	-0.048	0.111	-0.429	0.668
15	IVI	Direct+ Indirect	Pollution	a+(e*b)+ (f*d)+ (f+(i*b))	Total	0.508	0.121	4.199	**0.001**

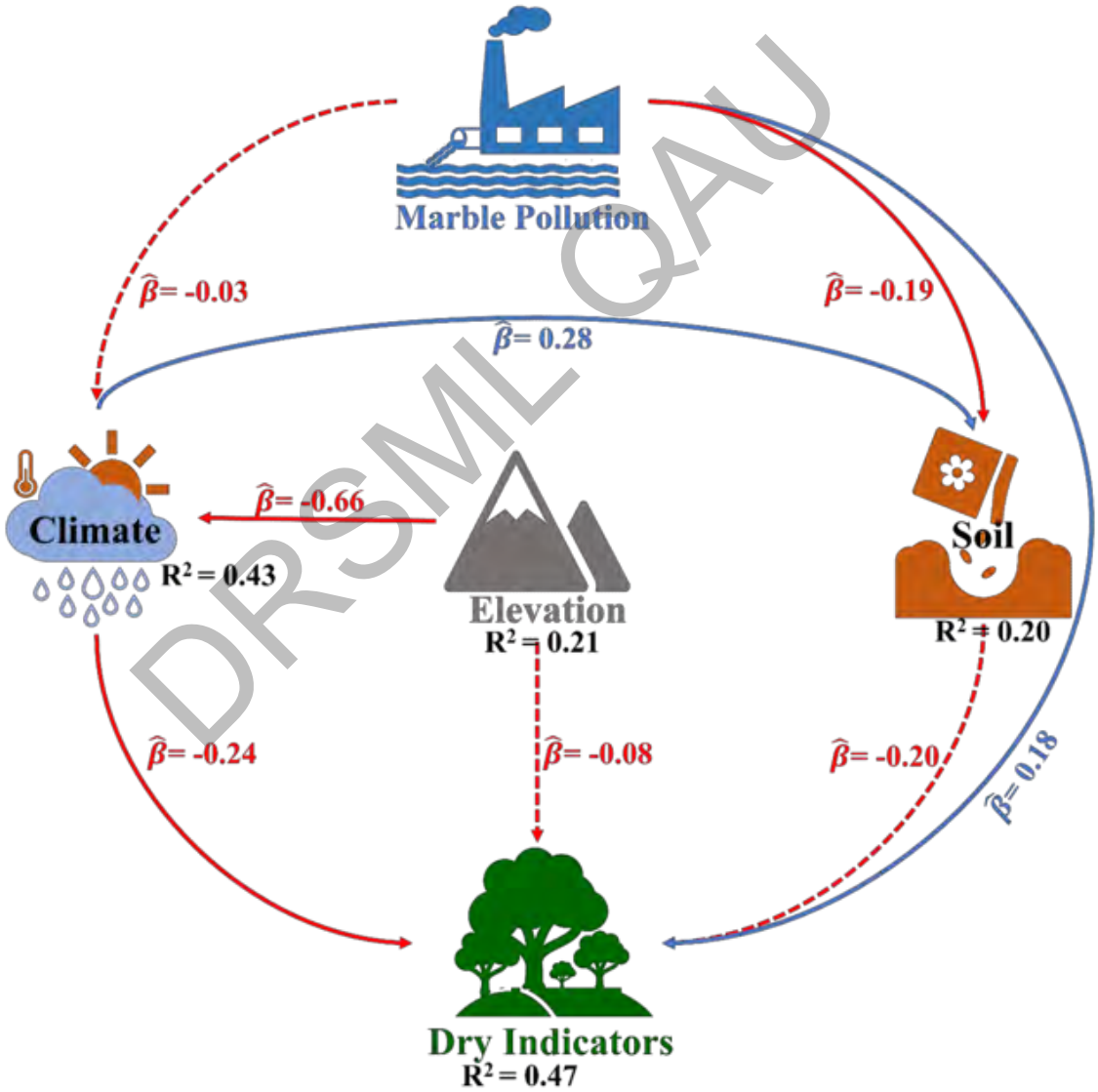


Fig. 3.17 SEM representing the influence of marble pollution, climate, soil and elevation on indicator species of dry subtropical MWPE, Khyber Pakhtunkhwa, Pakistan

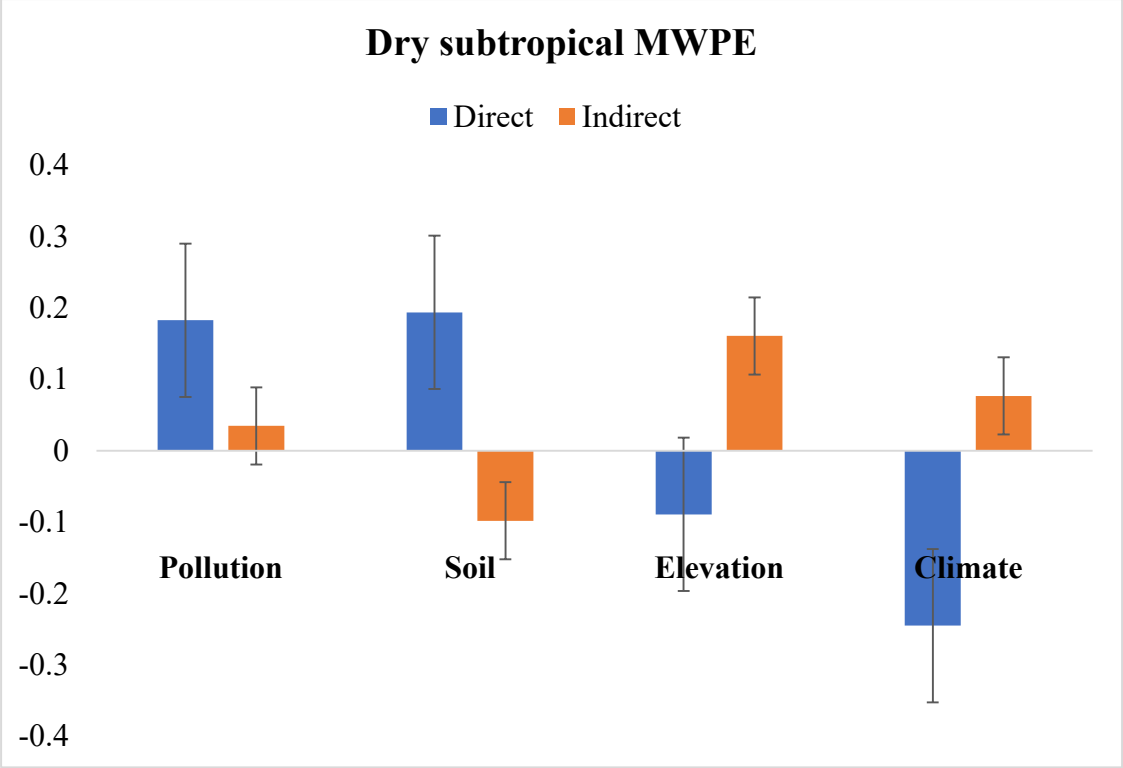


Fig. 3.18 The direct and indirect effects (standardized coefficient) acquired from structural equation modeling of marble pollution, soil, elevation and climate in the dry subtropical MWPE, KPK, Pakistan.

3.3.5.4 SEM and Goodness of the Model Fit for all identified MWPE indicators

SEM was carried out to further examine and verify the indicators of the whole subtropical MWPE that were identified based on ISA and CCA results. Our hypothesis of subtropical MWPE indicators is based on equations viii & ix. The SEM revealed that the pollution has a significant positive impact on the occurrence of identified indicators (β = 0.23) and significant negative effects on soil (β = -0.22) and climate (β = -0.58) of subtropical MWPE. While soil (β = -0.03), climate (β = 0.03) and elevation (β = 0.06) have insignificant influence on the identified indicators of the region. The elevation has a significant positive influence on soil (β = 0.16) and climate (β = 0.60). With an increase in elevation, soil condition and climate become better due to a decrease in marble pollution. At the same time, pollution has a negative impact on the climate (β = -0.58) of MWPE (Table 3.16; Fig. 3.19). The Goodness of Model Fit was recorded as Chi-square (26.435), P-value (0.153), Goodness of Fit Statistic (GFI) (0.960), Adjusted Goodness of Fit Statistic (AGFI) (0.943), Comparative Fit Index (CFI) (0.971), Standardized Root Mean Square Residual (SRMR) (0.049), Root Mean Square Error of Approximation (RMSEA) (0.079), Bentler-Bonett Index or Normed Fit Index (NFI) (0.960), Tucker Lewis Index or Non-normed Fit Index (NNFI) (0.959) and Akaike Information Criterion (AIC) (2534.551) through the most popular fit statistics which indicates our model is perfectly fit (Table 3.17). We have also determined the direct, indirect and combined effect of all measured variables on the occurrence of indicator species of MWPE. It showed that the pollution has a direct, indirect and combined effect (<0.0001) on the indicator species of subtropical MWPE, Khyber Pakhtunkhwa, Pakistan. Overall, marble pollution has a more significant impact on the indicator of MWPE than other explanatory variables (Fig. 3.20).

Table 3.16 The detail of SEM of identified plant indicators in the subtropical MWPE in relation to marble pollution, soil, climate and elevation explanatory variables.

S. No.	Response	Predictor	Unstandardized co-efficient (β)	Standardized co-efficient (β)	S.E of β	t-value	p-value
1	Indicators	Pollution	0.2308	0.2308	0.0655	3.5252	**0.0005*** **
2	Indicators	Soil	-0.0331	-0.0331	0.0608	-0.5442	0.5867
3	Indicators	Climate	0.0344	0.0344	0.0765	0.4498	0.6531
4	Indicators	Elevation	-0.0614	0.0614	0.068	-0.9024	0.3675
5	Elevation	Soil	0.1602	0.1602	0.0499	3.2101	**0.0015** **
6	Elevation	Climate	0.5955	0.5955	0.0499	11.9353	**0.0001*** **
7	Soil	Climate	0.2478	0.2478	0.0618	4.0132	**0.0001*** **
8	Soil	Pollution	-0.2245	-0.2245	0.0618	-3.635	**0.0003*** **
9	Climate	Pollution	-0.5773	-0.5773	0.0453	-12.7456	**0.0001*** **

Significant codes: 0 =***, 0.001=**, 0.01=*

Table 3.17 Summary of Goodness of Model Fit of SEM of indicators in the subtropical MWPE.

S. No.	Model Fit Indices	Value
1	Chi-sq	26.435
2	P-value	0.153
3	Goodness of Fit Statistic (GFI)	0.960
4	Adjusted Goodness of Fit Statistic (AGFI)	0.943
5	Comparative Fit Index (CFI)	0.971
6	Standardized root mean square residual (SRMR)	0.049
7	Root Mean Square Error of Approximation (RMSEA)	0.079
8	Bentler-Bonett Index or Normed Fit Index (NFI)	0.960
9	Tucker Lewis Index or Non-normed Fit Index (NNFI)	0.959
10	Akaike Information Criterion (AIC)	2534.551

Table 3.18 Summary of SEM representing the direct, indirect and combined impact of marble pollution, soil, climate and elevation on the indicators of subtropical MWPE, KPK.

S. No.	Response	Mediator	Predictor	Path label	Effect	β	S.E.	t-vale	p-value
1	Indicators	--	Pollution	a	Direct	0.231	0.064	3.612	**0.0001**
2	Indicators	--	Soil	b	Direct	-0.033	0.058	-0.571	0.568
3	Indicators	--	Elevation	c	Direct	-0.061	0.07	-0.88	0.379
4	Indicators	--	Climate	d	Direct	0.034	0.073	0.472	0.637
5	Soil	--	Pollution	e	Direct	-0.395	0.055	-7.192	**0.0001**
6	Climate	--	Pollution	f	Direct	-0.443	0.044	-10.151	**0.0001**
7	Soil	--	Elevation	g	Direct	0.076	0.055	1.39	0.164
8	Climate	--	Elevation	h	Direct	0.377	0.044	8.646	**0.0001**
9	Indicators	Soil	Pollution	eb	Indirect	-0.013	0.023	-0.569	0.569
10	Indicators	Climate	Pollution	fd	Indirect	-0.015	0.032	-0.471	0.637
11	Indicators	Climate	Elevation	hd	Indirect	0.013	0.028	0.471	0.637
12	Indicators	Soil	Elevation	gb	Indirect	-0.003	0.005	-0.528	0.597
13	Indicators	Direct + Indirect	Elevation	c+(h*d)+(g*b)	Total	0.033	0.058	0.575	0.565
14	Indicators	Direct + Indirect	Pollution	a+(e*b)+(f*d)	Total	-0.22	0.058	-3.829	**0.0001**

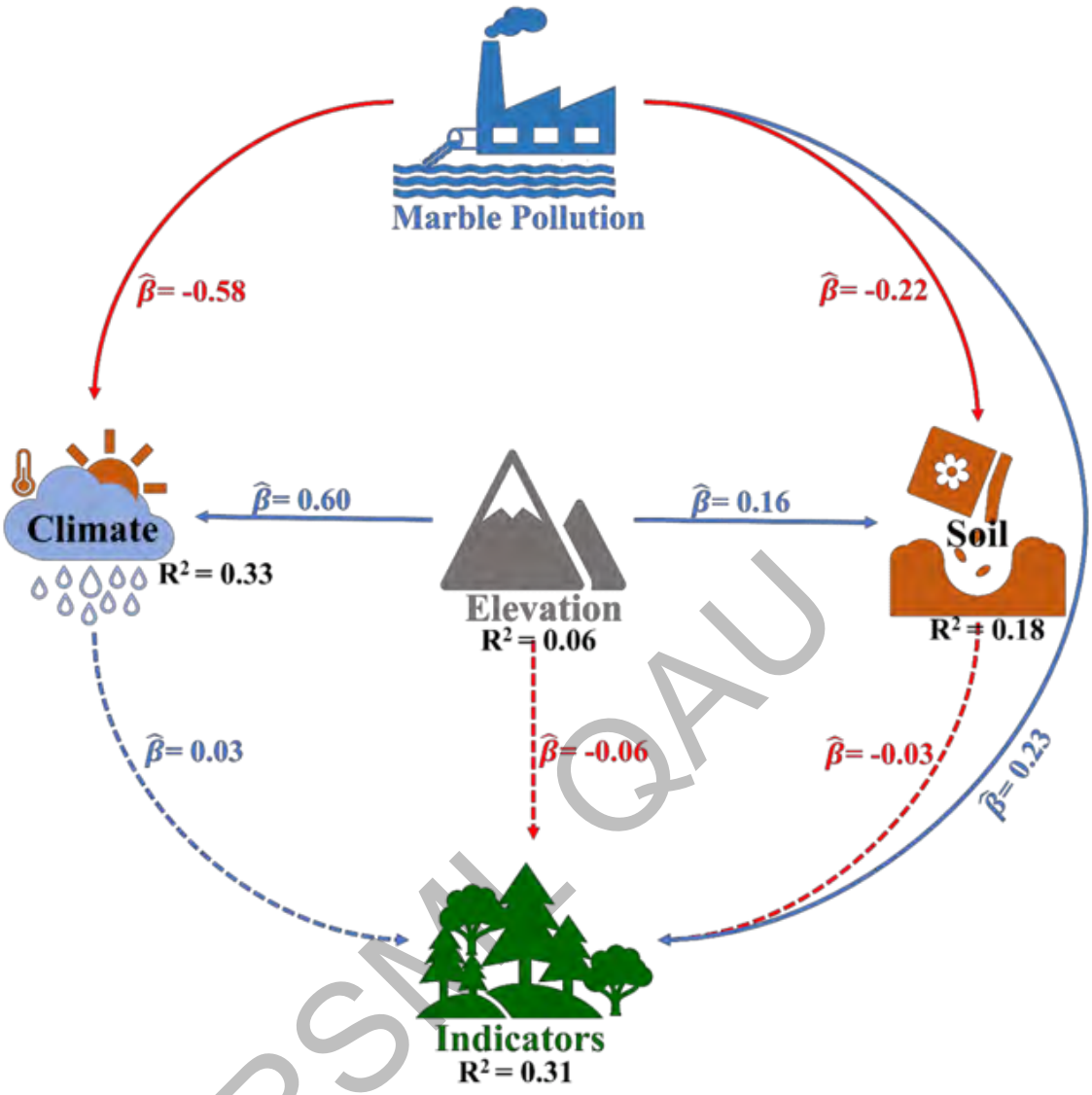


Fig. 3.19 Structural Equation Modeling of the indicator species in subtropical MWPE in relation to marble pollution, soil, climate and elevation environmental factors.

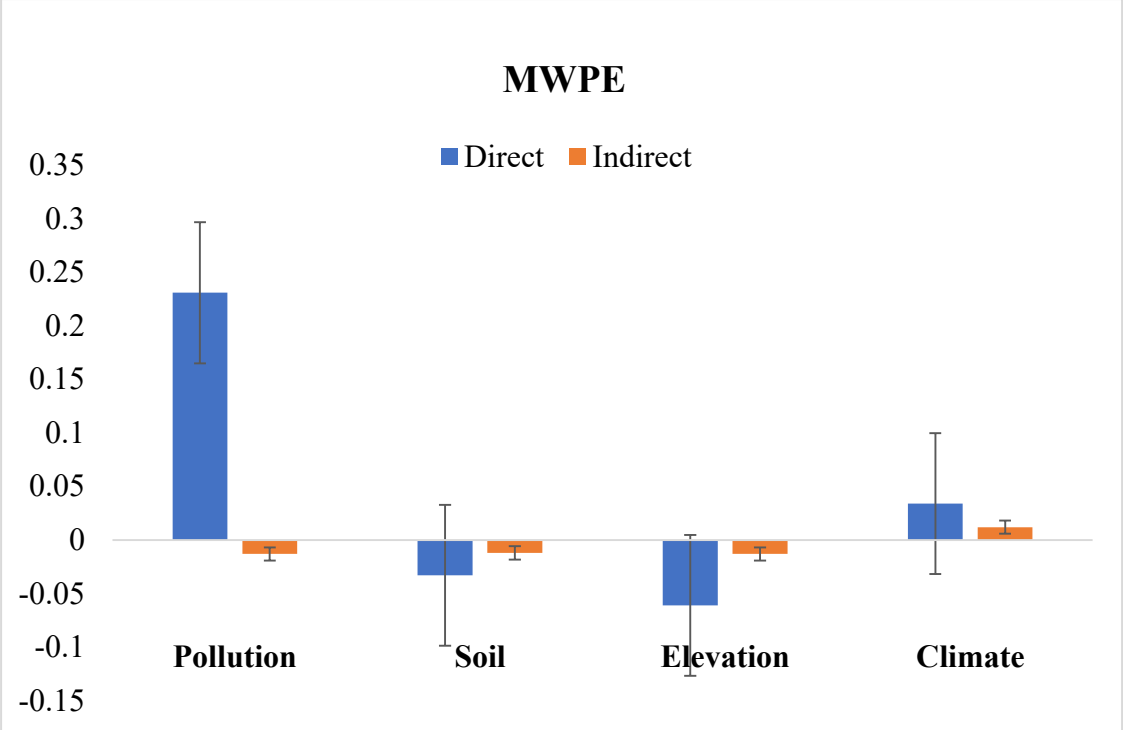


Fig. 3.20 The direct and indirect effects (standardized coefficient) obtained from SEM of marble pollution, soil, elevation and climate in the subtropical MWPE, KPK, Pakistan.

3.4 Discussion

The occurrence and behavior of a plant species are the result of combinations of all factors prevailing in any geographic region. Several plants, animals and microorganisms have certain specific environmental requirements which limit their distribution and composition. These specific geographic features mark particular communities/niches of flora and fauna. Hence, climatic and geographic gradients have a significant role in the determination of vegetation of a particular region (Pitt and Heady 1978; Xu et al. 2004).

This chapter revealed the impact of marble pollution, climate, elevation and edaphic factors on the formation of different types of subtropical marble waste polluted plant communities using multivariate statistical approaches. The TWCA further classified the subtropical vegetation into Humid, Semi-humid and Dry subtropical vegetation based on similar floristic composition and variation in climatic, edaphic and topographic factors. These environmental gradients have supreme importance in determining vegetation (Pitt and Heady 1978; Xu et al. 2004).

The humid subtropical MWPE indicators (*Ficus carica*, *Catharanthus roseus* and *Erigeron canadensis*) were under the impact of higher precipitation, marble pollution and edaphic (higher copper, cadmium, zinc, lower calcium, phosphorous concentration and neutral soil pH) variables in the current study. This means that marble pollution, higher precipitation and the above-mentioned edaphic features have a more significant role in the shape and structure of this humid subtropical vegetation. These phenomena were again reconfirmed two times with the help of CCA and SEM. The Humid subtropical vegetation has a higher Simpson Index as compared to other subtropical vegetation zones.

At the same time, the semi-humid subtropical MWPE indicators (Table 3.3.2-3) encompassed higher marble pollution, a moderate amount of precipitation (31mm), temperature, phosphorous, lower potassium, cadmium, manganese, and neutral soil pH compared to other subtropical MWPE with higher species richness, Shannon and Pielou's Evenness indices.

The indicator species (Table 3.3.2 & 3.3.4) of Dry subtropical MWPE was under the influence of higher temperature (38°C), electrical conductivity, moderate $CaCO_3$, low precipitation, pH, iron, copper, nickel and zinc concentration. Likewise, plant

indicators of mineral deposits were also reported by (Brooks 1979). According to him, *Krascheninnikovia ceratoides*, *Salsola nitraria* and *Limonium suffruticosum* were the boron mineral indicators. *Crotalaria cobalticola*, *Haumaniastrum robertii* and *Silene cobalticola* were the universal indicators of the cobalt and copper deposits (Duvigneaud 1959). *S. cobalticola* restricted to grow in copper and cobalt mine region of Savannah and the plant is also classified as critically endangered in the IUCN Red List of threatened species. *Acalypha dikuluwensis*, *Ocimum centraliafricanum*, *Ocimum metallorum*, *Commelina zigzag*, *Silene suecica* and *Polycarpaea spirostylis* were copper indicators. *S. suecica* was used to search for copper by the Scandinavian miner during the 17th century. *P. spirostylis* is one of the best-known copper indicators. Its reputation as an indicator was first described by (Skertchy, 1897) and later confirmed by field investigation (Brooks and Radford 1978; Groves et al. 1972; Nicolls et al. 1965). Whereas, *Agathis ovata* is more liable to be controlled by nickel, chromium and magnesium concentration. *Acacia athens*, *Gompholobium polyzygum*, *Eriachne pulchella subsp. dominii* and *Goodenia scaevolina* are the iron indicators (Cole 1965). (Nicolls et al. 1965) reported *Eriachne mucronata* as an indicator of copper, lead and zinc mineralization at Dugald River, Queensland. *Crotalaria florida var. congolensis* is a mangoanophytes (Duvigneaud 1959). *Hybanthus austrocaledonicus* (nickel indicator), *Astragalus pattersonii* and A. *preussii* (selenium/Uranium indicators), *Thlaspi caerulescens subsp. calaminare* and *Viola lutea subsp. calaminaria* are zinc indicators (Brooks 1979). (Madkour and Laurence 2002) reported clover, jute, garden rocket and alfalfa as a bioindicator for ozone in Egypt. (Johnston et al. 2007) worked on plant indicators of the Great Lake Coastal wetland and reported forty-eight hydrogeomorphic plant indicators and ninety soil indicators. Many of them are bog and fen species which are also considered as organic soil indicators and included *Carex lasiocarpa, Drosera rotundifolia, Myrica gale, Rhynchospora alba, Salix pedicellaris, Solidago uliginosa, Triadenum fraseri, Andromeda polifolia, Chamaedaphne calyculata, Menyanthes trifoliate, Pogonia ophioglossoides, Rhynchospora fusca, Sarracenia purpurea* and *Utricularia intermedia,* plants. Similar to our study these species were identified based on ISA. Some researchers also worked on the remediation ability of the plant of mining region as well (Barros et al. 2010; Chamaret et al. 2007; Sasmaz and Yaman 2006; Van der Walt et al. 2012). But little attention has been given to the identification of indicators

of environmental pollution using ISA at microhabitat level and their reconfirmation via CCA and SEM and hence we claimed it as a first ever study.

Further, structural equation modeling comprehended that the marble pollution has a positive significant (β = 0.26) impact on the occurrence of identified indicator species. Whereas, elevation (β = -0.19), soil (β = -0.20) and climate (temperature + precipitation) (β = -0.07) have an insignificant negative impact on indicators. The semi-humid subtropical vegetation decreases with an increase in marble pollution (β = -0.25). In contrast, elevation and soil are also harm indicators of this region. The marble pollution and soil have significant positive, climate and elevation have a negative influence on the indicators of dry MWPE. Comparing the finding above with previous theories and literature revealed that precipitation and temperature regulate vegetation's general distribution (Pitt and Heady 1978; Xu et al. 2004). It affects vegetation through atmospheric humidity and the soil's water content (Pielke Sr 2001). If the rainfall is moderate and occurs uniformly on a larger number of days, the plants are highly benefited, but heavy rains for a few days result in runoff and less available water. The small quantity of rain is of little significance for the plants, as most of it is lost to the atmosphere because of evaporation (Lazaro et al. 2001). In tropical regions, heavy rainfall throughout the year results in the evergreen rainforest. Heavy rainfall for few months results in deciduous forest vegetation (Hussain 1984). In the region of high rainfall in summer and low in winter, grassland prevails. Temperature is also considered one of the most important ecological factors as it regulates the plant's physiological process. Both extreme and low temperatures have an adverse effect on plant growth (Wahid et al. 2007). Low temperature cause cold injuries in which water is frozen into ice crystals in the intercellular spaces, causing injuries to cells (Ahmad et al. 2021). Similarly, extreme high temperature causes senescence of plants due to the adverse effect on many vital physiological processes such as respiration, transpiration, and protein metabolism (Feller and Vaseva 2014; Seleiman et al. 2021). Plants differ considerably in their temperature tolerance from species to species. Broad belts of different vegetation occur between the equator and the poles. They extend parallel with the equator and correspond roughly to temperature belts (Hussain 1984). The general climatic regions, edaphic factors are also crucial for the local differences in plant communities or geographical vegetation zones (Ahmad et al. 2016a; Khan et al. 2017a).

One of the important applications of the present research work was using multivariate statistical techniques, i.e., ISA, SEM, CCA and TWCA, to accurately indicate and interpret vegetation distribution patterns. It allows the researcher to compare multiple classification and their interrelationship for the factual information resulting from the analyses (Khan et al. 2016). TWCA was used to identify potential subtropical vegetation of MWPE based on pattern similarity via Sorenson distance measurements. ISA identified the significant indicators of each subtropical MWPE. It provides knowledge regarding species fidelity in a specific habitat (Dufrêne and Legendre 1997). ISA helps to relate indicators with environmental conditions (Baker and Wiley 2004; King et al. 2004). However, its features must be understood in describing the results. The ISA must have higher values for the relative abundance (RA_{kj}) and frequency (RF_{kj}) in a category (Mc-Cune and Grace 2002), which eliminated some plants from being the indicators. Identifying indicators does not impede plant species from occurring in another zone/association (Dufrêne and Legendre 1997; Ter Braak and Prentice 1988). It means that the species has a significant preference for the model group. The combination of RA_{kj} and RF_{kj} makes practical sense for the development, as it ensures a species is likely to be encountered once and has a high statistical probability value for the association with the environment characteristics for which it indicates (Johnston et al. 2007). The species are chosen as indicators that indicate the environmental change and predict the diversity of other species/taxa or communities within the area as they can reflect the physicochemical state of the environment (Dufrêne and Legendre 1997). In the current study, CCA was used to determine the relationship of plant species with different environmental variables (Ter Braak and Smilauer 2002). It is mostly used to explain covariation between two sets of variables and find canonical variates that are important for explaining covariation between sets of variables. Correlation of the canonical axes and explanatory matrix was reported, along with the significance of each correlation determined via permutation. Testing the hypothesized relationship between response and explanatory variables by standardizing the axis scores and centering on the unit variance and axes scale to optimize the representation of species. It reconfirms our observation regarding indicator species' significant consequence of distinct environmental variables. These techniques were also implemented by a different researcher for the classification of vegetation in the different regions of the world for various types of ecological observations like (Ahmad et al. 2016a; Iqbal et al. 2015; Khan et al. 2014;

Khan et al. 2012; Khan et al. 2016). Furthermore, Structural Equation Model was done using R- software in order to examine the complex relationship of vegetation structure/indicators plants and impact of marble pollution, climate, elevation and edaphic variables of three major subtropical vegetation zones of MWPE. It also examined the direct and indirect impact of measured environmental variables for clearer picture of the subtropical vegetation. It reconfirmed our observation of ISA and CCA through the Goodness of Model Fit statistics i.e., Chi-square Statistics (X^2), Goodness of Model Fit Index (GFI), Adjusted Goodness of Model Fit Index (AGFI), Root Mean Square Error of Approximation (RMSEA), Standard Root Mean Square Residual (SRMR), Normed Fit Index (NFI), Non-Normed Fit Index [NNFI or also known as Tucker Lewis Index (TLI)], Comparative Fit Index (CFI) and Akaike Information Criterion (AIC). GFI and AGFI is the measurement of fit between the observed covariance matrix and the hypothesized model. The GFI or AGFI values ≥0.90, indicating an acceptable model (Byrne 1994; Sharma et al. 2005). RMSEA tells us how well the model, with unknown but optimally chosen parameter estimates, would fit the population covariance matrix. Its value below 0.08 shows a good model fit (Hu and Bentler 1999; Steiger 2007). The CFI, also known as Bentler comparative fit index, compares the model of interest with relative independence or the null model (a model is accepted if it‘s CFI≥0.90) (Hooper et al. 2008). SRMR determines to mean covariance between observed and model-predicted correlation. A model should be considered acceptable if its SRMR <0.08 and ideally less than 0.05 (Hu and Bentler 1999). NFI and NNFI assess the model by comparing the chi-square (X^2) value of the model to the X^2 of the null model. Its recommending values greater than 0.90 indicating a good model fit (Hu and Bentler 1999). The aforementioned tool of SEM was also adopted by a number of a researcher in the field of vegetation ecology for the investigation of the complex relationship between vegetation dynamics and environmental gradients (Grace et al. 2010; Grace and Keeley 2006; Grace and Pugesek 1997; Iriondo et al. 2003; Lefcheck 2016; Vile et al. 2006).

3.5 Conclusion

It is concluded that marble pollution, climate, elevation and soil have a significant impact on the vegetation structure of the subtropical vegetation of MWPE. In more depth, the marble pollution and climate have a significant positive influence, while elevation and soil have a significant negative influence on subtropical vegetation and

their indicator plants. It is also concluded that ISA is one of the best and most effective techniques for the identification/selection of indicators. We claim that their reconfirmation via CCA and SEM analysis has been done for the first time and these indicators can further be used for multipurpose including reforestation drives and smart habitat plantation. Both SEM and CCA analysis identified the complex relation/impact of measured environmental factors on subtropical vegetation of MWPE. The statistical and modeling procedure adopted in the current study could be followed to classify vegetation and identify indicator plants of any geographic region or microhabitat type in any part of the world.

Chapter 4

Remediation of hazardous level of Potential Toxic Elements through phytoremediation and mycoremediation approaches

4.1 Introduction

In the study region, marble pollution has been one of the basic deteriorating sources of biosphere pollution with impacts at the species level. Rapid increases in the rate of marble industrialization have worsened the pollution problems (Hsiao et al. 2020; Lai et al. 2018; Lin et al. 2014; Yuanan et al. 2020). The marble industry gives rise to oxides of Ca, Mg, Fe, Si, Ti, P, Al and Na (Knoche et al. 1995). In addition, large amounts of water are used during marble processing, which, on discharge, directly affects local waterways. Without primary treatments and detoxification procedures, marble wastewater adds Potential Toxic Elements (PTEs)/heavy metals to the neighboring ecosystems (El-Maghraby et al. 2013). As a consequence, levels of pollutants increase in both water and soil that in turn adversely affect the natural biomes as well as the abiotic environment.

However, some of the organisms, and especially plants and microbes, can survive in such toxic conditions (Navarro-Cano et al. 2018). Such organisms can be identified as pollution indicators of such special habitats or biomes (Freeman et al. 2006; Li et al. 2017; Sepehri and Sarrafzadeh 2019). These plant species absorb or degrade PTEs well above levels present in the normal environment and hence act as a sink to remediate the polluted locations (Brückner et al. 2020; Paz-Alberto and Sigua 2013; Treesubsuntorn and Thiravetyan 2018). These species are able to develop phytoremediation and bioremediation strategies to cope with the polluted conditions (Bi et al. 2020; Ceschin et al. 2019).

4.1.1 Phytoremediation

The term phytoremediation was coined by (Raskin et al. 1994), and is derived from the Greek ‗Phyto' plants and the Latin ‗remedium' to restore. Phytoremediation is one of the strategies to restore polluted ecosystems at a low cost (Nascimento and Xing 2006; Wei et al. 2020; Yan et al. 2020). Certain plant species retain the inherent ability of bioaccumulation, translocation and degradation of different types of pollutants (Sepehri et al. 2020). They play a role as a sink for biologically hazardous

materials (Schwitzguébel 2017). This ability can be viewed as a low cost technology driven by natural sunlight energy and taking place in situ, where plants accumulate PTEs from the environment (Salt et al. 1998). Plants accumulate these PTEs in their tissues and release/extract out nutrients for their rapid growth. This proves includes removal of both organic and inorganic contaminants (Anderson and Coats 1995). Plants can act as an important means to reduce pollution irrespective of microbial assisted or chemical treatments. There is currently a research focus on the remediation of different pollutants through the use of plants.

The phytoremediation process involves different stages. These include phytoextraction and phytostabilization for inorganic pollution, and phytodegradation/ phytotransformation, rhizodegradation and rhizofiltration for organic pollution. Plant root exudates can demobilize and bind contamination within the soil matrix and hence reduce contaminant bioavailability; this is termed phytostabilization. Certain plant species absorb/accumulate PTEs from polluted ecosystems in roots or shoots through the process of phytoextraction. This approach is for organic contaminants, metals, nonmetals, metalloids and radionuclides in soil and sludge media (Moreno-Jiménez et al. 2011). Phytovolatilization describes the plant species‘ ability to absorb PTEs and subsequently volatilize it into the atmosphere. Phytodegradation or phytotransformation is the breakdown of PTEs taken up by plant species via metabolic procedures within plants or outside through various compounds released by plant roots (Parmar and Singh 2015). Rhizofiltration involves the use of plant roots to clean up polluted water by filtering out the contaminants from the aquatic ecosystem (Vara Prasad and de Oliveira Freitas 2003). Numerous aquatic species have been utilized for this purpose, for example duck weed (*Lemna minor* L.), sharp dock (*Polygonum amphibium* L.), penny wort (*Hydrocotyle umbellate* L.) (Vara Prasad and de Oliveira Freitas 2003). Rhizodegradation is the breakdown of PTEs via microorganisms that are boosted by rhizosphere. These microbes digest and consume organic compounds for energy and nutrition. Plant roots release sugars, acids and alcohols which provide the carbon source for the microbes. Hence, establish a dense root mass that takes large amount of water. Rhizodegradation approach is used for the removal of organic pollutants in the lithosphere.

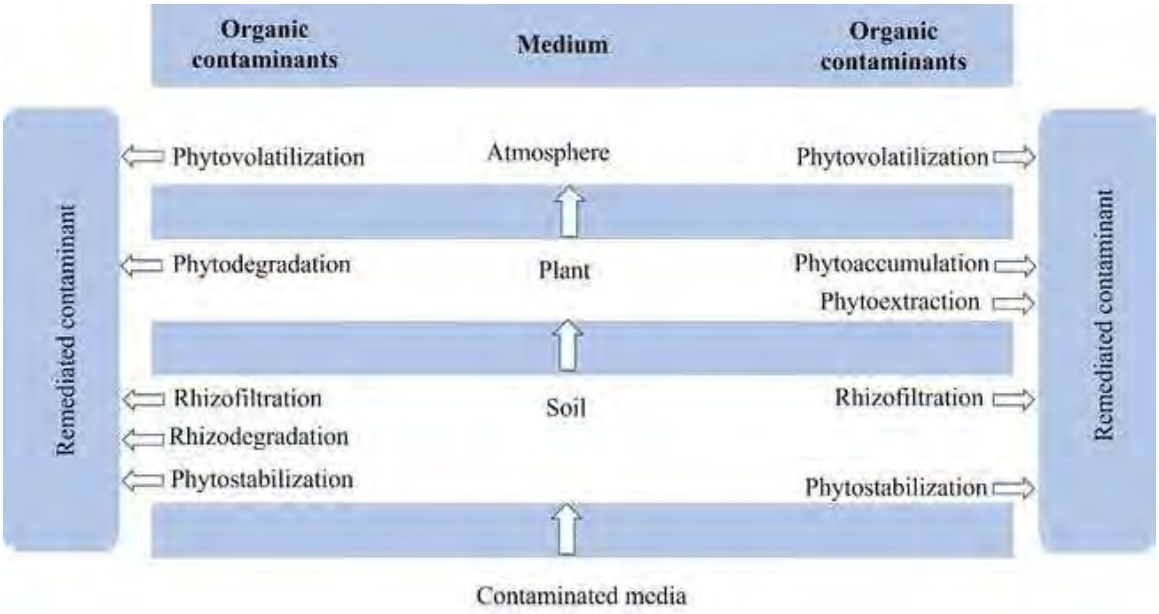


Fig. 4.1 Accumulation mechanism on phytoremediation technology (Tangahu et al., 2011).

4.1.2 Mechanisms of heavy metal uptake by plants

Plants have developed very efficient mechanisms to absorb both essential and non-essential nutrients from their surroundings. Plants produce chelating agents that stimulate pH changes and redox reactions to solubilize and accumulate nutrients even from very low levels and nearly insoluble precipitates in soils. This process is also involved in uptake, translocation and storage of PTEs whose chemical features simulate essential elements. Hence, the nutrient uptake procedure is of immense significance to phytoremediation.

Different transport mechanisms in the plant cell membrane are involved in ion uptake and translocation. These consist of channels (proteins that assist ions transport into the cell), proton pump (ATPases which utilize energy and produce electrochemical gradient) and co-and anti-transporters (proteins that utilize electrochemical gradient produced by ATPases to drive the active uptake of ions). Every transport system takes up an array of nutrients / ions. The plant uptake or translocation approach are usually closely regulated. They generally do not accumulate trace elements beyond their metabolic requirements. These needs range from 10-15 ppm for most trace elements. However, the exception are the hyperaccumulator plant species that can accumulate or

take up PTEs in thousands of ppm. Different mechanisms are adopted by hyper accumulator plants to avoid metal toxicity. One of these mechanisms is the storage of PTEs in vacuoles.

Evaporation from the leaves of plant species helps to absorb nutrients into plant roots. This development is responsible for transfers of contaminants into plant shoots. Some plant species that are used as phytoextraction are termed hyperaccumulators. Usually, the hyper accumulator requires little maintenance to remove toxic elements from its surroundings (Cluis 2004; Lasat 2000). The phytoremediators can concentrate or take up PTEs up to 100-1000 times greater than non-accumulator or excluder plant species.

4.1.3 Factors effecting the accumulation of PTEs

There are different factors which affect the accumulation of PTE, including type of plant species, bioavailability of metals, properties of medium/soil and root zone, vegetative uptake and addition of chelating agents etc. The plants with the greatest remediation ability need to be examined and carefully chosen (Prasad et al. 2010). The accumulation of PTEs is significantly affected by plant species characteristics (Rodriguez et al. 2005). Various practices are developed to change soil properties (i.e., change in pH, addition of fertilizer and chelators etc.) to enhance remediation ability. For example, lead accumulation in plants can be affected as a result of changes in soil pH, organic matter and phosphorus concentration (Ashraf et al. 2015). Furthermore, the rhizosphere possesses a special interest in phytoremediation. It can absorb or metabolize PTEs inside the plant tissue. An increase in diameter and reduction in root elongation are responses that can indicate morphological adaptation of plant species to the polluted soil (Huhle et al. 2008). The accumulation of PTEs is affected by environmental conditions. For example, temperature influences plant growth and subsequently the root length. Understanding the mass balance and metabolic fate of the contaminants are keys to demonstrating the application of phytoremediation (Mwegoha 2008). Accumulation of PTEs also depends on bioavailability in water phase, retention time and interaction with elements. When PTEs blend with soil, redox potential, pH and organic matter concentration will influence the tendency of metals to either exist in ionic or plant available form. The plant species will influence the soil via their ability to lower the pH and oxygenate the

sediment both of which will affect metal availability. The phytoremediation ability can be enhanced through addition of synthetic chelating agents. Chelating agents promote the leaching of PTEs into soil. The bioavailability of PTEs decreases over pH 5-6, therefore, chelating agents may be used in alkaline soil remediations. Exposure of plant species to Ethylenediaminetetraacetic acid (EDTA) for more than two weeks improves translocation of metals. The application of EDTA at 5 mmol/kg was found to give significant results (Roy et al. 2005). Plant root release of organic acids like oxalate and citrate also affect the availability of metals. The occurrence of ligands influences the accumulation of PTEs through the formation of metal ligand complexes and fluctuate to leach contaminants below rhizosphere (Seuntjens et al. 2004).

4.1.4 Advantages of Phytoremediation

Phytoremediation is considered a new technology that can remediate contamination of water, soil and ambient air. Phytoremediation is more publicly acceptable than other chemical and physical techniques. It can be considered an approach to contaminant reduction that is low-cost and environmentally friendly. It is the most suitable technology that can be used for the remediation of large numbers of hazardous elements and sites. It is inexpensive i.e., 60-80% less than other conventional technology. Therefore, it doesn‘t need highly specialized workers and expensive equipment. Phytoremediation is cost effective for large areas having moderately polluted surface soils (Liu et al. 2018b). It can be applied to a wide range of environmental contaminants either organic or inorganic. It is also applicable to a wide range of radionuclides and toxic elements with minimum environmental disturbance. The phytoextraction is regarded as significant method to degrade contaminants form soil in situ condition. The amount of soil disturbance is very low in *In-situ* application as compared to other conventional methods. It causes minimal environmental disturbance with topsoil left in a usable condition that can be reclaimed for agriculture use. Phytoremediation is an alternative to other much tougher remediation approaches like thermal vaporization, incineration, solvent and other soil washing techniques, all of which damage the biological components of the soil and hence change its physical and chemical features along with creating a comparatively nonviable waste. It is one of the most significant ecological clean up technologies for the remediation of contaminated soil also known as green technology or the green liver of the earth. An additional benefit of phytoremediation is the production of a recyclable metal rich

plant residue (Liu et al. 2000). Also, biomass generated through phytoremediation procedures can be economically valorized in the form of bioenergy. If soil phytoremediation occurs through oil crops, the biodiesel production from the resulting plant oil could be a viable option to generate bioenergy (Van Ginneken et al. 2007). On a larger scale, the stored potential energy can be used to generate thermal energy.

4.1.5 Limitations of Phytoremediation

Phytoremediation does have some limitations. It is time consuming since it may take several growing seasons to clean up a site. The polluted sites that pose severe risk may not be recommended to clean up through a phytoremediation approach. Phytoremediation technology takes time to clean the specific environment. This technology can be applied to those sites where there is no need for an urgent response and where human exposure is limited to that site. Numerous other factors may be a barrier to the path of phytoremediation e.g soil, root length, growth period of plants, environmental factors, magnitude of contaminants, soil and contaminant chemistry (Salido et al. 2003). The first and foremost barrier to phytoremediation is contact of roots to contaminants that must occur in two ways: either roots are deeper to be in contact or contaminants must be in vicinity of the roots (EPA 2000). The second important barrier for efficient phytoremediation is root age since this affects the process. Generally, roots of younger plants with active physiology have the ability to remediate at a faster rate compared to roots of older plants. Phytoremediation is also influenced by the magnitude of contaminants. Elevated levels of heavy metals are toxic to plants and causes inhibition of growth. The plants that accumulate the toxic metals at high levels are called as hyper accumulator plants. Among all above mentioned factors environmental factors also play an important role. The plants used for phytoremediation technology must be harvested and immediately disposed of to avoid further spread of contamination but still there are chances for toxic elements to become part of the food chain via animals and insects.

4.1.6 Plants physiological response to pollution

Various plant species respond to levels of pollution through the production of compatible osmolytes (i.e., glycine betaine, glycerol, sorbitol and proline, etc.) which in turn prevent plants from death and enable them to tolerate polluted, stressed environments (Ahmad et al. 2020; Patel and Parida 2020). Osmolyte molecules are

generally soluble in water at certain physiological pH, which usually accumulates in cytosols of the cells (Trovato et al. 2008). They protect plant species from different environmental stresses via regulating the cellular osmotic pressures, helping in detoxification of Reactive Oxygen Species (ROS) and through stabilization of protein and membrane protection reliability (Ghosh et al. 2020; Hayat et al. 2012; Pellegrini et al. 2019). Proline plays a significant role during different abiotic and biotic environmental stresses, drought, pathogen attack, salinity, nutrient deficiency, heavy metal accumulation, pollution and temperature fluctuation etc. (Gupta et al. 2020; Rehman et al. 2014; Rossi et al. 2020; Sreedevi et al. 2013).

Proline is a type of proteinogenic amino acid with an alpha-amino group, necessary for primary metabolism. Various types of plant species accumulate proline amino acids under certain types of environmental stresses including lithospheric pollution. The amount of proline level in plant cells is governed by transport between a cell‘s biosynthetic activities and catabolism (Szabados and Savoure 2010). Biosynthesis occurs via the glutamate or ornithine pathways. Glutamate encompasses proline synthesis from glutamic acid through Pyrroline-5-Carboxylate in the chloroplast or cytoplasm. It is an important biochemical pathway in osmotic stress, nitrogen deficiency and stressful physiological condition (Verslues and Sharma 2010). The P5C causes production of ROS, the initiation of apoptosis and harmful cellular constituents (Van Breusegem and Dat 2006). Excessive amounts of ROS cause programmed cell death (Patel and Parida 2020; Verslues and Sharma 2010). Therefore, plant species degrade it as soon as possible when the stress is relieved. ROS causes a reduction in chlorophyll content, membrane fluidity and lipid peroxidation (Verma and Mishra, 2005). In order to counteract ROS, plants have a variety of enzymatic and non-enzymatic detoxification processes (Sairam and Tyagi 2004). The lipid peroxidation is measured for oxidative damage in the form of malondialdehyde (Del Rio et al. 2005; Siddiqui et al. 2020).

Determination of the physiological characters, for example chlorophyll concentration is one of the approaches to measure the influence of environmental pollution on plant species. It is a tool that can detect whether their environmental pollution is interfering with photosynthesis, based on the loss of chlorophyll as a negative consequence of environmental pollution/stress. It has always been deliberated as one of the adaptive characters in growth of plant species growing in environmentally polluted or

physiologically stressed habitats. Rhizosphere has special interest in terms of phytoremediation. Microbial populations are usually higher in the rhizosphere than in the root free zone. Microorganisms living in the rhizosphere are directly associated with plant species. They contribute to mobilize PTE ions and hence increase the bioavailability fraction.

4.1.7 Mycoremediation

The use of fungi for the removal of waste or pollutants from the environment is termed mycoremediation. It is an economical and environmentally friendly approach to combat the ever-increasing challenge of environmental pollution. Mycoremediation is the safest means of contaminant remediation in terms of human health (Leonardi et al. 2007). As most of the contaminants are degraded by fungi rather than extracted, this minimizes the risk of pollutant transfer into the food chain (Haritash and Kaushik 2009). According to (Adenipekun and Lawal 2011), it is a distinctive method among other biological approaches like bacterial remediation, as there is no constraint for the preconditioning to a particular contamination. However, the proficiency of fungi remediation is influenced by several factors including sunlight, temperature, nutrients, oxygen level and moisture contents (Bhattacharya et al. 2012). The processes of mycoremediation are optimal at 25-30 °C temperature (Fletcher 2019). Other factors affecting the mycoremediation processes included are genetic and environmental, including pH, type of substrate, enzyme, ecology, fungi biomass content, mobilizing agents, life cycle of fungal agents, soil chemistry, and age of the mycelium (Amjad et al. 2017). Fungi have the broader ecological and biochemical capability to degrade environmental organic pollutants and hence reduce the risk associated with metalloids, metals and radionuclides (Harms et al. 2011). They are ideal species for the remediation of different types of pollutants due to their vigorous growth, immense hyphal network, high surface to volume ratio, production of extracellular enzymes, adaptability to changing pH and temperature (Koul et al. 2021). The fungal cell wall possesses polysaccharides and proteins containing hydroxyl, phosphate, amino, carboxyl and sulphate groups for binding with heavy metals/ metal(loid) ions/ pollutants (Maheswari and Murugesan, 2009). Fungi are tolerant to various heavy metals due to phytochelatin or metallothionein protein which can bind and deactivate toxic elements, or they may store potential toxic elements in the vacuoles (Anahid et al., 2011). They can adapt to extreme environmental conditions and are even able to

grow and to colonize soils affected by metal exploitation, such as mine soils, waste-rock dumps, and tailing deposits, which are characterized by extreme edaphic, physical, and chemical conditions.

In a nutshell, fungi can be regarded as pioneer organisms that can help to remediate, clean and prepare substances for eventual natural ecosystem colonization (Garcia et al., 2005). The use of native wild fungi for the accumulation of heavy metals from the polluted environment may represent an innovative, potentially cheap and sustainable remediation approach to rehabilitate the natural ecosystem.

4.1.8 Justification

Environmental pollution directly or indirectly affects more or less all organisms including plant species and microorganisms (Lin et al. 2010). However, the tolerant/indicator plant species and micro fungi develop strong defense mechanisms to survive in such unfavorable habitats. They develop phytoremediation or mycoremediation strategies and physiological changes to cope with the situation (Bi et al. 2020; Ceschin et al. 2019). This chapter determines the phytoremediation ability of the naturally occurring indicator plant species (discussed in the previous chapter) and their physiological responses in terms of proline accumulation and reduction in chlorophyll contents in the vicinities of the marble waste polluted ecosystem along with the bioremediation ability of micro fungi (isolated from MWPE) for potential toxic elements remediation. This study can be reproduced in other kinds of polluted systems as well. We are also of the opinion that naturally grown plants can be easily developed, propagated and forested if found beneficial in ecological and physiological terms. While the identified micro fungi can be grown in the bioreactor for the large scale removing of contaminants. Government and non-government organizations can also get guidelines for management of wastelands while devising policies and bylaws such as broader scale plantation drives and reforestation in the industrial zones.

4.2 Materials and Methods

As discussed in the previous chapter, the indicator plant species were identified based on indicator species analysis and confirmed through canonical correspondence analysis and structural equation modeling. These indicators were collected in replicates from near to vicinity (high polluted zone) and at a distance of 100 m interval (less polluted zone) from the Marble waste polluted ecosystem for the assessment of their phytoremediation ability and physiological response to marble pollution (Ahmad et al. 2021). Prior to the analysis, plant roots and shoots were carefully separated and washed with distilled water to remove any surface marble waste, soil or any other deposits. After that, the plant specimens were dried in the Oven (DSO-300D) and ground to a fine powder.

4.2.1 Quantification of Potential Toxic Elements (PTEs)/Heavy Metals

Chromium (Cr), nickel (Ni), copper (Cu), manganese (Mn), zinc (Zn), iron (Fe), cobalt (Co), cadmium (Cd), calcium (Ca), magnesium (Mg) and sodium (Na) heavy metals within the plant tissues were quantified using Atomic Absorption Spectrophotometry (Ahmad et al. 2019). One gram of sieved and dried marble waste, each indicator plant root and shoot samples were taken in a 250 mL conical flask. Ten mL of Per-chloric ($HCLO_4$) and Nitric acid (HNO_3) solution in 1:3 was added and placed for 24 hours. Then samples were digested by placing on a hot plate at an initial temperature of 150 °C for 1 hour and a final 235 °C till the red fumes of nitric acid disappear and white fumes become appear. The solution was filtered after cooling through filtered paper (Whatman No. 42) and 40 mL distilled water was added to raise its volume (Fig. 4.2-3). The blank reagents were also prepared. The atomic absorption spectrophotometer VARIAN, AA240FS, was used for the quantification of heavy metals (Fig. 4.4-5). The final metal concentrations were obtained using below formula:

$$Heavy\ metal\ concentration(mg/kg) = AA\ reading - Blank\ reagents/sample\ weight\ (kg) \times volume\ raised \times df$$

Where, AA= atomic absorption reading, df= dilution factor.

Fig. 4.2 Samples preparation for potential toxic elements/ heavy metals quantification.

Fig. 4.3 Samples filtration after its digestion on hotplate.

Fig. 4.4 Quantification of PTEs using atomic absorption spectrophotometer.

Fig. 4.5 A picture with atomic absorption spectrophotometer VARIAN, AA240FS after backbreaking work.

4.2.2 Phytoremediation and plant selection

Some of the selected indicator plants were examined for their phytoremediation ability (Table 4.1). The standard accumulation, transfer and concentration quotients were measured through Bioaccumulation Coefficient (BAC), Translocation Factor (TF) and Biological Concentration Factor (BCF) using the below equations/formulae (Malik et al. 2010). All the selected plants were tested for their phytoremediation capabilities.

$$BAC = Metal\ recorded\ in\ Shoot / Metal\ recorded\ in\ Soil \quad \text{(i)}$$

$$TF = Metal\ recorded\ in\ Shoot / Metal\ recorded\ in\ Root \quad \text{(ii)}$$

$$BCF = Metal\ recorded\ in\ Root / Metal\ recorded\ in\ Soil \quad \text{(iii)}$$

Table 4.1 The indicator species evaluated for their phytoremediation ability.

S. No.	Botanical Names	Family	Habit	IVI
1	*Adiantum capillus-veneris*	Pteridaceae	Herb	739.55
2	*Ailanthus altissima*	Simaroubaceae	Tree	1647.28
3	*Albizia lebbeck*	Leguminosae	Tree	784.25
4	*Calotropis procera*	Apocynaceae	Shrub	1799.81
5	*Cynodon dactylon*	Poaceae	Herb	2982.38
6	*Datura innoxia*	Solanaceae	Shrub	1050.27
7	*Debregeasia salicifolia*	Urticaceae	Shrub	766.67
8	*Desmostachya bipinnata*	Poaceae	Herb	596.84
9	*Dodonaea viscosa*	Sapindaceae	Shrub	620.56
10	*Erigeron bonariensis*	Compositae	Herb	993.83
11	*Ficus carica*	Moraceae	Tree	2441.65
12	*Morus alba*	Moraceae	Tree	1699.0
13	*Morus nigra*	Moraceae	Tree	1655.67
14	*Parthenium hysterophorus*	Compositae	Herb	1122.48
15	*Persicaria glabra*	Polygonaceae	Herb	752.36
16	*Ricinus communis*	Euphorbiaceae	Shrub	797.03
17	*Setaria viridis*	Poaceae	Herb	1090.93
18	*Tamarix aphylla*	Tamaricaceae	Tree	528.97
19	*Withania somnifera*	Solanaceae	Shrub	781.20

4.2.3 Determination of Photosynthetic pigments

The photosynthetic pigments i.e., chlorophyll-a, b and total carotenoids of each indicator plant species were measured both in high and less polluted zone of marble waste. Fresh leaf sample (0.5 g) was ground in 4 ml Dimethyl sulfoxide (DMSO) reagent using mortar and pestle. The extract was filtered by Whatman No 1 filter paper to get transparent (clear) supernatant (Fig. 4.6). Falcons containing samples were heated at 65 °C for 4 hours. Absorbance was measured at Optical Density 663 nm and 645 nm by spectrophotometer instrument (Hiscox and Israelstam 1979). The following standard formula were used for the determination of Chlorophyll-a, b and carotenoids (Arnon 1949).

$$\text{Chlorophyll a } \left(\frac{\text{mg}}{\text{g}}\right) = [1.07\ (\text{optical density } 663) - 0.09(\text{optical density } 645)] = Z$$

$$\text{Chlorophyll b } \left(\frac{\text{mg}}{\text{g}}\right) = [1.77\ (\text{optical density } 645) - 0.280(\text{optical density } 663)]$$

$$\text{Carotenoids content } \left(\frac{\text{mg}}{\text{g}}\right) = (Z \times 4)$$

Fig. 4.6 Samples prepared for photosynthetic determination.

4.2.4 Proline Quantification

The Proline concentration was determined in all the selected plant species collected near to vicinity and 100 m distance from MWPE using the method of (Bates et al. 1973a). Fresh plant leaf sample of 0.5g along with 3% (w/v) Sulphosalicylic was taken, ground and filtered using Whatman No. 1 filter paper to get transparent (clear) supernatant. The obtained 2 ml filtrated sample was mixed with 2 ml each of Ninhydrin, Glacial acetic acid and heated in a water bath at 100 °C for 1hr. The samples were kept at room temperature initially and then below 0 °C in the refrigerator to avoid any chemical reaction. Four ml of toluene was added to the sample and kept for 50 minutes. The upper organic layer of toluene was transferred into falcon tube (Fig. 4.7). Finally, the absorbance was measured at 520 nm optical density against toluene as blank using spectrophotometer. The amount of proline accumulation in each plant sample was calculated from the standard available curve of proline using the equation below.

$$\text{Proline } \left(\frac{\mu g}{g}\right) = \frac{\text{K value } \times \text{dilut. factor } \times \text{ optical density 520 nm}}{\text{weight of Sample}}$$

Where, K = 17.52, dilution factor = 2 and weight of sample = 0.5g

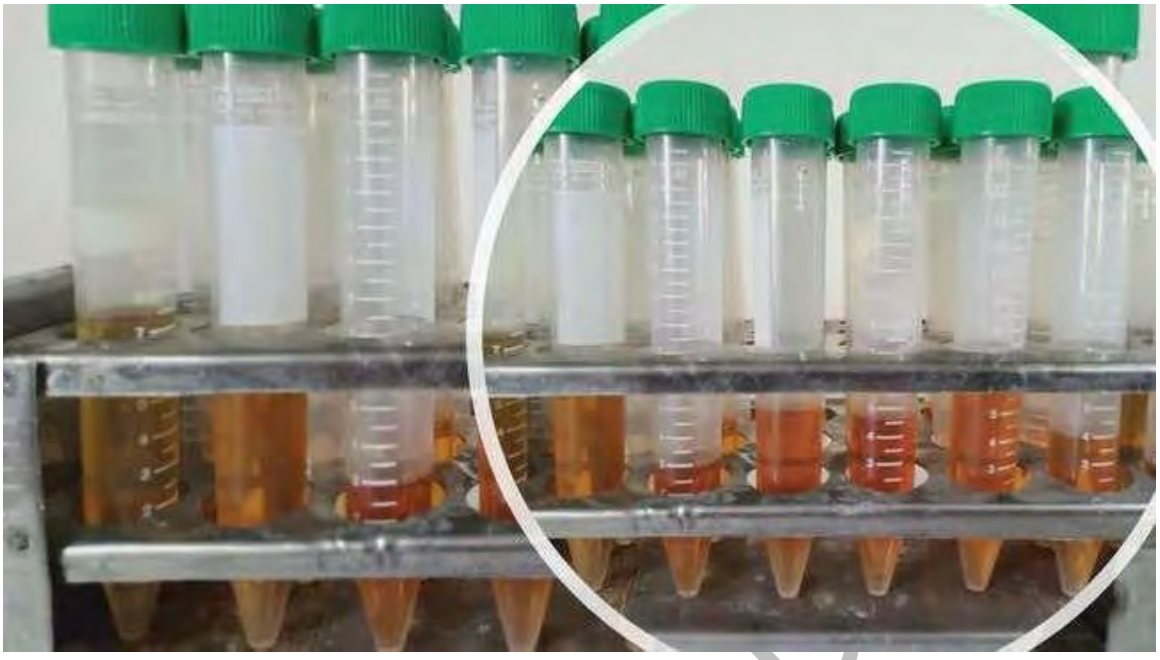


Fig. 4.7 Proline produced by plant species as response to marble waste pollution. Pink color show that plant come in stress and produce high level of Proline, while light yellow color indicate that plant cope up with pollution stress and produce very low level of Proline

4.2.5 Data Analyses

4.2.5.1 Mixed Effect Model

The study involved replicated measurement of the plant species within and across polluted zone of the marble waste polluted ecosystem. Such a design introduced the strong likelihood that the measurement within the same site would be influenced by phytoremediation. It was necessary to specify a model that attempts to explain the difference in phytoremediation capacity among proline, chlorophyll-a and chlorophyll-b contents in order to avoid systematic variation in residuals. The site and species were selected randomly rather than being of primary interest. The random component therefore included the categorical variable sites i.e., polluted and less polluted zone. The fixed components of the model were proline, chlorophyll-a, chlorophyll-b and phytoremediation. The final model fitted using the *nlme* package in R is expressed as:

Phytoremediation [pl] = $\alpha + \beta 1z[pl] + \beta 2\ p[pl] + \beta 3C\text{-}a[pl] + \beta 4C\text{-}b[pl] + a[p] + \varepsilon[pl]$

Here the response variable is phytoremediation, [pl] denotes polluted and less polluted region, respectively. [pl] zone with denoted by categorical variable 1 and 2 respectively. a[p] is random effect intercept and allowed variation between the sites. Whereas z[pl] is the categorical variable which represent the site of the study. the term p[pl], C-a[pl] and C-b[pl] are the explanatory variable of our study and Ɛ[pl] is the error term.

4.2.5.2 Regression /Bi-variate Analysis

The impact of phytoremediation on proline, chlorophyll-a, and chlorophyll-b was determined through the ordinary least square method. Phytoremediation was taken as a dependent variable and proline, chlorophyll-a, and chlorophyll-b as explanatory variables. R-software was used to determine regression coefficient of the following empirical model:

$$\text{Phytoremediation}_{[i]} = \alpha + \beta 1_{\text{proline}[i]} + \beta 2_{\text{chlorophyll-a}[i]} + \beta 3_{\text{chlorophyll-b}[i]} + \mu_i$$

Phytoremediation [i] is the dependent variable which indicates the remediation ability of plants, where [i] represent different polluted zones, β1, β2 and β3 were the coefficients of proline, chlorophyll-a and chlorophyll-b, respectively, μi is the disturbance/error term of the model.

4.2.6 Bioremediation / Myco-remediation

The micro fungi isolated and identified from the MWPE were further assessed for their bioremediation ability. Mineral Salts Medium (MSM) was used which contained 0.3g KNO_3, 0.01g of $FeSO_4.7H_2O$, 0.01g $MgSO_4$, and 3 g of glucose per liter. Ca, Cd, Co, Cu, Mg, Fe, Hg, Ni and $NaCl_2.6H_2O$ were used as the source of metals separately. The initial pH was adjusted to 7.0 with help of NaOH and HCl. All the media were sterilized via autoclaving at 121 °C for 15 minutes. After that the selected micro fungi i.e., *Aspergillus sydowii*, *Aspergillus brasilensi, Curvularia aeria* and *Alternaria alternata* were grown aerobically on a rotary shaker at 120 rpm at 30 °C. UV-vis spectrophotometer was used to calculate the absorption intensity at 200-600 nm at 7-, 14-, 21- and 28-days interval (Fig. 4.8).

Fig. 4.8 Samples preparation and processing for myco-remediation.

4.3 Results

A total of 85 plant species (out of 220) were recorded as indicators of the subtropical Marble Wastewater Ecosystem, Khyber Pakhtunkhwa, Pakistan. Out of which, 19 species i.e., *Adiantum capillus-veneris, Ailanthus altissima, Albizia lebbeck, Calotropis procera, Cynodon dactylon, Datura innoxia, Debregeasia salicifolia, Desmostachya bipinnata, Dodonaea viscosa, Erigeron bonariensis, Ficus carica, Morus alba, Morus nigra, Parthenium hysterophorus, Persicaria glabra, Ricinus communis, Setaria viridis, Tamarix aphylla* and *Withania somnifera* were further evaluated for Potential Toxic Elements (PTEs)/heavy metals remediation based on their availability, indicator and importance value index (IVI) data (already discussed in previous chapter). The detailed summary statistics are given in Table 4.2.

Table 4.2 Detailed summary statistics.

	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Na	Ni	Zn	Overall
BCF												
Mean (SD)	0.785 (0.382)	0.926 (0.0988)	0.871 (0.204)	1.05 (0.0979)	1.05 (0.221)	0.865 (0.202)	0.880 (0.291)	0.700 (0.337)	0.950 (0.194)	0.872 (0.196)	0.946 (0.144)	0.899 (0.249)
Median [Min, Max]	0.808 [0.0500, 2.17]	0.944 [0.683, 1.22]	0.895 [0.278, 1.33]	1.02 [0.842, 1.21]	1.02 [0.592, 1.45]	0.932 [0.168, 1.09]	0.937 [0.121, 1.30]	0.853 [0.0846, 1.04]	0.959 [0.407, 1.55]	0.916 [0.241, 1.44]	0.935 [0.712, 1.60]	0.943 [0.0500, 2.17]
TF												
Mean (SD)	0.824 (0.214)	0.934 (0.0642)	0.808 (0.184)	0.900 (0.0769)	0.914 (0.0777)	0.933 (0.0784)	0.854 (0.202)	0.859 (0.220)	0.940 (0.199)	0.883 (0.201)	0.914 (0.0785)	0.888 (0.163)
Median [Min, Max]	0.848 [0.0210, 1.38]	0.947 [0.722, 1.08]	0.824 [0.0645, 1.07]	0.913 [0.734, 1.13]	0.939 [0.701, 1.05]	0.971 [0.694, 1.01]	0.926 [0.139, 1.17]	0.923 [0.193, 1.37]	0.938 [0.587, 1.82]	0.940 [0.0888, 1.20]	0.941 [0.678, 1.04]	0.923 [0.0210, 1.82]
BAC												
Mean (SD)	0.637 (0.292)	0.866 (0.122)	0.711 (0.206)	0.944 (0.120)	0.959 (0.227)	0.817 (0.219)	0.775 (0.321)	0.628 (0.356)	0.892 (0.258)	0.767 (0.248)	0.861 (0.126)	0.805 (0.260)
Median [Min, Max]	0.693 [0.00306, 1.06]	0.878 [0.588, 1.11]	0.748 [0.0180, 1.01]	0.909 [0.738, 1.27]	0.974 [0.527, 1.30]	0.893 [0.132, 1.03]	0.807 [0.0567, 1.49]	0.772 [0.0808, 1.04]	0.878 [0.388, 1.81]	0.843 [0.128, 1.17]	0.870 [0.599, 1.30]	0.855 [0.00306, 1.81]

Phytoremediation												
Mean (SD)	0.749 (0.229)	0.909 (0.0850)	0.796 (0.168)	0.964 (0.0806)	0.973 (0.158)	0.872 (0.159)	0.836 (0.245)	0.729 (0.271)	0.928 (0.177)	0.841 (0.170)	0.907 (0.0901)	0.864 (0.192)
Median [Min, Max]	0.789 [0.0566, 1.24]	0.918 [0.708, 1.08]	0.831 [0.120, 1.01]	0.942 [0.826, 1.17]	0.985 [0.670, 1.22]	0.928 [0.363, 1.02]	0.868 [0.198, 1.31]	0.846 [0.271, 1.03]	0.918 [0.584, 1.54]	0.893 [0.349, 1.11]	0.912 [0.720, 1.24]	0.902 [0.0566, 1.54]
Proline												
Mean (SD)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.739)	-0.105 (0.730)
Median [Min, Max]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]	-0.381 [-1.01, 1.79]
Chlorophyll-a												
Mean (SD)	0.564 (0.310)	0.564 (0.310)	0.564 (0.310)	0.564 (0.310)	0.564 (0.310)	0.564 (0.310)	0.564 (0.310)	0.564 (0.310)	0.564 (0.310)	0.564 (0.310)	0.564 (0.310)	0.564 (0.306)
Median [Min, Max]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]	0.497 [0.128, 1.78]
Chlorophyll-b												

Mean (SD)	0.279 (0.229)	0.279 (0.229)	0.279 (0.229)	0.279 (0.229)	0.279 (0.229)	0.279 (0.229)	0.279 (0.229)	0.279 (0.229)	0.279 (0.229)	0.279 (0.229)	0.279 (0.229)	0.279 (0.226)
Median [Min, Max]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]	0.209 [0.0626, 1.06]
Carotenoid												
Mean (SD)	2.25 (1.24)	2.25 (1.24)	2.25 (1.24)	2.25 (1.24)	2.25 (1.24)	2.25 (1.24)	2.25 (1.24)	2.25 (1.24)	2.25 (1.24)	2.25 (1.24)	2.25 (1.24)	2.25 (1.22)
Median [Min, Max]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]	1.99 [0.511, 7.11]

4.3.1 Phytoremediation ability of identified indicators

The detailed phytoremediation ability of selected indicator plants are as follows:

4.3.1.1 Chromium (Cr)

The recorded chromium concentration varied from 11.04-15.88 in root and 10.28-16.62 mg/kg in shoot of all the selected plant species in the highly polluted zone (HPZ) of MWPE (Table 4.3). Several researchers used BAC, BCF and TF for the evaluation of phytoremediation ability in plant species. The plants with BCF and TF values > 1 are considered as phytoextractors. While BCF>1 and TF<1 had been used to evaluate plant potential for phytostabilization. Among the collected plant species *Ailanthus altissima*, *Datura innoxia, Parthenium hysterophorus* and *Ricinus communis* were identified as phytoextractors of Cr metal based on BCF and TF values in HPZ. While, *Albizia lebbeck, Calotropis procera, Cynodon dactylon, Debregeasia salicifolia, Desmostachya bipinnata, Dodonaea viscosa, Erigeron bonariensis, Persicaria glabra, Setaria viridis, Tamarix aphylla, Adiantum capillus-veneris* and *Withania somnifera* were recorded as phytostabilizers for chromium metal in HPZ of MWPE (Table 4.4).

At the same time, the chromium concentration varied between 8.04-9.84 mg/kg in shoot and 10.12-12.88 mg/kg in root of indicator species in less polluted zone (LPZ) of MWPE (Table 4.5). Based on BCF, TF and BAC values, *Debregeasia salicifolia*, *Desmostachya bipinnata* and *Withania somnifera* were the phytoextractor and *Albizia lebbeck*, *Dodonaea viscosa*, *Erigeron bonariensis*, *Persicaria glabra*, *Ricinus communis* and *Setaria viridis* were the phytostabilizers of potential toxic levels of chromium metal in the less polluted zone of the marble waste polluted ecosystem (Table 4.6).

4.3.1.2 Nickel (Ni)

The nickel concentration varied between 30.44-35.31 in root and 24.8-34.39 mg/kg in shoot of the studied plants of high polluted zone (Table 4.3). Based on BCF and TF values *Albizia lebbeck, Datura innoxia, Dodonaea viscosa, Erigeron bonariensis, Ficus carica, Parthenium hysterophorus, Ricinus communis, Setaria viridis* and *Withania somnifera* were the significant species for the phytoextraction of nickel heavy metal in highly polluted zone of MWPS. While *Morus alba, Morus nigra* and *Persicaria glabra*

were the best plant species for the phytostabilization of nickel pollution in HPZ (Table 4.4).

The nickel amount ranged from 2.64-15.76 in root and 1.40-12.80 mg/kg in shoot of indicator species in less polluted zone of MWPE (Table 4.5). The plant species *Parthenium hysterophorus*, *Ricinus communis* and *Withania somnifera* were determined as phytoextractors and *Persicaria glabra* as a phytostabilizer in the less marble waste polluted zone (Table 4.6).

4.3.1.3 Copper (Cu)

The copper concentration fluctuates in root and shoot between 31.94-54.46 and 30.47-48.98 mg/kg, respectively (Table 4.3). *Ailanthus altissima, Calotropis procera, Datura innoxia, Desmostachya bipinnata, Erigeron bonariensis, Morus alba, Morus nigra, Persicaria glabra, Ricinus communis, Tamarix aphylla* and *Withania somnifera* were the significant species for the phytoextraction of copper heavy metal in MWPS. The evaluated phytostabilizer plants for copper heavy metal in MWPS were *Adiantum capillus-veneris, Albizia lebbeck, Cynodon dactylon, Dodonaea viscosa, Ficus carica, Parthenium hysterophorus* and *Setaria viridis* (Table 4.4). At the same time, copper concentrations were recorded in the range 12.68-30.96 in root and 11.28-27.92 mg/kg in shoot of all the studied plants in LPZ of MWPE (Table 4.5). *Adiantum capillus-veneris*, *Ailanthus altissima*, *Calotropis procera*, *Morus alba* and *Morus nigra*, showed phytoextraction, while *Albizia lebbeck* and *Datura innoxia* demonstrated a phytostabilization ability against copper potential toxic element in LPZ (Table 4.6).

4.3.1.4 Manganese (Mn)

Manganese concentrations were between 11.99-42.95 in root and 16.44-43.02 mg/kg in shoot of the 19 studied plants (Table 4.3). Based on BCF and TF, *Ailanthus altissima, Cynodon dactylon, Desmostachya bipinnata, Dodonaea viscosa, Ficus carica, Persicaria glabra, Setaria viridis* and *Withania somnifera* were the significant phytoextractors of manganese in the subtropical of MWPS. While, *Calotropis procera, Datura innoxia* and *Morus alba* were recorded as phytostabilizers for manganese heavy metal in MWPS (Table 4.4). However, manganese concentration varied from 1.76-19.92 in root and 1.68-18.84 mg/kg in the shoots of indicator species in less polluted zone of MWPE (Table

4.5). Among the evaluated species *Ailanthus altissima* and *Persicaria glabra* showed a phytoextraction ability for manganese potential toxic element in LPZ (Table 4.6).

4.3.1.5 (Zn)

The zinc concentration in root and shoot material was between 40.74-91.68 and 40.27-74.42 mg/kg in high polluted zone of MWPE, respectively (Table 4.3). Among the studied plants *Albizia lebbeck, Parthenium hysterophorus, Persicaria glabra* and *Tamarix aphylla* were recorded as significant phytoextractors for zinc heavy metals in MWPS. At the same time, *Calotropis procera, Desmostachya bipinnata, Dodonaea viscosa, Erigeron bonariensis, Ficus carica, Morus nigra, Ricinus communis* and *Setaria viridis* showed significant phytostabilization ability for zinc heavy metal based on BCF and TF values in HPZ (Table 4.4). The zinc concentration was determined to be in the range 26.34 - 44.83 in root and 29.77 - 42.74 in shoot of plant indicators in the less polluted zone of MWPE (Table 4.5). *Dodonaea viscosa*, *Withania somnifera* were the phytostabilizers and *Morus nigra* was recorded as a phytoextractor of zinc heavy metal in the LPZ of MWPE (Table 4.6).

4.3.1.6 (Fe)

The iron concentration recorded in root material ranged between 86.36-102.74 and in shoot 78.28-97.5 mg/kg (Table 4.3). *Albizia lebbeck, Cynodon dactylon, Datura innoxia, Debregeasia salicifolia, Desmostachya bipinnata, Dodonaea viscosa, Erigeron bonariensis, Morus alba, Parthenium hysterophorus, Persicaria glabra, Ricinus communis, Setaria viridis* and *Withania somnifera* were recorded as phytoextractors of iron heavy metal in MWPS. *Ailanthus altissima* and *Calotropis procera* were recorded as phytostabilizers for iron heavy metal (Table 4.4). In the less polluted zone, iron varied from 9.56-70.24 in root and 33.52-69.68 in shoot of the studied indicators (Table 4.5). Among the studied plants none of the species showed a remediation ability for iron in the less polluted zone (Table 4.6).

4.3.1.7 Cobalt (Co)

The cobalt concentration fluctuates in root and shoot between 3.16-7.58 and 2.68-7.28 mg/kg in HPZ, respectively (Table 4.3). *Adiantum capillus-veneris* and *Morus nigra* were

identified as phytoextractors and *Cynodon dactylon, Datura innoxia, Ficus carica, Tamarix aphylla* and *Withania somnifera* were recorded as phytostabilizers of cobalt heavy metal in HPZ (Table 4.4). At the same time, cobalt varied from 1.24-5.92 in root and 0.08-4.25 mg/kg in shoot of the studied indicator species in the less polluted zone of MWPE (Table 4.5). Based on BCF, TF and BAC values, *Adiantum capillus-veneris, Desmostachya bipinnata, Erigeron bonariensis, Morus alba* and *Withania somnifera* were phytostabilizers of cobalt PTE in LPZ of MWPE (Table 4.6).

4.3.1.8 Cadmium (Cd)

The cadmium heavy metal concentration was revealed to be between 23.18-34.76 in root and 17.98-31.63 mg/kg in shoot of the 19 studied plants (Table 4.3). *Albizia lebbeck, Calotropis procera, Datura innoxia, Dodonaea viscosa, Morus alba, Morus nigra, Parthenium hysterophorus, Ricinus communis* and *Withania somnifera* were the significant phytoextracter species for cadmium non-biological heavy metal in the subtropical zone of MWPS. *Debregeasia salicifolia, Desmostachya bipinnata* and *Tamarix aphylla* were identified as accumulators for phytostabilization of cadmium metal (Table 4.4). In the less polluted zone, cadmium concentration ranged from 12.12 -68.64 in root and 10.44-18.21 mg/kg in shoot of indicator species (Table 4.5). *Dodonaea viscosa* was recorded as a hyperaccumulator of cadmium metal, while, *Calotropis procera* and *Withania somnifera* were reported as phytostabilizers of cadmium potential toxic element in LPZ of MWPE (Table 4.6).

4.3.1.9 Calcium

The calcium concentration was determined to be between 13.97 - 605.22 in root and 0.85 - 295.75 mg/kg in shoot of all the nineteen studied indicator species grown in the highly polluted ecosystem (Table 4.3). *Adiantum capillus-veneris, Ailanthus altissima, Cynodon dactylon, Debregeasia salicifolia, Erigeron bonariensis, Ficus carica, Morus alba, Morus nigra, Parthenium hysterophorus* and *Withania somnifera* were identified as contributing to the phytoremediation of calcium in the highly polluted marble waste ecosystem (Table 4.4). In contrast, the calcium concentration ranged between 37.08 - 172.20 in root and 36-164.04 mg/kg in shoot of selected indicators in the less polluted zone of MWPE (Table 4.5). *Desmostachya bipinnata* was identified as a phytoextractor,

while *Adiantum capillus-veneris*, *Erigeron bonariensis* and *Morus alba* were recorded as phytostabilizers of potential toxic levels of calcium in LPZ (Table 4.6).

4.3.1.10 Magnesium

In the highly polluted zone of MWPE, magnesium concentration was recorded between 59.61 - 248.76 in root and 11.42 - 284.22 mg/kg in shoot of the indicator species (Table 4.3). *Adiantum capillus-veneris* was recorded as a hyper accumulator, while *Ailanthus altissima, Albizia lebbeck, Calotropis procera, Cynodon dactylon, Debregeasia salicifolia, Desmostachya bipinnata, Erigeron bonariensis, Ficus carica, Morus alba, Morus nigra, Ricinus communis* and *Setaria viridis* were identified as phytostabilizers of toxic levels of magnesium in the highly polluted zone of MWPE (Table 4.4). Magnesium concentration was recorded between 16.72-135.92 in root and 7.80-133.58 mg/kg in shoot of indicators in the less polluted zone of MWPE (Table 4.5). Among all the studied plant species, none showed phytoremediation ability for magnesium metal in the less polluted zone of MWPE (Table 4.6).

4.3.1.11 Sodium

The recorded sodium element concentration varied from 14.37-54.75 mg/kg in root and 13.74-49.78 mg/kg in shoot of the indicator species in HPZ (Table 4.3). The plant species *Desmostachya bipinnata, Dodonaea viscosa* and *Erigeron bonariensis* were recorded as hyperaccumulators, while *Adiantum capillus-veneris, Albizia lebbeck, Calotropis procera, Cynodon dactylon, Datura innoxia, Debregeasia salicifolia, Ficus carica, Morus alba, Parthenium hysterophorus, Tamarix aphylla* and *Withania somnifera* were identified as phytostabilizers of sodium metal in the highly polluted zone of MWPE (Table 4.4). The sodium concentration fluctuates between 16.19-51.31 in root and 18.38-26.22 mg/kg in shoot of all the studied indicator species in the less polluted zone (Table 4.5). *Adiantum capillus-veneris*, *Erigeron bonariensis* were assessed as phytostabilizers, *Desmostachya bipinnata* as a hyper accumulator, and *Ricinus communis* and *Setaria viridis* as phytoextractors of sodium metal in LPZ (Table 4.6).

Table 4.3 The concentration of heavy metals recorded (mg/kg) in the root and shoot of plant species in the highly polluted zone of MWPE.

Plant Name	Part	Cr	Ni	Cu	Mn	Cd	Zn	Fe	Co	Ca	Mg	Na
	Root	13.06	32.21	54.46	33.84	26.78	50.35	89.36	6.96	297.20	242.20	37.34
Adiantum capillus-veneris	Shoot	11.06	31.61	48.98	31.44	24.22	49.14	89.02	6.67	234.48	284.22	36.66
	Root	13.12	32.11	43.70	42.02	23.58	51.19	102.7	5.92	283.76	216.63	32.50
Ailanthus altissima	Shoot	12.70	31.03	42.40	40.34	17.98	48.77	97.50	4.09	222.52	212.86	24.41
	Root	15.88	34.51	51.48	31.82	29.04	58.87	95.24	4.72	213.80	211.62	35.41
Albizia lebbeck	Shoot	14.81	33.64	45.67	31.44	31.37	56.58	93.81	3.22	138.80	197.56	34.96
	Root	13.99	31.10	45.36	40.59	27.91	54.52	92.53	5.88	13.97	235.36	39.43
Calotropis procera	Shoot	13.01	28.38	43.17	33.29	26.95	45.74	78.28	4.60	17.12	225.77	35.31
	Root	13.50	32.79	36.52	40.50	25.72	53.58	94.44	6.96	357.40	235.62	39.12
Cynodon dactylon	Shoot	11.98	31.88	34.52	39.07	23.72	40.27	92.79	5.18	289.30	192.79	31.85
	Root	15.76	34.83	45.56	40.04	28.04	53.16	97.14	7.58	143.98	173.83	46.72
Datura innoxia	Shoot	16.62	33.80	43.61	36.47	27.93	48.69	94.80	6.25	123.32	145.25	37.95
	Root	14.18	31.65	31.94	38.44	27.26	48.00	96.36	3.16	325.02	248.76	39.25
Debregeasia salicifolia	Shoot	13.14	29.42	30.47	35.57	25.52	43.36	94.50	2.68	295.75	234.13	36.80
	Root	14.67	30.45	46.76	42.95	27.79	63.60	94.19	5.28	40.71	247.38	35.57
Desmostachya bipinnata	Shoot	13.45	24.81	45.65	42.71	26.39	50.50	93.01	4.93	0.85	189.01	45.39
	Root	15.70	33.35	39.16	40.34	28.02	91.69	95.02	4.78	222.98	135.30	39.04
Dodonaea viscosa	Shoot	14.58	34.39	35.36	39.91	27.44	74.42	94.50	3.96	143.42	116.41	39.00
	Root	13.22	33.25	35.96	36.28	25.50	54.82	95.46	5.64	290.22	195.06	37.64
Erigeron bonariensis	Shoot	11.48	32.83	34.94	33.40	23.36	43.72	94.28	5.88	244.68	193.85	49.78
	Root	12.32	33.47	36.87	42.74	24.44	56.01	88.20	7.40	276.56	233.58	34.06
Ficus carica	Shoot	10.32	32.62	34.26	41.46	20.41	51.01	86.35	6.65	215.56	198.29	33.45
Morus alba	Root	12.72	35.31	37.58	40.62	27.84	47.01	94.70	3.92	288.72	197.21	42.34

	Shoot	12.24	33.27	36.76	37.30	26.84	42.96	93.68	3.36	217.56	193.78	31.87
	Root	11.04	33.59	37.98	38.96	27.82	56.18	87.30	7.52	605.22	157.80	30.12
Morus nigra	Shoot	10.28	31.77	36.92	36.46	27.76	51.57	86.66	7.28	294.14	133.34	28.52
	Root	14.92	33.30	53.99	38.55	28.31	57.67	95.16	6.40	38.91	82.45	33.49
Parthenium hysterophorus	Shoot	14.76	32.61	46.45	38.03	27.05	56.84	93.76	5.80	53.60	11.42	35.64
	Root	14.84	31.43	48.52	42.74	23.24	58.12	91.88	6.60	266.72	59.61	33.79
Persicaria glabra	Shoot	13.84	31.07	47.52	43.02	22.64	56.86	90.96	7.08	239.52	55.58	19.83
	Root	12.92	34.78	45.03	11.99	28.71	57.29	94.87	5.00	134.80	220.73	22.73
Ricinus communis	Shoot	14.60	33.94	47.20	16.45	27.85	52.01	94.39	5.23	92.95	204.47	16.57
	Root	14.41	33.41	49.03	42.35	23.19	63.29	95.51	5.65	119.20	217.75	14.37
Setaria viridis	Shoot	12.31	33.29	43.81	42.10	19.91	51.74	92.79	5.33	93.37	124.35	13.74
	Root	13.96	31.11	43.16	38.50	34.76	62.12	86.36	6.88	161.44	178.94	38.80
Tamarix aphylla	Shoot	12.69	28.94	41.49	32.60	31.63	59.58	87.19	5.58	127.43	175.40	35.27
	Root	15.20	35.18	46.32	42.17	28.67	40.75	95.08	7.45	277.92	121.44	54.75
Withania somnifera	Shoot	14.32	33.45	45.57	42.61	29.19	42.28	94.28	6.56	235.92	117.07	42.61

Table 4.4 Biological Concentration Factor (BCF), Translocation Factor (TF) and Bioaccumulation Coefficient (BAC) for different heavy metals of indicator species in the highly polluted zone of MWPE.

Plant Name	Factor	Cr	Ni	Cu	Mn	Cd	Zn	Fe	Co	Ca	Mg	Na
Adiantum capillus-veneris	BCF	1.00	0.94	1.44	0.82	0.94	0.88	0.94	0.97	1.06	1.27	1.06
	TF	0.85	0.98	0.90	0.93	0.90	0.98	1.00	0.96	0.79	1.17	0.98
	BAC	0.84	0.92	1.30	0.76	0.85	0.86	0.94	0.93	0.84	1.49	1.04
Ailanthus altissima	BCF	1.00	0.93	1.16	1.01	0.83	0.90	1.09	0.82	1.02	1.13	0.92
	TF	0.97	0.97	0.97	0.96	0.76	0.95	0.95	0.69	0.78	0.98	0.75
	BAC	0.97	0.90	1.12	0.97	0.63	0.85	1.03	0.57	0.80	1.11	0.69
Albizia lebbeck	BCF	1.21	1.00	1.37	0.77	1.02	1.03	1.01	0.66	0.77	1.11	1.00
	TF	0.93	0.97	0.89	0.99	1.08	0.96	0.98	0.68	0.65	0.93	0.99
	BAC	1.13	0.98	1.21	0.76	1.10	0.99	0.99	0.45	0.50	1.03	0.99
Calotropis procera	BCF	1.07	0.90	1.20	0.98	0.98	0.95	0.98	0.82	0.05	1.23	1.12
	TF	0.93	0.91	0.95	0.82	0.97	0.84	0.85	0.78	1.23	0.96	0.90
	BAC	0.99	0.82	1.15	0.80	0.94	0.80	0.83	0.64	0.06	1.18	1.00
Cynodon dactylon	BCF	1.03	0.95	0.97	0.98	0.90	0.94	1.00	0.97	1.28	1.23	1.11
	TF	0.89	0.97	0.95	0.96	0.92	0.75	0.98	0.74	0.81	0.82	0.81
	BAC	0.91	0.93	0.92	0.94	0.83	0.70	0.98	0.72	1.04	1.01	0.90
Datura innoxia	BCF	1.20	1.01	1.21	0.97	0.98	0.93	1.03	1.05	0.52	0.91	1.32
	TF	1.05	0.97	0.96	0.91	1.00	0.92	0.98	0.82	0.86	0.84	0.81
	BAC	1.27	0.98	1.16	0.88	0.98	0.85	1.00	0.87	0.44	0.76	1.07
Debregeasia salicifolia	BCF	1.08	0.92	0.85	0.93	0.95	0.84	1.02	0.44	1.16	1.30	1.11
	TF	0.93	0.93	0.95	0.93	0.94	0.90	0.98	0.85	0.91	0.94	0.94
	BAC	1.00	0.85	0.81	0.86	0.89	0.76	1.00	0.37	1.06	1.22	1.04
Desmostachya bipinnata	BCF	1.12	0.88	1.24	1.04	0.97	1.11	1.00	0.73	0.15	1.29	1.01
	TF	0.92	0.81	0.98	0.99	0.95	0.79	0.99	0.93	0.02	0.76	1.28
	BAC	1.03	0.72	1.21	1.03	0.92	0.88	0.98	0.69	0.00	0.99	1.28
Dodonaea viscosa	BCF	1.20	0.97	1.04	0.97	0.98	1.60	1.00	0.66	0.80	0.71	1.10
	TF	0.93	1.03	0.90	0.99	0.98	0.81	0.99	0.83	0.64	0.86	1.00
	BAC	1.11	1.00	0.94	0.96	0.96	1.30	1.00	0.55	0.51	0.61	1.10
Erigeron bonariensis	BCF	1.01	0.97	0.95	0.88	0.89	0.96	1.01	0.78	1.04	1.02	1.06
	TF	0.87	0.99	0.97	0.92	0.92	0.80	0.99	1.04	0.84	0.99	1.32
	BAC	0.88	0.95	0.93	0.81	0.82	0.77	1.00	0.82	0.88	1.01	1.41
Ficus carica	BCF	0.94	0.97	0.98	1.03	0.86	0.98	0.93	1.03	0.99	1.22	0.96
	TF	0.84	0.97	0.93	0.97	0.84	0.91	0.98	0.90	0.78	0.85	0.98
	BAC	0.79	0.95	0.91	1.00	0.71	0.89	0.91	0.92	0.77	1.04	0.95
Morus alba	BCF	0.97	1.03	1.00	0.98	0.98	0.82	1.00	0.54	1.03	1.03	1.20
	TF	0.96	0.94	0.98	0.92	0.96	0.91	0.99	0.86	0.75	0.98	0.75
	BAC	0.93	0.97	0.98	0.90	0.94	0.75	0.99	0.47	0.78	1.01	0.90

Morus nigra	BCF	0.84	0.98	1.01	0.94	0.97	0.98	0.92	1.05	2.17	0.83	0.85
	TF	0.93	0.95	0.97	0.94	1.00	0.92	0.99	0.97	0.49	0.84	0.95
	BAC	0.78	0.92	0.98	0.88	0.97	0.90	0.92	1.01	1.05	0.70	0.81
Parthenium hysterophorus	BCF	1.14	0.97	1.43	0.93	0.99	1.01	1.01	0.89	0.14	0.43	0.95
	TF	0.99	0.98	0.86	0.99	0.96	0.99	0.99	0.91	1.38	0.14	1.06
	BAC	1.13	0.95	1.23	0.92	0.95	0.99	0.99	0.81	0.19	0.06	1.01
Persicaria glabra	BCF	1.13	0.91	1.29	1.03	0.81	1.02	0.97	0.92	0.96	0.31	0.96
	TF	0.93	0.99	0.98	1.01	0.97	0.98	0.99	1.07	0.90	0.93	0.59
	BAC	1.06	0.90	1.26	1.04	0.79	1.00	0.96	0.98	0.86	0.29	0.56
Ricinus communis	BCF	0.99	1.01	1.19	0.29	1.01	1.00	1.00	0.70	0.48	1.15	0.64
	TF	1.13	0.98	1.05	1.37	0.97	0.91	0.99	1.05	0.69	0.93	0.73
	BAC	1.11	0.99	1.25	0.40	0.98	0.91	1.00	0.73	0.33	1.07	0.47
Setaria viridis	BCF	1.10	0.97	1.30	1.02	0.81	1.11	1.01	0.79	0.43	1.14	0.41
	TF	0.85	1.00	0.89	0.99	0.86	0.82	0.97	0.94	0.78	0.57	0.96
	BAC	0.94	0.97	1.16	1.02	0.70	0.91	0.98	0.74	0.33	0.65	0.39
Tamarix aphylla	BCF	1.06	0.90	1.15	0.93	1.22	1.09	0.91	0.96	0.58	0.94	1.10
	TF	0.91	0.93	0.96	0.85	0.91	0.96	1.01	0.81	0.79	0.98	0.91
	BAC	0.97	0.84	1.10	0.79	1.11	1.04	0.92	0.78	0.46	0.92	1.00
Withania somnifera	BCF	1.16	1.02	1.23	1.02	1.00	0.71	1.00	1.04	1.00	0.64	1.55
	TF	0.94	0.95	0.98	1.01	1.02	1.04	0.99	0.88	0.85	0.96	0.78
	BAC	1.09	0.97	1.21	1.03	1.02	0.74	1.00	0.91	0.84	0.61	1.21

Table 4.5 The concentration of heavy metals recorded (mg/kg) in the root and shoot of plant species in the less polluted zone of MWPE.

Botanical Names	Part	Cr	Ni	Cu	Mn	Cd	Zn	Fe	Co	Ca	Mg	Na
	Root	10.20	9.64	30.96	4.20	16.88	36.27	52.80	5.16	172.20	122.33	28.90
Adiantum capillus-veneris	Shoot	9.16	6.72	27.92	2.84	15.68	34.57	47.64	4.25	164.04	111.08	26.21
	Root	10.52	8.88	24.60	1.76	12.12	36.92	69.92	1.24	137.00	116.24	27.22
Ailanthus altissima	Shoot	9.84	6.92	24.28	1.88	11.52	33.15	58.36	0.08	116.00	111.08	25.55
	Root	12.88	10.26	21.48	13.29	14.72	41.35	65.00	3.28	103.34	111.88	25.25
Albizia lebbeck	Shoot	10.81	9.26	20.67	12.18	13.35	39.53	63.05	2.95	88.76	94.93	24.18
	Root	10.56	5.44	23.16	2.80	18.64	39.46	58.16	3.72	86.88	131.69	23.85
Calotropis procera	Shoot	9.04	2.00	23.01	2.20	16.80	37.34	52.96	2.64	60.76	110.36	23.25
	Root	10.23	8.35	20.07	5.96	15.33	39.76	64.82	4.29	68.29	135.73	27.27
Cynodon dactylon	Shoot	9.34	7.12	14.52	3.40	14.72	30.58	59.29	2.96	57.40	125.62	19.12
	Root	10.24	10.57	26.46	4.35	14.27	40.90	67.29	4.43	117.01	131.98	27.99
Datura innoxia	Shoot	9.64	8.96	20.80	2.56	13.76	38.75	59.20	3.48	106.20	81.32	25.83
	Soot	12.28	8.24	17.32	13.96	15.92	36.57	12.12	2.72	119.36	98.28	19.32
Debregeasia salicifolia	Shoot	11.08	4.28	15.56	12.72	15.60	33.44	9.56	2.60	110.68	80.76	16.19
	Root	12.72	6.28	16.52	5.04	16.20	38.87	17.00	5.20	163.56	72.17	28.26
Desmostachya bipinnata	Shoot	11.96	4.72	14.76	2.88	15.36	26.34	12.52	4.24	163.24	61.37	51.31
	Root	11.88	8.76	19.72	5.29	18.28	44.83	58.60	4.00	123.08	113.48	21.12
Dodonaea viscosa	Shoot	9.84	7.16	14.80	2.36	18.21	42.74	47.16	3.82	59.96	112.83	18.38
	Root	11.08	5.36	19.08	9.12	16.56	43.26	54.40	4.80	169.60	133.64	28.42
Erigeron bonariensis	Shoot	8.96	4.84	16.84	3.04	14.28	42.59	52.72	3.52	147.92	129.18	26.22
	Root	10.78	9.39	16.58	17.28	14.47	43.19	58.29	4.29	157.28	135.92	27.88
Ficus carica	Shoot	9.32	8.80	13.86	15.64	10.44	41.01	50.68	3.40	146.56	133.58	23.06
Morus alba	Root	10.26	9.64	22.48	19.52	16.84	43.68	64.08	5.92	168.72	129.21	25.34

	Shoot	9.24	6.60	21.76	18.20	15.68	42.28	59.16	3.36	152.56	123.78	23.87
	Root	10.76	7.60	23.88	19.92	16.76	41.04	70.24	4.32	163.68	126.81	25.87
Morus nigra	Shoot	9.84	7.48	20.96	18.84	15.56	40.97	69.68	3.88	162.72	104.68	24.34
	Root	10.12	2.64	15.20	2.32	16.24	42.80	59.44	4.16	119.40	54.17	23.01
Parthenium hysterophorus	Shoot	9.24	2.68	14.56	1.68	15.40	41.00	58.56	3.24	116.08	7.80	22.05
	Root	12.84	15.76	18.98	2.64	12.24	35.12	50.36	3.60	66.72	89.61	23.79
Persicaria glabra	Shoot	9.44	1.40	17.97	2.92	11.64	32.86	48.44	2.08	39.52	85.58	19.83
	Root	10.96	10.68	14.80	12.92	16.76	38.83	48.32	3.88	110.20	16.72	22.05
Ricinus communis	Shoot	8.04	12.80	13.80	11.20	15.28	35.64	33.52	1.76	103.20	11.49	22.83
	Root	12.84	7.20	12.68	11.84	16.48	31.33	45.80	3.52	37.08	55.04	21.44
Setaria viridis	Shoot	9.48	6.69	11.28	2.28	15.60	29.77	40.40	2.40	36.00	45.82	21.54
	Root	10.23	9.25	16.27	16.98	16.92	38.28	40.35	3.98	156.81	96.09	25.99
Tamarix aphylla	Shoot	8.96	8.44	13.16	12.40	15.76	35.12	38.84	3.88	131.44	88.94	22.80
	Root	12.08	9.28	19.80	19.88	17.84	38.31	57.64	4.51	143.08	132.27	21.33
Withania somnifera	Shoot	11.20	10.72	13.88	18.76	16.24	38.86	50.96	3.68	122.12	125.72	20.84

Table 4.6 BCF, TF and BAC for measured heavy metals of indicator species in the less polluted zone of MWPE.

Botanical Names	**Factor**	**Cr**	**Ni**	**Cu**	**Mn**	**Cd**	**Zn**	**Fe**	**Co**	**Ca**	**Mg**	**Na**
	BCF	0.94	0.88	1.45	0.20	0.95	0.82	0.73	1.16	1.03	0.89	1.02
	TF	0.90	0.70	0.90	0.68	0.93	0.95	0.90	0.82	0.95	0.91	0.91
Adiantum capillus-veneris	BAC	0.84	0.61	1.30	0.14	0.88	0.79	0.66	0.95	0.98	0.81	0.92
	BCF	0.97	0.81	1.15	0.08	0.68	0.84	0.97	0.28	0.82	0.84	0.96
	TF	0.94	0.78	0.99	1.07	0.95	0.90	0.83	0.06	0.85	0.96	0.94
Ailanthus altissima	BAC	0.90	0.63	1.13	0.09	0.65	0.75	0.81	0.02	0.69	0.81	0.90
	BCF	1.18	0.94	1.00	0.64	0.83	0.94	0.90	0.74	0.62	0.81	0.89
	TF	0.84	0.90	0.96	0.92	0.91	0.96	0.97	0.90	0.86	0.85	0.96
Albizia lebbeck	BAC	0.99	0.84	0.97	0.59	0.75	0.90	0.87	0.66	0.53	0.69	0.85
	BCF	0.97	0.50	1.08	0.13	1.05	0.90	0.81	0.83	0.52	0.96	0.84
	TF	0.86	0.37	0.99	0.79	0.90	0.95	0.91	0.71	0.70	0.84	0.98
Calotropis procera	BAC	0.83	0.18	1.08	0.11	0.95	0.85	0.73	0.59	0.36	0.80	0.82
	BCF	0.94	0.76	0.94	0.29	0.86	0.90	0.90	0.96	0.41	0.99	0.96
	TF	0.91	0.85	0.72	0.57	0.96	0.77	0.91	0.69	0.84	0.93	0.70
Cynodon dactylon	BAC	0.86	0.65	0.68	0.16	0.83	0.69	0.82	0.66	0.34	0.91	0.67
	BCF	0.94	0.96	1.24	0.21	0.80	0.93	0.93	0.99	0.70	0.96	0.99
	TF	0.94	0.85	0.79	0.59	0.96	0.95	0.88	0.79	0.91	0.62	0.92
Datura innoxia	BAC	0.89	0.82	0.97	0.12	0.77	0.88	0.82	0.78	0.63	0.59	0.91
	BCF	1.13	0.75	0.81	0.67	0.90	0.83	0.17	0.61	0.71	0.71	0.68
	TF	0.90	0.52	0.90	0.91	0.98	0.91	0.79	0.96	0.93	0.82	0.84
Debregeasia salicifolia	BAC	1.02	0.39	0.73	0.61	0.88	0.76	0.13	0.58	0.66	0.59	0.57
	BCF	1.17	0.57	0.77	0.24	0.91	0.88	0.24	1.17	0.98	0.52	1.00
	TF	0.94	0.75	0.89	0.57	0.95	0.68	0.74	0.82	1.00	0.85	1.82
Desmostachya bipinnata	BAC	1.10	0.43	0.69	0.14	0.86	0.60	0.17	0.95	0.97	0.45	1.81
	BCF	1.09	0.80	0.92	0.25	1.03	1.02	0.81	0.90	0.73	0.82	0.74
	TF	0.83	0.82	0.75	0.45	1.00	0.95	0.80	0.95	0.49	0.99	0.87
Dodonaea viscosa	BAC	0.90	0.65	0.69	0.11	1.03	0.97	0.65	0.86	0.36	0.82	0.65

	BCF	1.02	0.49	0.89	0.44	0.93	0.98	0.75	1.08	1.01	0.97	1.00
	TF	0.81	0.90	0.88	0.33	0.86	0.98	0.97	0.73	0.87	0.97	0.92
Erigeron bonariensis	BAC	0.82	0.44	0.79	0.15	0.80	0.97	0.73	0.79	0.88	0.94	0.92
	BCF	0.99	0.86	0.77	0.83	0.81	0.98	0.81	0.96	0.94	0.99	0.98
	TF	0.86	0.94	0.84	0.91	0.72	0.95	0.87	0.79	0.93	0.98	0.83
Ficus carica	BAC	0.86	0.80	0.65	0.75	0.59	0.93	0.70	0.76	0.87	0.97	0.81
	BCF	0.94	0.88	1.05	0.94	0.95	0.99	0.89	1.33	1.01	0.94	0.89
	TF	0.90	0.68	0.97	0.93	0.93	0.97	0.92	0.57	0.90	0.96	0.94
Morus alba	BAC	0.85	0.60	1.02	0.88	0.88	0.96	0.82	0.75	0.91	0.90	0.84
	BCF	0.99	0.69	1.12	0.96	0.94	0.93	0.97	0.97	0.98	0.92	0.91
	TF	0.91	0.98	0.88	0.95	0.93	1.00	0.99	0.90	0.99	0.83	0.94
Morus nigra	BAC	0.90	0.68	0.98	0.91	0.88	0.93	0.96	0.87	0.97	0.76	0.86
	BCF	0.93	0.24	0.71	0.11	0.91	0.97	0.82	0.93	0.71	0.39	0.81
	TF	0.91	1.02	0.96	0.72	0.95	0.96	0.99	0.78	0.97	0.14	0.96
Parthenium hysterophorus	BAC	0.85	0.24	0.68	0.08	0.87	0.93	0.81	0.73	0.69	0.06	0.78
	BCF	1.18	1.44	0.89	0.13	0.69	0.80	0.70	0.81	0.40	0.65	0.84
	TF	0.74	0.09	0.95	1.11	0.95	0.94	0.96	0.58	0.59	0.96	0.83
Persicaria glabra	BAC	0.87	0.13	0.84	0.14	0.66	0.75	0.67	0.47	0.24	0.62	0.70
	BCF	1.01	0.97	0.69	0.62	0.94	0.88	0.67	0.87	0.66	0.12	0.78
	TF	0.73	1.20	0.93	0.87	0.91	0.92	0.69	0.45	0.94	0.69	1.04
Ricinus communis	BAC	0.74	1.17	0.64	0.54	0.86	0.81	0.46	0.39	0.62	0.08	0.80
	BCF	1.18	0.66	0.59	0.57	0.93	0.71	0.63	0.79	0.22	0.40	0.76
	TF	0.74	0.93	0.89	0.19	0.95	0.95	0.88	0.68	0.97	0.83	1.00
Setaria viridis	BAC	0.87	0.61	0.53	0.11	0.88	0.68	0.56	0.54	0.21	0.33	0.76
	BCF	0.94	0.84	0.76	0.82	0.95	0.87	0.56	0.89	0.94	0.70	0.91
	TF	0.88	0.91	0.81	0.73	0.93	0.92	0.96	0.98	0.84	0.93	0.88
Tamarix aphylla	BAC	0.82	0.77	0.61	0.60	0.89	0.80	0.54	0.87	0.78	0.65	0.80
	BCF	1.11	0.85	0.93	0.96	1.00	0.87	0.80	1.01	0.85	0.96	0.75
	TF	0.93	1.16	0.70	0.94	0.91	1.01	0.88	0.82	0.85	0.95	0.98
Withania somnifera	BAC	1.03	0.98	0.65	0.90	0.91	0.88	0.71	0.83	0.73	0.91	0.73

4.3.2 Pattern in chlorophyll contents of indicator plants

The chlorophyll-a, b and total carotenoids were assessed in plant shoot (leaves) of indicator species both in highly and less polluted marble waste ecosytem. The results indicate that the chlorophyll-a, b and total caroteniods decrease with an increase in marble pollution. The chlorophyll contents increase when moving from high levels of marble pollution towards the less polluted zone (Fig. 4.9 & Table 4.7).

The maximum amount of chlorophyll-a was recorded in *Ailanthus altissima* (1.45 mg/g), followed by *Ficus carica* (0.71 mg/g), *Morus alba* (0.65 mg/g), *Debregeasia salicifolia* (0.52 mg/g), *Datura innoxia* (0.52 mg/g), *Persicaria glabra* (0.50 mg/g) and *Adiantum capillus-veneris* (0.48 mg/g) in the highly polluted marble waste ecosystem. While minimum amount were shown by *Desmostachya bipinnata* (0.13 mg/g), *Tamarix aphylla* (0.25 mg/g), *Setaria viridis* (0.31 mg/g), *Calotropis procera* and *Cynodon dactylon* (0.32 mg/g each) (Fig. 4.9 & Table 4.7). In the less polluted environment, the maximum concentration of chlorophyll-a was recorded from *Ailanthus altissima* (1.78 mg/g), *Morus alba* (1.03 mg/g), *Erigeron bonariensis* (0.82 mg/g), *Ficus carica* (0.81 mg/g), *Adiantum capillus-veneris* (0.71 mg/g), *Persicaria glabra* (0.70 mg/g) and *Morus nigra* (0.66 mg/g). At the same time, the lowest amount of chlorophyll-a was determined in *Desmostachya bipinnata* (0.29 mg/g), *Calotropis procera* (0.35 mg/g), *Dodonaea viscosa* (0.43 mg/g), *Cynodon dactylon* (0.44 mg/g), *Tamarix aphylla* (0.45 mg/g) and *Albizia lebbeck* (0.49 mg/g) (Fig. 4.9 & Table 4.7).

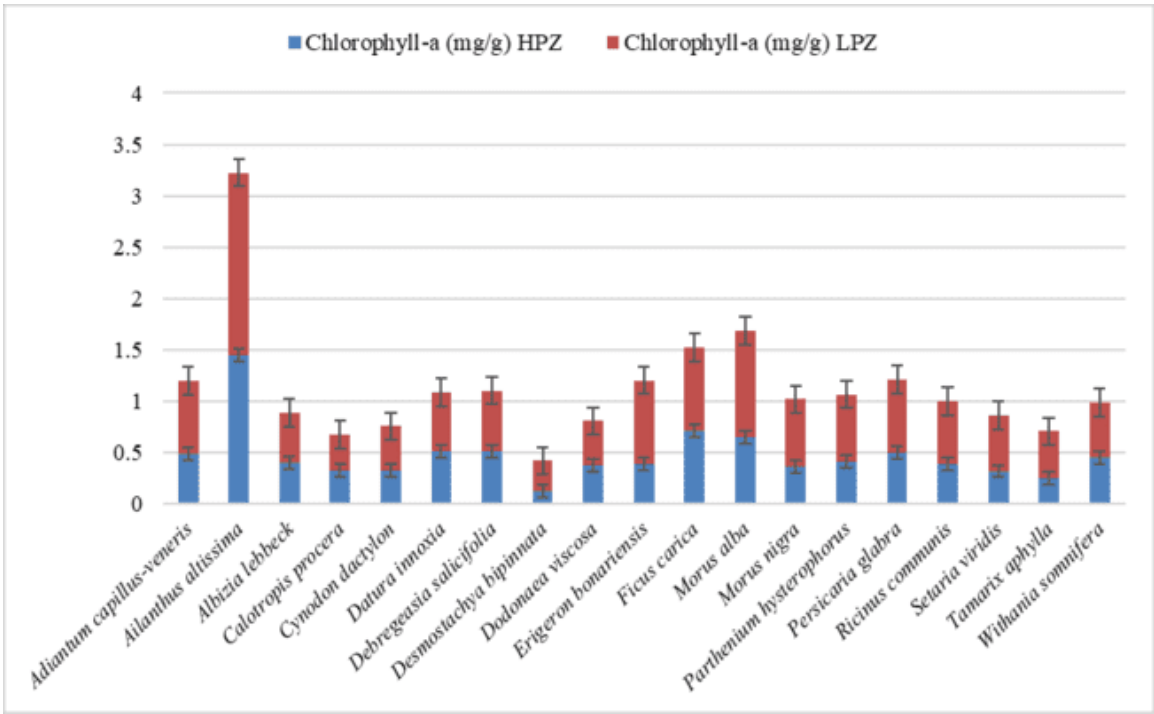


Fig. 4.9 Chlorophyll-a contents (mg/g) in the leaves of some identified indicator plants in HPZ and LPZ.

The highest amount of chlorophyll-b was observed in *Ailanthus altissima* (0.78 mg/g) acompanied by *Morus alba* (0.38 mg/g), *Ficus carica* (0.35 mg/g), *Persicaria glabra* (0.27 mg/g) and *Debregeasia salicifolia* (0.22 mg/g) in the highly MWPE. The lowest amount of chlorophyll-b was shown by *Desmostachya bipinnata* (0.063 mg/g), *Parthenium hysterophorus* (0.065 mg/g), *Ricinus communis* (0.075 mg/g), *Calotropis procera* (0.098 mg/g), *Erigeron bonariensis* (0.11 mg/g) and *Tamarix aphylla* (0.12 mg/g) (Fig. 4.10 & Table 4.7). Whereas, in the LPZ environment the maximum amount of chlorophyll-b was observed in *Calotropis procera* (1.06 mg/g) followed *Ailanthus altissima* (0.95 mg/g), *Ficus carica* (0.50 mg/g), *Morus alba* (0.48 mg/g), *Erigeron bonariensis* (0.43 mg/g) and *Persicaria glabra* (0.39 mg/g). *Desmostachya bipinnata* (0.06 mg/g), *Cynodon dactylon* (0.11 mg/g), *Parthenium hysterophorus* (0.14 mg/g), *Dodonaea viscosa* (0.19 mg/g) and *Tamarix aphylla* (0.20 mg/g) comprehended the miniumum amount of chlorophyll-b in the control enviroment (Fig. 4.10 & Table 4.7).

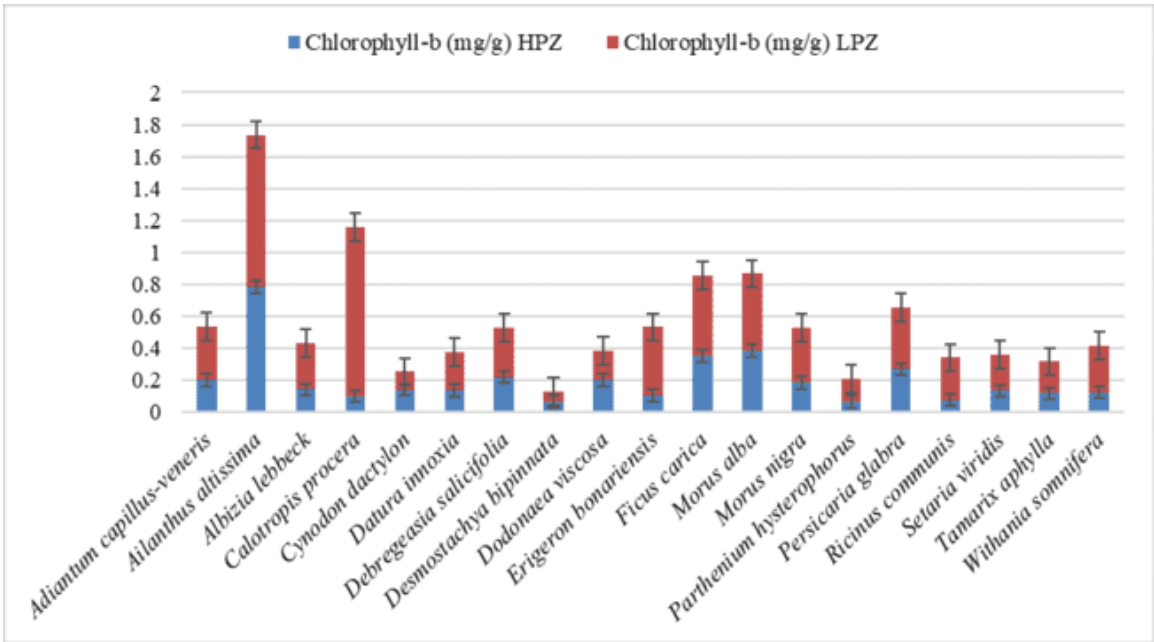


Fig. 4.10 Chlorophyll-b contents (mg/g) in leaves of some identified indicator plants in highly and less marble waste polluted ecosystem.

Regarding the amount of total carotenoids, the maximum concentrations were shown by *Ailanthus altissima* (5.79 mg/g), *Ficus carica* (2.85 mg/g), *Morus alba* (2.60 mg/g), *Debregeasia salicifolia* (2.07 mg/g), *Datura innoxia* (2.06 mg/g) and *Persicaria glabra* (2.01 mg/g) in the HPZ of MWPE. At the same time, the lowest amounts of total carotenoids were determined in *Cynodon dactylon* (1.28 mg/g), *Calotropis procera* (1.28 mg/g), *Setaria viridis* (1.27 mg/g), *Tamarix aphylla* (1.01 mg/g) and *Desmostachya bipinnata* (0.51 mg/g) in the MWPE (Fig. 4.11 & Table 4.7). While in the LPZ, *Ailanthus altissima* (7.11 mg/g), *Morus alba* (4.13 mg/g), *Erigeron bonariensis* (3.27 mg/g), *Ficus carica* (3.24 mg/g), *Adiantum capillus-veneris* (2.85 mg/g) showed maximum and *Desmostachya bipinnata* (1.81 mg/g), *Calotropis procera* (1.75 mg/g), *Dodonaea viscosa* (1.74 mg/g), *Cynodon dactylon* (1.41 mg/g) and *Tamarix aphylla* (1.16 mg/g) comprehended the minimum amount of total carotenoids (Fig. 4.11 & Table 4.7).

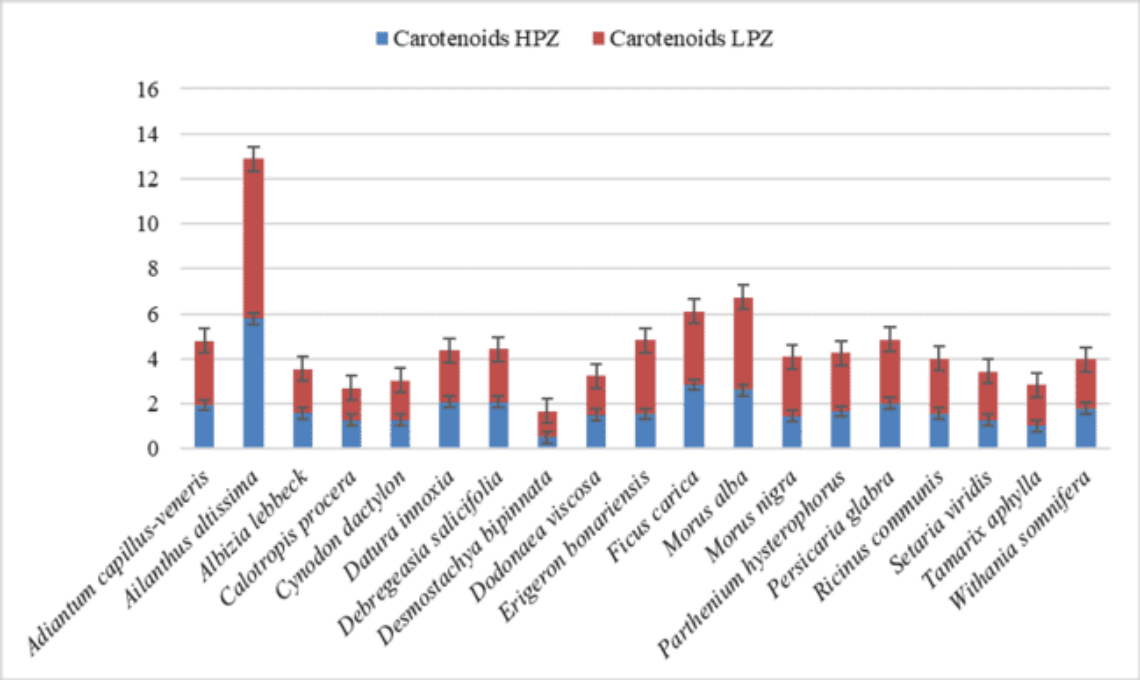


Fig. 4.11 Total carotenoids (mg/g) in leaves of identified indicator plants in the HPZ and LPZ of marble waste polluted ecosystem.

Table 4.7 Detailed description of Chlorophyll-a, b and total carotenoids determined in the indicator plant species in response to MWPE stress.

S. No	Plant Names	Chlorophyll-a (mg/g)		Chlorophyll-b (mg/g)		Carotenoids (mg/g)	
		HPZ	LPZ	HPZ	LPZ	HPZ	LPZ
1	*Adiantum capillus-veneris*	0.48393	0.71371	0.19995	0.33193	1.93572	2.85484
2	*Ailanthus altissima*	1.44787	1.77686	0.78076	0.95489	5.79148	7.10744
3	*Albizia lebbeck*	0.39409	0.49209	0.13963	0.28863	1.57636	1.96836
4	*Calotropis procera*	0.3202	0.35362	0.09784	1.06012	1.2808	1.41448
5	*Cynodon dactylon*	0.32027	0.43663	0.13799	0.11278	1.28108	1.74652
6	*Datura innoxia*	0.51579	0.57262	0.13398	0.24136	2.06316	2.29048
7	*Debregeasia salicifolia*	0.51789	0.58596	0.21726	0.31122	2.07156	2.34384
8	*Desmostachya bipinnata*	0.12773	0.29115	0.06263	0.06702	0.51092	1.1646

9	*Dodonaea viscosa*	0.3732	0.43392	0.19749	0.18684	1.4928	1.73568
10	*Erigeron bonariensis*	0.38635	0.81664	0.10498	0.4255	1.5454	3.26656
11	*Ficus carica*	0.71272	0.81072	0.3514	0.5004	2.85088	3.24288
12	*Morus alba*	0.65101	1.03219	0.38152	0.48436	2.60404	4.12876
13	*Morus nigra*	0.35921	0.66021	0.18194	0.34593	1.43684	2.64084
14	*Parthenium hysterophorus*	0.41374	0.65157	0.06463	0.14211	1.65496	2.60628
15	*Persicaria glabra*	0.50271	0.70771	0.26664	0.38764	2.01084	2.83084
16	*Ricinus communis*	0.38895	0.61264	0.07461	0.26407	1.5558	2.45056
17	*Setaria viridis*	0.31849	0.54169	0.13147	0.22675	1.27396	2.16676
18	*Tamarix aphylla*	0.25279	0.45335	0.11548	0.19922	1.01116	1.8134
19	*Withania somnifera*	0.4467	0.54264	0.12237	0.29112	1.7868	2.17056

4.3.3 Proline accumulation as a survival mechanism in indicator plants

The proline contents are directly proportional to the level of marble pollution in the indicator plants. The maximum amount of proline accumulation was recorded in plants grown in the HPZ of MWPE as compared to the LPZ (Fig. 4.12 & Table 4.8).

The maximum amount of proline accumulation was recorded in *Cynodon dactylon* (93.91 µg/g), followed by *Ailanthus altissima* (68.61 µg/g), *Withania somnifera* (57.54 µg/g), *Ficus carica* (43.52 µg/g), *Morus nigra* (35.53 µg/g), *Ricinus communis* (32.03 µg/g) and *Datura innoxia* (30.77 µg/g). The lowest amount of proline contents was shown by *Adiantum capillus-veneris* (9.95 µg/g), *Dodonaea viscosa* (11.35 µg/g), *Erigeron bonariensis* (12.96 µg/g), *Persicaria glabra* (13.74 µg/g), *Desmostachya bipinnata* (14.16 µg/g) and *Albizia lebbeck* (15.14 µg/g) in the HPZ of MWPE (Fig. 4.12 & Table 4.8).

At the same time, *Ailanthus altissima* (62.80 µg/g), *Ficus carica* (36.23 µg/g), *Morus nigra* (22.57 µg/g), *Parthenium hysterophorus* (21.87 µg/g), *Morus alba* (18.78 µg/g) comprehended maximum and *Dodonaea viscosa* (2.24 µg/g), *Calotropis procera* (4.20 µg/g), *Cynodon dactylon* (5.05 µg/g), *Adiantum capillus-veneris* (5.60 µg/g) and

Erigeron bonariensis (5.61 µg/g) showed the minimum accumulation of proline contents in the LPZ (Fig. 4.12 & Table 4.8).

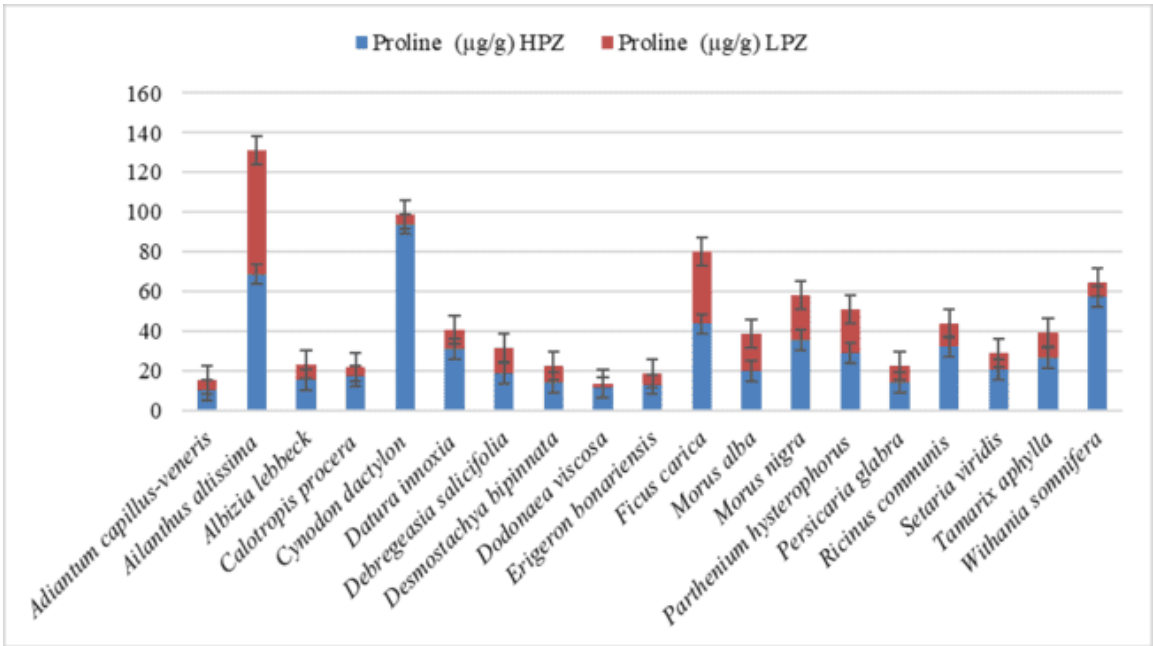


Fig. 4.12 Proline accumulation in the leaves of identified indicator plant species in the HPZ and LPZ of MWPE.

Table 4.8 Pattern of proline accumulation in the indicator plant in response to highly and less marble pollution.

S. No.	Plant Names	Proline (µg/g)	
		HPZ	LPZ
1	*Adiantum capillus-veneris*	9.95136	5.6064
2	*Ailanthus altissima*	68.60832	62.79168
3	*Albizia lebbeck*	15.13728	8.12928
4	*Calotropis procera*	17.23968	4.2048
5	*Cynodon dactylon*	93.9072	5.04576
6	*Datura innoxia*	30.76512	9.95136
7	*Debregeasia salicifolia*	18.29088	13.10496

8	*Desmostachya bipinnata*	14.15616	7.91904
9	*Dodonaea viscosa*	11.35296	2.24256
10	*Erigeron bonariensis*	12.9648	5.6064
11	*Ficus carica*	43.51968	36.23136
12	*Morus alba*	19.90272	18.78144
13	*Morus nigra*	35.53056	22.56576
14	*Parthenium hysterophorus*	28.94304	21.86496
15	*Persicaria glabra*	13.73568	8.68992
16	*Ricinus communis*	32.02656	11.70336
17	*Setaria viridis*	20.39328	8.26944
18	*Tamarix aphylla*	26.42016	12.82464
19	*Withania somnifera*	57.53568	7.07808

4.3.4 Mixed Effect Model

The mixed effect modeling showed both fixed and random effects simultaneously. Proline, chlorophyll-a and chlorophyll-b are fixed, while phytoremediation is a random component in the model. The results of the fixed effect model indicate a positive significant relationship between chromium phytoremediation and proline accumulation (0.3033) by indicator species in MWPE. At the same time, chlorophyll-a and b exhibit a significant negative (-0.4017 & -0.2882) relationship with phytoremediation of chromium metal (Fig. 4.3.5 & Table 4.9). The recorded inter-specific variance between highly and less polluted zone was 20%, and among chromium and other potential toxic elements was 70% (Fig. 4.13 & Table 4.3.8).

Nickel phytoremediation by the identified indicator species had a significant positive relationship with proline accumulation (0.3175) after the analysis of the fixed effect modeling. Chlorophyll-a showed an insignificant positive (0.1805) and chlorophyll-b a significant negative (-0.3489) relationship with the phytoremediation of nickel. The inter-specific variance between highly and less polluted zone was noted as 19%, and among nickel and other metals was 73% (Fig. 4.14 & Table 4.9).

The results of the mixed effect modeling demonstrated an insignificant positive (0.225) relation between copper phytoremediation and proline accumulation by indicator plants in MWPE. While chlorophyll-a showed insignificant (-0.24171) and chlorophyll-b significant negative (-0.1672) relationships with the phytoremediation of copper metal. The inter-specific variance between polluted zone and among copper plus other quantified metals were 22 and 63 %, respectively (Fig. 4.15 & Table 4.9)

The amount of proline accumulation increased (0.3822) significantly with increase in manganese phytoremediation. Both chlorophyll-a and b decrease (-0.3374 & -0.2107) with increase in accumulation of manganese metal by studied plant species. The recorded inter-specific variance between highly and less polluted zone was 22%, and among manganese and potential toxic elements was 66% (Table 4.9).

The amount of cadmium phytoremediation by identified indicators showed a significant positive relationship with proline accumulation (0.3023) after the analysis of the fixed effect modeling. The chlorophyll-a and chlorophyll-b illustrated significant negative (-1504 & -02199) relationships with the phytoremediation of cadmium toxic level. The inter-specific variance between highly and less polluted zone was observed as 32.1%, and among cadmium and other metals was 53% (Fig. 4.16 & Table 4.9).

The concentration of proline accumulation is directly proportional (0.3247) to phytoremediation of zinc heavy metal in marble waste polluted ecosystem. Initially, chlorophyll-a increased with an increase in zinc phytoremediation in the less polluted zone. However, it showed an inverse relation with zinc phytoremediation in the highly polluted zone. The chlorophyll-b has a significant negative (-0.3290) relationship with the phytoremediation of zinc heavy metal. The recorded inter-specific variance between highly and less polluted zone was 25%, and among zinc and potential toxic elements was 62 % (Fig. 4.17 & Table 4.9).

The amount of proline accumulation increases (0.3056) significantly with increase in iron phytoremediation. Both chlorophyll-a and b decreased (-0.1651 & -0.2653) with increase in accumulation of iron metal in the studied plant species. The inter-specific variance between polluted zone and among copper plus other quantified metals were 42 and 43 %, respectively (Fig. 4.18 & Table 4.9).

The mixed effect modeling illustrated that the proline has a positive (0.2183), while chlorophyll-a (-0.2013) and chlorophyll-b (-0.1507) have significant negative relationships with the cobalt phytoremediation of indicator species of marble waste polluted ecosystem. The inter-specific variance between polluted zone was 15 % and among potential toxic elements was 70% (Fig. 4.19 & Table 4.9).

The results of the fixed effect model demonstrated a positive significant relationship between calcium phytoremediation and proline accumulation (i.e., 0.4665) by indicator species in MWPE. At the same time, chlorophyll-a and b exhibited significant negative (-0.5671 & -0.2796) relationships with phytoremediation of calcium metal (Fig. 4.20). The recorded inter-specific variance between highly and less polluted zone was 22%, and among chromium and potential toxic elements was 60% (Fig. 4.20 Table 4.9).

Magnesium phytoremediation by identified indicator species had a significant positive relationship with proline accumulation (0.4445), chlorophyll-b (0.3191) and an insignificant positive correlation with chlorophyll-a (0.018) after the analysis of fixed effect modeling. The inter-specific variance between highly and less polluted zone was noted as 24%, and among magnesium and other metals was 62% (Table 4.9).

The results of the mixed effect modeling demonstrated an insignificant positive (0.221) relation between sodium phytoremediation and proline accumulation by indicator plants in MWPE. While chlorophyll-a and chlorophyll-b showed a significant negative relationship (-0.2778 & -0.3070) with the phytoremediation of sodium metal. The inter-specific variance between polluted zone and among copper plus other quantified metals were 22 and 63 %, respectively (Fig. 4.21 & Table 4.9)

Table 4.9 The detailed description of mixed effect model representing the impact of phytoremediation on proline and chlorophyll contents.

S. No.	Fixed Effect Model	Intercept	$\beta1_{proline}$	$\beta2$ Chlorophyll-a	$\beta3$ Chlorophyll-b
A.	**Chromium**				
1	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2$ Chlorophyll-a$+ \beta3$ Chlorophyll-b$+ \Upsilon_{ID}$	**1.09468** (0.03497)	**0.3125** (0.02284)	**-0.44065** (0.07779)	**-0.28794** (0.10063)
2	$Y_{TF} = \alpha + \beta1_{proline} + \beta2$ Chlorophyll-a$+ \beta3$ Chlorophyll-b$+ \Upsilon_{ID}$	**0.90825** (0.03527)	0.22736 (0.01979)	0.01901 (0.05968)	-0.25643 (0.07463)
3	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2$ Chlorophyll-a$+ \beta3$ Chlorophyll-b$+ \Upsilon_{ID}$	**0.98135** (0.05929)	**0.3069** (0.03143)	**-0.40175** (0.09371)	**-0.32842** (0.11673)
4	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2$ Chlorophyll-a$+ \beta3$ Chlorophyll-b$+ \Upsilon_{ID}$	**0.990125** (0.03951)	**0.303342** (0.021203)	**-0.401655** (0.06334)	**-0.288202** (0.078958)
Random Effect Model			**Variance**	**% of total**	
Inter-specific variance: among zone			3.2647	20.3084	
Inter-specific variance: among metal			21.345	70.507	
Residual (between zone)			0.09599	9.108	
Total			24.70569	100	
B.	**Nickel**				
5	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2$ Chlorophyll-a$+ \beta3$ Chlorophyll-b$+ \Upsilon_{ID}$	**0.83901** (0.115524)	**0.302936** (0.052652)	0.138943 (0.154324)	-0.163973 (0.191128)
6	$Y_{TF} = \alpha + \beta1_{proline} + \beta2$ Chlorophyll-a$+ \beta3$ Chlorophyll-b$+ \Upsilon_{ID}$	**0.93362** (0.07256)	**0.35451** (0.04573)	0.17765 (0.14606)	**-0.5192** (0.18585)
7	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2$ Chlorophyll-a$+ \beta3$ Chlorophyll-b$+ \Upsilon_{ID}$	**0.77849** (0.15136)	**0.33218** (0.05672)	0.17632 (0.16459)	**-0.38497** (0.20311)
8	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2$ Chlorophyll-a$+ \beta3$ Chlorophyll-b$+ \Upsilon_{ID}$	**0.83811** (0.103)	**0.31758** (0.03724)	0.18056 (0.10793)	**-0.34883** (0.13313)
Random Effect Model		**Variance**		**% of total**	

Inter-specific variance: among zone		2.1902		19.0103	
Inter-specific variance: among metal		25.434		73.197	
Residual (between zone)		0.1271		8.015	
Total		27.7513		100	
Copper					
9	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.95964** (0.16883)	**0.25681** (0.0573)	-0.28558 (0.16578)	-0.11641 (0.20437)
10	$Y_{TF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.89387** (0.04476)	0.22131 0.01932)	-0.22505 (0.05643)	**-0.12989** (0.0698)
11	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.85483** (0.18864)	**0.23316** (0.05315)	-0.25461 (0.15321)	-0.25103 (0.18861)
12	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.90027** (0.13111)	0.225 (0.03717)	-0.24171 (0.10715)	**-0.16725** (0.13191)
Random Effect Model		**Variance**		**% of total**	
Inter-specific variance: among zone		4.1573		22.042	
Inter-specific variance: among metal		20.334		63.170	
Residual (between zone)		0.1863		15.415	
Total		24.6776		100	
Manganese					
13	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	0.7645 (0.21264)	**0.37346** (0.07774)	**-0.34564** (0.22538)	-0.21232 (0.27805)
14	$Y_{TF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.83592** (0.1189)	**0.38829** (0.05409)	-0.01771 (0.15851)	0.1536 (0.1963)
15	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	0.68431 (0.23561)	**0.39964** (0.07568)	**-0.37235** (0.21868)	-0.21953 (0.26944)
16	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	0.75572 (0.18236)	**0.3822** (0.05658)	**-0.33748** (0.16336)	**-0.2107** (0.20123)

Random Effect Model		**Variance**		**% of total**	
Inter-specific variance: among zone		2.4762		22.1084	
Inter-specific variance: among metal		10.422		66.307	
Residual (between zone)		0.1638		12.008	
Total		13.062		100	
Cadmium					
17	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**1.03195** (0.03116)	**0.31394** (0.02035)	**-0.2199** (0.06931)	**-0.16966** (0.08966)
18	$Y_{TF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.96836** (0.0218)	**0.31189** (0.01424)	-0.01427 (0.0485)	-0.10055 (0.06274)
19	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.997331** (0.03774)	**0.305104** (0.02465)	**-0.217236** (0.08395)	-0.029071 (0.108604)
20	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.999216** (0.026253)	**0.302386** (0.017147)	**-0.150468** (0.058397)	**-0.219988** 0.075547)
Random Effect Model		**Variance**		**% of total**	
Inter-specific variance: among zone		5.1287		32.1	
Inter-specific variance: among metal		21.234		53.107	
Residual (between zone)		0.954		14.8	
Total		27.3167		100	
Zinc					
21	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.963936** (0.080954)	**0.342904** (0.039674)	**-0.244406** (0.11704)	**-0.307736** (0.145278)
22	$Y_{TF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.870509** (0.028313)	**0.303003** (0.018493)	0.054873 (0.062981)	-0.244762 (0.081477)
23	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.843198** (0.067044)	**0.3347** (0.035943)	**0.206547** (0.107353)	**-0.339291** (0.133816)

24	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.895093** (0.048301)	**0.324722** (0.025628)	**0.202298** (0.076408)	**-0.329097** (0.095186)
Random Effect Model		**Variance**		**% of total**	
Inter-specific variance: among zone		6.2647		25.407	
Inter-specific variance: among metal		22.345		62.819	
Residual (between zone)		0.12936		12.014	
Total		28.73906		100	
Iron					
25	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.770119** (0.156981)	**0.304023** (0.046032)	**-0.12631** (0.132769)	**-0.287381** (0.163486)
26	$Y_{TF} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.940864** (0.050711)	**0.307036** (0.019735)	-0.023819 (0.057346)	0.022648 (0.070805)
27	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	0.744674 (0.17681)	**0.308397** (0.046896)	-0.088757 (0.135065)	**-0.284095** (0.166223)
28	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2_{Chlorophyll\text{-}a} + \beta3_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.81751** (0.126926)	**0.305606** (0.034991)	**-0.165131** (0.100829)	**-0.265322** (0.124114)
Random Effect Model		**Variance**		**% of total**	
Inter-specific variance: among zone		3.7264		42.5667	
Inter-specific variance: among metal		26.518		43.546	
Residual (between zone)		0.1357		13.9732	
Total		29.74906		100	
Cobalt					

29	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**1.0136** (0.12106)	0.2689 (0.05665)	-0.14843 (0.16639)	**-0.18637** (0.20623)
30	$Y_{TF} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**1.016759** (0.062312)	0.203754 (0.037583)	**-0.313637** (0.116313)	-0.114441 (0.146636)
31	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.8907** (0.06934)	0.23264 (0.04518)	-0.22087 (0.15256)	**-0.18643** (0.19697)
32	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.95387** (0.05473)	**0.21838** (0.0357)	**-0.20138** (0.12092)	**-0.15074** (0.15624)
Random Effect Model		**Variance**		**% of total**	
Inter-specific variance: among zone		14.1793		15.2945	
Inter-specific variance: among metal		30.932		70.5675	
Residual (between zone)		0.15567		15.148	
Total		44.7667		100	
Calcium					
33	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.73466** (0.13784)	**0.44241** (0.09003)	**-0.37398** (0.30661)	**-0.28535** (0.39665)
34	$Y_{TF} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.757571** (0.086186)	**0.401685** (0.054672)	**-0.56297** (0.175859)	**-0.493501** (0.224199)
35	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.51582** (0.10394)	**0.47127** (0.06789)	**-0.56714** (0.23122)	**-0.27969** (0.29912)
36	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.66451** (0.08139)	**0.46658** (0.05316)	**-0.50778** (0.18105)	**-0.29309** (0.23422)
Random Effect Model		**Variance**		**% of total**	
Inter-specific variance: among zone		2.5658		22.001	
Inter-specific variance: among metal		25.528		60.107	
Residual (between zone)		0.18426		18.008	

Total		27.799		100	
Magnesium					
37	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.831425** (0.175743)	**0.404841** (0.079566)	**-0.190807** (0.233097)	**0.356132** (0.288639)
38	$Y_{TF} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.76074** (0.09286)	**0.44772** (0.05508)	0.04942 (0.16916)	**0.21538** (0.21275)
39	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	0.62456 (0.20714)	**0.46449** (0.08834)	0.06965 (0.25782)	**0.37449** (0.31881)
40	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.73238** (0.15334)	**0.44452** (0.06766)	0.01808 (0.19788)	**0.31918** (0.24488)
Random Effect Model		**Variance**		**% of total**	
Inter-specific variance: among zone		5.7425		24.2195	
Inter-specific variance: among metal		23.365		62.325	
Residual (between zone)		0.17936		13.167	
Total		28.78906		100	
Sodium					
41	$Y_{BCF} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**1.00187** (0.07537)	**0.38849** (0.04785)	**-0.24236** (0.1541)	**-0.26569** (0.19651)
42	$Y_{TF} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**0.99581** (0.07178)	**0.35937** (0.04688)	**-0.2675** (0.15967)	**-0.2856** (0.20656)
43	$Y_{BAC} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**1.00048** (0.09483)	**0.32291** (0.06194)	**-0.3071** (0.21094)	-0.16261 (0.27289)
44	$Y_{Phytoremediation} = \alpha + \beta1_{proline} + \beta2$ $_{Chlorophyll\text{-}a} + \beta3$ $_{Chlorophyll\text{-}b} + \Upsilon_{ID}$	**1.00353** (0.06516)	0.22084 (0.04256)	**-0.27781** (0.14494)	**-0.30707** (0.1875)
Random Effect Model		**Variance**		**% of total**	

Inter-specific variance: among zone	4.6356	22.6927
Inter-specific variance: among metal	20.881	63.0872
Residual (between zone)	0.1938	15.0586
Total	24.8033	100

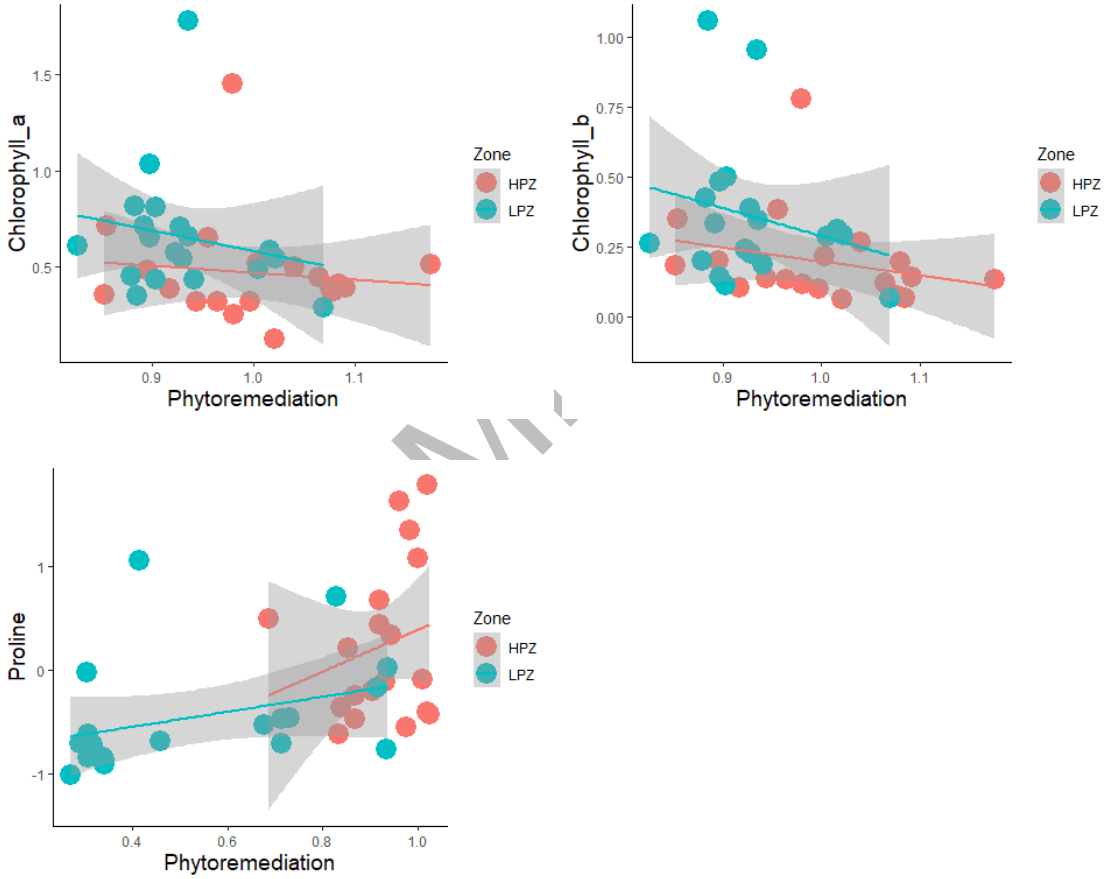


Fig. 4.13 The relationship between chromium phytoremediation along with proline, chlorophyll-a and chlorophyll-b in studied indicator plant species in the highly polluted zone (HPZ) and the less polluted zone (LZP) of MWPE, Khyber Pakhtunkhwa, Pakistan. The red and blue lines represent HPZ and LPZ, respectively.

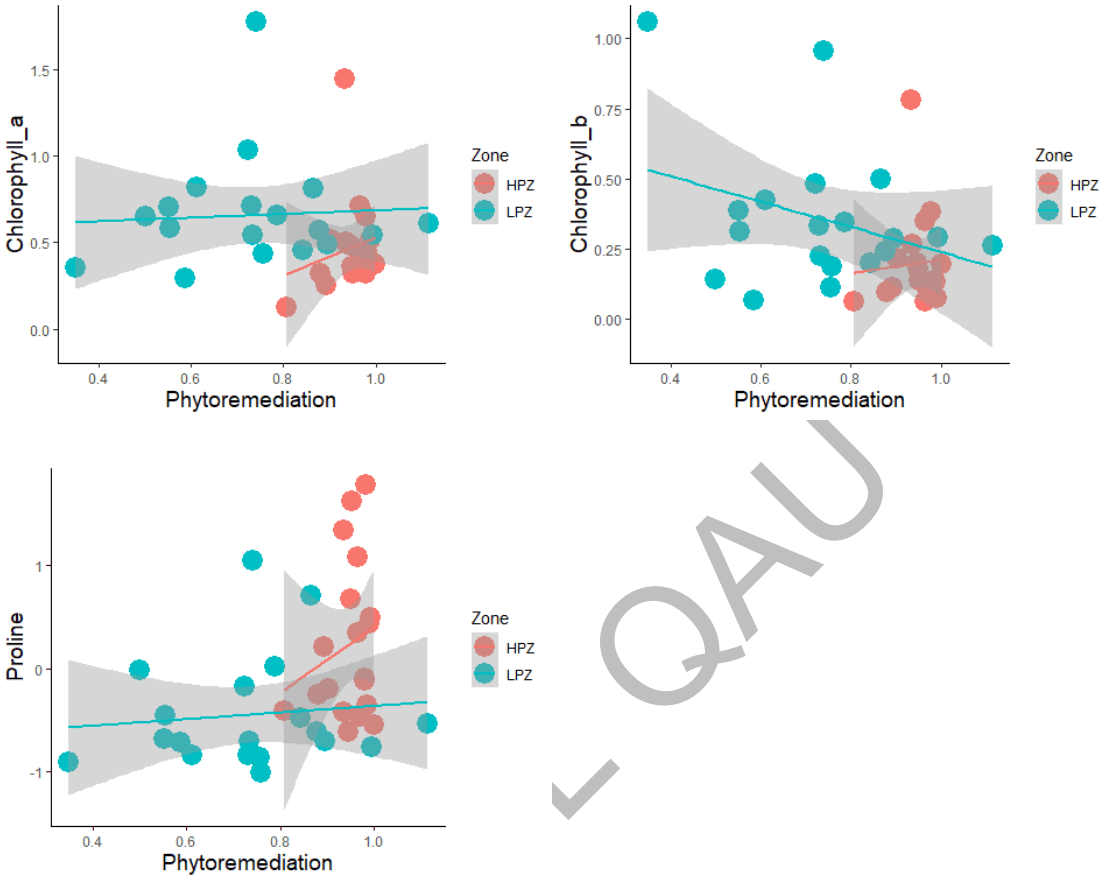


Fig. 4.14 The impact of nickel phytoremediation on proline accumulation, chlorophyll-a and chlorophyll-b in identified indicator plant species in the highly polluted zone (HPZ) and the less polluted zone (LZP) of MWPE, Khyber Pakhtunkhwa, Pakistan. The red and blue lines represent HPZ and LPZ, respectively.

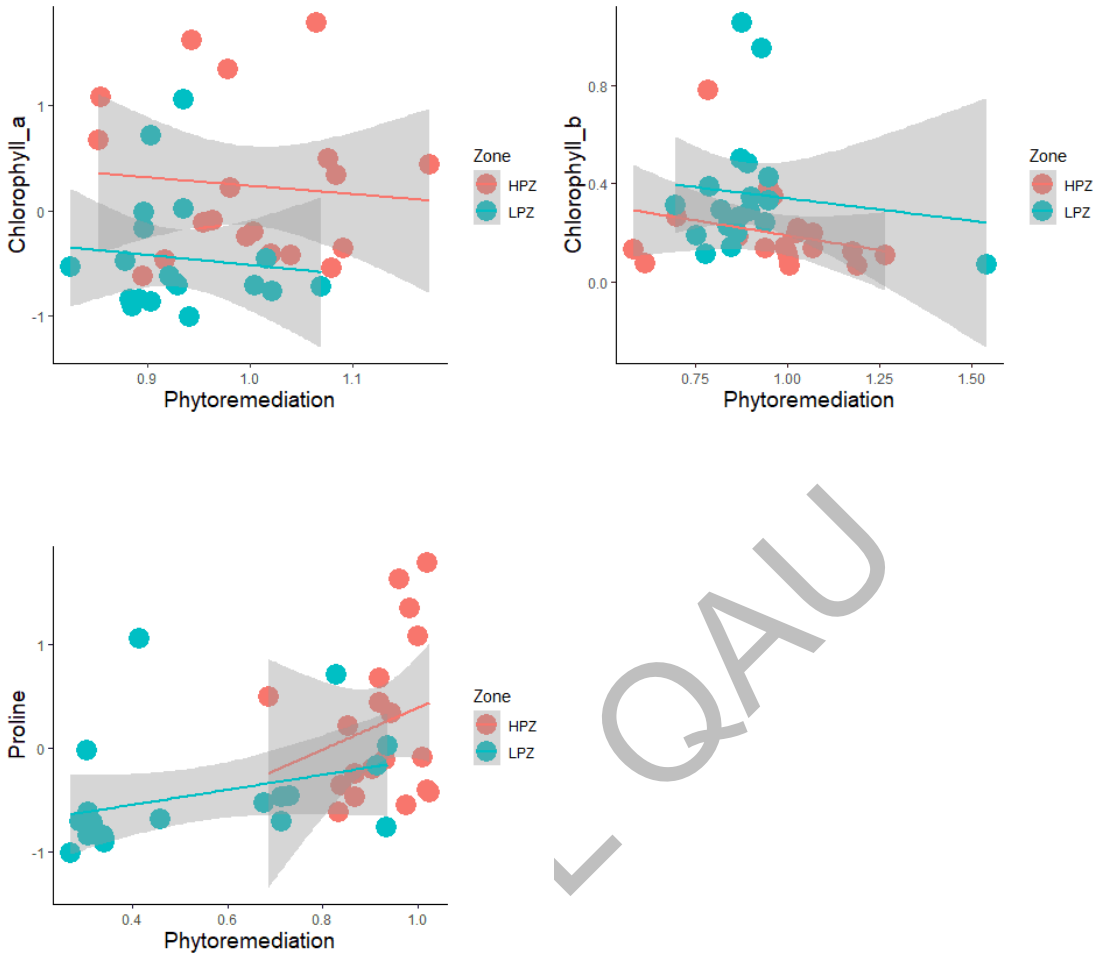


Fig. 4.15 The scattered plot for copper phytoremediation along with proline, chlorophyll-a and chlorophyll-b of indicator species in the highly polluted zone (HPZ) and the less polluted zone (LZP) of MWPE.

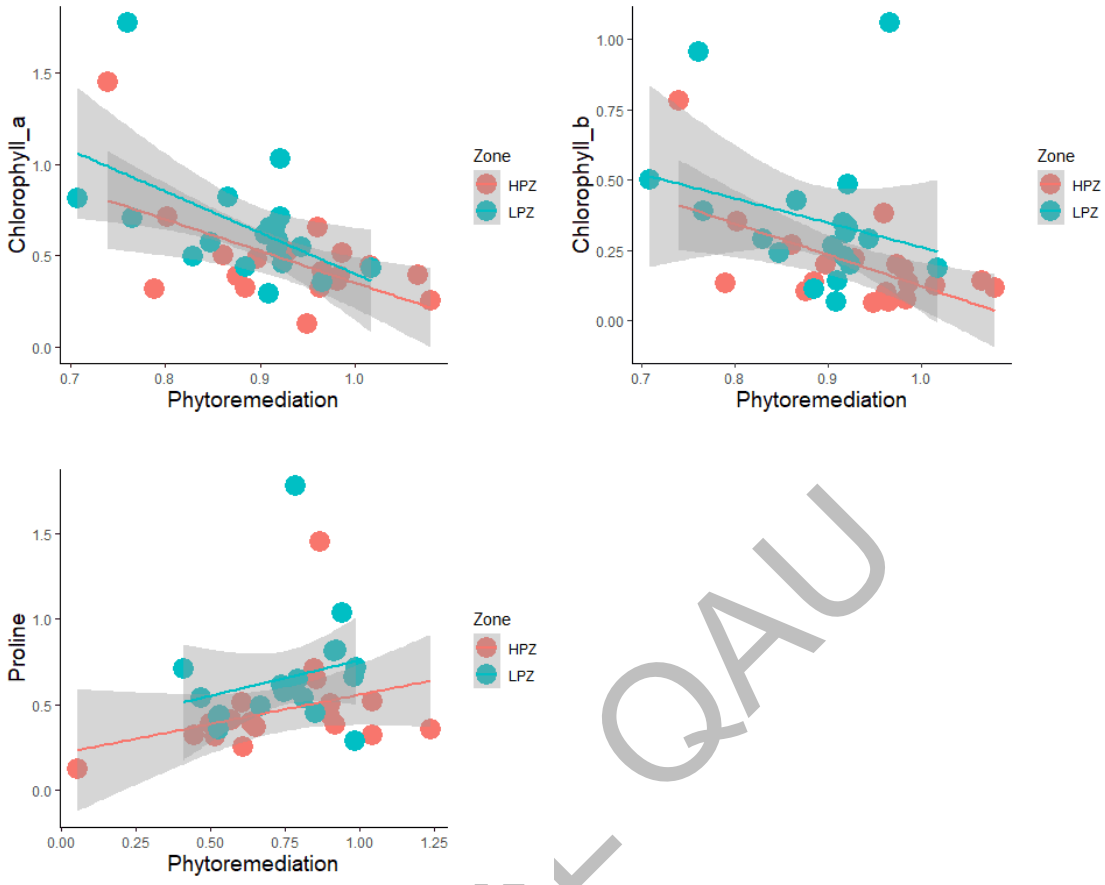


Fig. 4.16 The effect of cadmium phytoremediation leads to increase proline accumulation and decrease chlorophyll-a and chlorophyll-b in studied indicator plant species in the highly polluted zone (HPZ) and the less polluted zone (LZP) of MWPE.

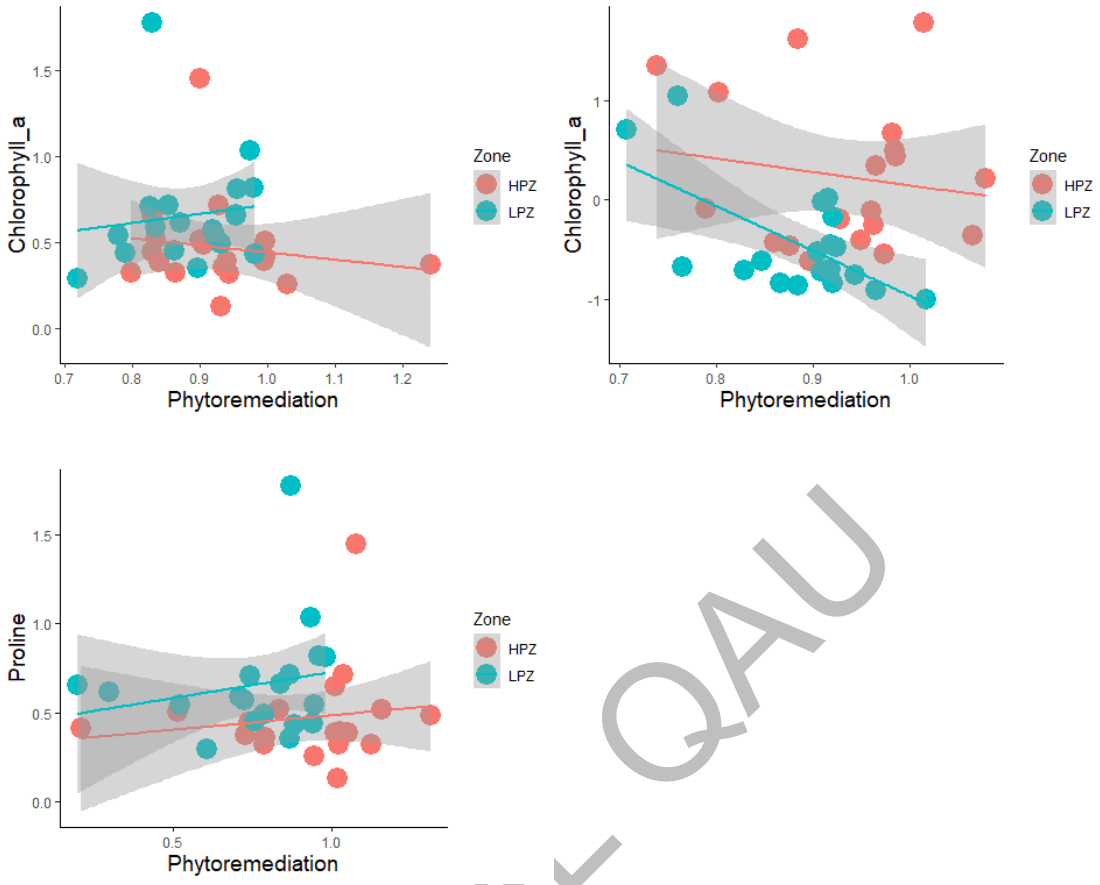


Fig. 4.17 The scattered plot for zinc phytoremediation along with proline, chlorophyll-a and chlorophyll-b of indicator species in the highly polluted zone (HPZ) and the less polluted zone (LZP) of MWPE.

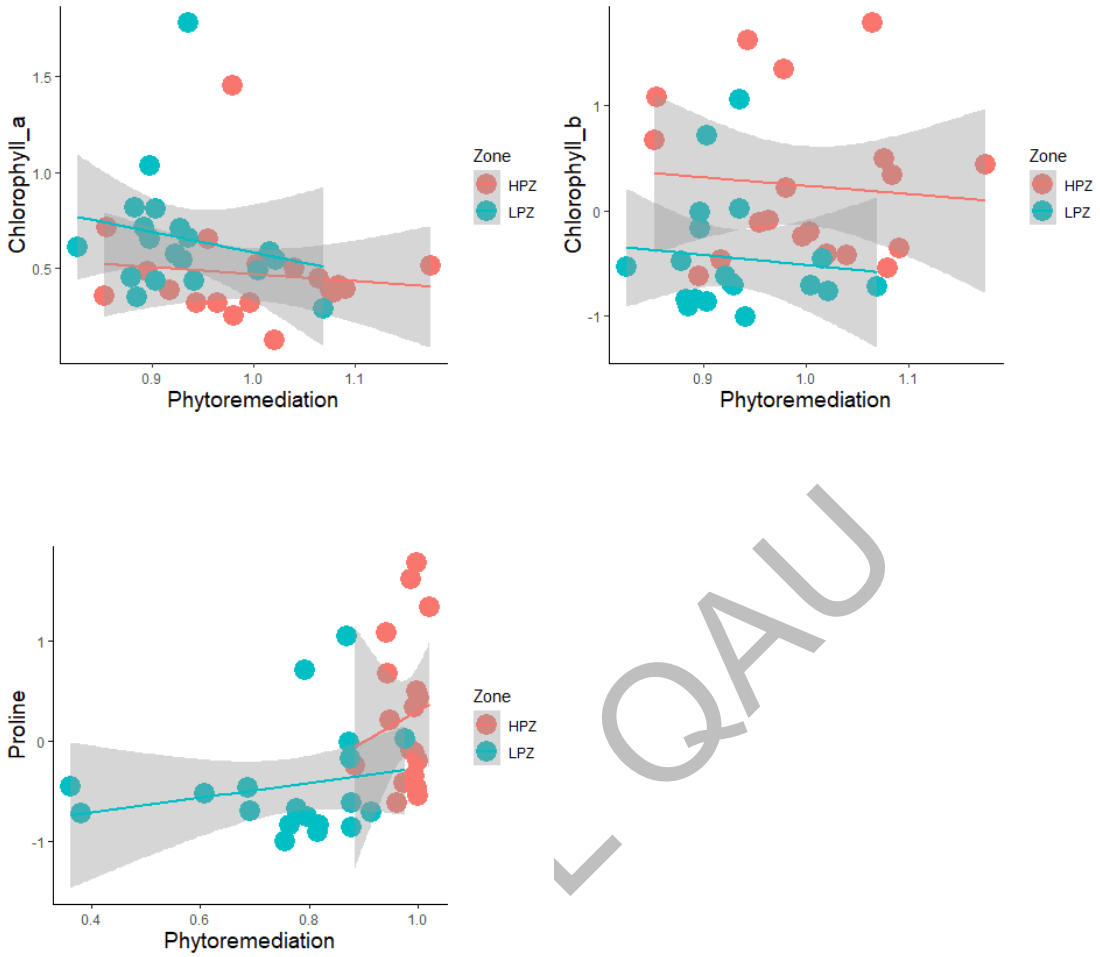


Fig. 4.18 The influence of iron phytoremediation on proline, chlorophyll-a and chlorophyll-b of indicator species in the highly and the less polluted zone of Marble waste polluted ecosystem.

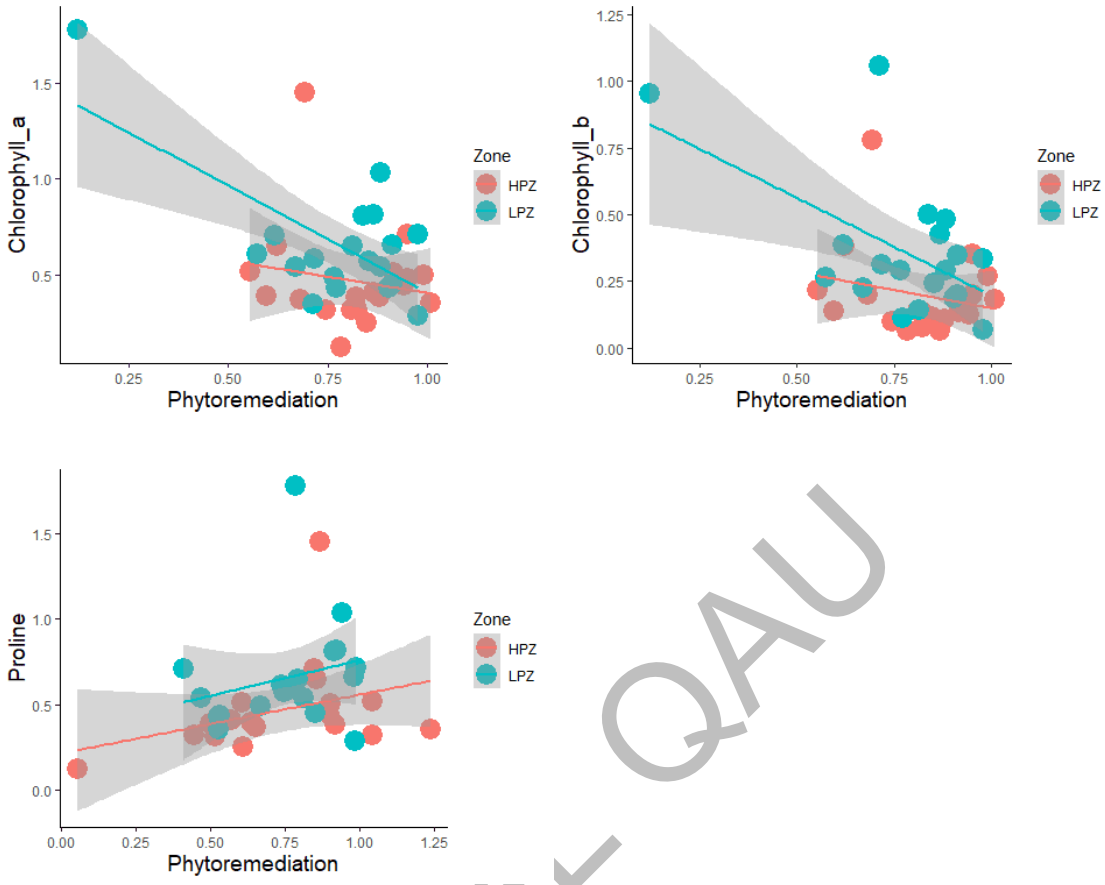


Fig. 4.19 The cobalt phytoremediation approach increases proline accumulation and reduce chlorophyll-a and chlorophyll-b in the indicator species in the highly and less polluted zones of Marble waste polluted ecosystem.

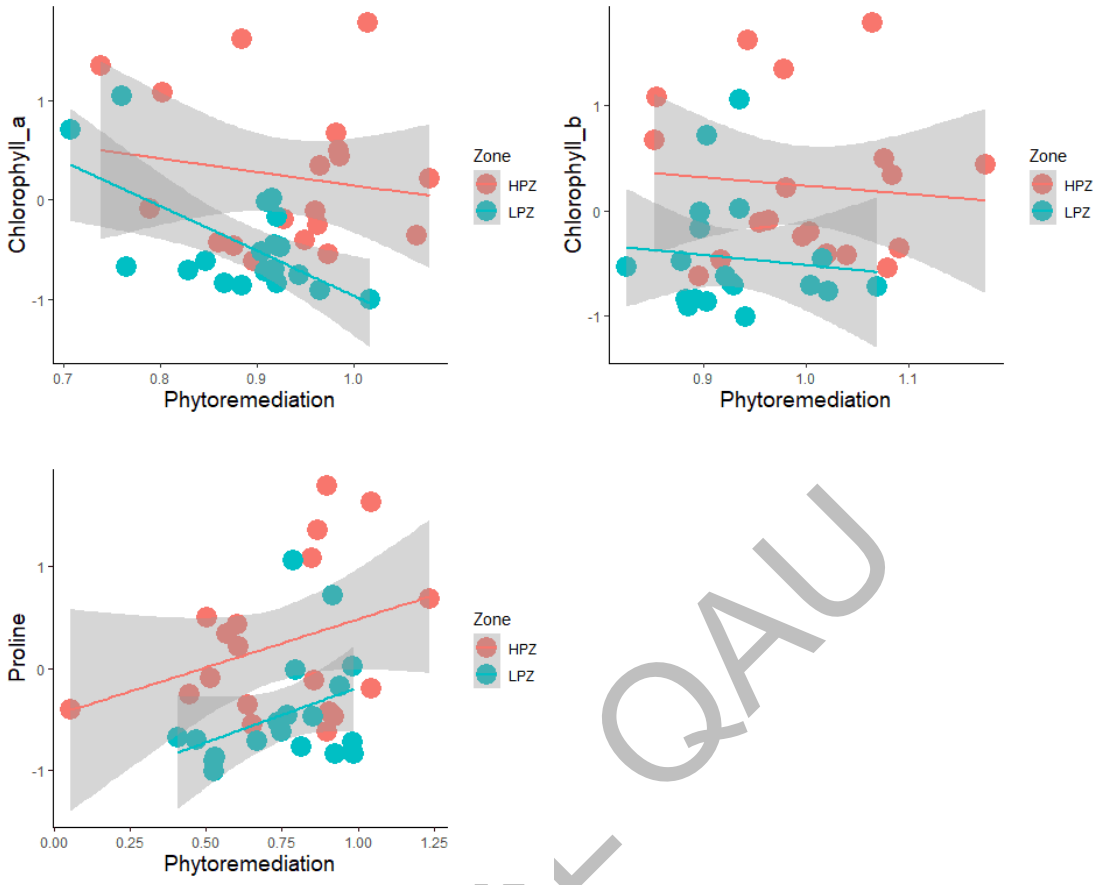


Fig. 4.20 The scattered plot for calcium phytoremediation along with proline, chlorophyll-a and chlorophyll-b of indicator species in the highly polluted zone (HPZ) and the less polluted zone (LZP) of MWPE.

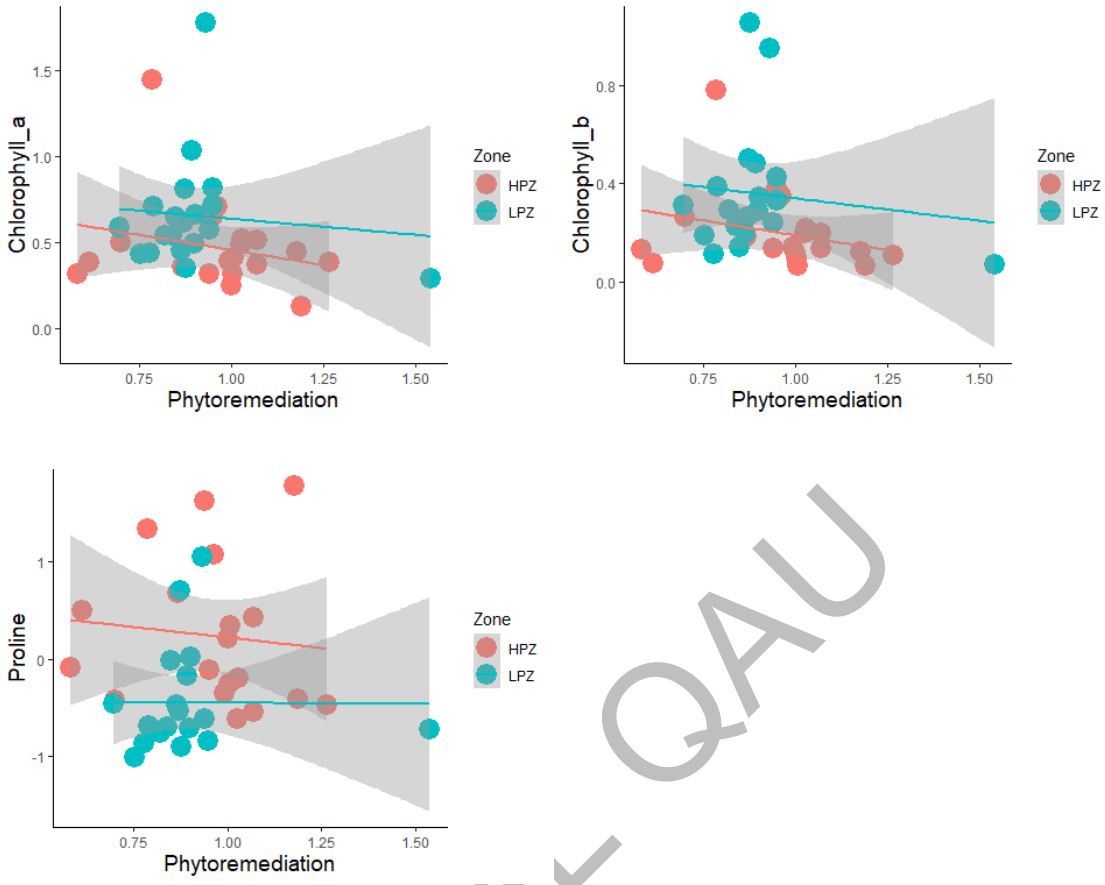


Fig. 4.21 The effect of sodium phytoremediation on proline, chlorophyll-a and chlorophyll-b in the studied plant species of the highly polluted zone (HPZ) and the less polluted zone (LZP) of MWPE, Khyber Pakhtunkhwa, Pakistan.

4.3.5 Myco-remediation / bioremediation ability of selected micro fungi

4.3.5.1 Magnesium

The biosorption or bioremediation ability of the selected micro fungi were assessed. Higher magnesium biosorption ability was observed in *Aspergillus sydowii* (average 0.25 mg/L; 50 %) followed by *Curvularia aeria* (0.178 mg/L; 35.5%), *Aspergillus brasilensi* (0.177 mg/L; 35.4 %) and *Alternaria alternata* (0.13 mg/L; 26 %) (Fig. 4.22 & Table 4.10). The detailed day wise absorption is given in Table 4.10.

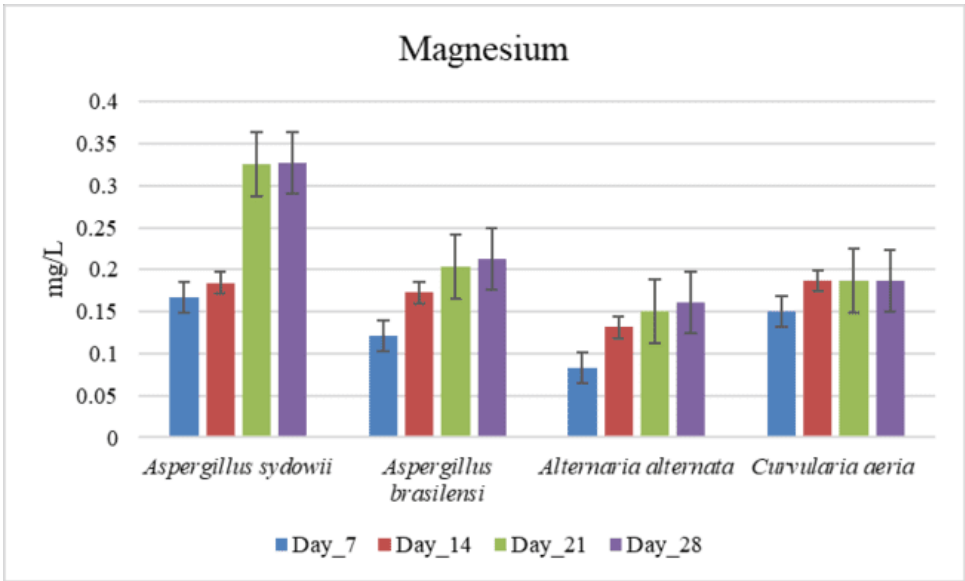


Fig. 4.22 Magnesium bioremediation by selected micro fungi over different time intervals. Error bar represent the standard error.

4.3.5.2 Calcium

Aspergillus brasilensi showed the maximum (0.17 mg/L; 34%) calcium remediation followed by *Alternaria alternata* (0.168 mg/L; 33.7 %) and *Aspergillus sydowii* (0.167; 33.6 %). While the minimum calcium remediation ability was demonstrated by *Curvularia aeria* (0.125 mg/L; 25 %) (Fig. 4.23 & Table 4.10).

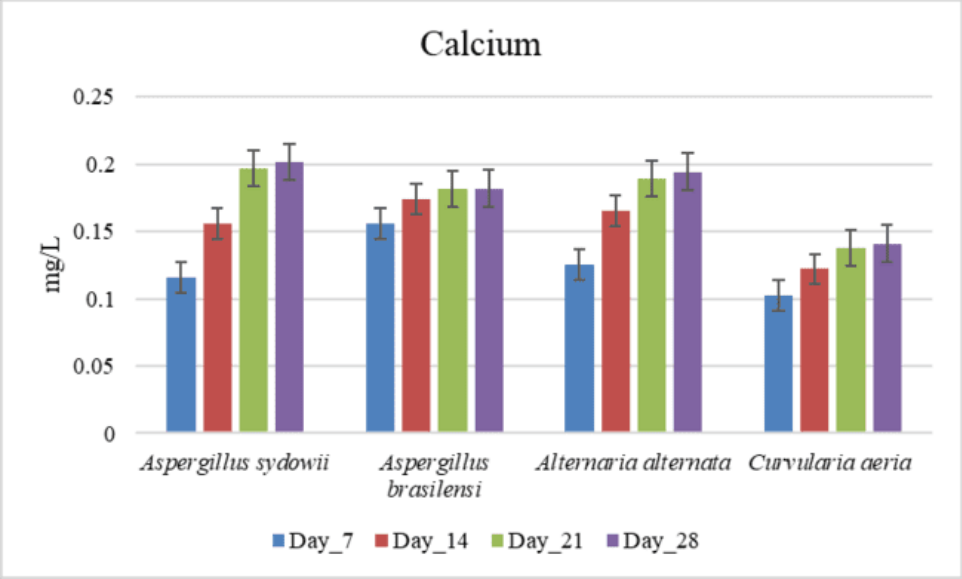


Fig. 4.23 Calcium remediation shown by selected micro fungi isolated from MWPE.

4.3.5.3 Cadmium

The highest cadmium concentration was remediated by *Aspergillus sydowii* (0.32 mg/L; 64 %) followed by *Aspergillus Brasilensi* (0.30 mg/L; 60 %). *Alternaria alternata* and *Curvularia aeria* remediated 0.18 mg/L (35 %) & 0.17 mg/L (33 %) cadmium, respectively (Fig. 4.24 & Table 4.10).

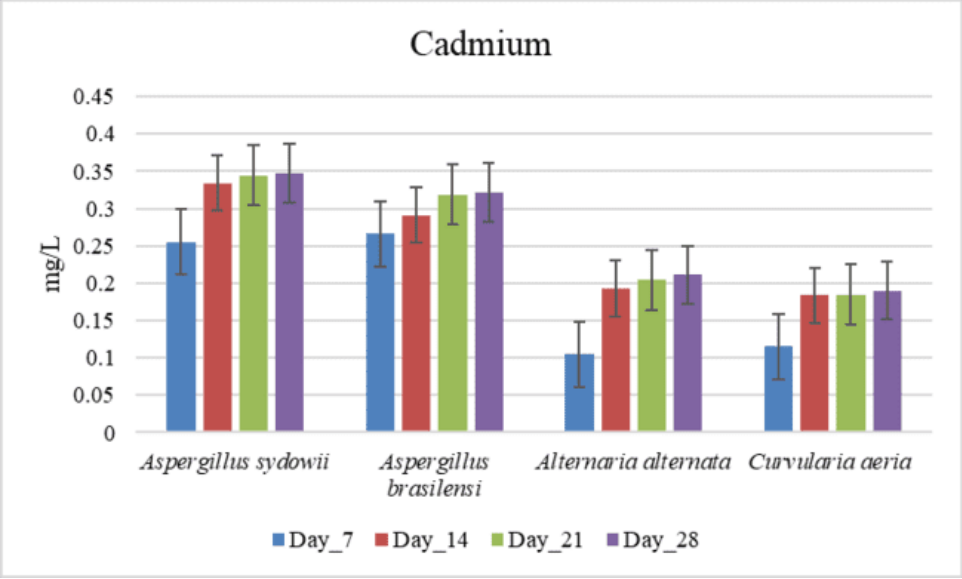


Fig. 4.24 Detail of cadmium heavy metal remediation through micro fungi.

4.3.5.4 Cobalt

Aspergillus brasilensi showed maximum cobalt bioremediation i.e., 0.0894 mg/L (17.89 %). While *Alternaria alternata* remediated 0.0892 mg/L (17.84 %), *Aspergillus sydowii* 0.0565 mg/L (11 %) and *Curvularia aeria* 0.0469 mg/L (9 %) (Fig. 4.25 & Table 4.10).

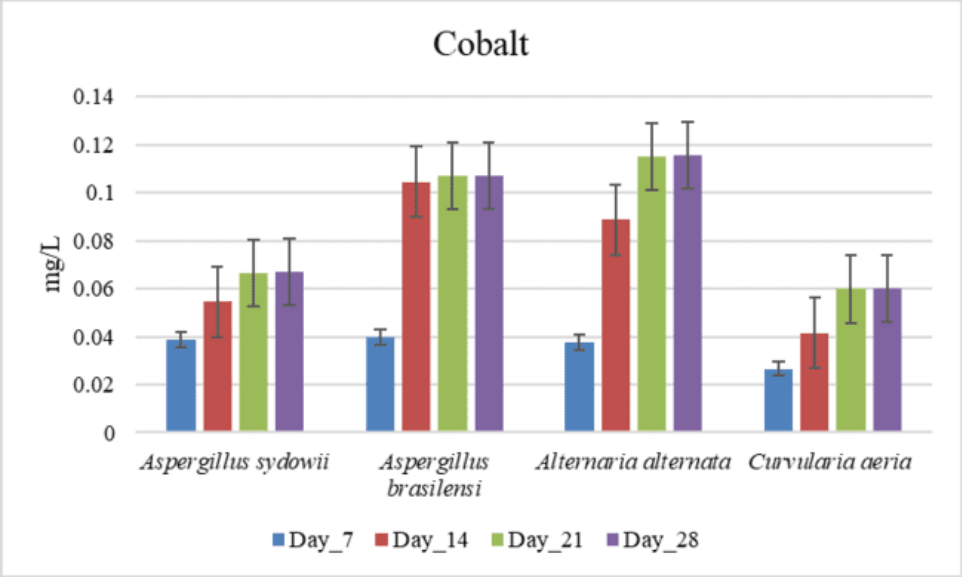


Fig. 4.25 Cobalt remediation by some of the selected micro fungi after specific time intervals.

4.3.5.5 Copper

The higher myco-remediation ability for copper heavy metal were shown by *Aspergillus sydowii* (0.175 mg/L; 35 %) followed by *Aspergillus brasilensi* (0.12 mg/L; 24 %), *Curvularia aeria* (0.114 mg/L; 22.8 %) and *Alternaria alternata* (0.113 mg/L; 22.7 %) (Fig. 4.26 & Table 4.10).

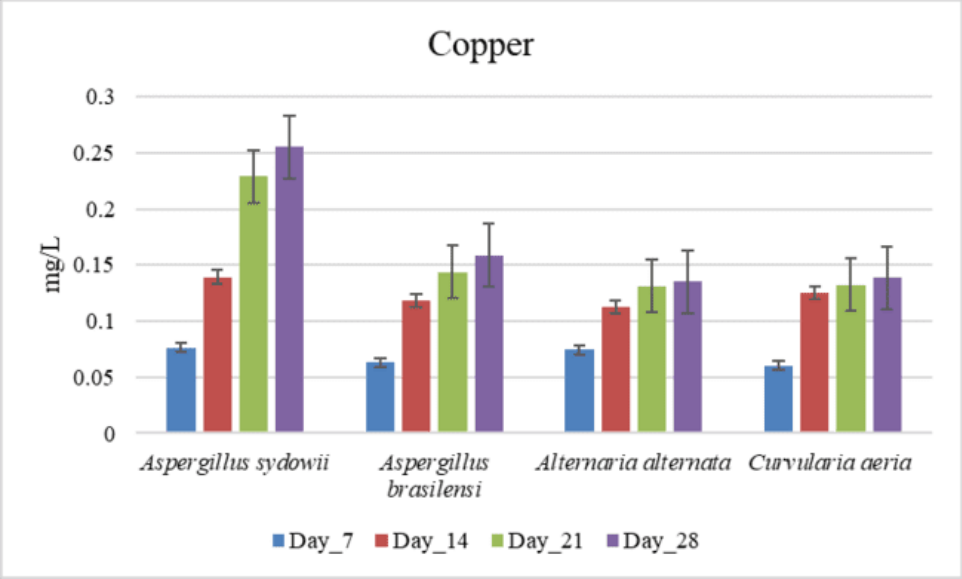


Fig. 4.26 Copper bioremediation by selected micro fungi over different time intervals.

4.3.5.6 Iron

Aspergillus sydowii showed maximum iron bioremediation i.e., 0.115 mg/L (23 %). While Aspergillus *brasilensi* remediated 0.106 mg/L (21 %), *Curvularia aeria* 0.103 mg/L (20.6 %) and *Alternaria alternata* 0.101 mg/L (20.2 %) (Fig. 4.27 & Table 4.10).

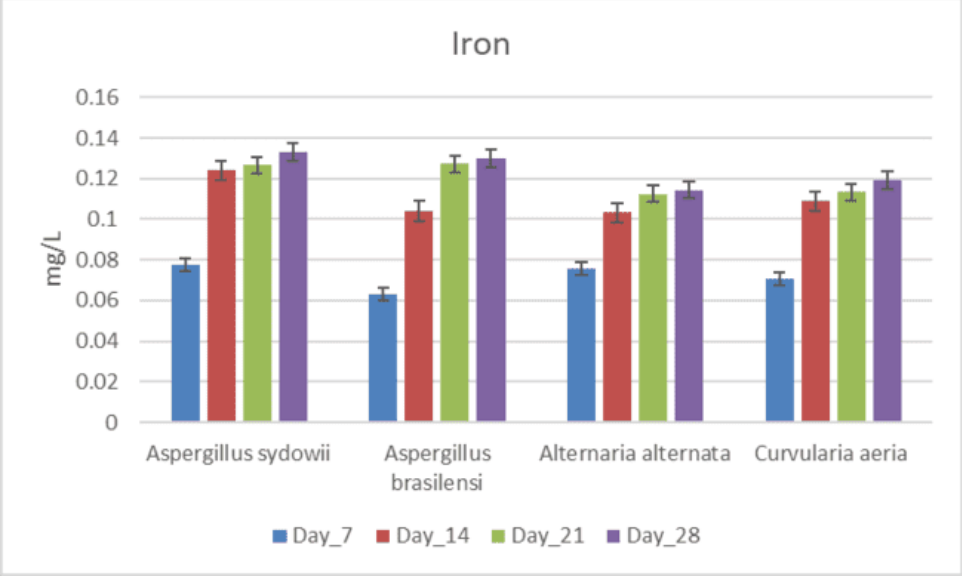


Fig. 4.27 Iron heavy metal remediation through micro fungi after different time intervals

4.3.5.7 Mercury

The maximum mycoremediation ability for mercury heavy metal was shown by *Aspergillus sydowii* i.e., 0.10 mg/L (21 %), accompanied by *Aspergillus brasilensi* 0.086 mg/L (17 %), *Curvularia aeria* 0.078 mg/L (16 %) and *Alternaria alternata* 0.075 mg/L (15 %) (18%) (Fig. 4.28 & Table 4.10).

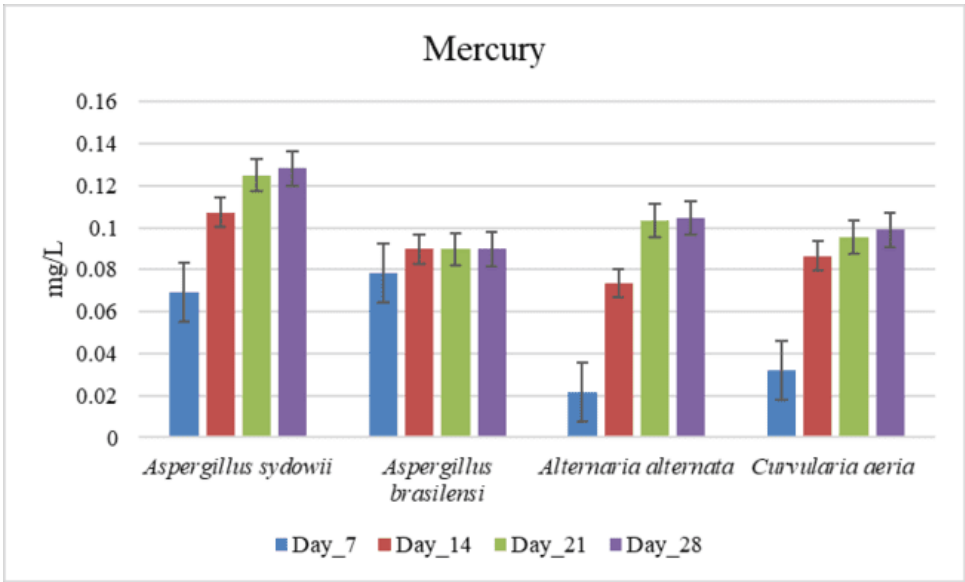


Fig. 4.28 Mercury mycoremediation ability of the studied species isolated from MWPE

4.3.5.8 Sodium

Aspergillus sydowii and *Alternaria alternata* showed the higher sodium remediation ability at 0.167 mg/L (33 %) each. While *Curvularia aeria* and *Alternaria alternata* remediated 0.17 mg/L (29 %) and 0.12 mg/L (23 %) sodium potential toxic element (Fig. 4.29 & Table 4.10).

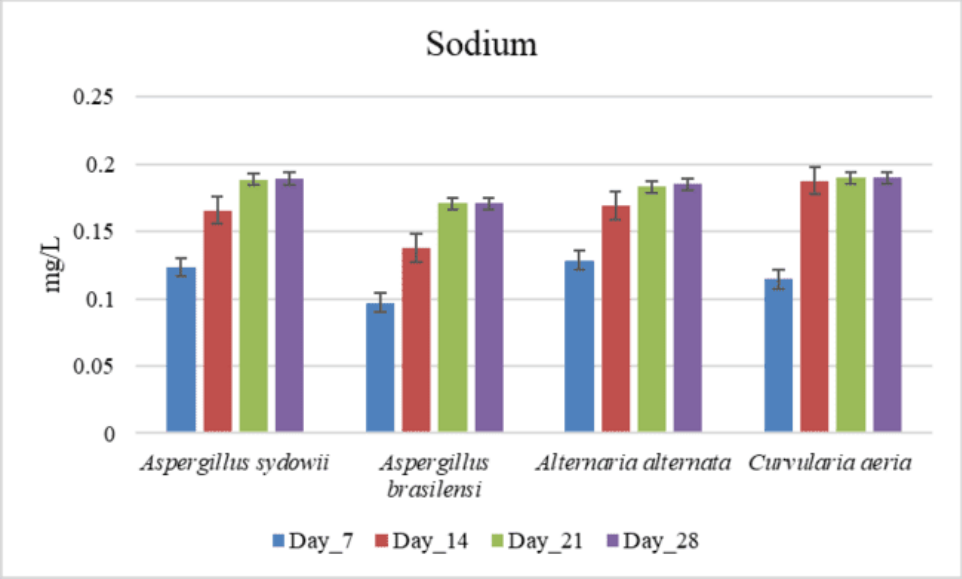


Fig. 4.29 Sodium bioremediation by micro fungal species.

4.3.5.9 Nickel

The maximum nickel remediation was shown by *Aspergillus brasilensi* (0.16 mg/L; 32 %) followed by *Curvularia aeria* (0.14 mg/L; 29%), *Aspergillus sydowii* (0.14 mg/L; 28 %) and *Alternaria alternata* (0.11 mg/L; 23 %) (Fig. 4.30 & Table 4.10).

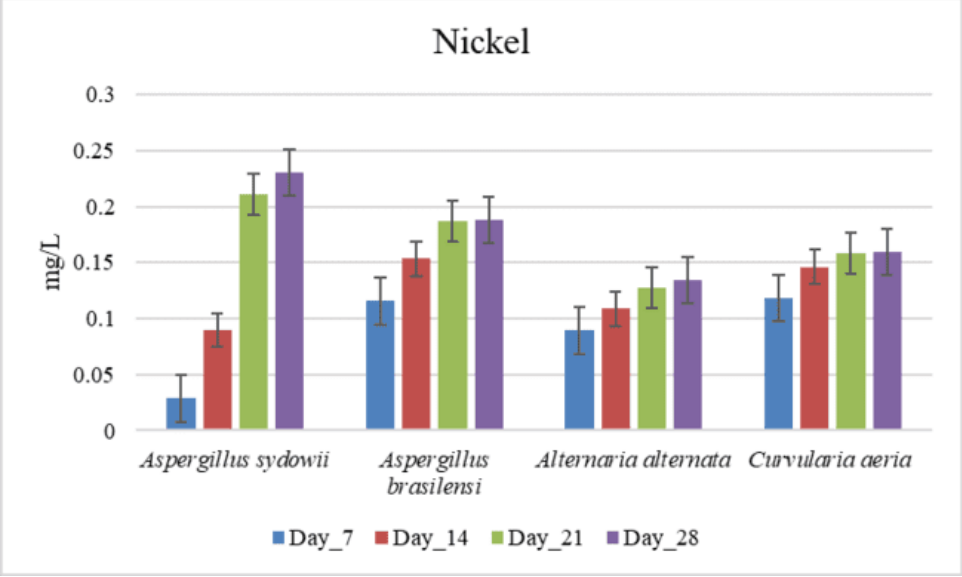


Fig. 4.30 The nickel remediation ability of micro fungi isolated from MWPE.

Table 4.10 Mycoremediation ability of selected micro fungi after specific time interval (7 days).

Species	Day_7 (mg/L)	Day_14 (mg/L)	Day_21 (mg/L)	Day_28 (mg/L)	Avg. removal efficiency (mg/L)	Day_7 (%)	Day_14 (%)	Day_21 (%)	Day_28 (%)	Avg. removal efficiency (%)
Magnesium										
Aspergillus sydowii	0.17	0.18	0.33	0.33	0.25	33.42	36.85	65.03	65.30	50.15
Aspergillus brasilensi	0.12	0.17	0.20	0.21	0.18	24.16	34.46	40.66	42.43	35.43
Alternaria alternata	0.08	0.13	0.15	0.16	0.13	16.75	26.29	30.01	32.03	26.27
Curvularia aeria	0.15	0.19	0.19	0.19	0.18	30.07	37.35	37.45	37.46	35.58
Calcium										
Aspergillus sydowii	0.12	0.16	0.20	0.20	0.17	23.24	31.22	39.44	40.34	33.56
Aspergillus brasilensi	0.16	0.17	0.18	0.18	0.17	31.24	34.72	36.26	36.40	34.66
Alternaria alternata	0.13	0.17	0.19	0.19	0.17	25.10	33.10	37.74	38.88	33.71
Curvularia aeria	0.10	0.12	0.14	0.14	0.13	20.41	24.42	27.46	28.21	25.12
Cadmium										
Aspergillus sydowii	0.26	0.33	0.34	0.35	0.32	51.06	66.82	68.88	69.51	64.07
Aspergillus brasilensi	0.27	0.29	0.32	0.32	0.30	53.25	58.23	63.76	64.28	59.88
Alternaria alternata	0.10	0.19	0.20	0.21	0.18	20.97	38.59	40.85	42.24	35.66
Curvularia aeria	0.11	0.18	0.18	0.19	0.17	23.00	36.72	36.98	38.04	33.69
Cobalt										
Aspergillus sydowii	0.04	0.05	0.07	0.07	0.06	7.73	10.89	13.27	13.38	11.31
Aspergillus brasilensi	0.04	0.10	0.11	0.11	0.09	7.93	20.88	21.38	21.40	17.90
Alternaria alternata	0.04	0.09	0.12	0.12	0.09	7.52	17.72	23.01	23.12	17.84
Curvularia aeria	0.03	0.04	0.06	0.06	0.05	5.32	8.31	11.97	11.99	9.40
Copper										
Aspergillus sydowii	0.08	0.14	0.23	0.26	0.17	15.27	27.89	45.79	51.02	34.99

Aspergillus brasilensi	0.06	0.12	0.14	0.16	0.12	12.64	23.59	28.77	31.75	24.19
Alternaria alternata	0.07	0.11	0.13	0.14	0.11	14.96	22.59	26.27	27.07	22.72
Curvularia aeria	0.06	0.13	0.13	0.14	0.11	12.03	25.10	26.43	27.72	22.82
Iron										
Aspergillus sydowii	0.08	0.12	0.13	0.13	0.12	15.54	24.80	25.32	26.58	23.06
Aspergillus brasilensi	0.06	0.10	0.13	0.13	0.11	12.65	20.82	25.42	26.02	21.23
Alternaria alternata	0.08	0.10	0.11	0.11	0.10	15.14	20.67	22.49	22.89	20.30
Curvularia aeria	0.07	0.11	0.11	0.12	0.10	14.15	21.80	22.66	23.87	20.62
Mercury										
Aspergillus sydowii	0.07	0.11	0.12	0.13	0.11	13.84	21.44	24.99	25.66	21.48
Aspergillus brasilensi	0.08	0.09	0.09	0.09	0.09	15.70	17.94	17.94	17.98	17.39
Alternaria alternata	0.02	0.07	0.10	0.10	0.08	4.31	14.70	20.66	20.92	15.15
Curvularia aeria	0.03	0.09	0.10	0.10	0.08	6.37	17.28	19.08	19.77	15.63
Sodium										
Aspergillus sydowii	0.12	0.17	0.19	0.19	0.17	24.64	33.14	37.66	37.86	33.33
Aspergillus brasilensi	0.10	0.14	0.17	0.17	0.14	19.40	27.58	34.13	34.17	28.82
Alternaria alternata	0.13	0.17	0.18	0.19	0.17	25.71	33.89	36.62	37.03	33.31
Curvularia aeria	0.11	0.19	0.19	0.19	0.17	22.88	37.54	37.96	37.98	34.09
Nickel										
Aspergillus sydowii	0.03	0.09	0.21	0.23	0.14	5.71	18.00	42.27	46.14	28.03
Aspergillus brasilensi	0.12	0.15	0.19	0.19	0.16	23.10	30.68	37.45	37.65	32.22
Alternaria alternata	0.09	0.11	0.13	0.13	0.12	17.85	21.75	25.50	26.90	23.00
Curvularia aeria	0.12	0.15	0.16	0.16	0.15	23.67	29.27	31.75	31.97	29.17

4.4 Discussion

Bioremediation is one of the better strategies to restore polluted ecosystems at a low cost (Nascimento and Xing 2006; Wei et al. 2020; Yan et al. 2020). Certain species of plants and microorganisms retain the inherent ability of bioaccumulation, translocation and degradation of different types of pollutants (Sepehri et al. 2020). In this way, they play a role as a sink for biologically hazardous materials (Schwitzguébel 2017).

The current study focused on evaluation of indicator plant species in relation to their tolerance levels of marble pollution. These species were selected based on the higher values of density, cover, frequency, importance value index in the region followed by indicator species analysis using phytosociological attributes and PCORD analyses. Phytoremediation abilities of these species were determined through atomic absorption spectrophotometry. The study shows that from 0.11 to 610 mg/kg of heavy metals can be detected in the soil of the less marble waste polluted zone and 0.91- 643 mg/kg in the heavily polluted zone. Heavy metal concentration varied from 12.1 to 299 mg/kg and 15-439 mg/kg in the less, moderate and heavily polluted marble wastewater, respectively. Edaphic factors i.e., soil pH, organic matter, phosphorous and potassium varied significantly among these three polluted zones. Likewise, (Mulk et al. 2017) examined the influence of marble effluents on water along with sediment quality. They concluded that the concentration of heavy metals considerably increased with the increase in marble wastewater pollution. (Noreen et al. 2019b) assessed heavy metal concentrations along different physico-chemical parameters in the marble industrial effluents in Mardan Khyber Pakhtunkhwa, Pakistan. They showed higher amounts of heavy metals in water and worker's blood than the permissible limit set by the World Health Organization, Occupational Health and Safety Act, Occupational Health & Safety Division and Agency for Toxic Substances & Disease Registry, USA. Whereas, (Zornoza et al. 2013) reported the effect of pig slurry combined and separately along with marble waste for heavy metals stabilization and organic matter mineralization. According to these researchers, the combined marble waste and pig slurry was the most significant treatment resulting in the highest reduction in the availability of metals which helped to reduce soil carbon loss and stabilize organic matter concentration. In other words, pig slurry

mixed with marble waste reduced heavy metal accumulation in plants (Kabas et al. 2012).

The current project revealed that the BCF, TF and BAC values recorded for roots and shoots of all the selected plants indicate that these species are significant phytostabilizors and phytoextractors of the marble waste polluted ecosystem. Similar to our study (Kumar and Thambavani 2012), investigated roadside vegetation exposed to pollution and observed *Pongamia pinnata, Polyalthia longifolia, Azadirachta indica* and *Ficus religiosa* helping in the remediation of pollution. Whereas, (Noor et al. 2015) worked on Air Pollution Tolerance Index and Anticipated Performance Index estimation of vegetation near to marble industrial region and concluded considerable impacts on the vegetation. Similarly, Malik et al. (2010) also examined the accumulation of different metals in various plant species. According to them, heavy metal accumulation and bioavailability significantly depends on the type of plant species, soil condition, climate, transfer progressions, sequestration, type of plant root system and their response to elements and seasonal cycles. Furthermore, higher soil pH resulted in a significant reduction in heavy metals and their leaching owing to their decreased solubility in less acidic soil. This reduced the absorption of heavy metals from the soil and their translocation into plant tissues (Liu et al. 2018a).

Pollution is a dynamic phenomenon that affects every aspect of plant chemistry, biology and physiology, varying in space and time. Proline accumulation is a typical physiological response/reaction of certain plant species to a wide range of environmental stresses/pollution. It has been recorded to accumulate in plant tissues or organs exposed to pollution, salt, temperature, drought, infection by different pathogens/insects and various gases like NO2, SO2 and NH3 etc. (Saradhi, 1991). The current research study has shown that the proline concentration increases when the concentration of marble wastewater pollution increases. The amount of proline concentration encompasses a highly significant relationship with the chlorophyll, biological concentration factors, translocation factor and bioaccumulation coefficient factor/ability of the examined plant species (*A. altissima, A. donax, C. dactylon, E. canadensis* L., *C. sativa, F. carica, L. aphaca* L, *M. alba* L., *P. alba* L, *R. pseudoacacia* and *V. negundo* L) in the marble wastewater pollution environment.

Proline has an important role to play in the protection of certain enzymes from denaturation, serving as a source of nitrogen and carbon, stabilizing protein synthesis, cytosolic acidity and scavenging hydroxyl radicals (Mateos et al. 2020). The amount

of proline concentration increases in plant species as a defense mechanism to cope with environmental stress and to improve survival (Akshita et al. 2018; Amiri et al. 2020; Bates et al. 1973b). Exposure of plant species to pollutants causes a reduction in the amount of photosynthetic pigments (Arellano et al. 2017; Kanwal et al. 2020; Li et al. 2017; Lin and Jin 2018). These and other physiological variations help plants to maximize their efficiency for resource utilization under environmental stress. (Zouari et al. 2016) also described the role of exogenous proline in cadmium heavy metal stress alleviation. According to them, proline supplemented the plant‘s antioxidant defense and mineral uptake, while diminishing heavy metal (cadmium) oxidative damage in a young date palm. Proline increased photosynthetic activities and mineral nutrition under salt stress in *Olea europaea* (olive tree) (Ahmed et al., 2011). Similarly, (Xu et al. 2009) also reported that the exogenous proline increased heavy metal tolerance in *Solanum nigrum* by improving the activities of antioxidant enzymes. The application of proline reduces the toxic effects of arsenate by reducing arsenate accumulation and oxidative stress in *Solanum melongena* (Singh et al. 2015a). Plants exposed to adverse environmental conditions exhibit variation in functions for the accumulation of proline i.e., sustaining osmotic and cell turgor equilibrium, scavenging reactive oxygen species and preventing electrolyte leakage by stabilizing the cell membrane (Hayat et al. 2012; Shahid et al. 2014b). The beneficial effect of proline is either direct i.e., increase in photosynthetic rate and mineral nutrition, or indirect in the form of tolerance against diseases in the plant species. (Shahid et al. 2014b) reported that the exogenous proline concentration amplified fresh plant weight by enhancing CO_2 absorption for photosynthesis in *Pisum sativum*. The protective effect of exogenous proline may be linked to improved mineral uptake (Dawood et al. 2014). On the other hand, a significant proline amount has also been found in the reproductive organs of various plant species for developmental purposes (Mattioli et al. 2009). For example, (Chiang and Dandekar 1995) reported proline accounts for up to 26% of the whole pool of amino acids in reproductive tissues (pollen, seeds, siliques and florets) as compared to 1-3% in the vegetative parts of *Arabidopsis thaliana*. (Schwacke et al. 1999) also reported a higher concentration of proline in tomato flowers as compared to other vegetative organs.

The current study has revealed that the amount of chlorophyll content decreased in all selected plant species when moving from the highly marble waste polluted zones

towards the moderate and less polluted zones. Exposure of plants to various types of pollution decreases the concentration of their photosynthetic pigments, namely carotenoids and chlorophyll. It also affects pedicle length, plant yield, seed germination and inflorescence number (Nithamathi and Indira 2005). Pollution and heavy metal stress decrease the activity of enzymes involved in chlorophyll synthesis and hence reduce photosynthetic activity (de Filippis and Pallaghy, 1994), disruption of membrane (Caspi et al. 1999), metal ion exchange for chlorophyll molecule and decrease in the concentration of leaf chlorophyll (Kastori et al. 1998). A significant decrease in the chlorophyll content was observed during drought stress in *Catharanthus roseus, Gossypium hirsutum, Helianthus annuus* and *Vaccinium myrtillus* (Jaleel et al. 2008; Kiani et al. 2008; Massacci et al. 2008; Tahkokorpi et al. 2007). When relative water content and leaf water capacity reduce the foliar photosynthetic rate of higher plants decreases (Lawlor and Cornic 2002). This may be due to metabolic impairment or stomatal closure (Lawson et al. 2003).

Furthermore, this chapter also assessed the mycoremediation ability of *Aspergillus sydowii, Aspergillus brasilensi, Curvularia aeria* and *Alternaria alternata.* All these species showed efficient bioremediation ability against cadmium, copper, cobalt, magnesium, iron, mercury, nickel, sodium and calcium heavy metals. Similar to the current finding (Joseph et al. 2011) also worked on the mycoremediation of some fungal species and assessed *Aspergillus sydowii*, *A. brasilensi* and *A. alliaceus* for copper, zinc and tin remediation. Likewise, (Kisielowska et al. 2012) reported *Aspergillus* species for heavy metal remediation. Our results are in close harmony with the findings of (Brunner et al. 2018; El-Morsy et al. 2017; Ojha et al. 2017), where they reported that *Aspergillus sydowii* and *Aspergillus brasensli* were effective strains for the remediation of different types of metal pollution. Different *Aspergillus* species were isolated from heavy metal contaminated sites showing high tolerance against arsenic, copper, zinc, magnesium heavy metals (Singh et al. 2015b; Vickers 2017; Wu et al. 2016). These fungal species also improve soil physio chemical properties and plant growth as well (Abu-Elsaoud et al. 2017). Similar to the current findings, (Khan et al. 2019) worked on the mycoremediation of heavy metal polluted sites at Hattar Industrial Estate, Pakistan through indigenous metallotolerant fungal isolates and reported the *Aspergillus* species for the remediation of cadmium and chromium heavy metals. In addition to, the evaluation of polluted ecosystem has led to the identification of different fungi that can remove heavy metals. These

indigenous species are not only resistant to the harmful effects of heavy metal but also show adaptations to the polluted ecosystem (Khan et al. 2019).

4.5 Conclusion

It is concluded that the identified indicator plant species i.e., *Adiantum capillus-veneris, Ailanthus altissima, Albizia lebbeck, Calotropis procera, Cynodon dactylon, Datura innoxia, Debregeasia salicifolia, Desmostachya bipinnata, Dodonaea viscosa, Erigeron bonariensis, Ficus carica, Morus alba, Morus nigra, Parthenium hysterophorus, Persicaria glabra, Ricinus communis, Setaria viridis, Tamarix aphylla, Withania somnifera* and micro fungi i.e., *Aspergillus sydowii, Aspergillus brasilensi, Curvularia aeria* and *Alternaria alternata* have a significant role in the remediation of heavy metals present in marble waste polluted ecosystems and hence could be used for the phytoremediation and mycoremediation purposes. The proline accumulation increases in plant species with the increase in marble waste pollution while chlorophyll decreases with an increase in pollution. The fluctuation in concentration of both proline and chlorophyll was due to the phytoremediation property of the plant species. It is recommended that these plant species could be grown to remediate the MWPS in the marble processing industries and its catchments. Such findings can also be applied on the broader scale in drives to revegetate and reforest polluted industrial zones.

Mapping of heavy metals and temporal changes in NDVI in the MWPE - (1986-2021)

5.1 Introduction

The rapid growth of technology can help to identify and solve environmental problems. The elemental interactions of heavy metals and their chemical distribution in the soil have been studied (Eze et al. 2010; Ren et al. 2017; Udeigwe et al. 2015). However, there is still a need for site-specific studies of the distribution of heavy metals under different pollution and environmental conditions. Site specific studies can play an essential role in understanding the distribution of pollutants and in remediation planning.

A Geographic Information System (GIS) can be used to create, manage, analyze, and map all types of data. A GIS links data to maps and integrates location data with descriptive information. This information can provide a foundation for mapping and analysis. GIS helps users to understand patterns, geographical context, and relationships. The benefits include improved communication, efficiency, better management and decision-making (O'Looney 2000).

GIS provides the ability to relate previously unrelated information through the location as a key index variable. Earth spacetime can record location and extent through date and time of occurrence and three coordinates (x, y, and z). The x coordinate represents longitude, y latitude and z elevation. The actual physical location or extent are identified through all earth-based spatial-temporal sites and extent reference should be relatable to one another (Naidu 2015). Based on this, GIS has begun to open new scientific inquiry and study avenues. Hundreds of thousands of organizations in virtually every field use GIS to make maps that communicate, perform analysis, share information, and solve complex problems worldwide.

GIS has various applications in multiple domains, including benefit-risk management and urban planning by creating awareness and sharing knowledge about the environment, natural resources, and potential disasters (Pierce and Clay 2007). Organizations like ESRI, Here Maps, and Leidos work on various models regarding

the nations' environmental assets, advanced operating systems, and even security systems. Applications of GIS also allow people and organizations to manage geological interpretations and evaluate the spatial data in a granular format (Singh 2019).

The findings of spatial analysis research have led to clear conclusions for several decades (Fotheringham and Charlton 1994). Many researchers and practitioners adopt GIS as a front end and a back end to georeferenced databases. GIS serves as a spatial database management system for managing georeferenced data and a spatial decision support system for mapping and communicating geographic information to colleagues, decision-makers, and stakeholders.

In recent decades, GIS and remote sensing tools have been extensively used to identify and quantify changes in vegetation and the spatial distribution of heavy metals. The assessment and mapping of heavy soil metals can assist the development of strategies to promote sustainable use of soil resources, decrease soil degradation and expand crop production. This will include identifying contamination levels and assessing associated impacts on the environment and human health. Remediation of soils polluted by heavy metals is a major global ecological issue. Remote sensing is one of the most important methods for environmental investigation, mapping, and soil survey (Lillesand et al. 2015). In addition, image investigation by remote sensing can directly record short- and long-term effects on vegetation cover. For example, Landsat data can be used to provide a precise classification of vegetation cover changes over time (Tsarouchi and Buytaert 2013). The change detection by remote sensing relies on the difference in spectral signatures corresponding with the variation in vegetation cover. Change detection can be accurately determined by using GIS due to its high volume of spatial and non-spatial data handling abilities (Sakthivel et al. 2010). Numerous change detection methods have been developed to use remotely sensed imageries. A range of change detection techniques have been developed and studied for their advantages and disadvantages. Normalized Difference Vegetation Index, supervised classification, fuzzy classification, unsupervised classification or PCA/ clustering and hybrid classification are the most frequently applied methods used for classification (Zhang et al. 1999).

5.1.1 Normalized Difference Vegetation Index (NDVI)

The vegetation index is a simple and effective measurement parameter used in remote sensing to designate the vegetation cover and crop growth status (Ahmadi and Nusrath 2010). There are many indices for highlighting vegetation-bearing areas in remote sensing. Among the most important ones is the Normalized Difference Vegetation Index (NDVI), widely used in research on the global environment and climate change (Hacihaliloglu and Karta 2004). NDVI is calculated as the ratio difference between measured canopy reflectance in the red and near-infrared bands. The values of NDVI vary with the absorption of red light by plant chlorophyll and the reflection of infrared radiation by water-filled leaf cells (Hacihaliloglu and Karta 2004). The leaf area index, biomass, plant productivity, fractional vegetation cover, chlorophyll concentration in leaves, accumulated rainfall, and other vegetation properties have all been estimated using the NDVI value. Such relationships are derived by comparing space-derived NDVI values to ground-measured values of these variables. The multispectral remote sensing data technology is used to find vegetation index, land cover classification, water bodies, open area, agriculture area, hilly area, thick forest and thin forest with few band combinations of remote sensed data. The multispectral remote sensing images (MSRS) can be employed to provide a better understanding of the earth's environment (Hacihaliloglu and Karta 2004; Karaburun 2010). The science and art of acquiring information and extracting features in the form of spatial, spectral and temporal information about some objects, areas, or phenomena, such as vegetation, land cover classification, agricultural land, urban areas and water resources, without coming into physical contact with these objects is known as multispectral remote sensing (Chouhan and Rao 2011).

Remote sensing data has diverse applications, including forest classification, fire and snow mapping, land cover classification, environmental pollution and crop prediction (Hacihaliloglu and Karta 2004). Multispectral remote sensing images convey important spatial features (Ramachandra and Kumar 2004). Remote sensing is classified into thermal infrared, visible and reflective infrared, and microwave remote sensing (Xie et al. 2010). The digital image processing of satellite data gives a tool for analyzing images via various mathematical indices and algorithms. The features are based on reflectance characteristics and indices formulated to highlight the feature of interest in the image (Gao 1996). Recently, researchers have employed new space

remote sensing, which provides opportunities to monitor environmental applications and give up to date information in the space and time domain. Optical remote sensing is a commonly used data source for acquiring biophysical variables (Thiruvengadachari 1988), crop variables (Jeyaseelan and Venkataratnam 2003), crop type mapping (Yang et al. 2011) and biomass estimation (Kogan 2000) due to the sensitivity of crop leaves to visible and infrared bands (Aparicio et al. 2002; Ayyangar et al. 1980). Contradictory to optical sensors, Synthetic Aperture Radar (SAR) sensors can acquire images under all weather conditions, making them suitable for long-term and multi-seasonal monitoring (Lan et al. 2009). Current SAR systems are becoming an increasingly valuable source for environmental monitoring (Carlson and Ripley 1997; El-Shikha et al. 2007). The ability to discriminate crops enables production of reliable and accurate crop maps for cultivated areas using multi-temporal analysis. Finally, multi-temporal environment mapping is achieved via object-based image analysis. Proper extraction of environmental pollution data and spatial-temporal monitoring are crucial for long-term ecological management (Kim et al. 2008). Different tools and techniques are used to analyze satellite images including normalized difference vegetation index, artificial neural network, singular value decomposition, and satellite image contrast enhancement. Among them, the NDVI is very useful in detecting surface features. It can help us to understand how natural and anthropogenic pressure affects the flora or vegetation cover.

This chapter evaluates the spatial distribution of the concentrations of heavy metals present in the marble waste polluted ecosystem of Khyber Pakhtunkhwa province and an assessment of temporal changes in the NDVI for the last forty years in the region.

5.2 Materials and Methodology

5.2.1 Spatial distribution of heavy metals

Spatial interpolation is usually used when the data is collected at various locations to generate continuous information (Moghanum, 2013). An interpolation method, i.e., Inverse distance weighted (IDW), measures the surrounding prediction location. Using this approach, the geostatistical relationships among the known points (IDW) of ArcGIS 10.8 were used to interpolate the heavy metal concentrations in the studied region. The heavy metal concentration data were used as input for GIS mapping. The spatial distribution pattern for each heavy metal was created using ArcGIS software.

5.2.2 Spatial autocorrelation

Spatial autocorrelation was used to describe the spatial pattern formed by heavy metals. This method can help to understand the degree of similarity and differences among variables. Moran‘s Index (MI) was used for the determination of spatial autocorrelation.

5.2.3 Determination of Normalized Difference Vegetation Index (NDVI)

The NDVI is a vegetation index calculated from satellite data bands. It approximates the density of vegetation at a pixel based on various intensities of reflected sunlight. It ranges from -1 to 1. The general formula for NDVI calculation is as follows:

NDVI= Near Infrared – Red / Near Infrared + Red

Google Earth Engine (GEE) was used for the calculation/ extraction of NDVI from 1986 to 2021. The general script for GEE consists of feature collection, buffer points, three NDVI functions for Landsat 5, 7 & 8, Fmask, image collection etc. (Appendix X). Finally, the NDVI data were downloaded in CSV file format.

5.2.4 Statistical Analyses

The bivariate statistical analysis and structural equation modelling were used to explore the impact of marble pollution (calcium + $CaCO_3$), precipitation and temperature on NDVI of the marble polluted and non-polluted ecosystems. The marble pollution, precipitation and temperature were treated as explanatory variables,

while NDVI was a dependent variable. R software was used for these statistical evaluations.

5.3 Results

The soil of marble waste polluted ecosystem was analyzed for the quantification of heavy metals or potential toxic elements. The contaminated soil has a chromium content ranging from 0.11-32.11 (average 13.11) mg/kg, nickel 17.13-50.55 (34.42) mg/kg, copper 16.37-91.68 (38.57) mg/kg, manganese 0.67-131.65 (42.45) mg/kg, zinc 30.33-86.03 (57.60) mg/kg, iron 0.38-225.9 (94.61) mg/kg, cobalt 2.23-12.99 (7.19) mg/kg, cadmium 0.59-56.93 (20.50) mg/kg, magnesium 13.49-471.72 (191.23) mg/kg and calcium 2.764-643.85 (279.27) mg/kg (Fig. 5.1-5.11; Appendix table X). On the basis of average metal concentration, calcium has the highest concentration followed by Mg> Fe>Zn>Mn>Cu>Ni>Cd>Cr>Co in the region. The spatial distribution of these potential toxic elements is illustrated in the following figures (Fig. 5.1– 5.11).

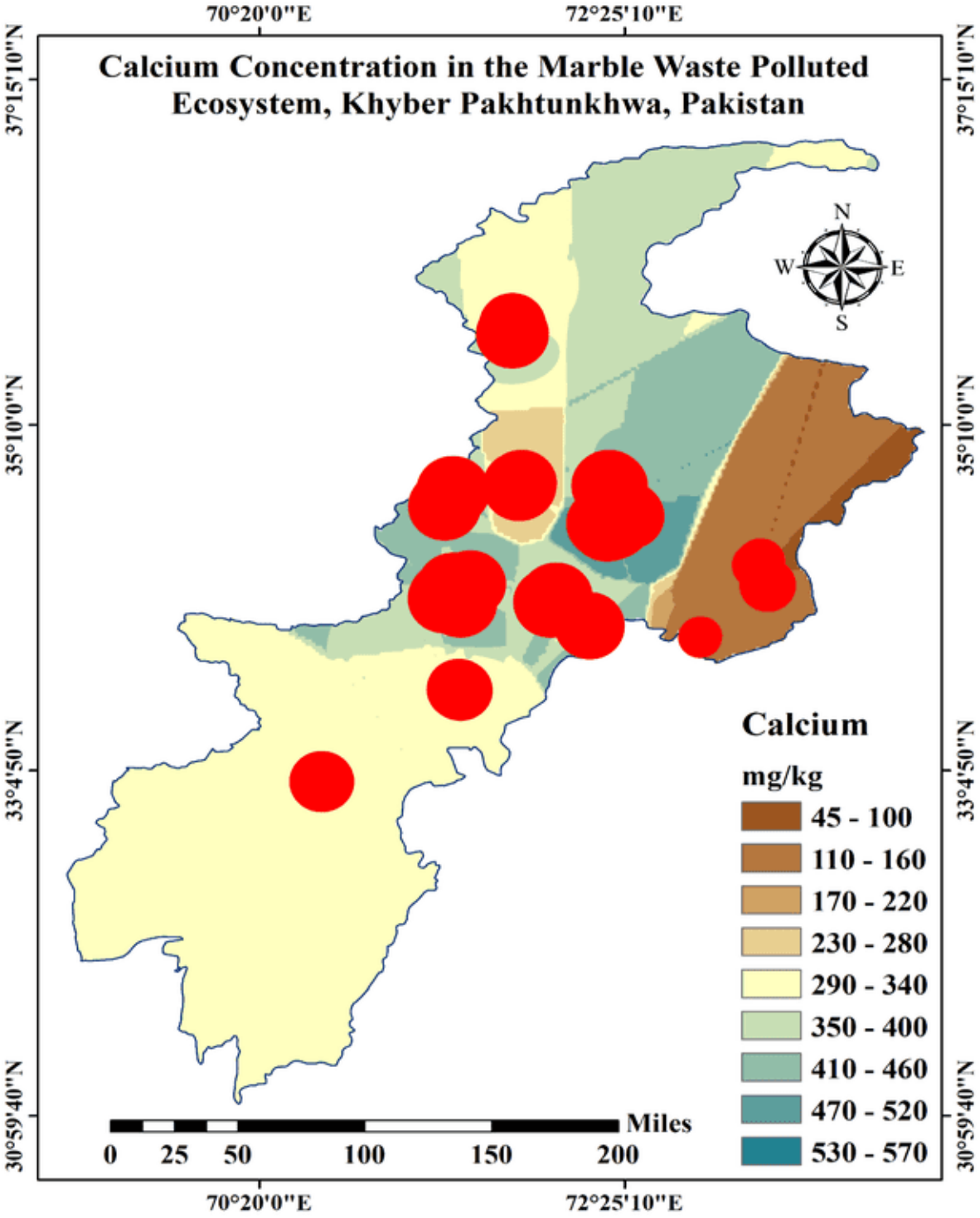


Fig. 5.1 Spatial distribution pattern of calcium element in the marble waste polluted ecosystem, Khyber Pakhtunkhwa, Pakistan. The red circle represents the concentration of calcium element.

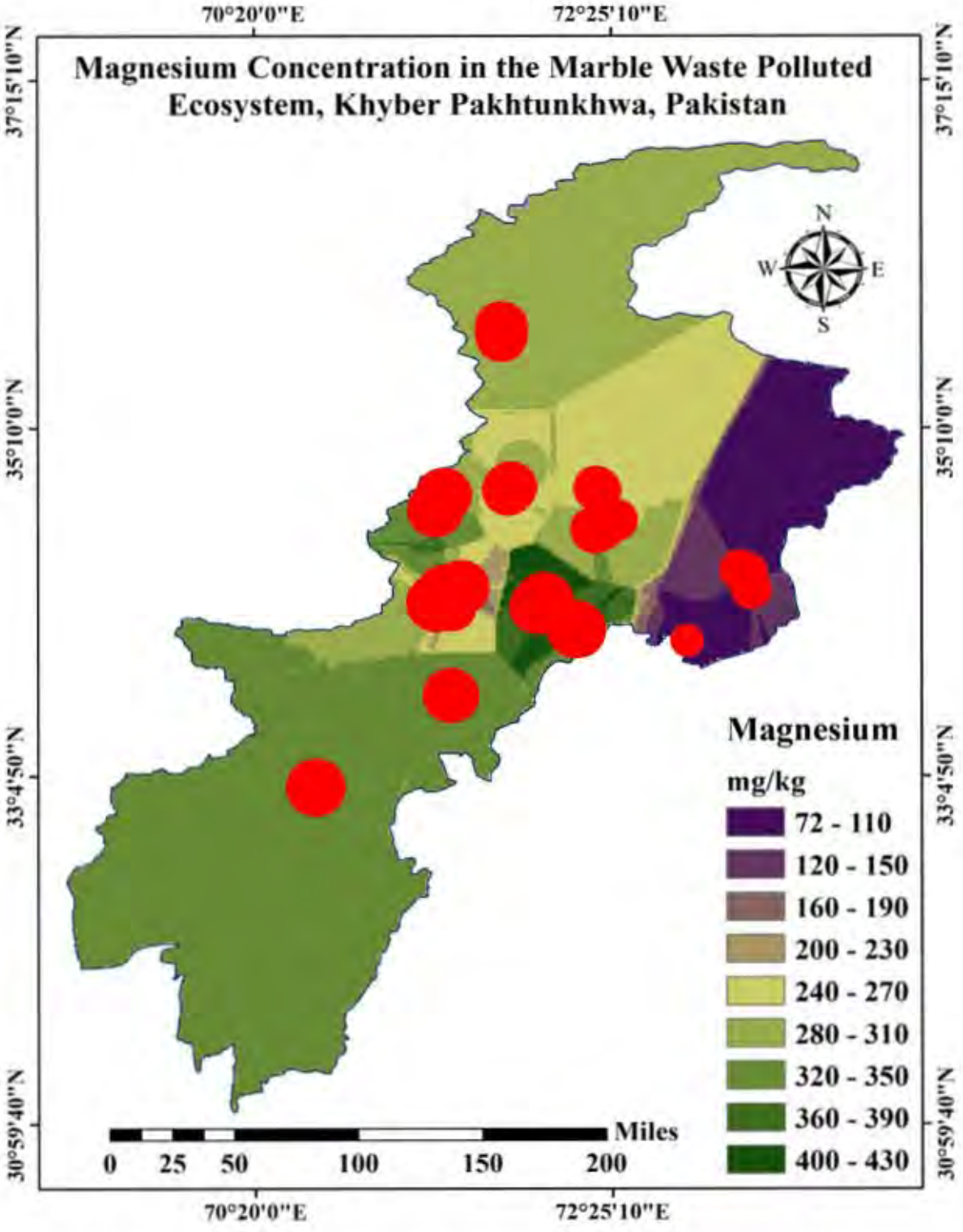


Fig. 5.2 Spatial distribution of magnesium heavy metal secreted from marble factories in the KPK province.

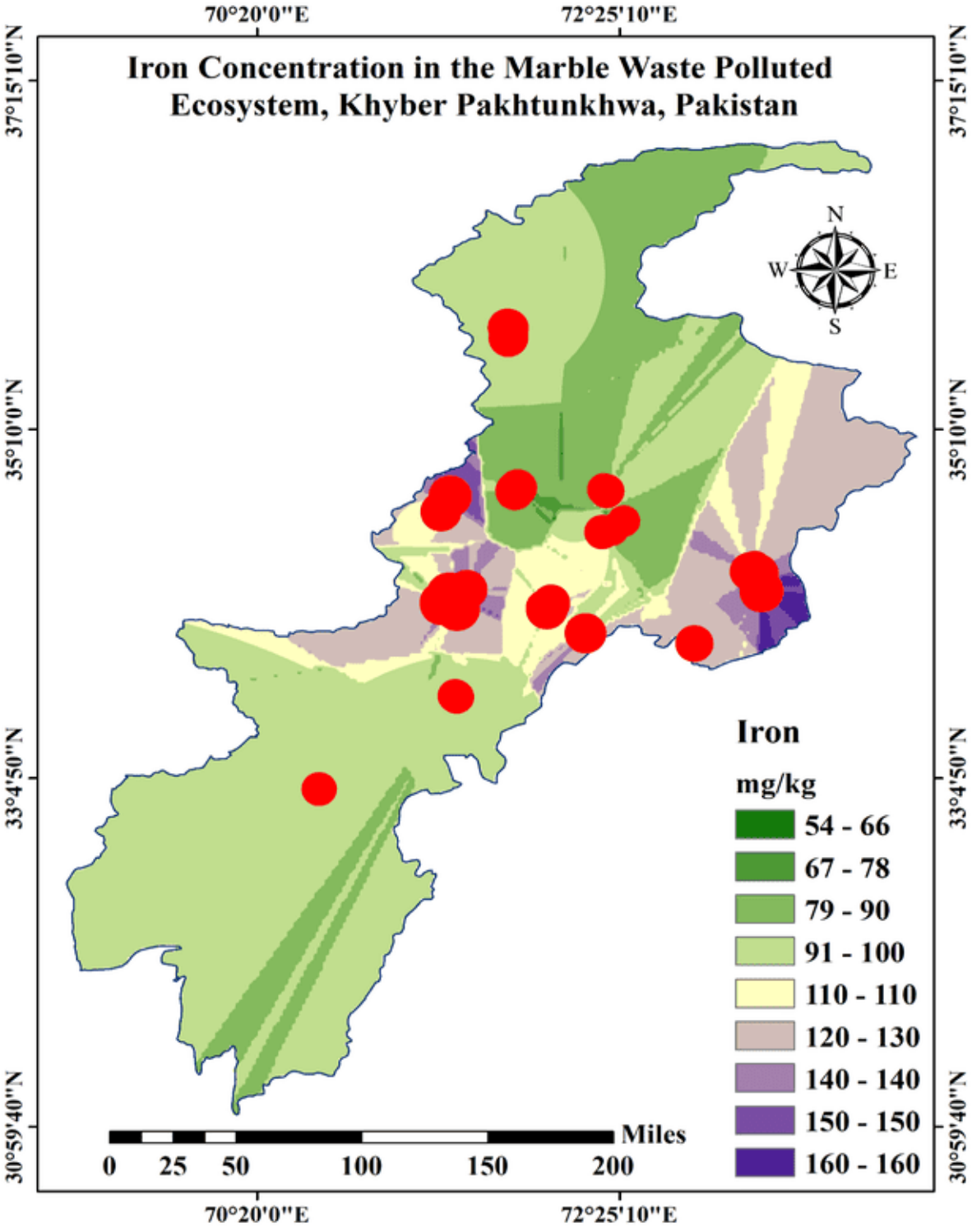


Fig. 5.3 Mapping the distribution of iron heavy metal in the marble waste polluted ecosystem.

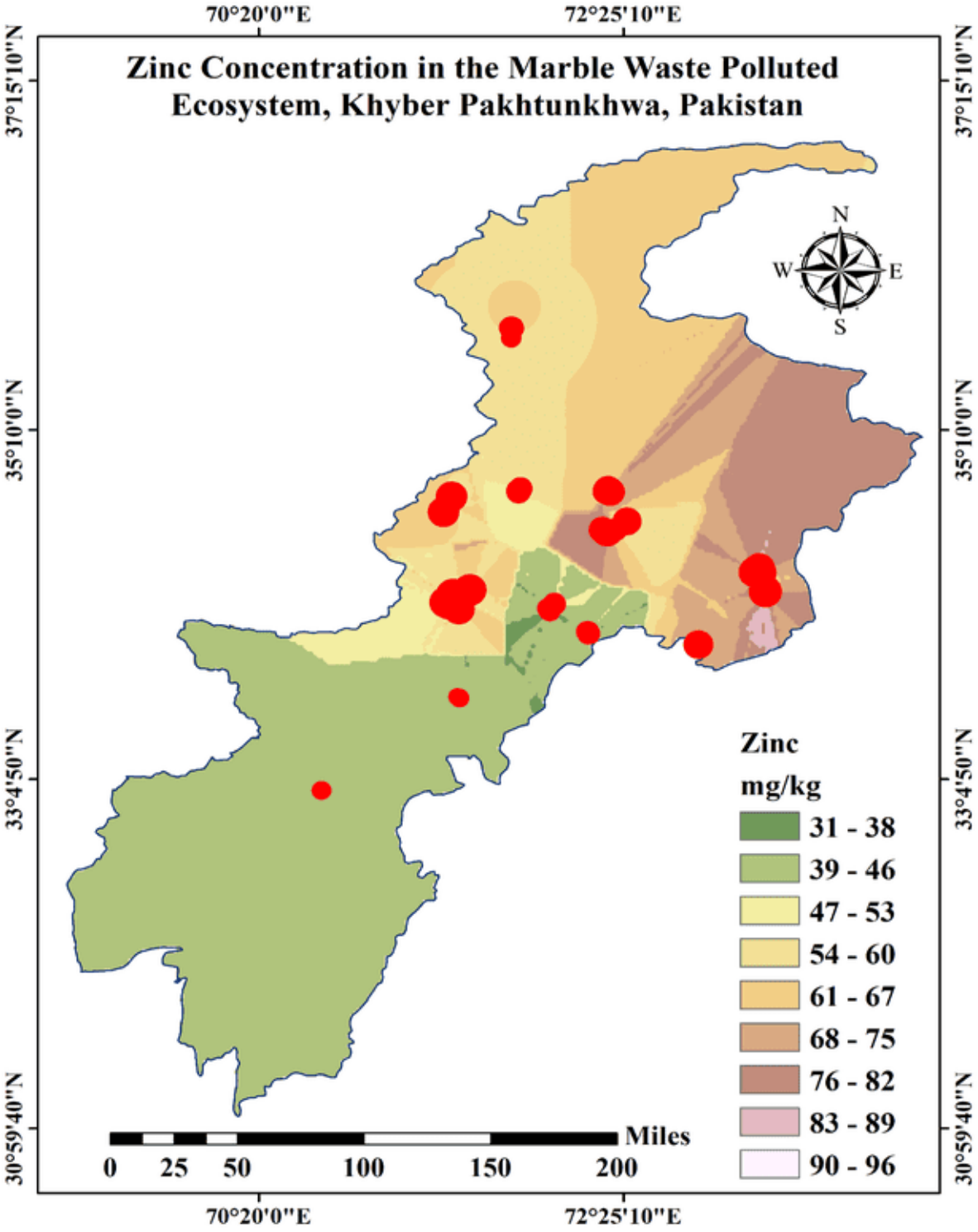


Fig. 5.4 Spatial distribution of zinc heavy metal in the MWPE, Khyber Pakhtunkhwa.

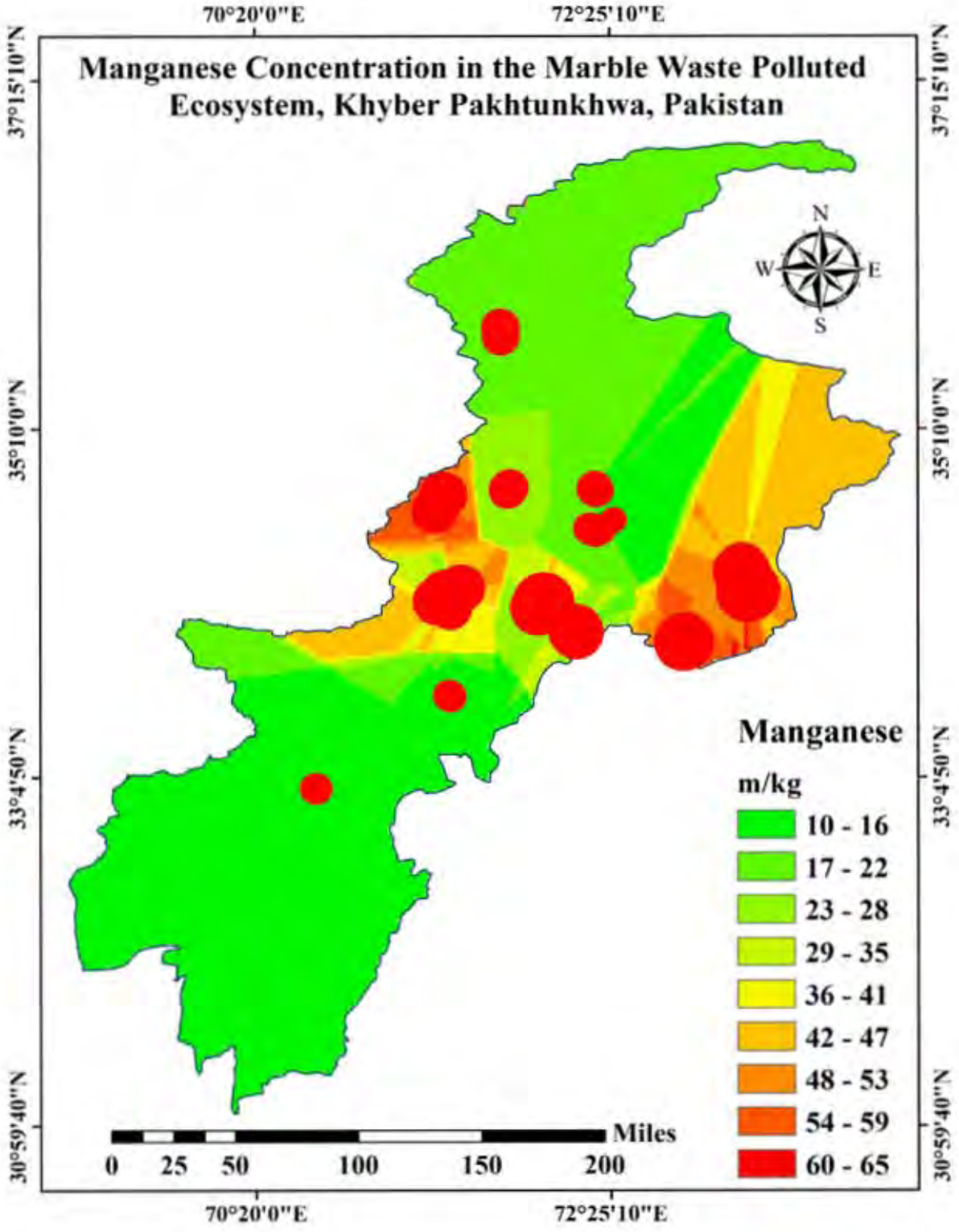


Fig. 5.5 Spatial distribution of manganese heavy metal in the MWPE, Khyber Pakhtunkhwa.

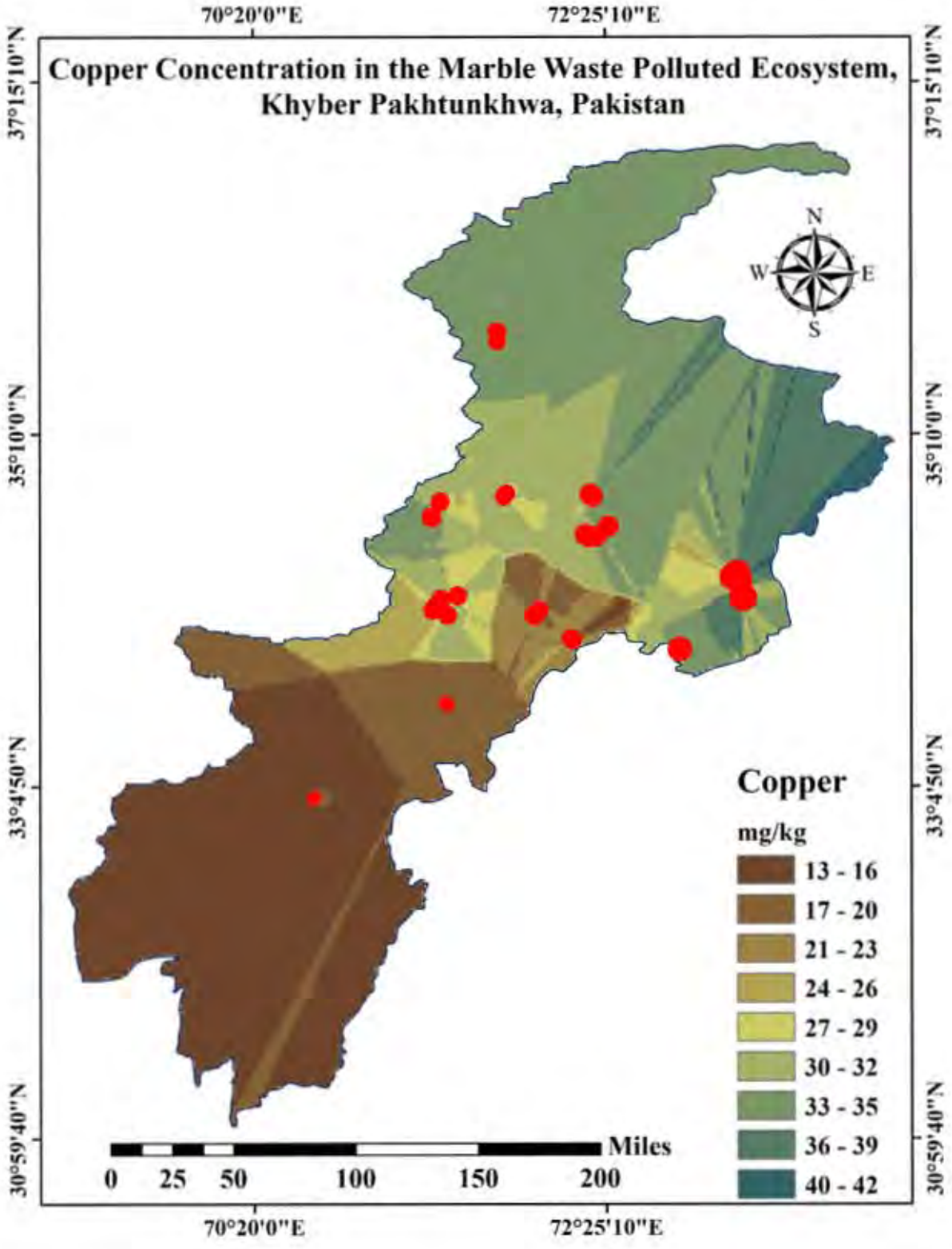


Fig. 5.6 Spatial distribution of copper heavy metal in the studied region.

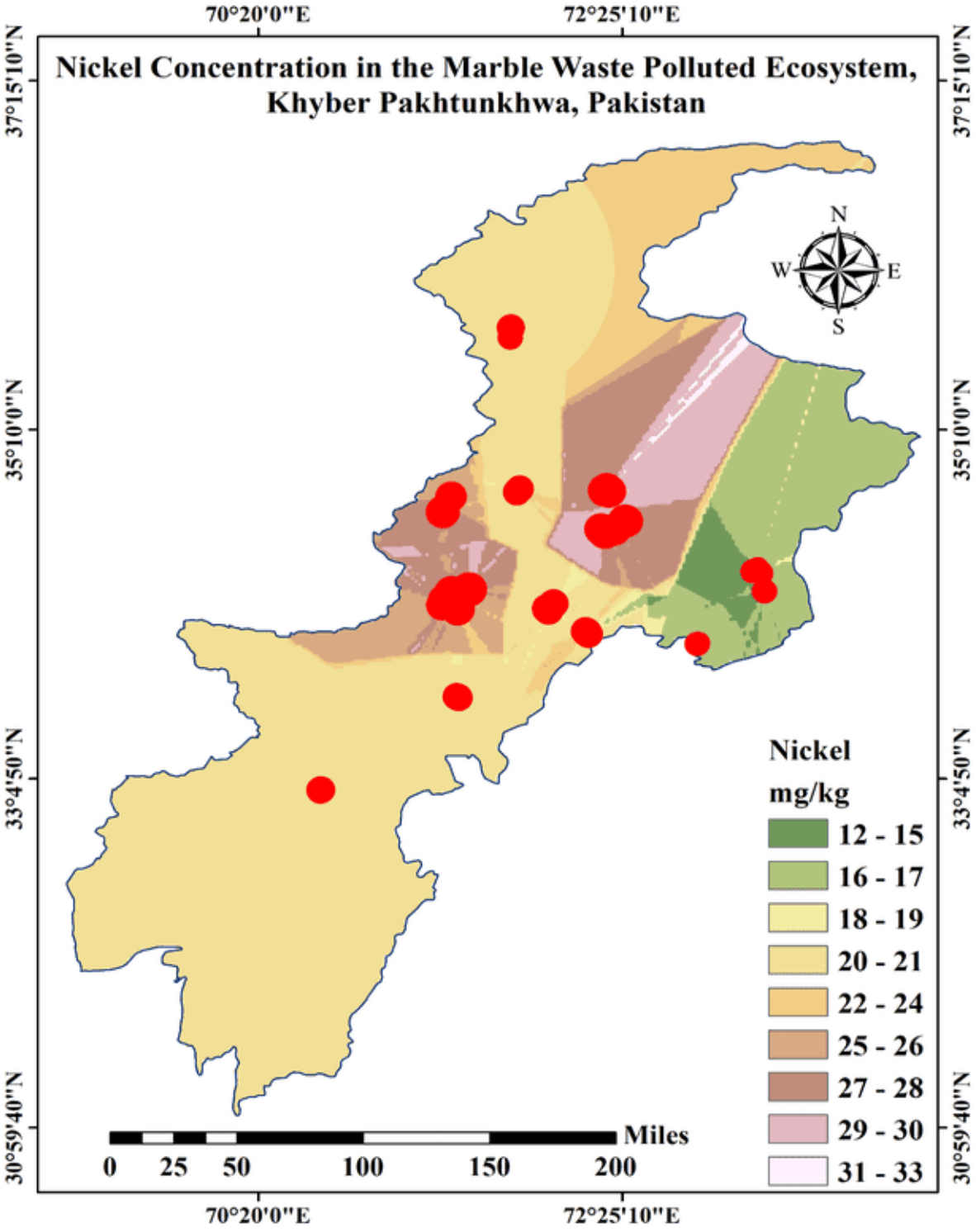


Fig. 5.7 Mapping of nickel concentration in the marble waste polluted ecosystem.

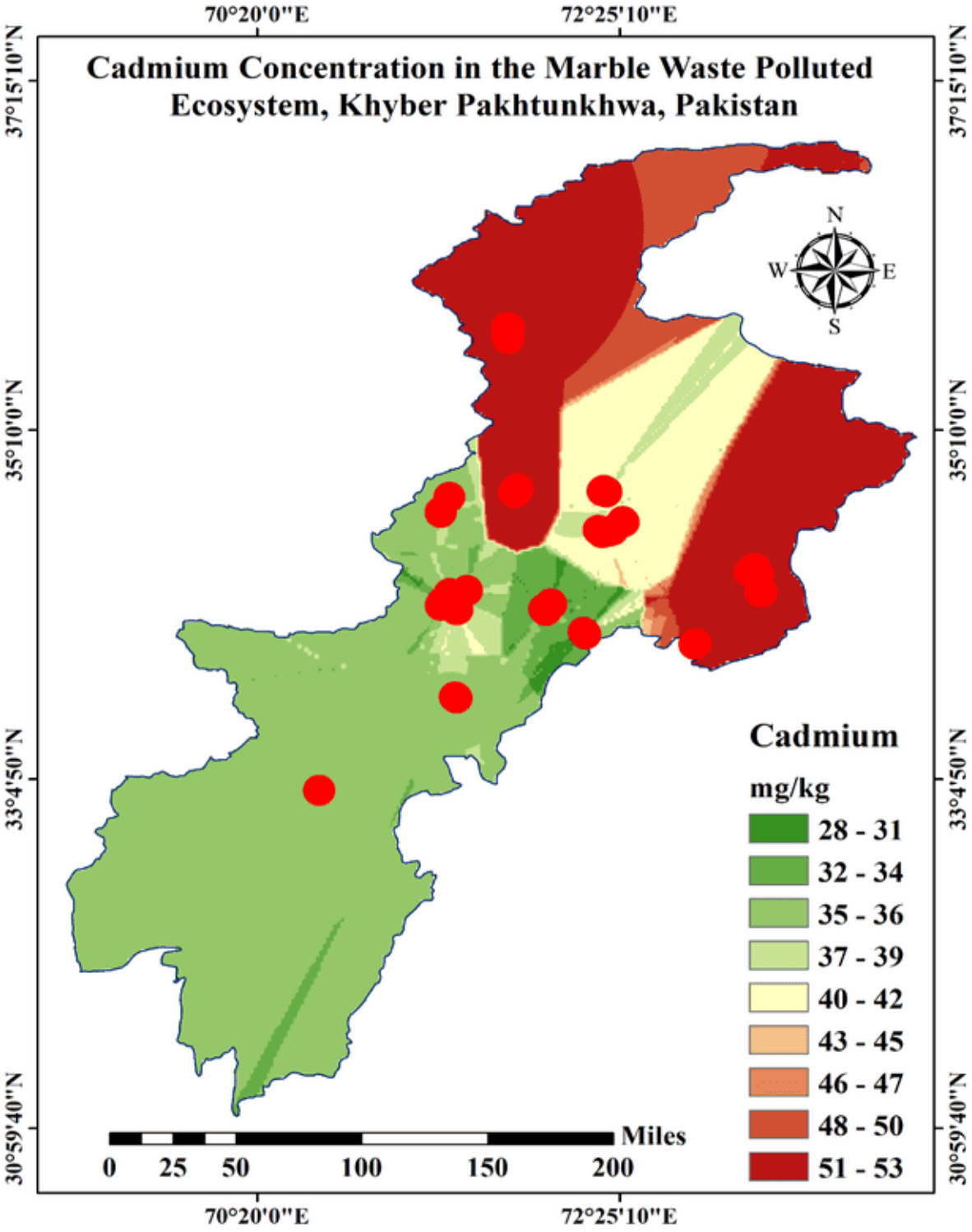


Fig. 5.8 Cadmium heavy metal in the MWPE Khyber Pakhtunkhwa Pakistan

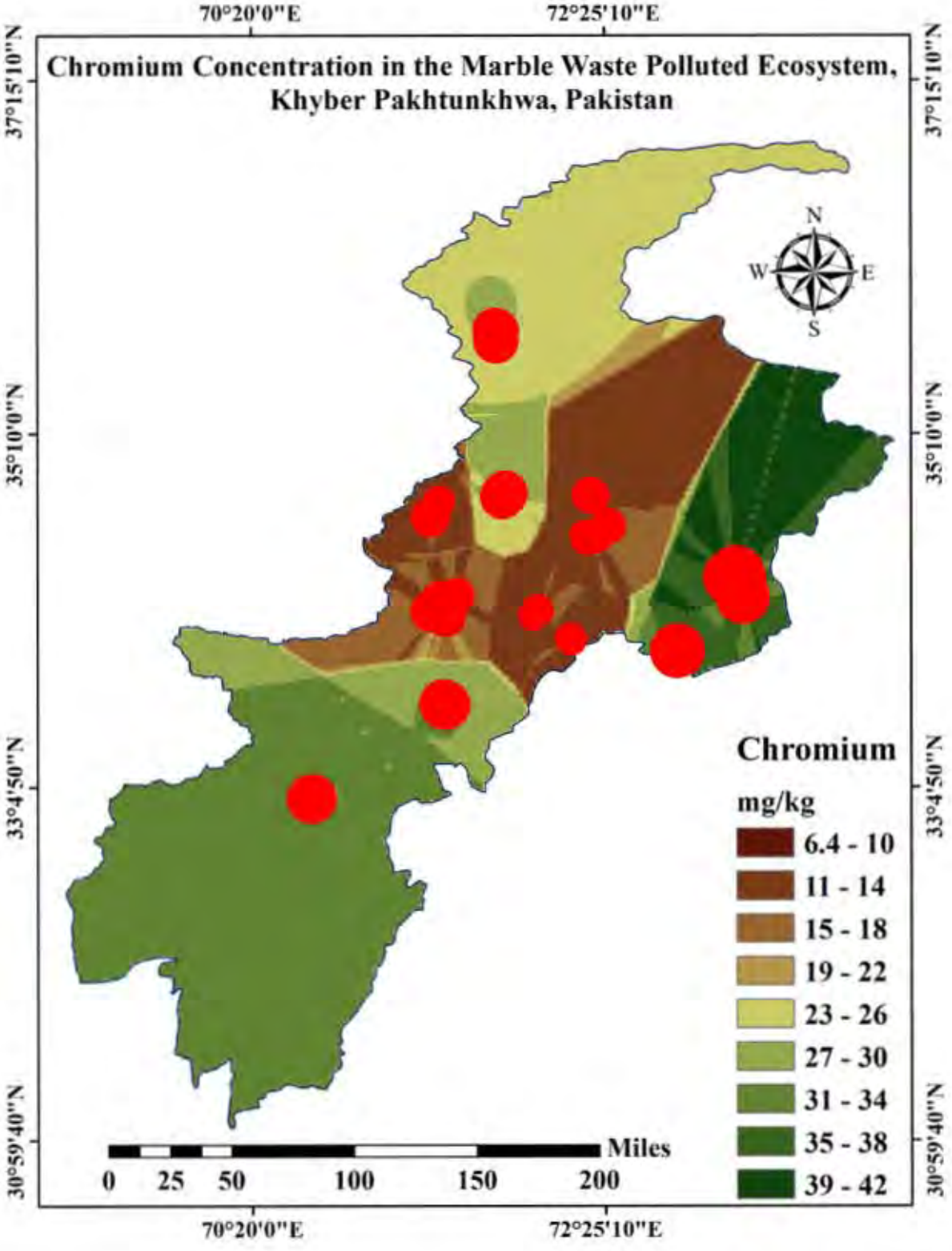


Fig. 5.9 Spatial distribution of chromium heavy metal in the studied region.

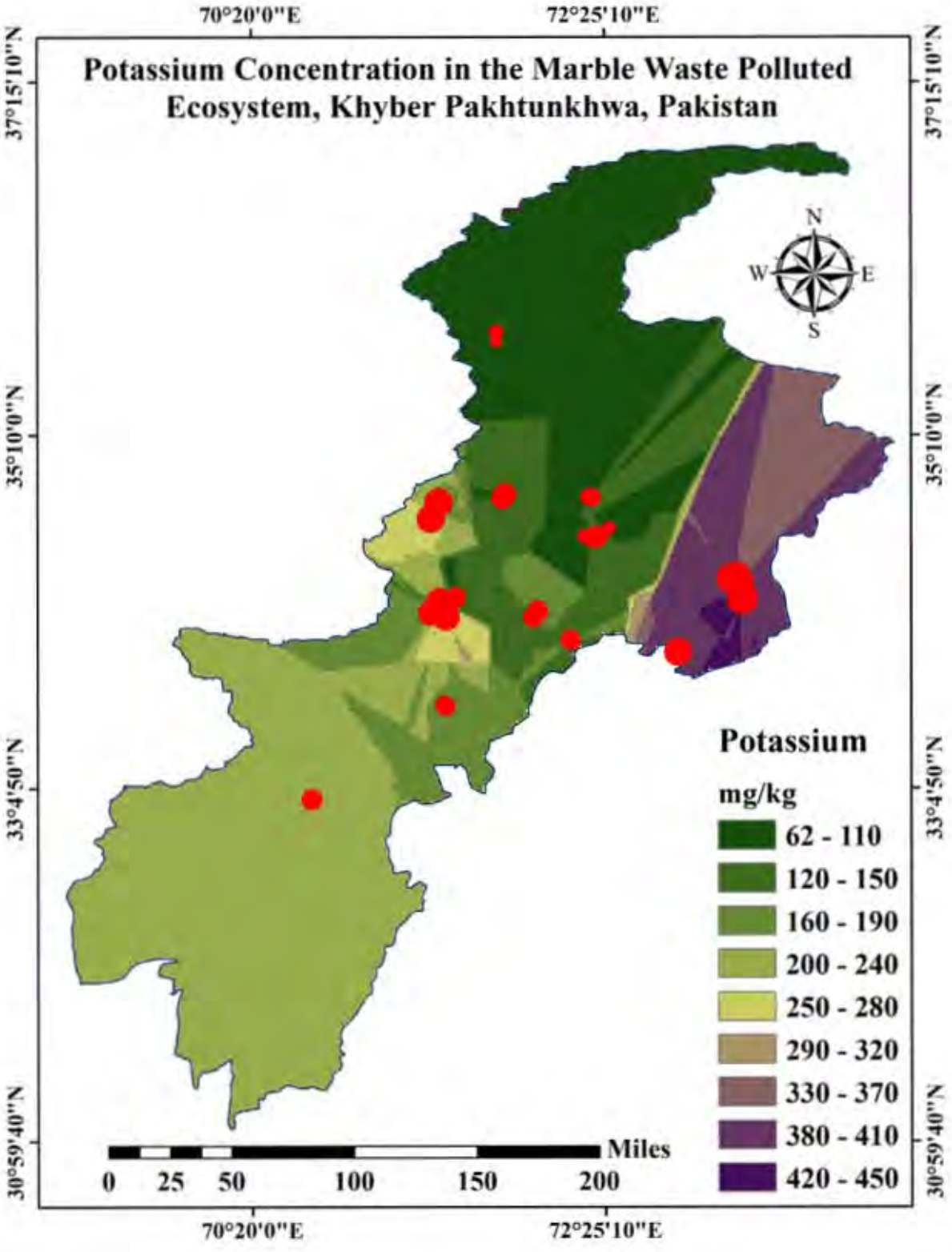


Fig. 5.10 Spatial distribution potassium element in the studied region.

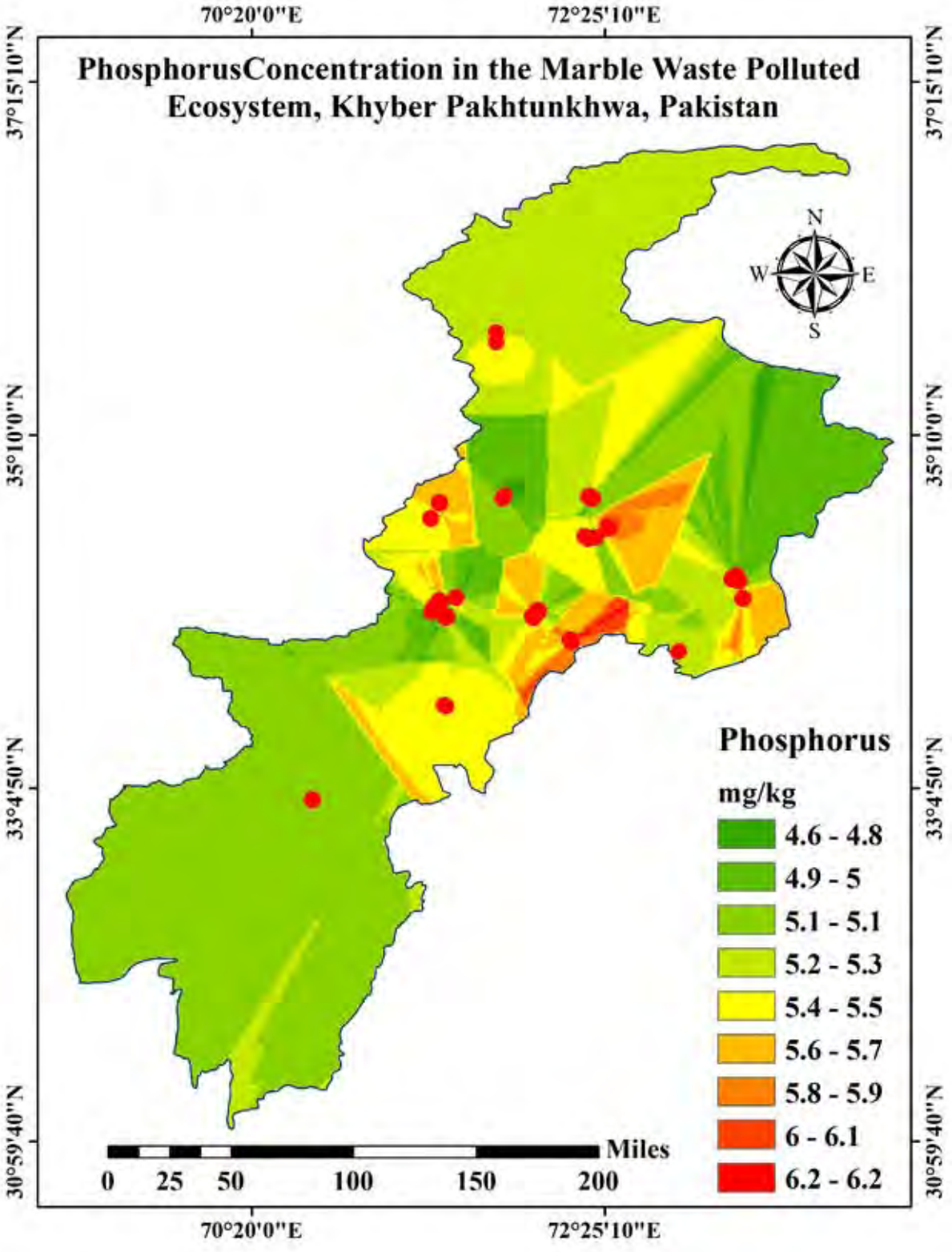


Fig. 5.11 Spatial distribution of phosphorous element in the studied area.

5.3.1 Spatial Autocorrelation Moran's Index

The results of spatial autocorrelation revealed that calcium (Moran's I=0.48, z-score=11.18, p-value=0.0001), chromium (Moran's I=0.29, z-score=6.79, p-value=0.0001), potassium (Moran's I=0.74, z-score=17.29, p-value=0.0001), magnesium (Moran's I=0.31, z-score=7.35, p-value=0.0001), manganese (Moran's I=0.074, z-score=1.79, p-value=0.07) and zinc (Moran's I=0.09, z-score=2.16, p-value=0.03) all showed a clustered distribution pattern. $CaCO_3$ (Moran's I= -0.03, z-score= -0.62, p-value=0.53), cadmium (Moran's I=0.02, z-score=0.12, p-value=0.89), iron (Moran's I=0.003, z-score=0.14, p-value=0.88), nickel (Moran's I=0.04, z-score=1.10, p-value=0.27), and phosphorus (Moran's I= -0.07, z-score= -1.5, p-value=0.12) all showed a random distribution pattern, while copper (Moran's I= -0.13, z-score= -3.05, p-value=0.002) had a dispersed pattern in the region (Fig. 5.12-5.23 & Table 5.1).

Table 5.1 Global Moran's Index (MI) summary of the measured variables.

S. No.	Variable	MI	Z-score	p-value
1	Calcium	0.484	11.183	0.0001
2	Chromium	0.292	6.793	0.0001
3	Potassium	0.749	17.29	0.0001
4	Magnesium	0.317	7.351	0.0001
5	Manganese	0.074	1.792	0.072
6	Zinc	0.091	2.164	0.030
7	CaCO3	-0.030	-0.621	0.534
8	Cadmium	0.002	0.129	0.896
9	Iron	0.003	0.143	0.886
10	Nickel	0.044	1.101	0.270
11	Phosphorous	-0.070	-1.548	0.121
12	Copper	-0.135	-3.050	0.002

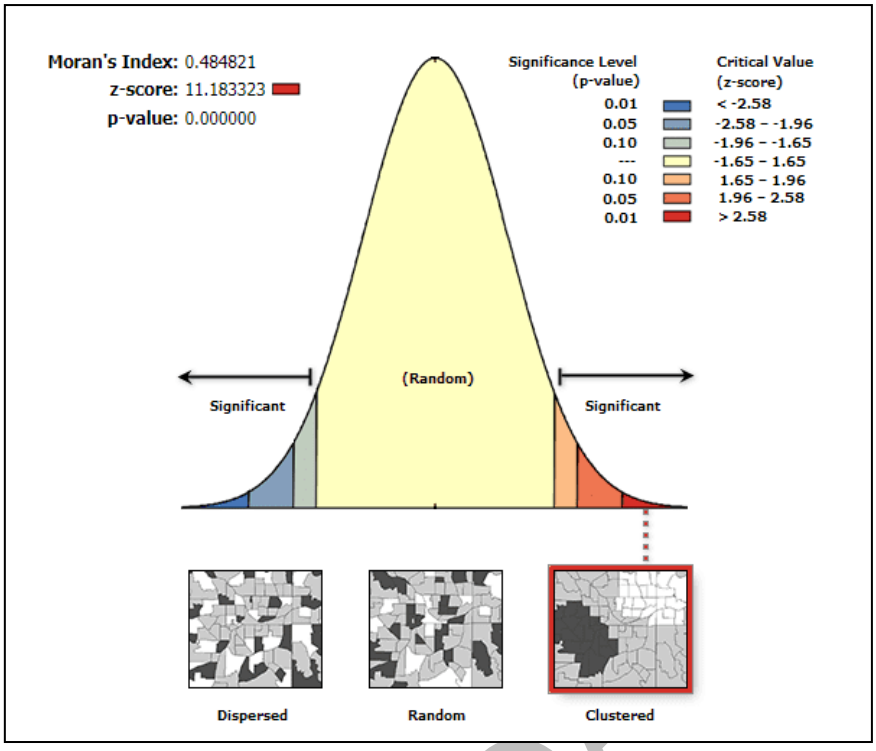


Fig. 5.12 Spatial autocorrelation of calcium.

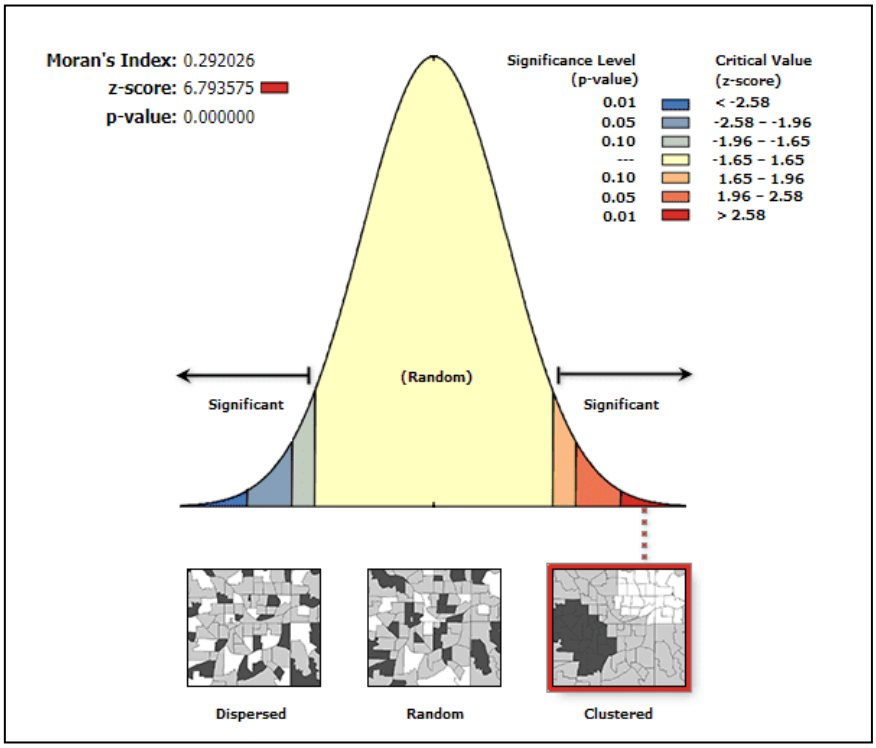


Fig. 5.13 Spatial autocorrelation of chromium.

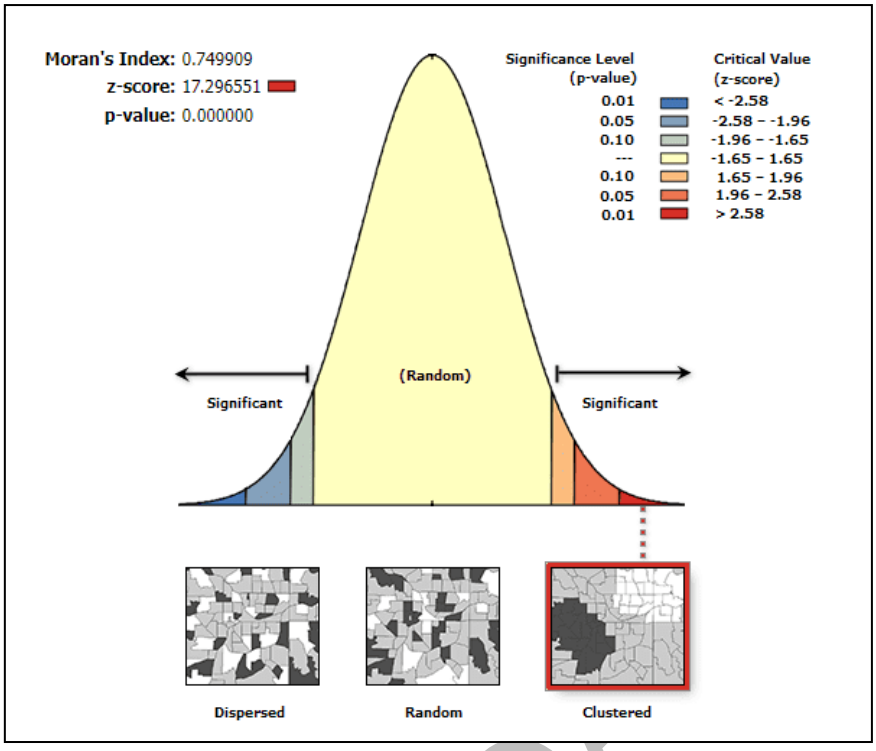


Fig. 5.14 Spatial autocorrelation of potassium

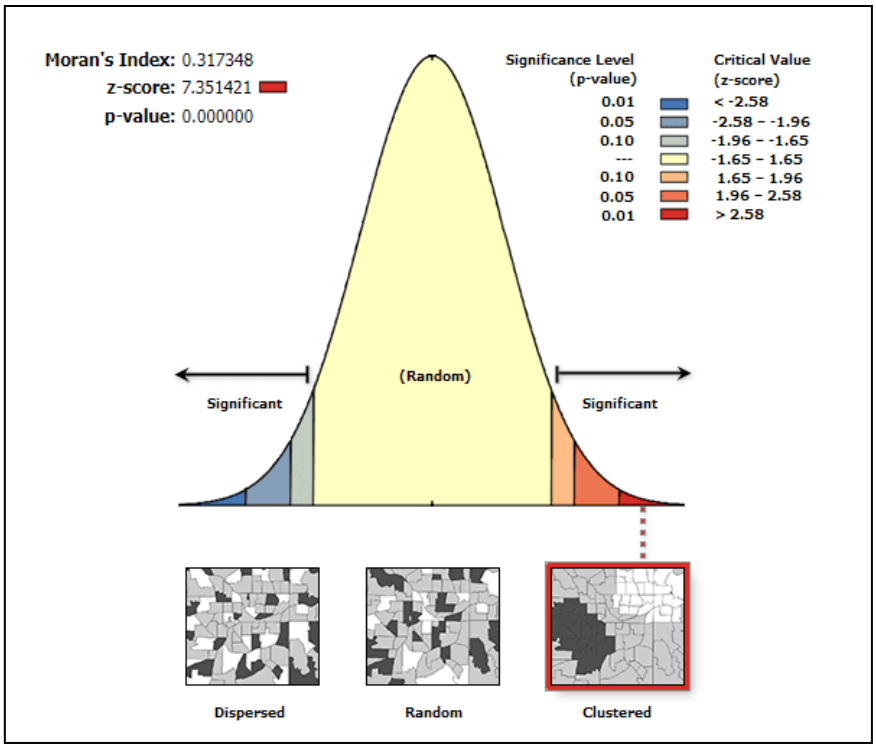


Fig. 5.15 Spatial autocorrelation of magnesium

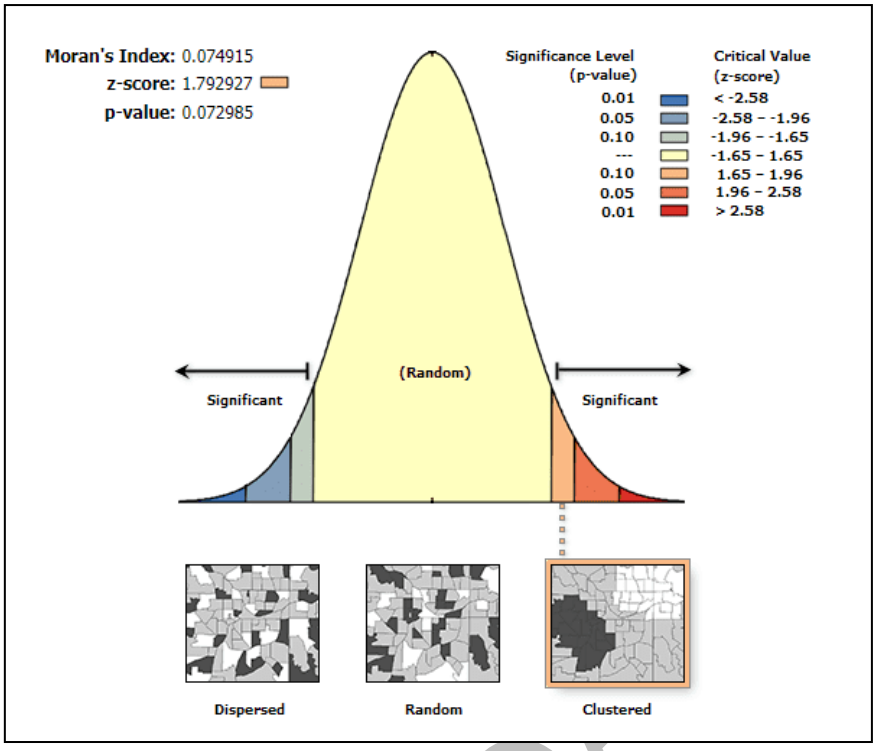


Fig. 5.16 Spatial autocorrelation of manganese

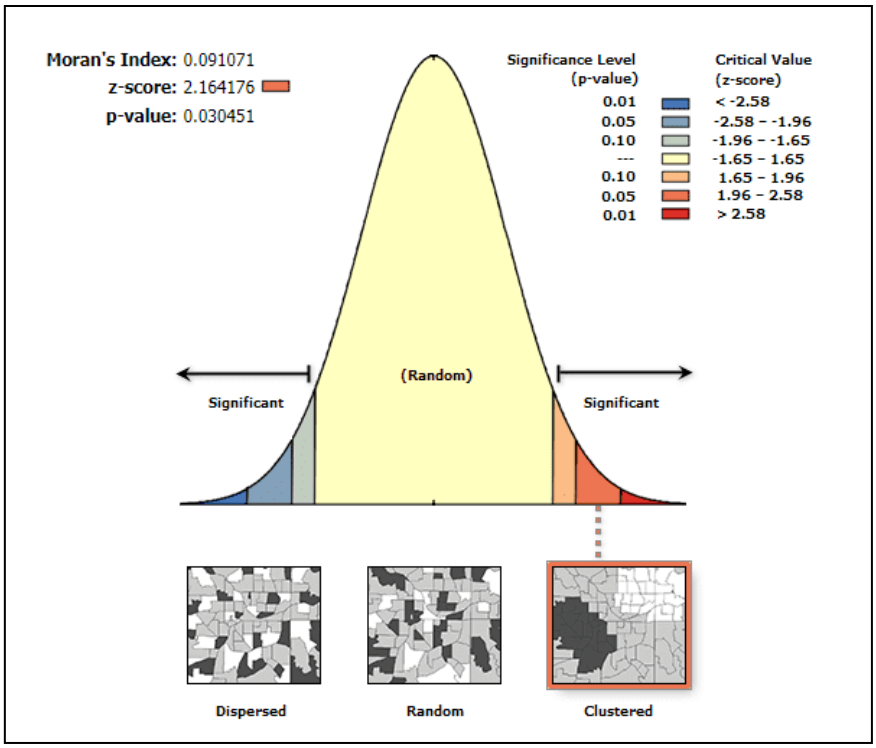


Fig. 5.17 Spatial autocorrelation of zinc

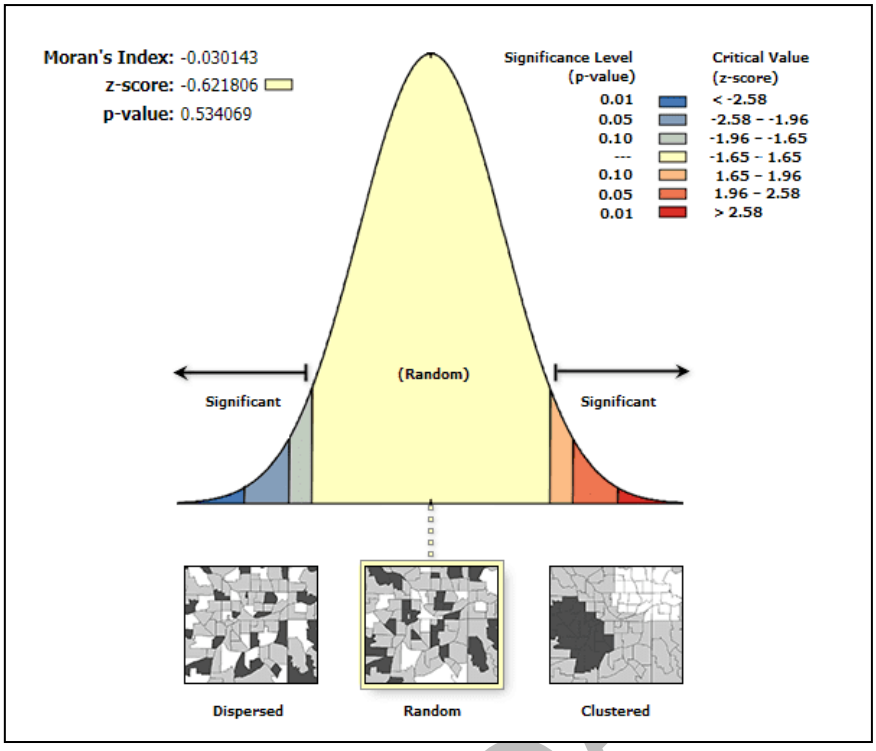


Fig. 5.18 Spatial autocorrelation of CaCO3

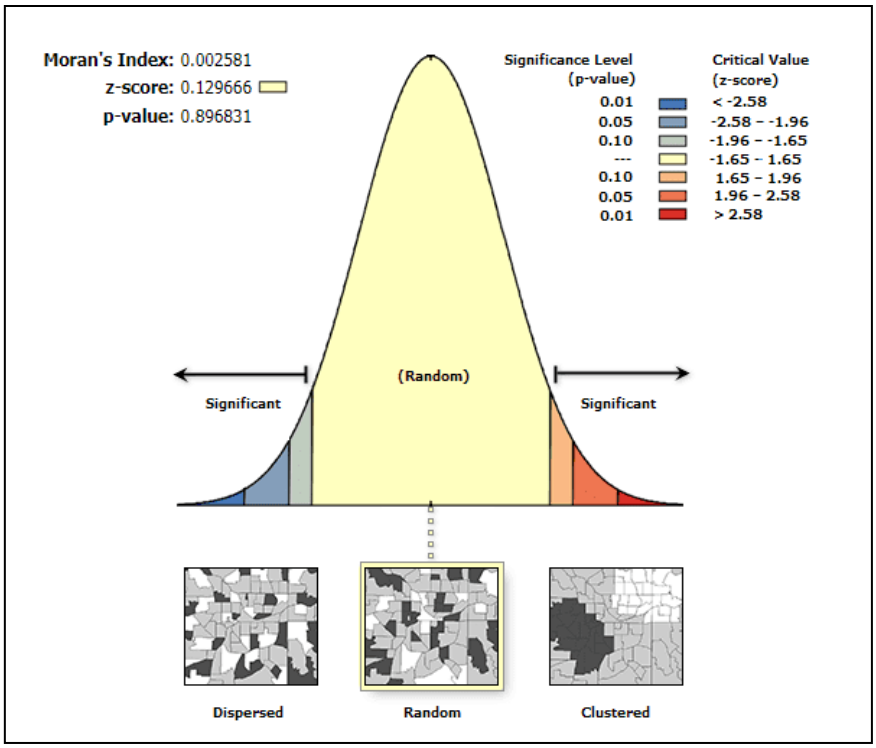


Fig. 5.19 Spatial autocorrelation of cadmium

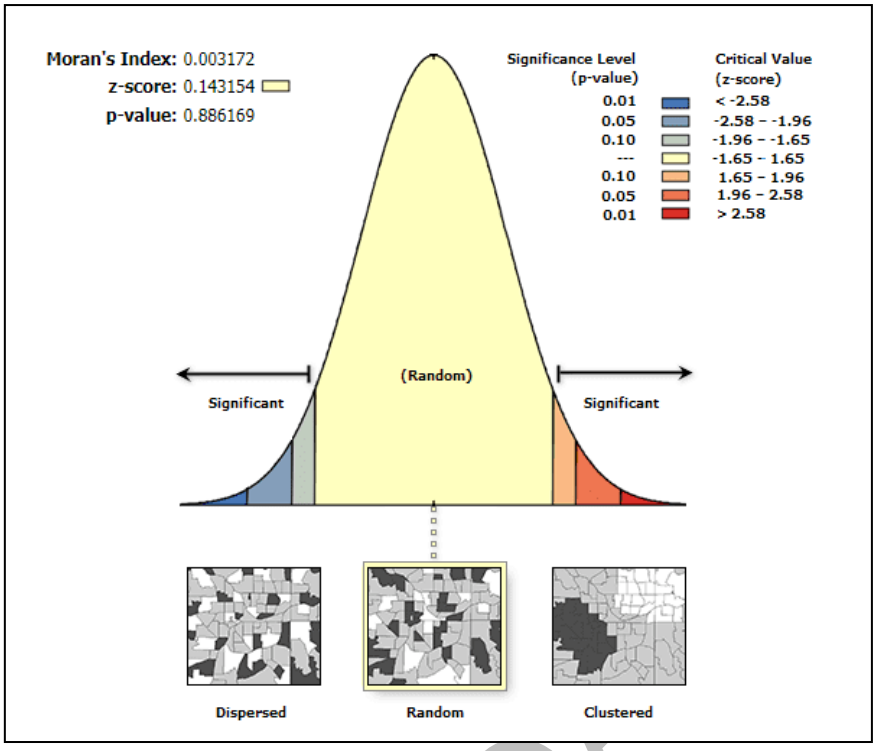


Fig. 5.20 Spatial autocorrelation of iron

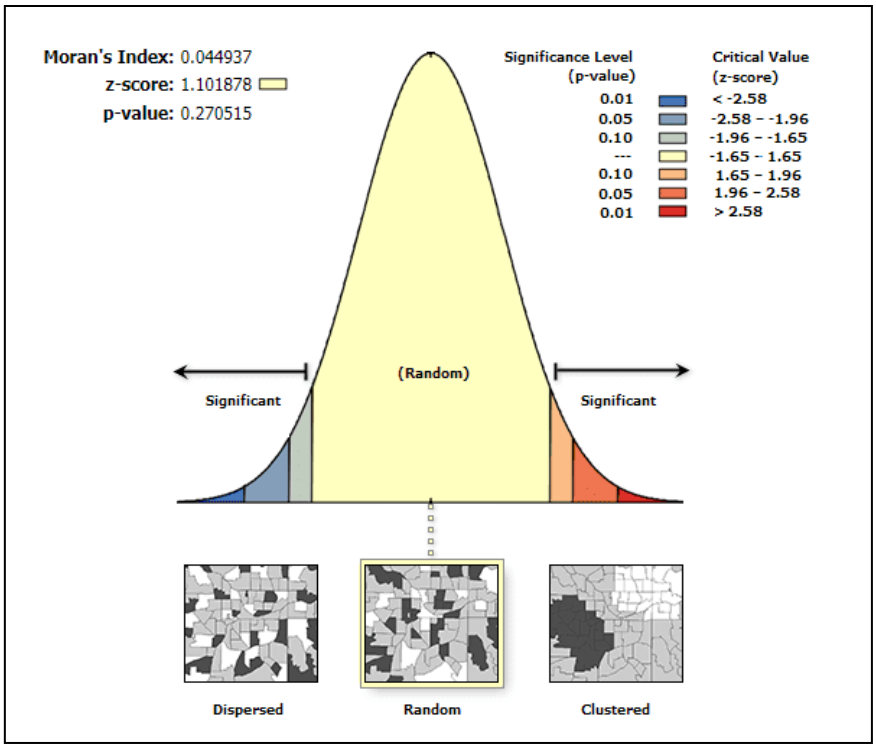


Fig. 5.21 Spatial autocorrelation of nickel

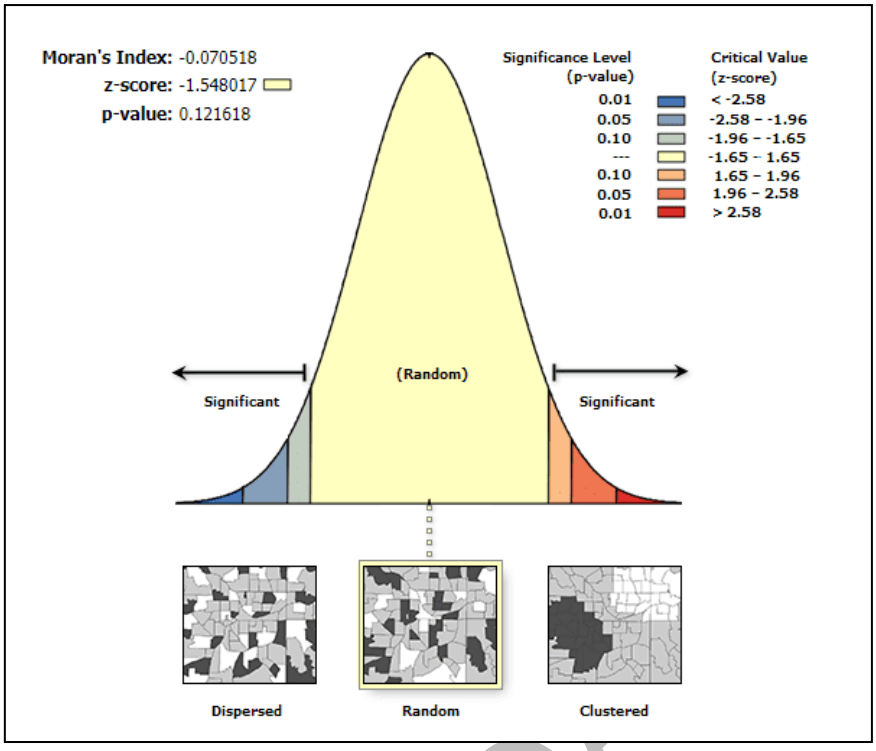


Fig. 5.22 Spatial autocorrelation of phosphorous

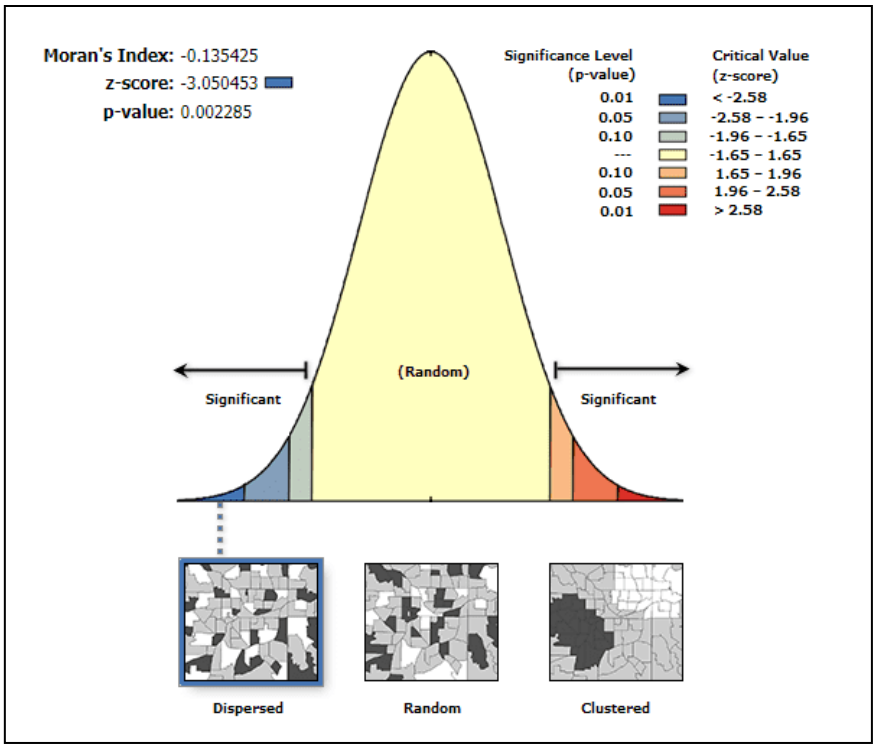


Fig. 5.23 Spatial autocorrelation of copper

5.3.2 Normalised Difference Vegetation Index (NDVI) 1986-2021

The Normalised Difference Vegetation Index was calculated for the marble waste polluted (contaminated) and non-polluted (noncontaminated) regions from 1986 to 2021. The results comprehend a significant NDVI difference in the polluted and non-polluted areas. The non-polluted areas have higher NDVI than the marble polluted regions (Fig. 5.24 & Table 5.2). The overall average NDVI in the marble polluted and non-polluted regions were 0.263 and 0.382, respectively.

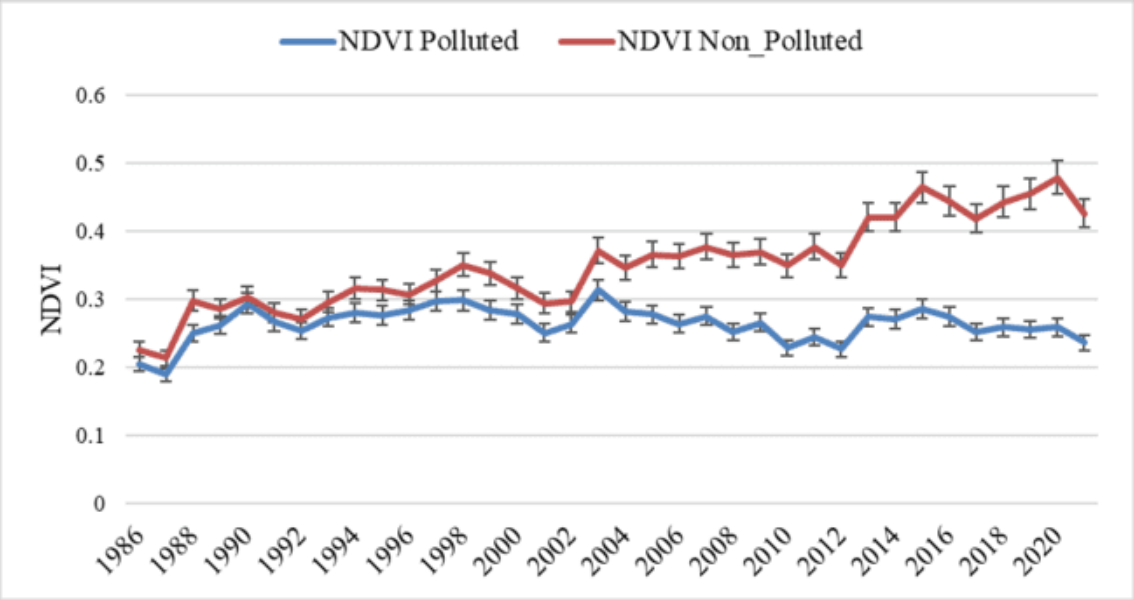


Fig. 5.24 Variation in NDVI between marble polluted and non-polluted regions during 1986-2021.

Table 5.2 Detailed average NDVI in the marble polluted and non-polluted area from 1986 to 2021.

Year	NDVI Polluted	NDVI Non-polluted
1986	0.204336688	0.226378924
1987	0.188986831	0.213528827
1988	0.250632311	0.297899236
1989	0.26214269	0.286399353
1990	0.294199344	0.302905593

1991	0.266286087	0.279649062
1992	0.253422547	0.270551815
1993	0.273329627	0.295741585
1994	0.280758879	0.316042934
1995	0.275994568	0.313438885
1996	0.283088406	0.307059645
1997	0.297386927	0.327831704
1998	0.298622885	0.350886986
1999	0.283410913	0.338439929
2000	0.278242048	0.316728829
2001	0.250780129	0.294206787
2002	0.263796486	0.29631534
2003	0.313332039	0.371303329
2004	0.281351302	0.346417775
2005	0.27744804	0.365844691
2006	0.263486445	0.362715949
2007	0.275312289	0.376599112
2008	0.252169308	0.365362332
2009	0.265543107	0.369382947
2010	0.228680873	0.349482086
2011	0.24462357	0.376621723
2012	0.226679372	0.349861227
2013	0.274032826	0.420702617
2014	0.270940227	0.420341023
2015	0.286029519	0.464221186
2016	0.274638838	0.444207039
2017	0.252315008	0.418323965
2018	0.258949629	0.443290201
2019	0.255727879	0.455078525
2020	0.258808601	0.479053876
2021	0.236001005	0.426229336
Grand Total	0.2638469	0.38249676

5.3.3 NDVI at a province level

NDVI at the province level was determined and mapped at ten year intervals, i.e., 1990, 2000, 2010 and 2020. There is a significant reduction in the NDVI from 1990 to 2020, i.e., -0.945 to 0.834 and -0.727 to 0.603, respectively. The highest NDVI recorded during 1990 was 0.834, which was reduced to 0.71 (in 2000), 0.57 (in 2010) and 0.60 (in 2020) (Fig. 5.25).

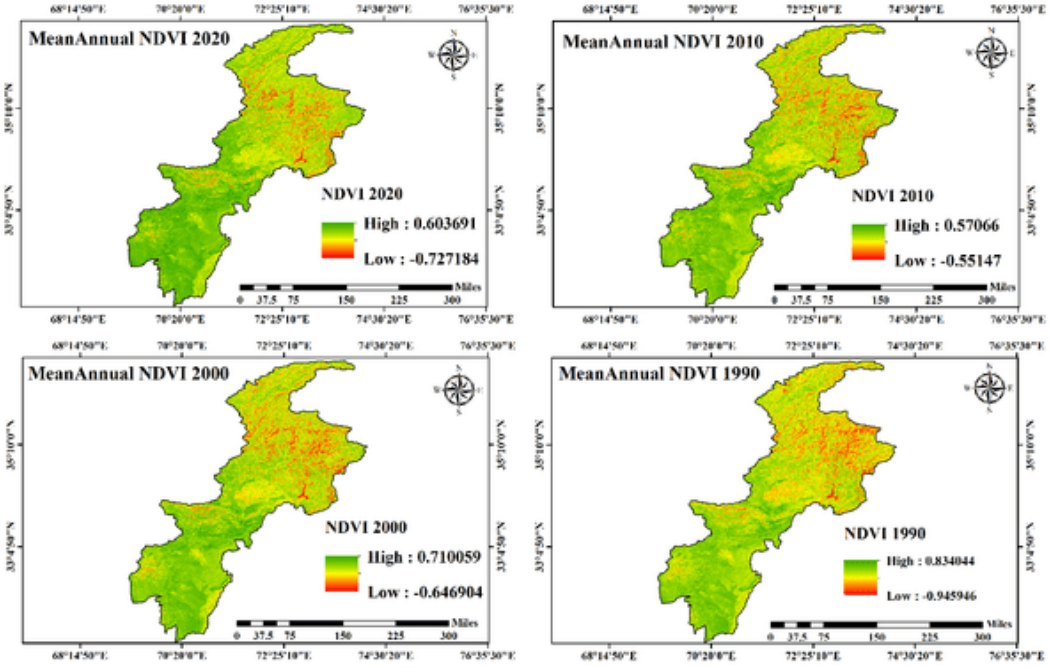


Fig. 5.25 Mean Annual NDVI maps of the Khyber Pakhtunkhwa, Pakistan (1990-2020).

5.3.4 NDVI at Factory Level

NDVI at the factory/station/quadrat level was assessed and compared with the non-polluted station/quadrat from 1987 to 2021 (Fig. 5.26-5.32; Table 5.3 Table 5.3 NDVI at polluted (contaminated) and non-polluted plots during 1990-2020.& Appendix 6). The NDVI at marble polluted quadrats significantly decreased with passage of time, while increasing gradually in the non-polluted/non-contaminated quadrats. For example, the recorded NDVI in Abbottabad Marble Factory-1 (AB-F1) was 0.33 during 1990, reduced to 0.29, 0.17 and 0.14 in 2000, 2010 and 2020, respectively. At the same time, in the non-polluted quadrat i.e., AB-F1-NC, NDVI increased from 0.35

to 0.47, 0.48 and 0.54 in 1990, 2000, 2010 and 2020, respectively. The detailed NDVI values for all the marble polluted and non-polluted quadrats is given in table 5.3.

Table 5.3 NDVI at polluted (contaminated) and non-polluted plots during 1990-2020.

Station	Zone	1990	2000	2010	2020
AB-F1	C	0.33879	0.29706	0.17529	0.14024
AB-F1-NC	NC	0.35461	0.47150	0.47726	0.54611
AB-F10	C	0.21289	0.25537	0.21725	0.21579
AB-F10- NC	NC	0.43326	0.51606	0.42382	0.51331
AB-F2	C	0.35289	0.33996	0.21818	0.16505
AB-F2-NC	NC	0.36579	0.49078	0.46775	0.54144
AB-F3	C	0.30103	0.27250	0.17052	0.16167
AB-F3-NC	NC	0.44959	0.56311	0.52820	0.59342
AB-F4	C	0.28130	0.29465	0.21924	0.17512
AB-F4-NC	NC	0.36966	0.51557	0.47388	0.57581
AB-F5	C	0.28701	0.22680	0.13880	0.13206
AB-F5-NC	NC	0.32950	0.51155	0.53107	0.66786
AB-F6	C	0.30085	0.27494	0.19730	0.22857
AB-F6-NC	NC	0.24030	0.41771	0.45169	0.59630
AB-F7	C	0.20546	0.19323	0.13999	0.11583
AB-F7-NC	NC	0.24501	0.42518	0.51100	0.65316
AB-F8	C	0.29076	0.26227	0.20889	0.26961
AB-F8-NC	NC	0.30382	0.55226	0.58349	0.69248
AB-F9	C	0.21206	0.23695	0.18689	0.18287
AB-F9-NC	NC	0.42019	0.55595	0.54287	0.68168
BA-F1	C	0.25973	0.32991	0.22265	0.25105
BA-F1-NC	NC	0.16747	0.23380	0.36843	0.49263
BA-F2	C	0.19365	0.30190	0.19540	0.26945
BA-F2-NC	NC	0.19057	0.29088	0.46340	0.46261
BA-F3	C	0.31033	0.38175	0.29550	0.35239
BA-F3-NC	NC	0.34647	0.38300	0.35003	0.43376
BA-F4	C	0.15609	0.18019	0.15465	0.22413
BA-F4-NC	NC	0.23804	0.28975	0.31715	0.38302
BA-F5	C	0.13795	0.14499	0.16308	0.22084
BA-F5-NC	NC	0.14937	0.12832	0.17080	0.22647
BN-F1	C	0.22419	0.20732	0.21618	0.15517
BN-F1-NC	NC	0.48034	0.44371	0.45298	0.57325
BN-F2	C	0.19715	0.17255	0.20466	0.17129
BN-F2-NC	NC	0.49130	0.48822	0.49938	0.57240
BN-F3	C	0.20626	0.16014	0.17906	0.18218
BN-F3-NC	NC	0.52384	0.51041	0.49032	0.57116

BU-F1	C	0.37765	0.33929	0.31999	0.41050
BU-F1-NC	NC	0.44640	0.37802	0.50883	0.58454
BU-F2	C	0.42863	0.35654	0.28727	0.38284
BU-F2-NC	NC	0.35421	0.43348	0.44324	0.62305
BU-F3	C	0.42920	0.39116	0.29787	0.38782
BU-F3-NC	NC	0.31938	0.36945	0.38066	0.51737
BU-F4	C	0.37854	0.39194	0.34609	0.47067
BU-F4-NC	NC	0.32587	0.45578	0.48778	0.64735
BU-F5	C	0.27501	0.34479	0.28835	0.37132
BU-F5-NC	NC	0.31183	0.36515	0.34585	0.49413
BU-F6	C	0.30222	0.33208	0.33070	0.39506
BU-F6-NC	NC	0.28071	0.33519	0.33950	0.46015
BU-F7	C	0.29760	0.34846	0.36073	0.40938
BU-F7-NC	NC	0.34992	0.42278	0.45192	0.58787
BU-F8	C	0.17609	0.16891	0.15520	0.24326
BU-F8-NC	NC	0.26051	0.28363	0.33751	0.44649
CH-F1	C	0.41308	0.40301	0.21585	0.19261
CH-F1-NC	NC	0.38609	0.31999	0.33195	0.35027
CH-F2	C	0.36846	0.37489	0.18550	0.14890
CH-F2-NC	NC	0.10218	0.10483	0.13978	0.23110
CH-F3	C	0.40281	0.40307	0.17425	0.17471
CH-F3-NC	NC	0.10469	0.09573	0.17637	0.35530
CH-F4	C	0.41868	0.40681	0.21713	0.19433
CH-F4-NC	NC	0.10787	0.08482	0.14405	0.19931
CH-F5	C	0.44425	0.44163	0.21085	0.19912
CH-F5-NC	NC	0.11391	0.09950	0.21750	0.26832
CH-F6	C	0.46356	0.39520	0.30286	0.15718
CH-F6-NC	NC	0.19961	0.16931	0.20801	0.28280
CH-F7	C	0.40863	0.39395	0.23483	0.24124
CH-F7-NC	NC	0.21775	0.17355	0.21787	0.29903
Chi-F1	C	0.05436	0.04196	0.07373	0.10194
Chi-F1-NC	NC	0.42494	0.40220	0.53425	0.58573
Chi-F2	C	0.03166	0.04637	0.07784	0.09320
Chi-F2-NC	NC	0.37081	0.29564	0.42782	0.49353
Chi-F3	C	0.11132	0.15933	0.21749	0.30118
DL-F1	C	0.43751	0.38973	0.35755	0.42911
DL-F1-NC	NC	0.29410	0.34436	0.35140	0.50802
DL-F2	C	0.33168	0.29618	0.26773	0.26734
DL-F2-NC	NC	0.30094	0.30110	0.42619	0.56461
DL-F3	C	0.39962	0.34678	0.30896	0.34021
DL-F3-NC	NC	0.30602	0.30686	0.42550	0.54880
DL-F4	C	0.47208	0.40790	0.16729	0.18700
DL-F4-NC	NC	0.39346	0.35952	0.39611	0.55016
DL-F5	C	0.45241	0.45656	0.22527	0.13589

DL-F5-NC	NC	0.31075	0.36008	0.42410	0.51719
HR-F1	C	0.34716	0.34634	0.28585	0.33460
HR-F1-NC	NC	0.30958	0.28995	0.25872	0.42841
HR-F2	C	0.27460	0.28103	0.24877	0.34016
HR-F2-NC	NC	0.30388	0.28716	0.26550	0.41983
KB-F1	C	0.25782	0.21364	0.22938	0.29258
KB-F1-NC	NC	0.17978	0.15886	0.25316	0.33802
KB-F2	C	0.31259	0.25788	0.14015	0.12151
KB-F2-NC	NC	0.12456	0.09802	0.16767	0.24242
KB-F3	C	0.31215	0.34109	0.19105	0.13822
KB-F3-NC	NC	0.11484	0.10236	0.17276	0.24543
KB-F4	C	0.35913	0.24089	0.19391	0.21552
KB-F4-NC	NC	0.12609	0.15130	0.21069	0.31120
KB-F5	C	0.13095	0.13834	0.21219	0.27083
KB-F5-NC	NC	0.12376	0.14099	0.20969	0.29393
KB-F6	C	0.08537	0.08127	0.11925	0.22218
KB-F6-NC	NC	0.10273	0.08675	0.13850	0.18610
KB-F7	C	0.06356	0.06278	0.11541	0.15815
KB-F7-NC	NC	0.07823	0.06577	0.10531	0.15899
KB-F8	C	0.28586	0.29985	0.30803	0.43558
KB-F8-NC	NC	0.27128	0.21618	0.25822	0.35838
KO-F1	C	0.35113	0.29591	0.28790	0.28985
KO-F1-NC	NC	0.28600	0.47982	0.52503	0.62271
KO-F2	C	0.34823	0.30649	0.29942	0.31422
KO-F2-NC	NC	0.37116	0.27103	0.34559	0.46646
KO-F3	C	0.32982	0.33025	0.29720	0.29066
MM-F1	C	0.33879	0.36816	0.33897	0.31667
MM-F1-NC	NC	0.13513	0.10797	0.17582	0.19224
MM-F2	C	0.27077	0.32528	0.33469	0.28024
MM-F2-NC	NC	0.14872	0.10454	0.18533	0.19986
MM-F3	C	0.17361	0.17071	0.20330	0.30408
MM-F3-NC	NC	0.11701	0.09005	0.15521	0.17982
MM-F4	C	0.40132	0.39442	0.40962	0.37433
MM-F4-NC	NC	0.11100	0.26290	0.29495	0.31834
MM-F5	C	0.30394	0.24203	0.16314	0.17676
MM-F5-NC	NC	0.34251	0.32947	0.40422	0.53743
MM-F6	C	0.24023	0.23064	0.19775	0.18514
MM-F6-NC	NC	0.18344	0.19112	0.23960	0.30916
MM-F7	C	0.13764	0.19373	0.19176	0.38169
MM-F7-NC	NC	0.12508	0.11333	0.18349	0.24137
MM-F8	C	0.14118	0.11782	0.11044	0.26164
MM-F8-NC	NC	0.07062	0.10495	0.14223	0.19653
MN-F1	C	0.23503	0.26155	0.21808	0.26091
MN-F10	C	0.31099	0.39083	0.26261	0.25051

Mn-F10-NC	NC	0.42637	0.45244	0.46337	0.55109
Mn-F1-NC	NC	0.29876	0.40920	0.46901	0.57470
MN-F2	C	0.37072	0.37522	0.34802	0.37747
Mn-F2-NC	NC	0.31547	0.43992	0.46982	0.60016
MN-F3	C	0.37121	0.39773	0.35123	0.36126
Mn-F3-NC	NC	0.33359	0.44740	0.44735	0.54355
MN-F4	C	0.32847	0.34887	0.31839	0.31719
Mn-F4-NC	NC	0.32098	0.43857	0.44201	0.58006
MN-F5	C	0.31415	0.30352	0.27589	0.20417
Mn-F5-NC	NC	0.37274	0.47778	0.47930	0.58438
MN-F6	C	0.42233	0.39893	0.37203	0.40709
Mn-F6-NC	NC	0.32970	0.47531	0.47255	0.59868
MN-F7	C	0.33094	0.35037	0.25744	0.29094
Mn-F7-NC	NC	0.33383	0.46433	0.46635	0.57356
MN-F8	C	0.36303	0.35111	0.25608	0.25368
Mn-F8-NC	NC	0.33043	0.47452	0.48671	0.60841
MN-F9	C	0.21606	0.24951	0.19550	0.23297
Mn-F9-NC	NC	0.36188	0.39636	0.41841	0.51089
MR-F1	C	0.14773	0.16535	0.16284	0.16091
MR-F1-NC	NC	0.21126	0.22023	0.24800	0.37820
MR-F2	C	0.14817	0.14751	0.15608	0.12683
MR-F2-NC	NC	0.21660	0.27764	0.31044	0.50592
MR-F3	C	0.16320	0.13686	0.14473	0.12630
MR-F3-NC	NC	0.23288	0.28692	0.28875	0.44437
MR-F4	C	0.23894	0.22260	0.19840	0.18462
MR-F4-NC	NC	0.29745	0.27169	0.30342	0.48441
MR-F5	C	0.22028	0.18378	0.17981	0.12933
MR-F5-NC	NC	0.37769	0.39634	0.42858	0.52688
MR-F6	C	0.26020	0.22020	0.23994	0.28384
MR-F6-NC	NC	0.32138	0.45477	0.49085	0.65599
MR-F7	C	0.17929	0.16283	0.15171	0.13051
MR-F7-NC	NC	0.37796	0.46856	0.49307	0.66298
MR-F8	C	0.22505	0.24443	0.25063	0.24253
MR-F8-NC	NC	0.39655	0.46023	0.50795	0.67679
NW-F1	C	0.24183	0.19089	0.25450	0.25001
NW-F1-NC	NC	-0.08382	0.05306	0.16835	0.55286
NW-F2	C	0.14703	0.12486	0.07061	0.15193
NW-F2-NC	NC	0.30577	0.07992	0.25849	0.47615
NW-F3	C	0.14153	0.09640	0.12798	0.15155
NW-F3-NC	NC	0.38143	0.41169	0.46252	0.64357
NW-F4	C	0.25294	0.14793	0.17974	0.21250
NW-F4-NC	NC	0.35943	0.37513	0.45022	0.64717
NW-F5	C	0.34898	0.33522	0.18402	0.18087
NW-F5-NC	NC	0.29381	0.33000	0.36032	0.57981

NW-F6	C	0.43932	0.35384	0.23705	0.19905
NW-F6-NC	NC	0.34248	0.32011	0.40633	0.63165
P-F1	C	0.39822	0.35599	0.33814	0.30463
P-F1-NC	NC	0.29880	0.25764	0.36110	0.53036
P-F2	C	0.35359	0.33537	0.24958	0.28419
P-F2-NC	NC	0.32818	0.19939	0.27879	0.42904
P-F3	C	0.37252	0.36584	0.29780	0.30549
P-F3-NC	NC	0.32053	0.35010	0.32031	0.49958
P-F4	C	0.37245	0.37184	0.30788	0.33725
P-F4-NC	NC	0.30830	0.19802	0.30747	0.45747
P-F5	C	0.42819	0.34054	0.30378	0.29305
P-F5-NC	NC	0.30867	0.19310	0.29749	0.44627
SA-F1	C	0.19531	0.24855	0.16335	0.22938
SA-F10	C	0.31481	0.28477	0.20493	0.36694
SA-F10-NC	NC	0.21683	0.21592	0.22833	0.40936
SA-F1-NC	NC	0.31157	0.26523	0.23395	0.38810
SA-F2	C	0.13480	0.11900	0.13975	0.19036
SA-F2-NC	NC	0.22427	0.22813	0.20560	0.38722
SA-F3	C	0.23501	0.24643	0.20972	0.26317
SA-F3-NC	NC	0.35041	0.33785	0.22376	0.40498
SA-F4	C	0.26159	0.18667	0.13933	0.17332
SA-F4-NC	NC	0.32388	0.22128	0.20494	0.27759
SA-F5	C	0.33827	0.24921	0.21929	0.24250
SA-F5-NC	NC	0.41702	0.38004	0.34849	0.40892
SA-F6	C	0.33669	0.23541	0.19077	0.33210
SA-F6-NC	NC	0.42572	0.38441	0.35853	0.41171
SA-F7	C	0.28681	0.19970	0.14877	0.20416
SA-F7-NC	NC	0.25617	0.24394	0.31349	0.40825
SA-F8	C	0.36112	0.28557	0.23200	0.30955
SA-F8-NC	NC	0.27135	0.26454	0.33567	0.44451
SA-F9	C	0.33275	0.25767	0.15574	0.28604
SA-F9-NC	NC	0.24826	0.23308	0.25390	0.43988
ST-F1	C	0.27690	0.29404	0.28606	0.30569
ST-F1-NC	NC	0.25113	0.33038	0.42480	0.53964
ST-F2	C	0.36852	0.37261	0.32083	0.33425
ST-F2-NC	NC	0.31016	0.39427	0.43808	0.66400
ST-F3	C	0.37288	0.34952	0.33284	0.38780
ST-F3-NC	NC	0.45950	0.47820	0.45867	0.52613
ST-F4	C	0.37708	0.36089	0.35633	0.41266
ST-F4-NC	NC	0.39305	0.44774	0.49808	0.63647
ST-F5	C	0.42474	0.39796	0.24581	0.32278
ST-F5-NC	NC	0.47533	0.48121	0.35964	0.52598
ST-F6	C	0.39644	0.41745	0.19068	0.29210
ST-F6-NC	NC	0.45138	0.50036	0.32996	0.53672

ST-F7	C	0.41478	0.40116	0.23417	0.27920
ST-F7-NC	NC	0.45888	0.47031	0.38440	0.48215
ST-F8	C	0.39744	0.43270	0.22566	0.26116
ST-F8-NC	NC	0.43863	0.47759	0.35471	0.59387

C=contaminated, NC=noncontaminated

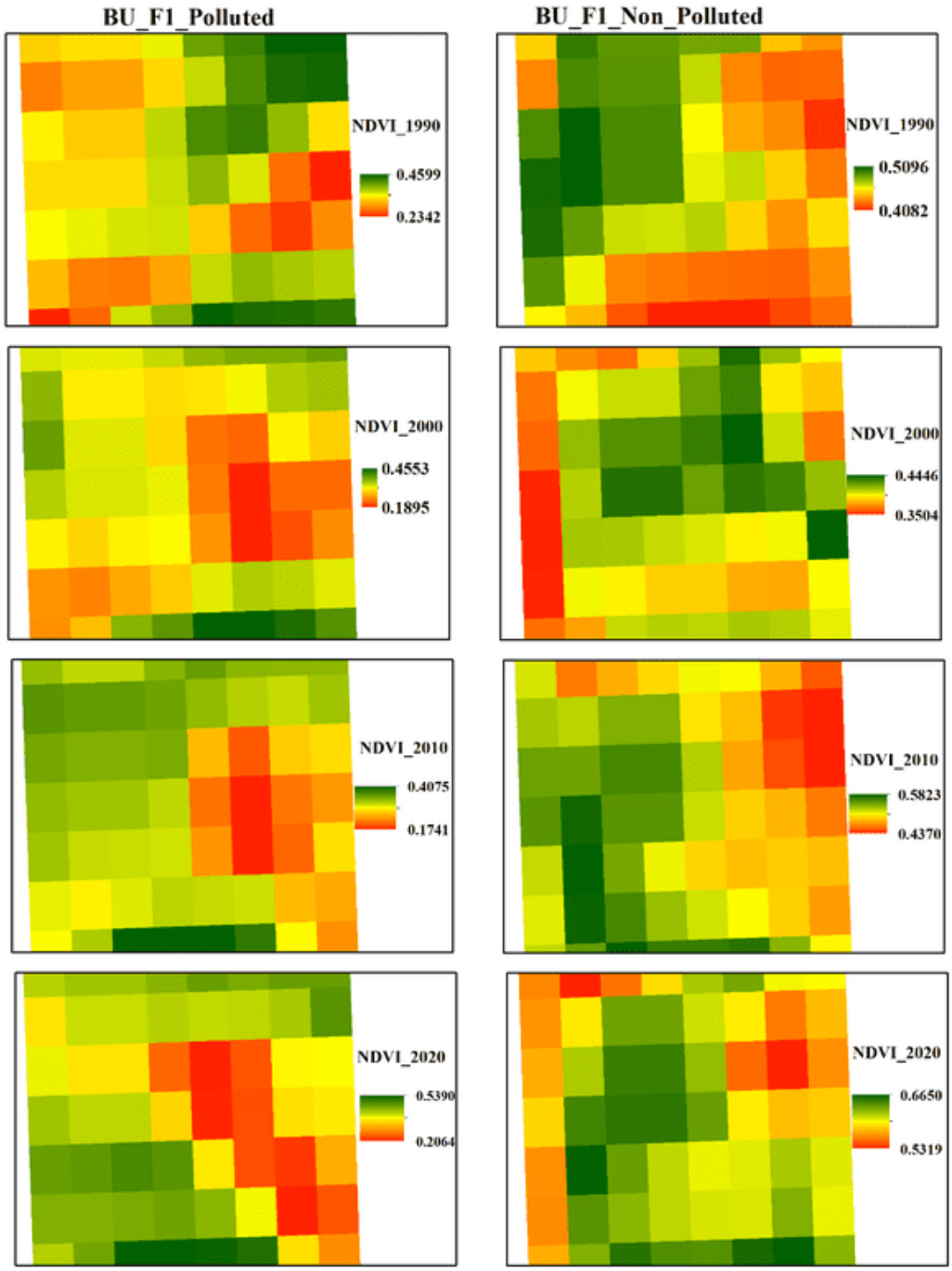


Fig. 5.26 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble Factory-1 and non-polluted plot.

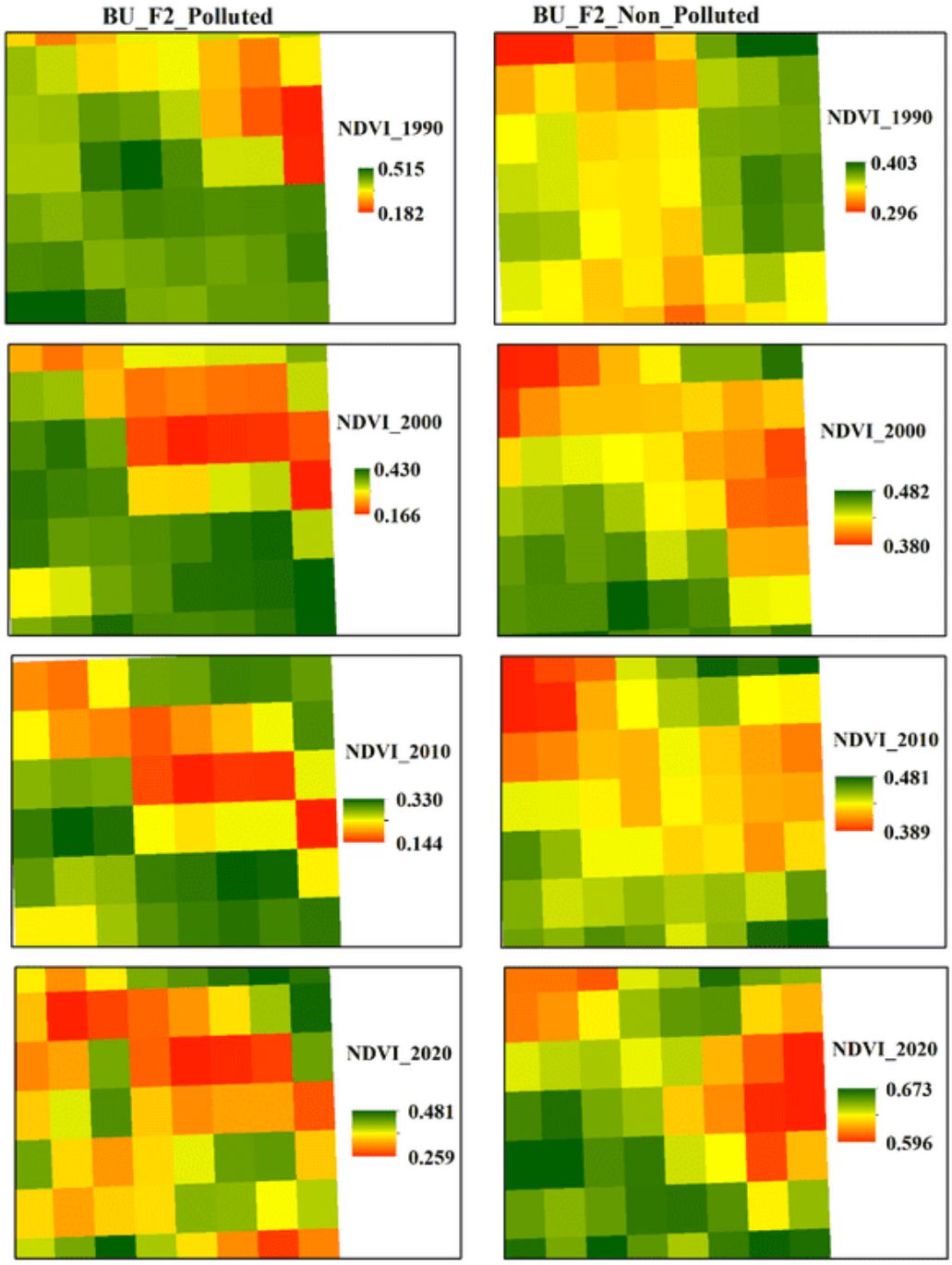


Fig. 5.27 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble Factory-2 and non-polluted quadrat.

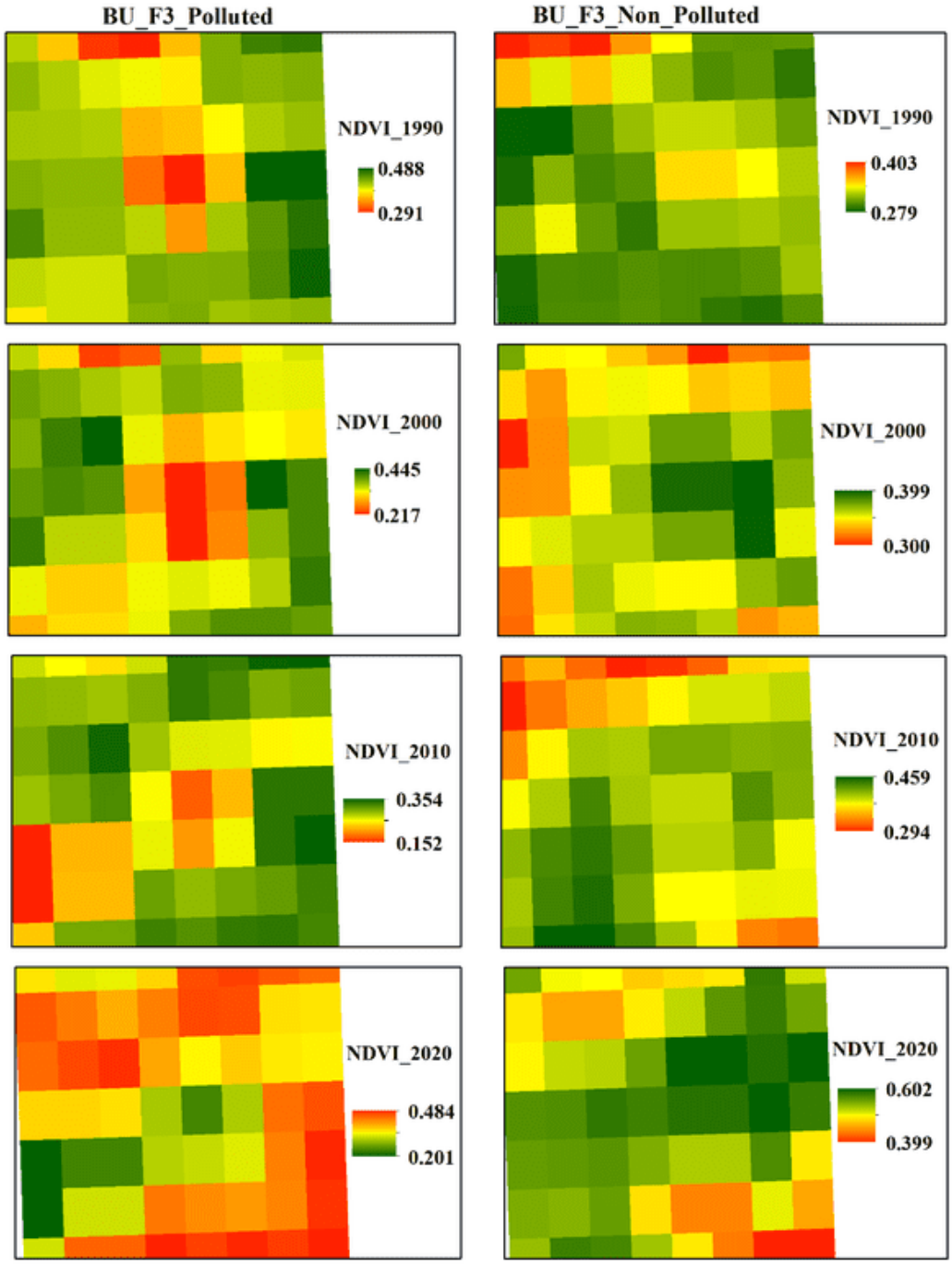


Fig. 5.28 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble Factory-3 and non-polluted plot.

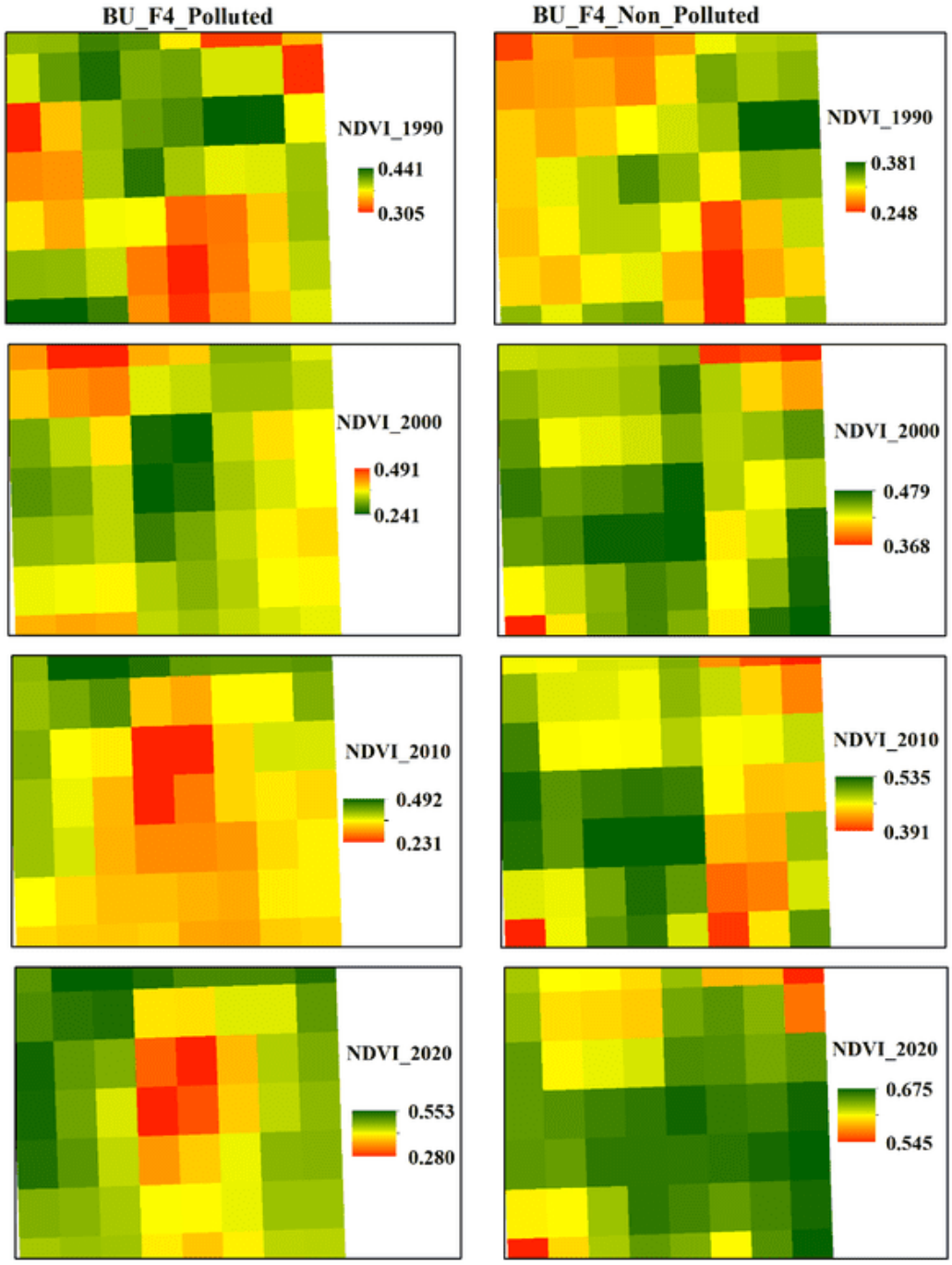


Fig. 5.29 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble Factory-4 and non-polluted quadrat.

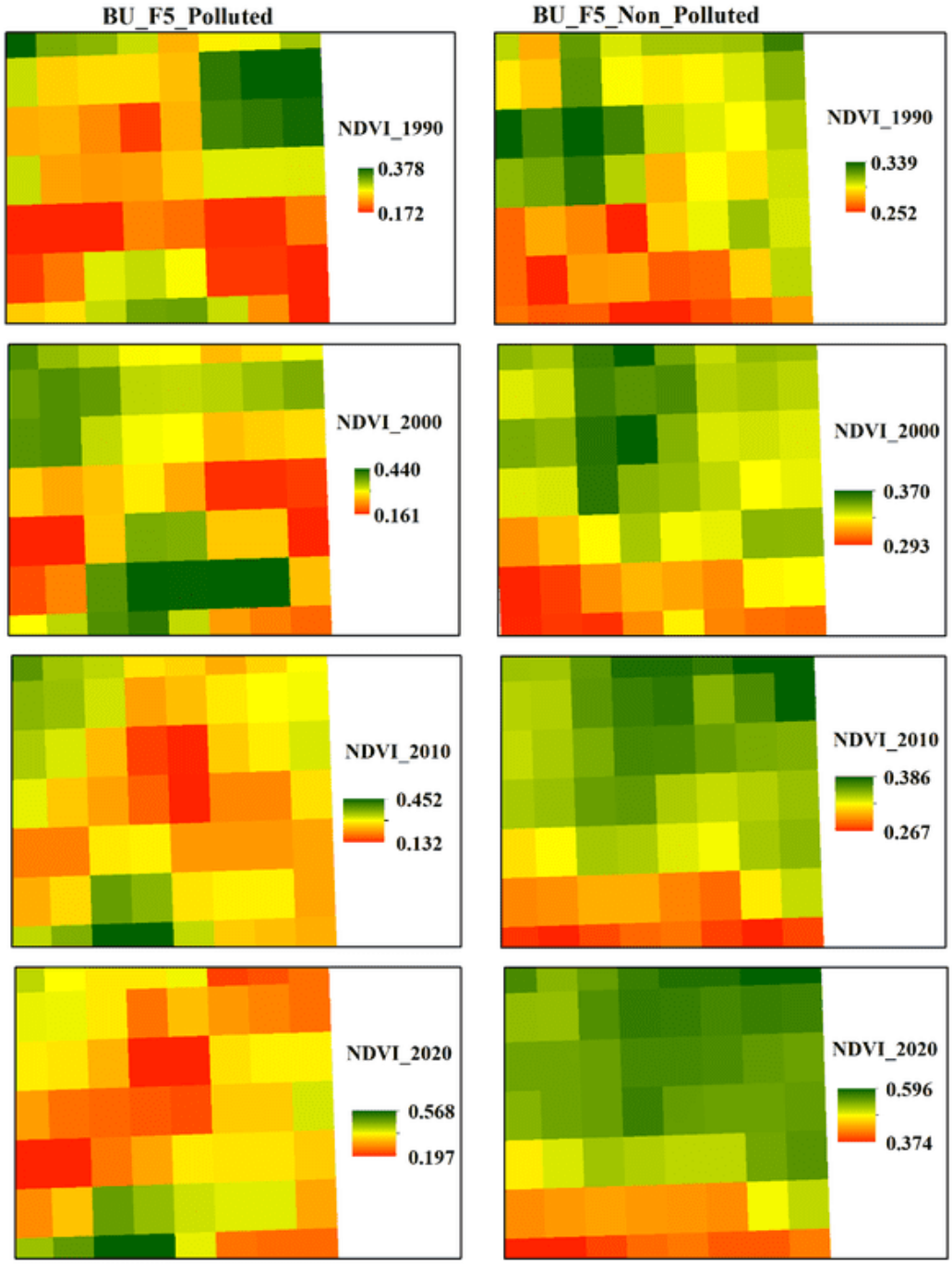


Fig. 5.30 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble Factory-1 and non-polluted plot.

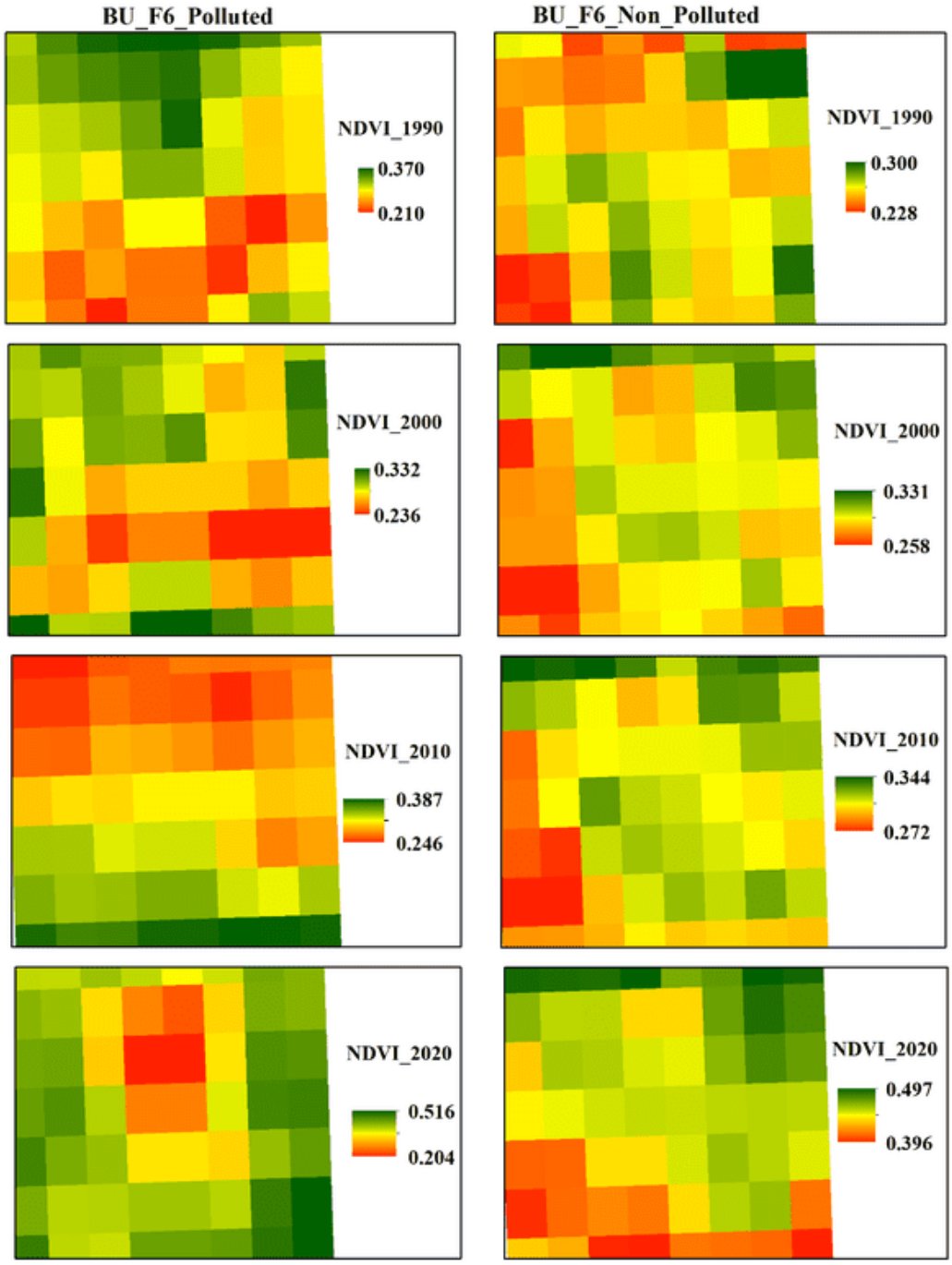


Fig. 5.31 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble Factory-6 and non-polluted quadrat.

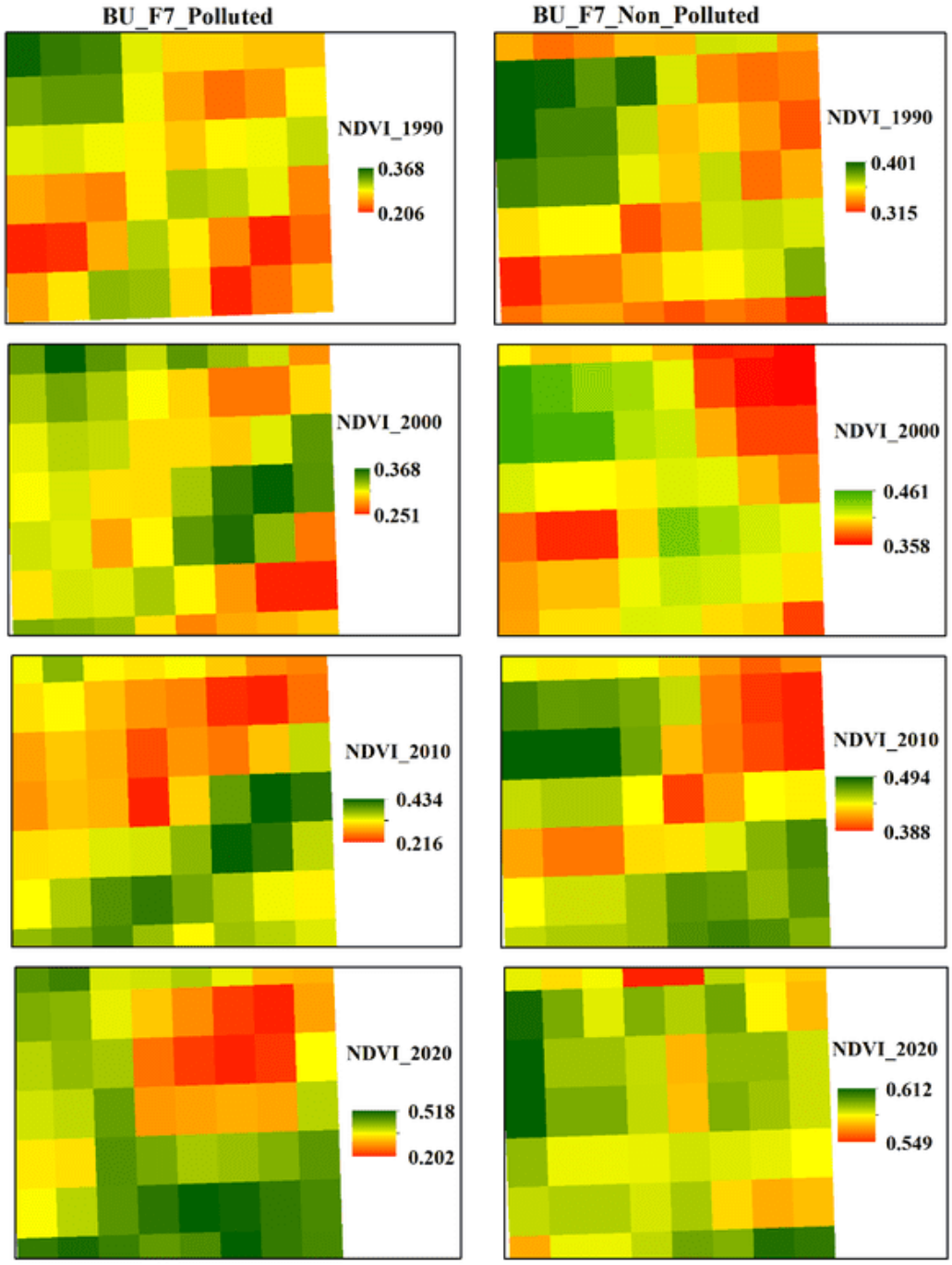


Fig. 5.32 Mean annual NDVI (1990, 2000, 2010 & 2020) in the Buner Marble Factory-7 and non-polluted plot.

5.3.5 Impact of Marble pollution on NDVI through bivariate analysis

Following the assessment of NDVI in the marble polluted and non-polluted regions, an evaluation was undertaken of the impact of precipitation, temperature, and marble pollution concerning NDVI in order to better understand the role of pollution in driving differences in NDVI, and to discount any effect of changes in regional climate over the time period of the study. The NDVI decreases with an increase in marble pollution in the marble contaminated region (Fig. 5.33). In comparison, NDVI increases in the non-contaminated area. At the same time, NDVI strongly correlates with precipitation in the marble contaminated compared to the non-contaminated regions (Fig. 5.34). NDVI also decreases faster with increased temperature in the marble polluted ecosystem than in the non-polluted area (Fig. 5.35).

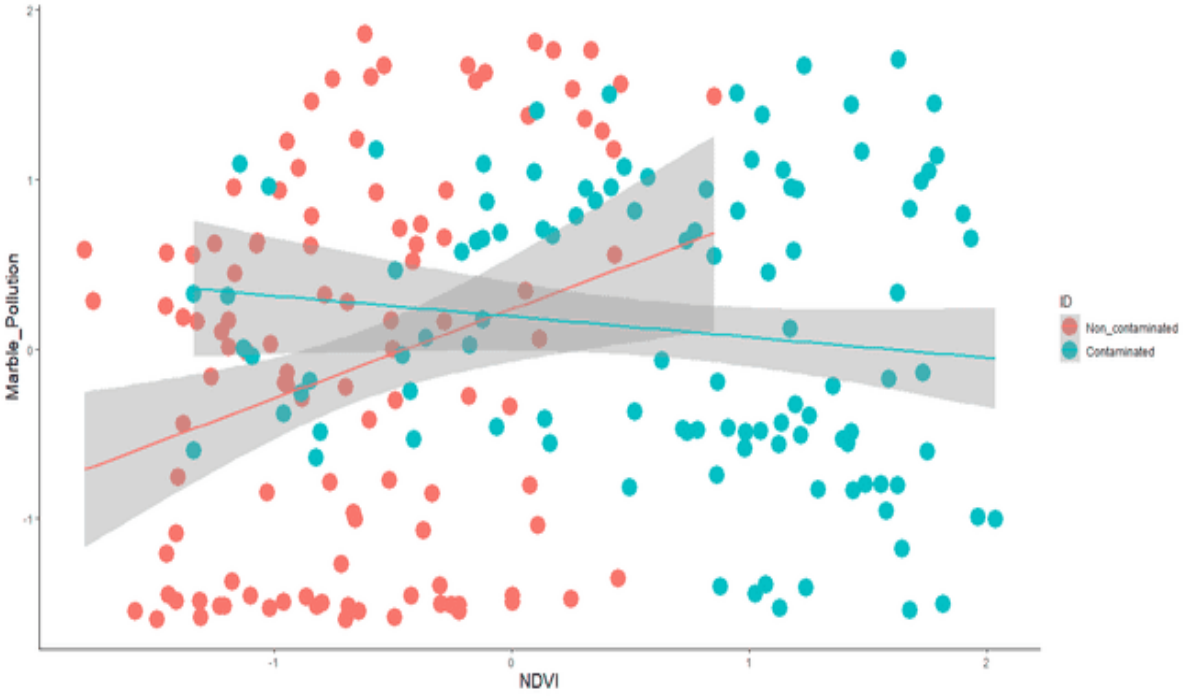


Fig. 5.33 Impact of marble pollution on NDVI in the contaminated and non-contaminated regions.

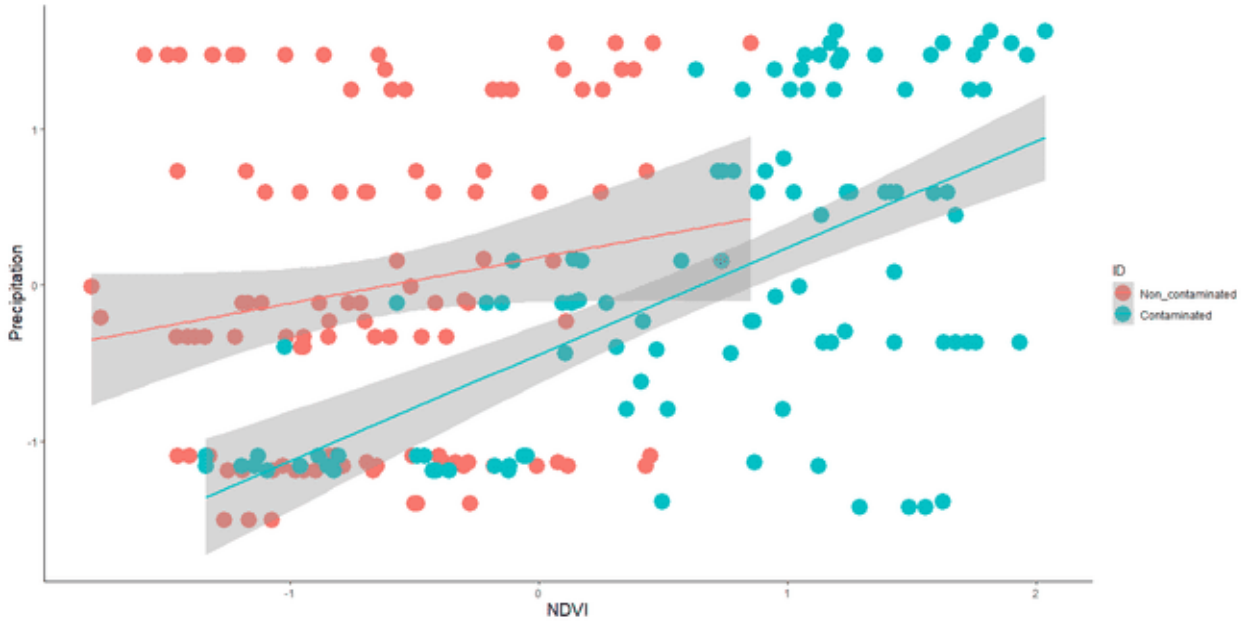


Fig. 5.34 Impact of precipitation on NDVI in the marble contaminated and non-contaminated regions.

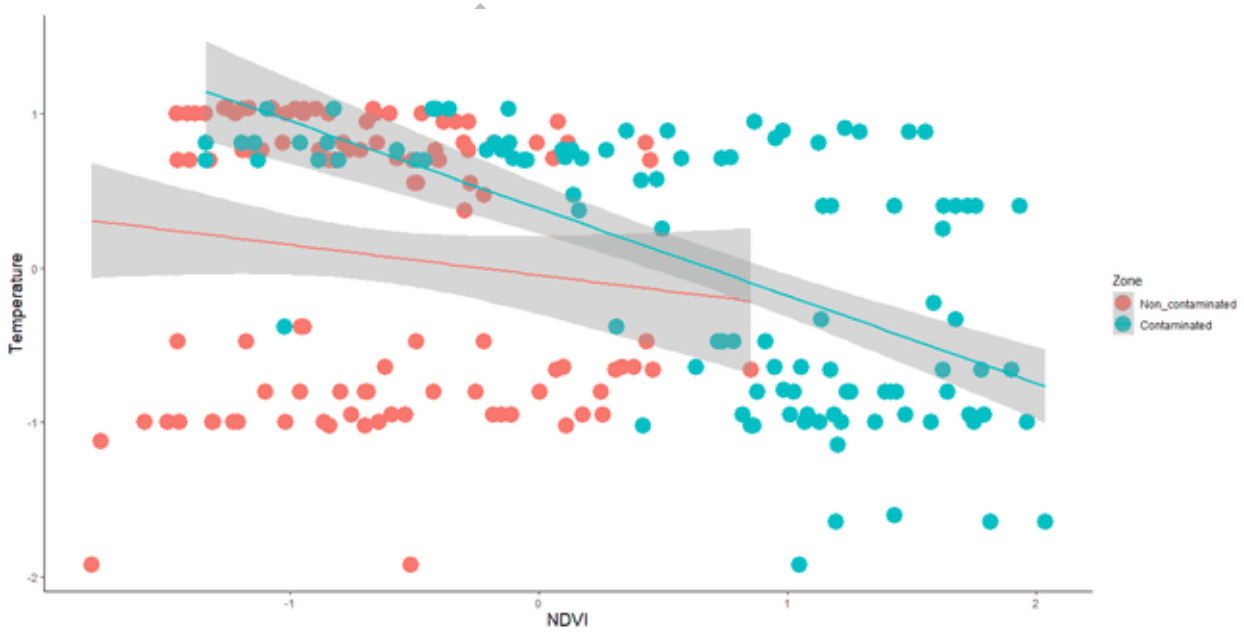


Fig. 5.35 The influence of temperature on NDVI in the MWPE.

5.3.6 Impact of Marble pollution viz NDVI on Structural Equation Modelling

Structural equation modelling was carried out to determine whether NDVI changes are due to marble pollution or precipitation and temperature in the studied area. SEM showed that the marble pollution has a significant negative (β = -0.27) and precipitation has a significant positive (β = 0.24) effect on the NDVI in the polluted region (Fig. 5.36a; Table 5.4). While in the non-contaminated region, the precipitation has a significant positive (β = 0.46) influence on the NDVI compared with marble pollution (Fig. 5.36b & Table 5.4). Hence, marble pollution has a significant role in determining the NDVI of the studied region. The measured variables' direct and indirect effects in marble polluted and non-polluted zones are given in Table 5.5.

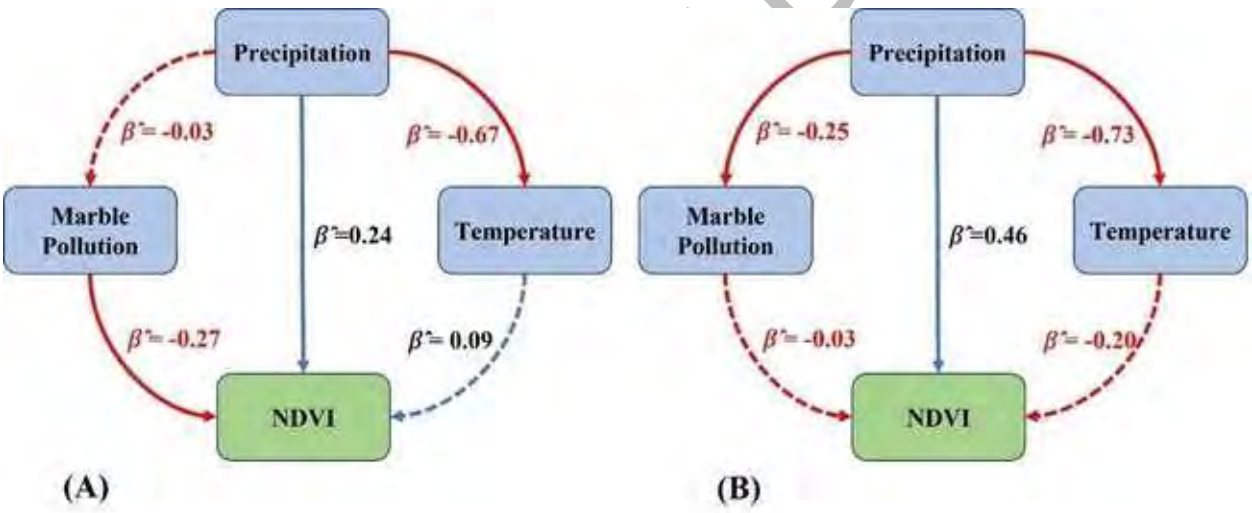


Fig. 5.36 SEM representing the impact of marble pollution, precipitation and temperature on NDVI in the (A) marble polluted and (B) non-polluted regions.

Table 5.4 Summary of SEM represents the impact of marble pollution, precipitation, and temperature on NDVI in the marble polluted and non-polluted regions.

Response	Predictor	Beta	S. Beta	S.E.	t-value	p-value
Marble Polluted zone						
NDVI	**Marble Pollution**	**-0.1407**	**-0.2727**	**0.0477**	**-2.9531**	**0.0039**
NDVI	Temperature	0.0546	0.0983	0.0694	0.7872	0.4329
NDVI	**Precipitation**	**0.1403**	**0.2446**	**0.0715**	**1.9631**	**0.0523**
Marble Pollution	Temperature	0.1098	0.102	0.141	0.7785	0.438
Marble Pollution	Precipitation	-0.0355	-0.032	0.1456	-0.244	0.8077
Temperature	**Precipitation**	**-0.6958**	**-0.6738**	**0.0738**	**-9.433**	**0.0001**
Non-polluted zone						
NDVI	**Precipitation**	**0.4142**	**0.4616**	**0.1019**	**4.064**	**0.0001**
Marble Pollution	**Temperature**	**0.4165**	**0.4702**	**0.1199**	**3.4731**	**0.0008**
NDVI	Marble Pollution	-0.033	-0.0314	0.085	-0.3889	0.6981
NDVI	Temperature	-0.1912	-0.2054	0.1098	-1.7414	0.0846
Marble Pollution	Precipitation	-0.2212	-0.259	0.1156	-1.9133	0.0585
Temperature	**Precipitation**	**-0.704**	**-0.7303**	**0.0643**	**-10.9534**	**0.0001**

Table 5.5 The measured variables' direct and indirect effect in marble polluted and non-polluted zones.

S. No.	Response	Mediator	Predictor	label	Beta	S.E	z-value	p-value
Marble Poluted Zone								
1	**NDVI**	**-**	**Marble Pollution**	**c**	**-0.141**	**0.047**	**-3.017**	**0.003**
2	**NDVI**	**-**	**Precipitation**	**a**	**0.14**	**0.07**	**2.00**	**0.046**
3	NDVI	-	Temperature	b	0.055	0.068	0.804	0.421
4	**Temperature**	**-**	**Precipitation**	**D**	**-0.696**	**0.073**	**-9.521**	**0.0001**
5	Marble Pollution	-	Precipitation	e	-0.041	0.106	-0.384	0.701
10	NDVI	Temperature	Precipitation	bd	-0.038	0.047	-0.801	0.423
11	NDVI	MP	Precipitation	ec	-0.006	0.015	-0.381	0.703
12	**Total**	**Direct + Indirect**	**a+(b*d)+(e*c)**		**0.097**	**0.054**	**1.785**	**0.074**
Non-polluted zone								
1	**NDVI**	**-**	**Precipitation**	**a**	**0.414**	**0.098**	**4.207**	**0.0001**

2	NDVI	-	Temperature	b	-0.191	0.102	-1.875	0.061
3	NDVI	-	Marble Pollution	c	-0.033	0.079	-0.419	0.675
4	**Temperature**	-	**Precipitation**	**d**	**-0.704**	**0.064**	**-11.057**	**0.0001**
5	Marble Pollution	-	Precipitation	e	-0.072	0.082	-0.876	0.381
10	NDVI	Temperature	Precipitation	bd	0.135	0.073	1.849	0.065
11	NDVI	Marble Pollution	Precipitation	ec	0.002	0.006	0.378	0.706
12	**Total**		**Direct + Indirect**		**0.551**	**0.068**	**8.071**	**0.0001**

Table 5.6 Model Fit values after the SEM.

Test	**Marble polluted zone**	**Non-polluted zone**
Chi-sq.	8.621	11.742
p-value	0.431	0.481
GFI	0.997	0.961
AGFI	0.97	0.912
RMSEA	0.01	0.017
NFI	0.992	0.921
RMR	0.021	0.057
CFI	0.986	0.925
SRMR	0.018	0.071
AIC	776.476	719.381

5.4 Discussion

Environmental pollution has become one of the main problems globally. Increase in industrialization has worsen this situation by adding heavy metals or potential toxic elements (PTE) into the natural environment (He et al. 2013). PTEs don‘t easily break down through natural degradation procedures. Unfortunately, these PTEs can accumulate in the soil, which directly or indirectly influence the chemical and physical characteristics of the surrounding ecosystem (Khan et al. 2008; Nicholson et al. 2003). The presence of a large amount of these elements in the soil poses an increasing risk to all organisms. Understanding the spatial distribution of PTE is an important prerequisite for monitoring and evaluating the environment (Facchinelli et al. 2001; Pan et al. 2016). The current chapter evaluates the spatial distribution pattern of potential toxic elements in the marble waste polluted ecosystem of Khyber Pakhtunkhwa, Pakistan. On the basis of average metal concentration, calcium has the highest concentration followed by magnesium, iron, zinc, manganese, copper, nickel, cadmium, chromium and cobalt. Similar to our study, (Li et al. 2014) summarized the data of soil metal contamination from 72 mining regions. They reported eight heavy metals concentration exceeding the grade II environmental quality standard for soil in the China (SEPAC, 1995). Interpolation methods, i.e., IDW of ArcGIS 10.8 were used to interpolate the heavy metal concentrations in the marble waste polluted ecosystem. The PTE concentration /density was indicated through red circle on the maps. Similar to the current study, the geo-statistics multivariate and GIS approaches are powerful analyses that have been carried out in various research studies for the assessment of spatial distribution of pollution (Gong et al. 2010; Guagliardi et al. 2012; Imperato et al. 2003; Lee et al. 2006; Morton-Bermea et al. 2009; Rodríguez-Salazar et al. 2011; Yuan et al. 2013). The IDW approach has been used in various soil quality survey that integrates GIS with multivariate statistical analysis (Huang et al. 2015; Iñigo et al. 2011; Lee et al. 2006; Zhang 2006). This method is one of the most frequent used spatial interpolation approaches due to its fast implementation, easy use and interpretation (Lu and Wong 2008).

Similar to the current study (Mihailović et al. 2015) also assessed the spatial distribution of heavy metals in the Novi Sad, Serbia. They applied the GIS mapping tools and techniques for the spatial distribution of heavy metal contamination in the region. They also reported the arsenic, cobalt, manganese and nickel as natural origin

in the region while copper, lead and zinc originated from anthropogenic activities (Mihailović et al. 2015). Furthermore, (Pan et al. 2016) worked on the heavy metals (arsenic, chromium, lead, zinc, mercury, nickel, copper and cadmium) origin and spatial distribution pattern in the Xiangfen, Shanxi province, China. The sources of these heavy metals were industrial practices (chromium and mercury), agricultural, vehicle emission (cadmium, lead, zinc and copper) and parent materials (arsenic and nickel). The spatial distribution of these metals varied significantly and closely correlated to the local anthropogenic activities (Pan et al. 2016). The ArcGIS spatial distribution of PTE was related to human disturbance in the Weifang region (Liu et al. 2016). ArcGIS has become an important approach for the spatial distribution of heavy metals and pollution assessment. (Shi et al. 2007) used ArcGIS software for the assessment of spatial distribution of heavy metals in the Changxin Zhejiang region of China. They suggested ArcGIS for spatial distribution of heavy metals or pollutants and health risk assessment.

In the current study spatial autocorrelation was used to describe the spatial pattern formed by heavy metals. This method can help to understand the degree of similarity and differences among variables. Moran‘s Index (MI) was used for the determination of spatial autocorrelation. The spatial autocorrelation was considered as problem requiring correction as compared to inherent ability of spatial data in the early days of GIS research (Goodchild et al. 1992). Nevertheless, researchers have discovered the spatial autocorrelation as ubiquitous, occurring at spatial scales up to hundreds of kilometers (F. Dormann et al. 2007). Spatial autocorrelation could be taken to derive values for a given attribute factors in the areas between observed samples (F. Dormann et al. 2007). (Eze et al. 2019) worked on the turning bands conditional co-simulation approach has been successfully used to quantify and map spatial uncertainty of heavy metals in a semiarid Ni–Cu exploration field in Botswana. In the case of heavy metals dataset with strong positive correlation, multivariate mapping of the cross-correlated variables is highly encouraged rather than univariate mapping of each variable separately because the former method considers the intrinsic dependency among variables, and so the post-processing outputs are more reliable. The co-simulation maps of the heavy metals show spatial variations in Co, Mn and Fe distribution.

In the current study, the Normalised Difference Vegetation Index was calculated for the marble waste polluted (contaminated) and non-polluted (noncontaminated) regions from 1986 to 2021. The results comprehend a significant NDVI difference in the polluted and non-polluted areas. The non-polluted areas have higher NDVI than the marble polluted regions. The overall average NDVI in the marble polluted and non-polluted regions were 0.263 and 0.382, respectively.

Normalize difference vegetation index (NDVI) is the measurement of quantitative estimation from the surface reflectance to calculate vegetation cover‘s growth and biomass (Arabameri and Pourghasemi 2019). It monitors vegetation of an area from space in the visible and infrared portion of the spectrum (Bannari et al. 1995; Baret and Guyot 1991; Justice et al. 1986; Tucker et al. 1991; Tucker et al. 1985). Understanding human disturbance particularly industrialization enables us to predict productivity changes under different climatic scenarios, hence NDVI has been recognized as a good indicator for terrestrial vegetation productivity (Wang et al. 2001). Vegetation performs a baseline for all living things and carrying out a crucial role in global equilibrium, therefore vegetation structuring is very important to manage all the natural resources and plan land uses. Vegetation mapping provides a valued information to investigate natural and semi-natural ecosystem for quantification of the vegetation cover both locally and globally, for a given time over a continuous period (He et al. 2005; Malatesta et al. 2013; Xiao et al. 2004). The arrival of recent technology and the use of remote sensing provides very efficient tools used to monitor the spatial and temporal changes upon the surface of land. Similar findings have also been reported by (Sun et al. 2019), who worked on the different pollution concentration in relation to NDVI changes in the Beijing, Tianjin, & Hebei, China. They concluded that the high concentration of pollution has negative correlation with NDVI in the studied regions. The lower NDVI values, the more obvious interference of human disturbance in the form of environmental pollution (Sun et al. 2019). NDVI reflects the land use, industrial layout to certain extent and these factors directly or indirectly estimates the level of environmental pollution. (Prakasam et al. 2022) worked on the estimation of NDVI as a precursor for monitoring air pollution. They concluded that increases in environmental pollution decreases the forest vegetation quality (NDVI) between 2001-2021 (Prakasam et al. 2022).

In the current study Google Earth Engine (GEE) was used to assess the NDVI from 1986-2021. Remotely sensed NDVI values were used for the assessment of NDVI temporal changes in the marble waste polluted ecosystem. GEE, the cloud computing platform can be used to extract a number of parameters such as Normalized Difference Vegetation Index (NDVI) utilizing images taken by satellite. GEE may be used to show and plot data in time series graphs or download data for external processing. It was successfully implied for analyzing forests losses and gains on global scale (Kim et al. 2014; Townshend et al. 2021). GEE can also be used for identification of archaeological heritage sites (Agapiou 2017), risk mapping for spread of malaria, population mapping (Patel et al. 2015), automated mapping of cropland (Xiong et al. 2017) and soil mapping (Padarian, Minasny, & McBratney, 2015). In addition to researchers throughout the world, GEE is also used by millions of regular users as the Google Maps is put together (i.e mosaicked) by GEE. GEE is easy to read, well documented and high level dynamic programming language (Schmid 2017). Google datacenters get scripts that run in the Code Editor, get executed and the results are shared with the users. In this way, the workflow becomes fast in such a way that the scripts can be tested, revised and tested again to obtain desired performance. An image obtained as a layer from Google Maps base map, the one used in this study, a just in time principle for computation will be utilized as this will compute the data necessary for current map zoom. This increases the speed of visualization of results by GEE (Ferrari and Cribari-Neto 2004). Remote sensing alone is not enough to assess the current and past status of the forests in a comprehensive manner. A combination of remote sensing and *in situ* observations about land use (indicator sets) can bring about comprehensiveness in the analysis. Thus, the utilization of indicator sets in analyses is necessary. In the future, indicator sets- as automated and standardized models- should be part of every remote sensing analysis. Further improvement in the indicator sets can be brought about by incorporating information about local plant and animal species from open access data sites such as Global Biodiversity Information Facility (GBIF).

5.5 Conclusion

It is concluded that the use of GIS technology is an effective approach for the mapping of heavy metals contamination. The results comprehend a significant NDVI difference in the marble polluted and non-polluted areas between 1986-2021. Increase in marble pollution with time decreases the average NDVI of the region. The non-polluted areas have higher NDVI than the marble polluted regions. The overall average NDVI in the marble polluted and non-polluted regions were 0.263 and 0.382, respectively.

6.1 Discussion and Synthesis

Environmental pollution is a substantial issue of the world, causing huge damage to natural ecosystems. Approximately, 2.01 billion metric tons per annum waste is produced worldwide. According to World Bank‘s estimations, waste generation will increase up to 3.4 billion metric tons by 2050. An estimate of 13.5% of today‘s waste is recycled and 5.5% is composted. One-third or 40% of the waste generated is not managed appropriately and is dumped or openly burned. Among one of them is the improper use of marble industry. Globally, Pakistan is 6th largest extractor of the marble mineral and granite. According to Pakistan Federal Boards of Revenue Directorate of Training and Research, there are 160.2 million tons of the marble reserves in the country. Out of these 158 million ton (98%) is present in province of Khyber Pakhtunkhwa (KP) Pakistan. Marble industry or pollution has been one of the basic deteriorating sources of biosphere pollution with impacts at the species level. Rapid increases in the rate of marble industrialization have worsened the pollution problems (Lin, Panchangam et al. 2014; Lai, Lin et al. 2018; Hsiao, Lin et al. 2020; Yuanan, He et al. 2020). Levels of contamination or pollutants increase in the surrounding ecosystem (water, soil and air) that in turn adversely affect natural environment in the form of physical and chemical changes. As a result, it affects people, animals and plants directly in the form of diseases and indirectly in the form of food safety and habitat destruction. In such situations it becomes imperative for young students and researchers to study environmental issues including pollutant, microbial strains, bioremediation procedure and heavy metals accumulation. For the first time, this thesis evalautes in depth the marble waste poluted ecosytem of Khyber Pakhtunkhwa, Pakistan. This study determines the physio-chemical properties of the marble waste polluted ecosystems, their holistic impacts on local vegetation & their classification, identification and role of specific indicators (plants and Fungi) in remediation, internal physiological changes in indicator plants, spatial distribution of potential toxic elements (heavy metals), NDVI changes and corrective measures to treat marble pollution in the Khyber Pakhtunkhwa province, Pakistan.

6.1.1 Vegetation dynamics; abundance and rare plant species of the MWPE

The ordinary least square, logistic and probabilistic models were used for the analysis of binary outcome variables i.e., abundant and rare plant species (Kwak and Clayton-Matthews 2002; Moffat 2014). It gives us why some of the plant species are abundant, while some of them are rare in the marble waste polluted ecosystem. The explanatory variables that were taken including soil major characteristics and binary variables were abundant and rare plant species. The abundant tree species based on higher importance value index were *Ficus carica*, *Morus alba* (2441 IVI), *Morus nigra* (1699 IVI), *Ailanthus altissima* (1655 IVI), *Populus alba* (1647 IVI), *Broussonetia papyrifera* (1624 IVI), *Eucalyptus globulus* (1308 IVI), *Dalbergia sissoo* (970 IVI), *Azadirachta indica* (898 IVI) and *Salix tetrasperma* (724 IVI). The foremost abundant shrub species were *Calotropis procera* (1799 IVI), followed by *Datura innoxia* (1050 IVI), *Ricinus communis* (797 IVI), *Withania somnifera* (781 IVI), *Lantana camara* (444 IVI), *Ziziphus nummularia* (392 IVI), *Rosa indica* (382 IVI), *Senna occidentalis* (332 IVI), *Dodonaea viscosa* (320 IVI) and *Vitis vinifera* (317 IVI). The top ten most abundant herb species were *Cynodon dactylon* (2982 IVI), *Parthenium hysterophorus* (1122 IVI), *Erigeron canadensis* (993 IVI), *Arundo donax* (795 IVI), *Adiantum capillus-veneris* (739 IVI), *Cannabis sativa* (664 IVI), *Xanthium strumarium* (633 IVI), *Taraxacum officinale* (627 IVI), *Amaranthus viridis* (621 IVI) and *Eleusine indica* (619 IVI). Whereas, the top ten rarest tree species were *Pyrus communis* (6.78 IVI) followed by *Cupressus sempervirens* and *Araucaria heterophylla* (8.46 IVI each), *Litchi chinensis* (12.22 IVI), *Juglans regia* (13.09 IVI), *Citrus limon* (16.67 IVI), *Sapium sebiferum* (17.42 IVI), *Ficus macrophylla* (17.56 IVI), *Ficus benjamina* (27.5 IVI) and *Citrus reticulata* (56 IVI). At the same time, *Rumex hastatus*, *Combretum indicum* (13.88 IVI), *Bougainvillea spectabilis* (17.78 IVI), *Duranta stenostachya* (22.72 IVI), *Datura metel* (33.34), *Nannorrhops ritchieana*, *Indigofera heterantha*, *Duranta erecta*, *Debregeasia saeneb* (each with 66.67 IVI) and *Parthenocissus inserta* (120.74 IVI) were recorded as rare shrub species of the MWPE. *Bidens bipinnata* and *Pteris cretica* (3.67 IVI each) were the rare herb species accompanied by *Aloe vera* (4.18 IVI), *Dichanthium annulatum* (4.53 IVI), *Capsella bursa-pastoris* (5.04), *Malva sylvestris* (5.09 IVI), *Aerva javanica* (5.27 IVI), *Brassica campestris* (5.45 IVI), *Artemisia scoparia* (5.78 IVI) and

Sisymbrium irio (6 IVI) in the MWPE. Based on OLS, logistic and probabilistic models the abundance and rareness of these plant species were due to $CaCO_3$, magnesium, soil pH, electrical conductivity, chromium, copper, zinc, iron, organic matter, potassium, phosphorous, manganese, nickel, cadmium, calcium, temperature, precipitation and elevation (Yee and Mitchell 1991; Wu and Huffer 1997; Guisan, Theurillat et al. 1998; Augustin, Cummins et al. 2001; Carl and Kühn 2007; Kubota, Shiono et al. 2015). Our model is best fit based on R^2, Akaike Information Criteria, Chi-square and Probability values. This quantitative modeling approaches i.e., OLS, probabilistic and logistic to dominant and rare plant species of the MWPE not only fulfills the methodological deficiencies and gap in the literature regarding the impact of marble pollution on vegetation. But it also provides a firm basis for extending this approach for the impact of other types of pollution on vegetation in different region of the world.

6.1.2 Multivariate statistical approach Modelling

One of the important applications of this research project was the use of multivariate statistical techniques, i.e., Cluster Analysis, Two-way Cluster Analysis, Indicator Species Analysis, Species Area Curves, Detrended Correspondence Analysis, Canonical Correspondence Analysis, Structural Equation Modeling, Ordinary Least Square, Probabilistic & Logistic Models, Bivariate regression etc., to accurately indicate and interpret vegetation distribution patterns in the marble waste polluted ecosystem. It allows the researcher to compare multiple classification and their interrelationship for the factual information resulting from the analyses (Khan, Khan et al. 2016). TWCA was used to identify potential subtropical vegetation of MWPE based on pattern similarity via Sorenson distance measurements. ISA identified the significant indicators of each subtropical MWPE (Dufrêne and Legendre 1997). ISA helps to relate indicators with environmental conditions (Baker and Wiley 2004; King, Richardson et al. 2004). In the current study, CCA was used to determine the relationship of plant species with different environmental variables (Ter Braak and Smilauer 2002). It is mostly used to explain covariation between two sets of variables and find canonical variates that are important for explaining covariation between sets of variables. Correlation of the canonical axes and explanatory matrix was reported, along with the significance of each correlation determined via permutation. Testing the hypothesized relationship between response and explanatory variables by

standardizing the axis scores and centering on the unit variance and axes scale to optimize the representation of species. These techniques were also implemented by a different researcher for the classification of vegetation in the different regions of the world for various types of ecological observations like (Khan, Page et al. 2012; Khan, Page et al. 2014; Iqbal, Khan et al. 2015; Ahmad, Khan et al. 2016; Khan, Khan et al. 2016). Furthermore, Structural Equation Model was done using R- software in order to examine the complex relationship of vegetation structure/indicators plants and impact of marble pollution, climate, elevation and edaphic variables of three major subtropical vegetation zones of MWPE. It also examined the direct and indirect impact of measured environmental variables for clearer picture of the subtropical vegetation (Byrne 1994; Sharma, Mukherjee et al. 2005). These statistical tool and techniques can be used in the field of vegetation ecology for the investigation of the complex relationship between vegetation dynamics and pollution or environmental gradients.

6.1.3 Concept of Indicator species in MWPE

This vegetation study of the MWPE is distinctive as, unlike other vegetation assessments along the pollution and environmental gradients which simply compare vegetation indices among different zones and treat all species equally without surveying their ecological position (Oommen and Shanker 2005; Gould, González et al. 2006; Crimmins, Crimmins et al. 2008; Wazir, Dasti et al. 2008; Siddiqui, Ahmed et al. 2009). Indicator species analysis was used for the identification of indicator plant species in the three major subtropical marble waste polluted ecosystem.

It provides knowledge about species fidelity with the habitat of specific subtropical vegetation zone of MWPE. The indicator values were calculated using data about species abundance in PCORD and at least one indicator species (statistically significant) was selected for each of the tree, shrub and herb layers for each marble polluted zone using ISA. The Monte Carlo Test was carried out for statistical significance after determining Indicator Values (%age of perfect indication established on combing values of relative abundance and frequency) of respective indicators (Dufrêne and Legendre 1997). A threshold level of 25% indication and 95% significance ($p \leq 0.05$) was deliberated as a cutoff value for determining the indicators. The topmost indicators of Humid subtropical MWPE were *Ficus carica*, *Catharanthus roseus* and *Erigeron canadensis*. Other indicators of humid subtropical

zone were *Ailanthus altissima*, *Salix tetrasperma*, *Diospyros lotus*, *Punica granatum*, *Prunus persica*, *Pinus wallichiana*, *Amaranthus viridis*, *Eleusine indica*, *Brachiaria ramosa*, *Persicaria barbata*, *Solanum americanum*, *Echinochloa colona*, *Paspalum distichum*, *Tagetes erecta*, *Setaria viridis*, *Sorghum halepense*, *Oenothera rosea*, *Setaria pumila*, *Plantago major*, *Digera muricata*, *Rumex nepalensis, Arundo donax* and *Artemisia vulgaris*. While, the topmost indicator species of semi-humid subtropical MWPE were *Morus nigra*, *Datura innoxia* and *Persicaria glabra*. The other characteristic species of this vegetation zone were *Populus alba*, *Albizia lebbeck*, *Mangifera indica*, *Ziziphus jujuba*, *Celtis australis*, *Ficus palmata, Senna occidentalis, Adiantum capillus-veneris, Chenopodium album, Dactyloctenium aegyptium, Achyranthes aspera, Persicaria maculosa, Adiantum incisum, Euphorbia hirta, Juncus maritimus, Cortaderia selloana, Cheilanthes acrostica, Rumex dentatus, Poa annua, Verbena officinalis, Saccharum spontaneum* and *Colocasia esculenta*. The identified topmost indicators of dry subtropical MWPE were *Dalbergia sissoo*, *Withania somnifera* and *Saccharum bengalense*. Other characteristics species included *Prosopis juliflora* (Sw.) DC., *Tamarix aphylla*, *Tribulus pentandrus*, *Ricinus communis*, *Dodonaea viscosa*, *Rumex hastatus*, *Cynodon dactylon*, *Desmostachya bipinnata*, *Artemisia persica*, *Dysphania nepalensis*, *Euphorbia prostrata*, *Sonchus oleraceus*, *Chenopodium murale*, *Heliotropium europaeum*, *Boerhavia diffusa*, *Solanum surattense*, *Cucumis melo var agrestis* and *Dysphania ambrosioides*.

The identified indicator species after the ISA was further reconfirmed through structural equation modeling. This reconfirmation of indicator species via SEM analysis has been done for the first time which can be used for the multipurpose including restoration of polluted ecosystem, reforestation drives and smart habitat plantation. This statistical and modeling procedure adopted in the current study could be followed to classify vegetation and identify indicator plants of any geographic region or microhabitat type in any part of the world.

6.1.4 Physiological response as defense mechanism

Some of the indicator species were selected for assessment of physiological response or changes as a defense mechanism against marble pollution based on their

importance value index and indicator values. Literature reviews showed that majority of such experimentations had generally been performed solely in vitro and there are very few examples from the natural sites (Pál, Horváth et al. 2006; Zobayed, Afreen et al. 2007; Hayat, Khalique et al. 2012; Ghatak, Chaturvedi et al. 2018; Arif, Singh et al. 2020). This thesis evaluated the physiological response of naturally occurring indicator species of the marble polluted ecosystem. The amount of proline accumulation increases with increase in the marble pollution. While chlorophyll-a, chlorophyll-b and total carotenoids decrease with increases in marble pollution. The proline has highly significant negative relation with chlorophyll a, chlorophyll-b, total carotenoids and significant positive correlation with biological concentration factors, translocation factor and bioaccumulation coefficient factor. Proline accumulation is a typical physiological response/reaction of certain plant species to a wide range of environmental pollution. The amount of proline concentration increases in plant species as a defense mechanism to cope with environmental stress and to improve survival (Bates, Waldren et al. 1973; Akshita, Nandini et al. 2018; Amiri, Nafez et al. 2020). Exposure of plant species to pollutants causes a reduction in the amount of photosynthetic pigments (Arellano, Tansey et al. 2017; Li, Feng et al. 2017; Lin and Jin 2018; Kanwal, Farhan et al. 2020). These and other physiological variations help plants to maximize their efficiency for resource utilization under environmental stress. This evaluation of internal physiological changes of indicator plant species can also be checked for abundant and rare plant species of any polluted ecosystem in order to understand the physiological factors responsible for the survival and death of plant species in the polluted environment. This study can be performed for other kinds of polluted systems as well. The naturally grown plants can be easily developed, propagated and forested if found beneficial in ecological and physiological terms.

6.1.5 Bioremediation of indicator plants and Micro Fungi

The bioremediation ability was determined of the naturally occurring indicator plant species and micro fungi of the MWPE. The standard accumulation, transfer and concentration quotients for each indicator plant species were measured through Bioaccumulation Coefficient (BAC), Translocation Factor (TF) and Biological Concentration Factor (BCF) (Malik, Husain et al. 2010). All the selected plant indicators i.e., *Adiantum capillus-veneris, Ailanthus altissima, Albizia lebbeck, Calotropis procera, Cynodon dactylon, Datura innoxia, Debregeasia salicifolia,*

Desmostachya bipinnata, Dodonaea viscosa, Erigeron bonariensis, Ficus carica, Morus alba, Morus nigra, Parthenium hysterophorus, Persicaria glabra, Ricinus communis, Setaria viridis, Tamarix aphylla and *Withania somnifera* have significant phytoremediation ability against chromium, nickel, copper, manganese, zinc, iron, cobalt, cadmium, calcium, magnesium and sodium potential toxic elements in the marble waste polluted ecosystem. Certain plant species retain the inherent ability of bioaccumulation, translocation and degradation of different types of pollutants (Sepehri, Sarrafzadeh et al. 2020). They play a role as a sink for biologically hazardous materials (Schwitzguébel 2017). This ability can be viewed as a low cost technology driven by natural sunlight energy and taking place in situ, where plants accumulate PTEs from the environment (Salt, Smith et al. 1998). Similarly, the mycoremediation ability of *Aspergillus sydowii*, *Aspergillus brasilensi, Curvularia aeria* and *Alternaria alternata* (isolated from MWPE) was assesses. All these species showed efficient bioremediation ability against cadmium, copper, cobalt, magnesium, iron, mercury, nickel, sodium and calcium heavy metals. Fungi have the broader ecological and biochemical capability to degrade environmental organic pollutants and hence reduce the risk associated with metalloids, metals and radionuclides (Harmus et al., 2011). They are ideal species for the remediation of different types of pollutants due to their vigorous growth, immense hyphal network, high surface to volume ratio, production of extracellular enzymes, adaptability to changing pH and temperature (Khan et al., 2019; Singh et al., 2015; Kapahi et al., 2017; Bhattacharya et al., 2011).

6.1.6 Spatial distribution pattern of PTE and temporal changes in NDVI

The spatial distribution of potential toxic elements (heavy metals) released from the marble waste polluted ecosystem were assessed using ArcGIS software. The assessment and mapping of heavy soil metals can assist the development of strategies to promote sustainable use of soil resources, decrease soil degradation and conserve natural vegetation. This will include identifying contamination levels and assessing associated impacts on the environment and human health. Remote sensing is one of the most important methods for environmental investigation, mapping, and soil survey (Lillesand and Kiefer 2003). The GEE was used for the determination of temporal

changes in NDVI for the last forty years in the MWPE. The vegetation index is a simple and effective measurement parameter used in remote sensing to designate the vegetation cover and impact of pollution on flora (Ahmadi and Nusrath 2010). NDVI reflects the land use, industrial layout to certain extent and these factors directly or indirectly estimates the level of environmental pollution.

6.1.7 Concept of Water tank for initial treatment of marble wastewater

Large quantity of water consumed during marble processing i.e., cutting, cleaning, washing, cooling of saws and polishing that results enormous quantity of the wastewater. The production of marble process involves water use for cool down the cuttings, equipment‘s and control particulate matter emission. During marble processing process 30-40% of block weight is wasted into fine particles (powder) of around <0.2 mm in size. Hence, a large amount of water is used to capture the produced particles, resulting in the generation of high solid effluent (Taşdemir and Kurama 2013). Initially, wastewater coming from the marble industry can be stored in sedimentation or settlement tank separated by walls. The layout will include 3-4 sedimentation tanks, where in the first one more sludge will be settled down and the last one will contain more clean water. These can be reused by marble factory directly or may discarded into constructed wetland via growing the identified plant species and micro fungi. At the end water from the constructed wetland may be directly released into rivers or streams (Fig. 6.1)

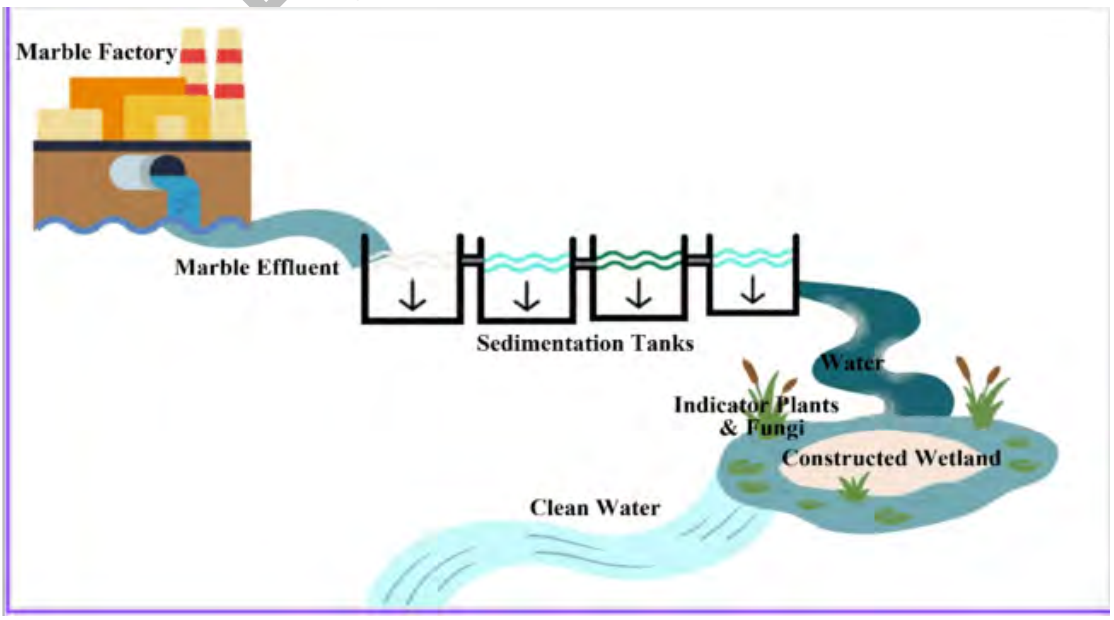


Fig. 6.1 A recommended sketch for the treatment of marble waste water.

Conclusions

It is concluded that the MWPE of Khyber Pakhtunkhwa province, Pakistan mostly contains 220 different plant species. Family Poaceae was the topmost dominant family of the polluted region. *Ficus carica, Calotropis prosera, Cynodon dactylon* were the dominant and *Pyrus communis, combretum indicum and Biden bippinta* were the rare plant species of MWPE. Based on OLS, logistic and probabilistic all the measured variables have significant role in the occurrence of dominant and rare plant species in the region. While CCA concluded that the $CaCO_3$, electrical conductivity, calcium, soil pH, magnesium, temperature, precipitation, elevation and organic matter have significant role in the distribution pattern of plant species in the region. The marble wastewater mostly revealed six different types of micro fungal strains in Buner, Pakistan. Their molecular identification and phylogeny resulted *Aspergillus sydowii* (ZAF 02 and ZAF 03), *Aspergillus brasilensi* (ZAF 04 and ZAF 05), *Fusarium petrophilium* (ZAF 06), *Aureobasidium leucosper* (ZAF 07), *Alternaria alternata* (ZAF 08) and *Curvularia aeria* (ZAF 09) species. Morphologically many of these strains exhibited aseptate hyphae and varying colors i.e., black, brown, green and white. Anatomically, these strains range from cylindrical to round, hyaline in lacto-phenol blue, thick to thin walled, smooth to ornamented surface with sharp scale and fusoid to ellipsoid in shape.

The marble pollution, climate, elevation and soil have a significant impact on the vegetation structure of the subtropical vegetation of MWPE. In more depth, the marble pollution and climate have a significant positive influence, while elevation and soil have a significant negative influence on subtropical vegetation and their indicator plants. The ISA is one of the best and most effective techniques for the identification/selection of indicators. We claim that their reconfirmation via CCA and SEM analysis has been done for the first time and these indicators can further be used for multipurpose including reforestation drives and smart habitat plantation. Both SEM and CCA analysis identified the complex relation/impact of measured environmental factors on subtropical vegetation of MWPE.

Among the identified indicator plant species i.e., *Adiantum capillus-veneris, Ailanthus altissima, Albizia lebbeck, Calotropis procera, Cynodon dactylon, Datura innoxia, Debregeasia salicifolia, Desmostachya bipinnata, Dodonaea viscosa, Erigeron bonariensis, Ficus carica, Morus alba, Morus nigra, Parthenium hysterophorus,*

Persicaria glabra, Ricinus communis, Setaria viridis, Tamarix aphylla, Withania somnifera and micro fungi i.e., *Aspergillus sydowii, Aspergillus brasilensi, Curvularia aeria* and *Alternaria alternata* have a significant role in the remediation of heavy metals present in marble waste polluted ecosystems and hence could be used for phytoremediation and mycoremediation purposes. The proline accumulation increases in plant species with the increase in marble waste pollution while chlorophyll decreases with an increase in pollution. The fluctuation in concentration of both proline and chlorophyll was due to the phytoremediation property of the plant species. It is also concluded that the use of GIS technology is an effective approach for the mapping of heavy metals contamination. The results comprehend a significant NDVI difference in the marble polluted and non-polluted areas between 1986-2021. Increase in marble pollution with time decreases the average NDVI of the region. The non-polluted areas have higher NDVI than the marble polluted regions. The overall average NDVI in the marble polluted and non-polluted regions were 0.263 and 0.382, respectively.

Future Recommendations

In order to continue the the study on marble waste polluted ecosystem following prospects could be worked on in the future. It is recommended that-

- Identified indicator plants can further be used for multipurpose including reforestation drives and smart habitat plantation in the MWPE.
- The statistical and modeling procedure adopted in the current study could be followed to classify vegetation and identify indicator plants of any geographic region or microhabitat type in any part of the world.
- To evaluate molecular mechanisms adopted by these plants and micro fungi for marble waste bio solubilization.
- Environmental Management System should be materialized, and environment friendly Eco Industrial Cluster (EIC) should be developed.
- Filter press should be used in MPUs for separation of water and marble powder from slurry.
- Government should regulate and inspect the private sector by enforcing the regulations for collecting waste.
- Awareness should be created among mine owners and marble processors to manage marble waste effectively and efficiently.
- To evaluate the pathogenicity of certain pathogenic strains of fungi.
- To introduce and implement Marble Waste Water Treatments in the region.

References

Abu-Elsaoud, A.M., N.A. Nafady, and A.M. Abdel-Azeem. 2017. Arbuscular mycorrhizal strategy for zinc mycoremediation and diminished translocation to shoots and grains in wheat. PloS one 12(11):e0188220.

Adenipekun, C., and Y. Lawal. 2011. Mycoremediation of crude oil and palm kernel contaminated soils by Pleurotus pulmonarius Fries (Quelet). Nature and Science 9(9):125-131.

Adewole, M., and M. Adesina. 2011. Impact of marble mining on soil properties in a part of Guinea Savanna zone of southwestern Nigeria. Ethiopian Journal of environmental studies and management 4(2):1-8.

Ahmad, P., M.N. Alyemeni, L. Wijaya, M.A. Ahanger, M. Ashraf, P. Alam, B.A. Paray, and J. Rinklebe. 2020. Nitric oxide donor, sodium nitroprusside, mitigates mercury toxicity in different cultivars of soybean. Journal of Hazardous Materials:124852.

Ahmad, Z., S.M. Khan, E.F. Abd_Allah, A.A. Alqarawi, and A. Hashem. 2016a. Weed species composition and distribution pattern in the maize crop under the influence of edaphic factors and farming practices: A case study from Mardan, Pakistan. Saudi journal of biological sciences 23(6):741-748.

Ahmad, Z., S.M. Khan, M.I. Ali, N. Fatima, and S. Ali. 2019. Pollution indicandum and marble waste polluted ecosystem; role of selected indicator plants in phytoremediation and determination of pollution zones. Journal of Cleaner Production 236:117709.

Ahmad, Z., S.M. Khan, S. Ali, I. Rahman, H. Ara, I. Noreen, and A. Khan. 2016b. Indicator species analyses of weed communities of maize crop in district Mardan, Pakistan. Pak. J. Weed Sci. Res 22(2):227-238.

Ahmad, Z., S.M. Khan, and S. Page. 2021. Politics of the natural vegetation to balance the hazardous level of elements in marble polluted ecosystem through phytoremediation and physiological responses. Journal of Hazardous Materials 414:125451.

Akbulut, H., and C. Gürer. 2007. Use of aggregates produced from marble quarry waste in asphalt pavements. Building and environment 42(5):1921-1930.

Akshita, C., T. Nandini, M. Sumedha, and U. Trishang. 2018. Variation in Reducing Sugar and Proline Content of Saraca asoca Due to Air Pollution. Universal Journal of Environmental Research & Technology 7(2).

Ali, H. 2010. Biodegradation of synthetic dyes—a review. Water, Air, & Soil Pollution 213(1-4):251-273.

Ali, S. 2008. Significance of flora with special reference to Pakistan. Pak. J. Bot 40(3):967-971.

Ali, S., and J. Nasir. 1990. Flora of Pakistan. Department of Botany, University of Karachi. Shamim Printing Press, Karachi.

Ali, S., and M. Qaiser. 1995. 2005. Flora of Pakistan.

Alker, A.P., G.W. Smith, and K. Kim. 2001. Characterization of Aspergillus sydowii (Thom et Church), a fungal pathogen of Caribbean sea fan corals. Hydrobiologia 460(1-3):105-111.

Amiri, S., A.H. Nafez, and N. Amiri. 2020. Effects of water pollution on some physiological parameters and phytoremediation performance of watercress (Nasturtium officinale). Environmental Quality Management.

Amjad, A., G. Di, A. Mahar, W. Ping, S. Feng, L. Ronghua, and Z. Zhang. 2017. Mycoremediation of potentially toxic trace elements—a biological tool for soil cleanup: a review. Pedosphere 27(2):205-222.
Anderson, M.J., K.E. Ellingsen, and B.H. McArdle. 2006. Multivariate dispersion as a measure of beta diversity. Ecology letters 9(6):683-693.
Anderson, T., and J. Coats. 1995. An overview of microbial degradation in the rhizosphere and its implications for bioremediation. Bioremediation: science and applications(bioremediation):135-143.
André, A., J. de Brito, A. Rosa, and D. Pedro. 2014. Durability performance of concrete incorporating coarse aggregates from marble industry waste. Journal of cleaner Production 65:389-396.
Andrews, J.H., R.N. Spear, and E.V. Nordheim. 2002. Population biology of Aureobasidium pullulans on apple leaf surfaces. Canadian journal of microbiology 48(6):500-513.
Antunes, J., T. Capucho, A. Pereira, and C. Sequeira. 1998. Electrochemical Remediation: A Modern Option for Cleaning up Contaminated Land. SPECIAL PUBLICATION-ROYAL SOCIETY OF CHEMISTRY 217:407-416.
Anwar, S., S.M. Khan, Z. Ahmad, Z. Ullah, R. Afza, Z. Abbas, A. Abdullah, and M. Hussain. 2022. Plant diversity and communities pattern with special emphasis on the indicator species of a dry temperate forest: A case study from Liakot area of the Hindu Kush mountains, Pakistan. Tropical Ecology:1-16.
Appannagari, R.R. 2017. Environmental pollution causes and consequences: a study. North Asian Int Res J Soc Sci Humanit 3(8):151-161.
Arabameri, A., and H.R. Pourghasemi. 2019. Spatial modeling of gully erosion using linear and quadratic discriminant analyses in GIS and R. P. 299-321 in Spatial Modeling in GIS and R for Earth and Environmental Sciences. Elsevier.
Arellano, P., K. Tansey, H. Balzter, and M. Tellkamp. 2017. Plant family-specific impacts of petroleum pollution on biodiversity and leaf chlorophyll content in the Amazon rainforest of Ecuador. PloS one 12(1):e0169867.
Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology 24(1):1.
Ashraf, U., A.S. Kanu, Z. Mo, S. Hussain, S.A. Anjum, I. Khan, R.N. Abbas, and X. Tang. 2015. Lead toxicity in rice: effects, mechanisms, and mitigation strategies—a mini review. Environmental Science and Pollution Research 22(23):18318-18332.
Aukour, F.J., and M.I. Al-Qinna. 2008a. Marble production and environmental constrains: case study from Zarqa Governorate, Jordan. Jordan J Earth Environ Sci 1(3):11-21.
Aukour, F.J., and M.I. Al-Qinna. 2008b. Marble production and environmental constrains: case study from Zarqa Governorate, Jordan. Jordan J Earth Environ Sci 1(1):11-21.
Austin, D. 2007. Character or caricature? Concluding discussion. Landscapes 8(2):92-105.
Austin, M. 1968. An ordination study of a chalk grassland community. The Journal of Ecology:739-757.
Austin, M. 1976. On non-linear species response models in ordination. Vegetatio 33(1):33-41.

Autry, A.R., and G.M. Ellis. 1992. Bioremediation: An effective remedial alternative for petroleum hydrocarbon-contaminated soil. Environmental Progress & Sustainable Energy 11(4):318-323.
Badshah, L., F. Hussain, and Z. Sher. 2013. Floristic inventory, ecological characteristics and biological spectrum of rangeland, District Tank, Pakistan. Pak. J. Bot 45(4):1159-1168.
Baillie, J., C. Hilton-Taylor, and S.N. Stuart. 2004. 2004 IUCN red list of threatened species: a global species assessment.
Baker, M.E., and M.J. Wiley. 2004. Characterization of woody species distribution in riparian forests of lower Michigan, USA using map-based models. Wetlands 24(3):550.
Bannari, A., D. Morin, F. Bonn, and A. Huete. 1995. A review of vegetation indices. Remote sensing reviews 13(1-2):95-120.
Bano, S., S.M. Khan, J. Alam, A.A. Alqarawi, E.F. Abd_Allah, Z. Ahmad, I.U. Rahman, H. Ahmad, A. Aldubise, and A. Hashem. 2018. Eco-Floristic studies of native plants of the Beer Hills along the Indus River in the districts Haripur and Abbottabad, Pakistan. Saudi journal of biological sciences 25(4):801-810.
Barbour, M.G., J.H. Burk, W.D. Pitts, F.S. Gilliam, and M.W. Schwartz. 1987. Methods of sampling the plant community. Ecology. Menlo Park: Benjamin/Cummings Publishing Co.
Baret, F., and G. Guyot. 1991. Potentials and limits of vegetation indices for LAI and APAR assessment. Remote sensing of environment 35(2-3):161-173.
Barros, Y.J., V.d.F. Melo, K.D. Sautter, B. Buschle, E.B. de Oliveira, J. de Azevedo, L.d.P. Souza, and L. Kummer. 2010. Soil quality indicators in lead mining and metalurgy area: II-Mesofauna and plants. Revista Brasileira de Ciencia do Solo 34(4):1413-1426.
Bates, L., R. Waldren, and I. Teare. 1973a. Rapid determination of free proline for water-stress studies. Plant and soil 39(1):205-207.
Bates, L.S., R.P. Waldren, and I. Teare. 1973b. Rapid determination of free proline for water-stress studies. Plant and soil 39(1):205-207.
Beals, E.W. 1984. Bray-Curtis ordination: an effective strategy for analysis of multivariate ecological data. Advances in ecological research 14:1-55.
Bhattacharya, S., J. Angayarkanni, A. Das, and M. Palaniswamy. 2012. Mycoremediation of Benzo [a] pyrene by Pleurotus ostreatus isolated from Wayanad district in Kerala, India. Int J Pharm Bio Sci 2(2):84-93.
Bi, J., X. Liu, S. Liu, Y. Wang, and M. Liu. 2020. Microstructural and physiological responses to cadmium stress under different nitrogen forms in two contrasting Populus clones. Environmental and experimental botany 169:103897.
Bini, C., L. Maleci, and M. Wahsha. 2017. Mine waste: assessment of environmental contamination and restoration. P. 89-134 in Assessment, restoration and reclamation of mining influenced soils. Elsevier.
Blackwell, M. 2011. The Fungi: 1, 2, 3… 5.1 million species? American journal of botany 98(3):426-438.
Brandt, C.J., and R.W. Rhoades. 1973. Effects of limestone dust accumulation on lateral growth of forest trees. Environmental Pollution (1970) 4(3):207-213.
Braun-Blanquet, J. 1932. Plant sociology. The study of plant communities. Plant sociology. The study of plant communities. First ed.
Brooks, R. 1979. Indicator plants for mineral prospecting—a critique. Journal of Geochemical Exploration 12:67-78.

Brooks, R., and C. Radford. 1978. An evaluation of background and anomalous copper and zinc concentrations in the" copper plant" Polycarpaea spirostylis and other Australian species of the genus. P. 33-37 *in* PROC AUSTRALAS INST MIN METALL.

Brückner, I., S. Classen, M. Hammers-Wirtz, K. Klaer, J. Reichert, and J. Pinnekamp. 2020. Tool for selecting indicator substances to evaluate the impact of wastewater treatment plants on receiving water bodies. Science of the Total Environment 745:140746.

Brunner, I., M. Fischer, J. Rüthi, B. Stierli, and B. Frey. 2018. Ability of fungi isolated from plastic debris floating in the shoreline of a lake to degrade plastics. PloS one 13(8):e0202047.

Bryson, R.A. 1974. A perspective on climatic change. Science 184(4138):753-760.

Burgass, M.J., B.S. Halpern, E. Nicholson, and E. Milner-Gulland. 2017. Navigating uncertainty in environmental composite indicators. Ecological Indicators 75:268-278.

Byrne, B.M. 1994. Structural equation modeling with EQS and EQS/Windows: Basic concepts, applications, and programming. Sage.

Cannon, H.L. 1971. The use of plant indicators in ground water surveys, geologic mapping, and mineral prospecting. Taxon 20(2-3):227-256.

Caspi, V., M. Droppa, G. Horváth, S. Malkin, J.B. Marder, and V.I. Raskin. 1999. The effect of copper on chlorophyll organization during greening of barley leaves. Photosynthesis Research 62(2):165-174.

Celik, M.Y., and E. Sabah. 2008. Geological and technical characterisation of Iscehisar (Afyon-Turkey) marble deposits and the impact of marble waste on environmental pollution. Journal of environmental management 87(1):106-116.

Ceschin, S., V. Sgambato, N.T.W. Ellwood, and V. Zuccarello. 2019. Phytoremediation performance of Lemna communities in a constructed wetland system for wastewater treatment. Environmental and experimental botany 162:67-71.

Chamaret, A., M. O'Connor, and G. Recoche. 2007. Top-down/bottom-up approach for developing sustainable development indicators for mining: application to the Arlit uranium mines (Niger).

Champion, S.H., S.K. Seth, and G. Khattak. 1965. Forest types of Pakistan. Forest types of Pakistan.

Chaney, R., S. Slonim, and S. Slonim. 1982. Determination of calcium carbonate content in soils. in Geotechnical properties, behavior, and performance of calcareous soils. ASTM International.

Cheevaporn, V., and P. Menasveta. 2003. Water pollution and habitat degradation in the Gulf of Thailand. Marine pollution bulletin 47(1-6):43-51.

Chiang, H.H., and A. Dandekar. 1995. Regulation of proline accumulation in Arabidopsis thaliana (L.) Heynh during development and in response to desiccation. Plant, Cell & Environment 18(11):1280-1290.

Clements, F.E. 1916. Plant succession: an analysis of the development of vegetation. Carnegie Institution of Washington.

Cluis, C. 2004. Junk-greedy greens: phytoremediation as a new option for soil decontamination. BioTeach Journal 2(6):l-67.

Cole, M.M. 1965. Biogeography in the Service of Man: With Particular Reference to the Underdeveloped Lands. Bedford College.

Cox, G. 1990. Laboratory manual of general ecology 6th Ed. Dubuque, Iowa: WIlliam C. Brown 248.

Critchfield, W.B. 1985. The late Quaternary history of lodgepole and jack pines. Canadian Journal of Forest Research 15(5):749-772.
Cunningham, S.D., T.A. Anderson, A.P. Schwab, and F. Hsu. 1996. Phytoremediation of soils contaminated with organic pollutants. Advances in agronomy 56(1):55-114.
da Cruz, R.H.S.F., I.G. Baseia, and K. Hosaka. 2018. Rediscovery of Cyathus badius, an _extinct‘species from the Bonin Islands, Japan. Mycoscience 59(3):193-199.
Da Cunha, L.V.F.C., and U.P. De Albuquerque. 2006. Quantitative ethnobotany in an Atlantic Forest fragment of Northeastern Brazil–Implications to conservation. Environmental Monitoring and Assessment 114(1):1-25.
Davis, M., C. Douglas, R. Calcote, K.L. Cole, M.G. Winkler, and R. Flakne. 2000. Holocene climate in the western Great Lakes national parks and lakeshores: implications for future climate change. Conservation Biology 14(4):968-983.
Dawood, M., H. Taie, R. Nassar, M. Abdelhamid, and U. Schmidhalter. 2014. The changes induced in the physiological, biochemical and anatomical characteristics of Vicia faba by the exogenous application of proline under seawater stress. South African Journal of Botany 93:54-63.
De Carolis, E., B. Posteraro, C. Lass-Flörl, A. Vella, A. Florio, R. Torelli, C. Girmenia, C. Colozza, A. Tortorano, and M. Sanguinetti. 2012. Species identification of Aspergillus, Fusarium and Mucorales with direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clinical Microbiology and infection 18(5):475-484.
Del Rio, D., A.J. Stewart, and N. Pellegrini. 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, metabolism and cardiovascular diseases 15(4):316-328.
Dentinger, B., S. Margaritescu, and J. MONCALVO. 2010. Rapid and reliable high-throughput methods of DNA extraction for use in barcoding and molecular systematics of mushrooms. Molecular Ecology Resources 10(4):628-633.
Dhanapandian, S., B. Gnanavel, and T. Ramkumar. 2009. Utilization of granite and marble sawing powder wastes as brick materials. Carpathian journal of earth and environmental sciences 4(2):147-160.
Di Francesco, A., L. Ugolini, L. Lazzeri, and M. Mari. 2015. Production of volatile organic compounds by Aureobasidium pullulans as a potential mechanism of action against postharvest fruit pathogens. Biological Control 81:8-14.
Dufrêne, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological monographs 67(3):345-366.
Duvigneaud, P. 1959. Plant cobaltophytes in Upper Katanga (in Fr.). Bull Soc. R. Bot. Belg. 91:111-134.
Dyer, R.J., S. Gillings, R.F. Pywell, R. Fox, D.B. Roy, and T.H. Oliver. 2017. Developing a biodiversity-based indicator for large-scale environmental assessment: a case study of proposed shale gas extraction sites in Britain. Journal of Applied Ecology 54(3):872-882.
Dymytrova, L. 2009. Epiphytic lichens and bryophytes as indicators of air pollution in Kyiv city (Ukraine). Folia Cryptogamica Estonica 46:33-44.
Ein-Gil, N., M. Ilan, S. Carmeli, G.W. Smith, J.R. Pawlik, and O. Yarden. 2009. Presence of Aspergillus sydowii, a pathogen of gorgonian sea fans in the marine sponge Spongia obscura. The ISME journal 3(6):752-755.
El-Maghraby, A., M.F.M. El-Kady, N.A. Taha, and M.A.A. El-Hamied. 2013. BENEFICIAL REUSE OF WASTE PRODUCTS. Handbook of Environment

and Waste Management: Volume 2: Land and Groundwater Pollution Control 2:425.
El-Morsy, E., H. Hassan, and E. Ahmed. 2017. Biodegradative activities of fungal isolates from plastic contaminated soils. Mycosphere 8(8):1071-1087.
El Haggar, S. 2010. Sustainable industrial design and waste management: cradle-to-cradle for sustainable development. Academic Press.
Elliot, R., N. Singhal, and S. Swift. 2010. Surfactants and bacterial bioremediation of polycyclic aromatic hydrocarbon contaminated soil—unlocking the targets. Critical Reviews in Environmental Science and Technology 41(1):78-124.
EPA, U. 2000. Introduction to phytoremediation. EPA/600/R-99/107.
Erlat, E., and M. Türkeş. 2013. Observed changes and trends in numbers of summer and tropical days, and the 2010 hot summer in Turkey. International Journal of Climatology 33(8):1898-1908.
Ewald, J. 2003. A critique for phytosociology. Journal of Vegetation Science 14(2):291-296.
Feller, U., and I.I. Vaseva. 2014. Extreme climatic events: impacts of drought and high temperature on physiological processes in agronomically important plants. Frontiers in Environmental Science 2:39.
Ferreira-Pinto, M., M. Moura-Guedes, M. Barreiro, I. Pais, M. Santos, and M. Silva. 2006. Aureobasidium pullulans as a biocontrol agent of blue mold in" Rocha" pear. Communications in agricultural and applied biological sciences 71:973-978.
Fiorentino, N., M. Mori, V. Cenvinzo, L.G. Duri, L. Gioia, D. Visconti, and M. Fagnano. 2018. Assisted phytoremediation for restoring soil fertility in contaminated and degraded land. Ital. J. Agron 13(1S):34-44.
Firn, J., J.M. McGree, E. Harvey, H. Flores-Moreno, M. Schütz, Y.M. Buckley, E.T. Borer, E.W. Seabloom, K.J. La Pierre, and A.M. MacDougall. 2019. Leaf nutrients, not specific leaf area, are consistent indicators of elevated nutrient inputs. Nature Ecology & Evolution 3(3):400-406.
Fletcher, I. 2019. Effect of temperature and growth media on mycelium growth of Pleurotus ostreatus and Ganoderma lucidum strains. Cohesive journal of microbiology and infectious disease 2(5).
Freedman, B., and T. Hutchinson. 1980. Long-term effects of smelter pollution at Sudbury, Ontario, on forest community composition. Canadian Journal of Botany 58(19):2123-2140.
Freeman, J.L., L.H. Zhang, M.A. Marcus, S. Fakra, S.P. McGrath, and E.A. Pilon-Smits. 2006. Spatial imaging, speciation, and quantification of selenium in the hyperaccumulator plants Astragalus bisulcatus and Stanleya pinnata. Plant Physiology 142(1):124-134.
Fu, W., K. Zhao, P. Jiang, and G. Zhou. 2013. Using ArcGIS and Geostatistics to Study Spatial Pattern of Forest Litter Carbon Density in Zhejiang Province, China. P. 419-423 in Functions of Natural Organic Matter in Changing Environment. Springer.
Ganjurjav, H., E.S. Gornish, G. Hu, M.W. Schwartz, Y. Wan, Y. Li, and Q. Gao. 2020. Warming and precipitation addition interact to affect plant spring phenology in alpine meadows on the central Qinghai-Tibetan Plateau. Agricultural and Forest Meteorology 287:107943.
García, J., S. Suárez-Seoane, D. Miguélez, P.E. Osborne, and C. Zumalacárregui. 2007. Spatial analysis of habitat quality in a fragmented population of little

bustard (Tetrax tetrax): Implications for conservation. Biological conservation 137(1):45-56.
Gaston, K.J. 2000. Global patterns in biodiversity. nature 405(6783):220-227.
Gazi, A., G. Skevis, and M. Founti. 2012. Energy efficiency and environmental assessment of a typical marble quarry and processing plant. Journal of cleaner Production 32:10-21.
Ghosh, S., N.U. Kamble, and M. Majee. 2020. A protein repairing enzyme, PROTEIN L-ISOASPARTYL METHYLTRANSFERASE is involved in salinity stress tolerance by increasing efficiency of ROS-scavenging enzymes. Environmental and experimental botany 180:104266.
Gilmore, S.A., S. Naseem, J.B. Konopka, and A. Sil. 2013. N-acetylglucosamine (GlcNAc) triggers a rapid, temperature-responsive morphogenetic program in thermally dimorphic fungi. PLoS genetics 9(9):e1003799.
Gomez, F. 2014. Assessment and optimization of ex-situ bioremediation of petroleum contaminated soil under cold temperature conditions, Université d'Ottawa/University of Ottawa.
Goovaerts, P. 1999. Geostatistics in soil science: state-of-the-art and perspectives. Geoderma 89(1-2):1-45.
Gouleta, E. 2015. Educational Assessment in Khyber Pakhtunkhwa Pakistan's North-West Frontier Province: Practices, Issues, and Challenges for Educating Culturally Linguistically Diverse and Exceptional Children. Global Education Review 2(4):19-39.
Grace, J.B., T.M. Anderson, H. Olff, and S.M. Scheiner. 2010. On the specification of structural equation models for ecological systems. Ecological monographs 80(1):67-87.
Grace, J.B., and J.E. Keeley. 2006. A structural equation model analysis of postfire plant diversity in California shrublands. Ecological Applications 16(2):503-514.
Grace, J.B., and B.H. Pugesek. 1997. A structural equation model of plant species richness and its application to a coastal wetland. The American Naturalist 149(3):436-460.
Greig-Smith, P. 1983. Quantitative plant ecology. Univ of California Press.
Groves, R., G. RW, S. BG, S. EA, and T. RG. 1972. Geochemical and geobotanical studies in the Emuford district of the Herberton tin field, North Queensland, Australia.
Guisan, A., and N.E. Zimmermann. 2000. Predictive habitat distribution models in ecology. Ecological modelling 135(2-3):147-186.
Guo, L.D., G.R. Huang, Y. Wang, W.H. He, W.H. Zheng, and K.D. Hyde. 2003. Molecular identification of white morphotype strains of endophytic fungi from Pinus tabulaeformis. Mycological research 107(6):680-688.
Gupta, A., M. Patil, A. Qamar, and M. Senthil-Kumar. 2020. ath-miR164c influences plant responses to the combined stress of drought and bacterial infection by regulating proline metabolism. Environmental and experimental botany 172:103998.
Haller, H., A. Jonsson, and M. Fröling. 2018. Application of ecological engineering within the framework for strategic sustainable development for design of appropriate soil bioremediation technologies in marginalized regions. Journal of Cleaner Production 172:2415-2424.

Haq, F., H. Ahmad, and Z. Iqbal. 2015. Vegetation composition and ecological gradients of subtropical-moist temperate ecotonal forests of Nandiar Khuwar catchment, Pakistan. Bangladesh Journal of Botany 44(2):267-276.
Haritash, A., and C. Kaushik. 2009. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. Journal of Hazardous Materials 169(1-3):1-15.
Harms, H., D. Schlosser, and L.Y. Wick. 2011. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nature Reviews Microbiology 9(3):177-192.
Hasan, Z., M. Khan, Z. Ali, Q. Zia, Z. MASOOD, and W. Khan. 2016. Fish Diversity of Sharki Dam, District Karak, Khyber Pakhtunkhwa, Pakistan. Sindh University Research Journal-SURJ (Science Series) 47(1).
Hawksworth, D.L., and T. Iturriaga. 2006. Lichenicolous fungi described from Antarctica and the sub-Antarctic islands by Carroll W. Dodge (1895–1988). Antarctic Science 18(3):291-301.
Hawksworth, D.L., and R. Lücking. 2017. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. Microbiology spectrum 5(4).
Hayat, S., Q. Hayat, M.N. Alyemeni, A.S. Wani, J. Pichtel, and A. Ahmad. 2012. Role of proline under changing environments: a review. Plant signaling & behavior 7(11):1456-1466.
Heath, L.S., P.E. Kauppi, P. Burschel, H.-D. Gregor, R. Guderian, G.H. Kohlmaier, S. Lorenz, D. Overdieck, F. Scholz, and H. Thomasius. 1993. Contribution of temperate forests to the world's carbon budget. Water, Air, & Soil Pollution 70(1):55-69.
Hester, A., and R. Brooker. 2007. Threatened habitats: marginal vegetation in upland areas. Issues in Environmental Science and Technology 25:107-134.
Hill, M.O., and H.G. Gauch. 1980. Detrended correspondence analysis: an improved ordination technique. P. 47-58 in Classification and ordination. Springer.
Hiscox, J.T., and G. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany 57(12):1332-1334.
Hoekstra, J.M., T.M. Boucher, T.H. Ricketts, and C. Roberts. 2005. Confronting a biome crisis: global disparities of habitat loss and protection. Ecology letters 8(1):23-29.
Hooper, D., J. Coughlan, and M. Mullen. 2008. Evaluating model fit: a synthesis of the structural equation modelling literature. P. 195-200 *in* 7th European Conference on research methodology for business and management studies.
Hsiao, T.-C., A.Y.-C. Lin, W.-C. Lien, and Y.-C. Lin. 2020. Size distribution, biological characteristics and emerging contaminants of aerosols emitted from an urban wastewater treatment plant. Journal of Hazardous Materials 388:121809.
Hu, L.t., and P.M. Bentler. 1999. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. Structural equation modeling: a multidisciplinary journal 6(1):1-55.
Huai, H.-Y., and S.-J. Pei. 2004. Plants used medicinally by folk healers of the Lahu people from the autonomous county of Jinping Miao, Yao, and Dai in southwest China. Economic Botany 58(1):S265-S273.
Huhle, B., H. Heilmeier, and B. Merkel. 2008. Potential of Brassica juncea and Helianthus annuus in phytoremediation for uranium. P. 307-318 in Uranium, mining and hydrogeology. Springer.

Hussain, M., Z. Ahmad, M. Iqbal, B. Zuhra, S. Rasheed, and S. Khan. 2022. Plants and Plant Communities of the Kurram Valley, Pakistan. P. 241-265 in Biodiversity, Conservation and Sustainability in Asia. Springer.

Hussain, S.S. 1984. Pakistan manual of plant ecology. National Book Foundation.

Hussain, T. 2009. A floristic description of flora and ethnobotany of Samahni Valley (AK), Pakistan. Ethnobotanical Leaflets 2009(7):6.

Ilyas, M., R. Qureshi, M. Arshad, and S.N. Mirza. 2013. A Preliminary checklist of the vascular flora of Kabal Valley, Swat, Pakistan. Pak. J. Bot 45(2):605-615.

Ippolito, A., A. El Ghaouth, C.L. Wilson, and M. Wisniewski. 2000. Control of postharvest decay of apple fruit by Aureobasidium pullulans and induction of defense responses. Postharvest Biology and Technology 19(3):265-272.

Iqbal, M., S. Khan, M. Khan, I. Rahman, and Z. Abbas. 2015. Zahidullah. 2015. Exploration and inventorying of weeds in wheat crop of the district Malakand, Pakistan. Pak. J. Weed Sci. Res 21(3):435-452.

Iqbal, M., S.M. Khan, M.A. Khan, Z. Ahmad, and H. Ahmad. 2018. A novel approach to phytosociological classification of weeds flora of an agro-ecological system through Cluster, Two Way Cluster and Indicator Species Analyses. Ecological Indicators 84:590-606.

Iriondo, J.M., M.J. Albert, and A. Escudero. 2003. Structural equation modelling: an alternative for assessing causal relationships in threatened plant populations. Biological conservation 113(3):367-377.

Ivanov, L., D. Ronzhina, and L. Ivanova. 2008. Changes in leaf characteristics as indicator of the alteration of functional types of steppe plants along the aridity gradient. Russian Journal of Plant Physiology 55(3):301-307.

Jaleel, C.A., R. Gopi, and R. Panneerselvam. 2008. Growth and photosynthetic pigments responses of two varieties of Catharanthus roseus to triadimefon treatment. Comptes Rendus Biologies 331(4):272-277.

Jennings, M., O. Loucks, D. Glenn-Lewin, R. Peet, D. Faber-Langendoen, D. Grossman, A. Damman, M. Barbour, R. Pfister, and M. Walker. 2004. Guidelines for describing associations and alliances of the US National Vegetation Classification. The Ecological Society of America Vegetation Classification Panel 46.

Johnston, C.A., B.L. Bedford, M. Bourdaghs, T. Brown, C. Frieswyk, M. Tulbure, L. Vaccaro, and J.B. Zedler. 2007. Plant species indicators of physical environment in Great Lakes coastal wetlands. Journal of Great Lakes Research 33:106-124.

Joseph, E., A. Simon, S. Prati, M. Wörle, D. Job, and R. Mazzeo. 2011. Development of an analytical procedure for evaluation of the protective behaviour of innovative fungal patinas on archaeological and artistic metal artefacts. Analytical and bioanalytical chemistry 399(9):2899-2907.

Joshi, P., and A. Swami. 2007. Physiological responses of some tree species under roadside automobile pollution stress around city of Haridwar, India. The Environmentalist 27(3):365-374.

Justice, C., B. Holben, and M. Gwynne. 1986. Monitoring East African vegetation using AVHRR data. International Journal of Remote Sensing 7(11):1453-1474.

Kabas, S., A. Faz, J. Acosta, R. Zornoza, S. Martínez-Martínez, D. Carmona, and J. Bech. 2012. Effect of marble waste and pig slurry on the growth of native vegetation and heavy metal mobility in a mine tailing pond. Journal of Geochemical Exploration 123:69-76.

Kabir, M., M.Z. Iqbal, Z. Farooqi, and M. Shafiq. 2010. Vegetation pattern and soil characteristics of the polluted industrial area of Karachi. Pak. J. Bot 42(1):661-678.
Kamran, S., S.M. Khan, Z. Ahmad, A.U. Rahman, M. Iqbal, F. Manan, Z.U. Haq, and S. Ullah. 2020. The role of graveyards in species conservation and beta diversity: a vegetation appraisal of sacred habitats from Bannu, Pakistan. Journal of Forestry Research 31(4):1147-1158.
Kanwal, A., M. Farhan, F. Sharif, M.U. Hayyat, L. Shahzad, and G.Z. Ghafoor. 2020. Effect of industrial wastewater on wheat germination, growth, yield, nutrients and bioaccumulation of lead. Scientific reports 10(1):1-9.
Kapoor, A., T. Viraraghavan, and D.R. Cullimore. 1999. Removal of heavy metals using the fungus Aspergillus niger. Bioresource technology 70(1):95-104.
Karaşahin, M., and S. Terzi. 2007. Evaluation of marble waste dust in the mixture of asphaltic concrete. Construction and Building Materials 21(3):616-620.
Kastori, R., M. Plesničar, Z. Sakač, D. Panković, and I. Arsenijević-Maksimović. 1998. Effect of excess lead on sunflower growth and photosynthesis. Journal of Plant Nutrition 21(1):75-85.
Kelley, A.P. 1922. Plant indicators of soil types. Soil Sci 13:411-423.
Khan, I., M. Aftab, S. Shakir, M. Ali, S. Qayyum, M.U. Rehman, K.S. Haleem, and I. Touseef. 2019. Mycoremediation of heavy metal (Cd and Cr)–polluted soil through indigenous metallotolerant fungal isolates. Environmental Monitoring and Assessment 191(9):1-11.
Khan, M., S.M. Khan, M. Ilyas, A.A. Alqarawi, Z. Ahmad, and E.F. Abd_Allah. 2017a. Plant species and communities assessment in interaction with edaphic and topographic factors; an ecological study of the mount Eelum District Swat, Pakistan. Saudi journal of biological sciences 24(4):778-786.
Khan, S.M. 2012. Plant communities and vegetation ecosystem services in the Naran Valley, Western Himalaya, University of Leicester.
Khan, S.M., D. Harper, S. Page, and H. Ahmad. 2011. Species and community diversity of vascular flora along environmental gradient in Naran Valley: A multivariate approach through indicator species analysis. Pak. J. Bot 43(5):2337-2346.
Khan, S.M., S. Page, H. Ahmad, and D. Harper. 2013a. Identifying plant species and communities across environmental gradients in the Western Himalayas: Method development and conservation use. Ecological Informatics 14:99-103.
Khan, S.M., S. Page, H. Ahmad, and D. Harper. 2014. Ethno-ecological importance of plant biodiversity in mountain ecosystems with special emphasis on indicator species of a Himalayan Valley in the northern Pakistan. Ecological Indicators 37:175-185.
Khan, S.M., S. Page, H. Ahmad, H. Shaheen, and D. Harper. 2012. Vegetation dynamics in the Western Himalayas, diversity indices and climate change. Sci., Tech. and Dev 31(3):232-243.
Khan, S.M., S. Page, H. Ahmad, Z. Ullah, H. Shaheen, M. Ahmad, and D. Harper. 2013b. Phyto-climatic gradient of vegetation and habitat specificity in the high elevation Western Himalayas. Pakistan Journal of Botany 45:223-230.
Khan, S.M., S.E. Page, H. Ahmad, and D.M. Harper. 2013c. Sustainable utilization and conservation of plant biodiversity in montane ecosystems: the western Himalayas as a case study. Annals of botany:mct125.
Khan, W., S.M. Khan, H. Ahmad, Z. Ahmad, and S. Page. 2016. Vegetation mapping and multivariate approach to indicator species of a forest ecosystem: A case

study from the Thandiani sub Forests Division (TsFD) in the Western Himalayas. Ecological Indicators 71:336-351.
Khan, W., S.M. Khan, H. Ahmad, A. Shakeel, and S. Page. 2017b. Ecological gradient analyses of plant associations in the Thandiani forests of the Western Himalayas, Pakistan. Turkish Journal of Botany 41(3):253-264.
Kiani, S.P., P. Maury, A. Sarrafi, and P. Grieu. 2008. QTL analysis of chlorophyll fluorescence parameters in sunflower (Helianthus annuus L.) under well-watered and water-stressed conditions. Plant science 175(4):565-573.
King, R.S., C.J. Richardson, D.L. Urban, and E.A. Romanowicz. 2004. Spatial dependency of vegetation–environment linkages in an anthropogenically influenced wetland ecosystem. Ecosystems 7(1):75-97.
Kisielowska, E., R. Wojtasik, E. Kasińska-Pilut, and A. Hołda. 2012. The bioleaching of silver from the" Żelazny Most" disposal after-flotation wastes in Lubin with application of microfungi from genus Aspergillus niger. AGH Journal of Mining and Geoengineering 36(4):31-37.
Knoche, R., D. Dingwell, and S. Webb. 1995. Melt densities for leucogranites and granitic pegmatites: Partial molar volumes for SiO2, Al2O3, Na2O, K2O, Li2O, Rb2O, Cs2O, MgO, CaO, SrO, BaO, B2O3, P2O5, F2O− 1, TiO2, Nb2O5, Ta2O5, and WO3. Geochimica et Cosmochimica Acta 59(22):4645-4652.
Köppen, W. 1900. Versuch einer Klassifikation der Klimate, Vorzugsweise nach ihren Beziehungen zur Pflanzenwelt [Attempted climate classification in relation to plant distributions]. Geographische Zeitschrift 6:593–611.
Köppen, W., E. Volken, and S. Brönnimann. 2011. The thermal zones of the earth according to the duration of hot, moderate and cold periods and to the impact of heat on the organic world (Translated from: Die Wärmezonen der Erde, nach der Dauer der heissen, gemässigten und kalten Zeit und nach der Wirkung der Wärme auf die organische Welt betrachtet, Meteorol Z 1884, 1, 215-226). Meteorologische Zeitschrift 20(3):351-360.
Kore, S.D., and A. Vyas. 2016. Impact of marble waste as coarse aggregate on properties of lean cement concrete. Case Studies in Construction Materials 4:85-92.
Koul, B., W. Ahmad, and J. Singh. 2021. Mycoremediation: A novel approach for sustainable development. P. 409-420 in Microbe Mediated Remediation of Environmental Contaminants. Elsevier.
Krüger, E., E. Suzuki, and A. Matoski. 2013. Evaluation of a Trombe wall system in a subtropical location. Energy and Buildings 66:364-372.
Kumar, R.S., and D.S. Thambavani. 2012. Biological monitoring of roadside plants exposed to vehicular pollution in an urban area. Asian Journal of Research in Chemistry 5(10):1262-1267.
Kwatra, S., A. Kumar, P. Sharma, S. Sharma, and S. Singhal. 2016. Benchmarking sustainability using indicators: An Indian case study. Ecological Indicators 61:928-940.
Lai, W.W.-P., Y.-C. Lin, Y.-H. Wang, Y.L. Guo, and A.Y.-C. Lin. 2018. Occurrence of emerging contaminants in aquaculture waters: cross-contamination between aquaculture systems and surrounding waters. Water, Air, & Soil Pollution 229(8):249.
Larsen, R., J. Bell, P. James, P. Chimonides, F. Rumsey, A. Tremper, and O. Purvis. 2007. Lichen and bryophyte distribution on oak in London in relation to air pollution and bark acidity. Environmental pollution 146(2):332-340.

Lasat, M.M. 2000. The use of plants for the removal of toxic metals from contaminated soils. US Environmental Protection Agency.
Laughlin, D.C., and S.R. Abella. 2007. Abiotic and biotic factors explain independent gradients of plant community composition in ponderosa pine forests. ecological modelling 205(1-2):231-240.
Lawlor, D.W., and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant, Cell & Environment 25(2):275-294.
Lawson, T., K. Oxborough, J.I. Morison, and N.R. Baker. 2003. The responses of guard and mesophyll cell photosynthesis to CO2, O2, light, and water stress in a range of species are similar. Journal of Experimental Botany 54(388):1743-1752.
Lazaro, R., F. Rodrigo, L. Gutiérrez, F. Domingo, and J. Puigdefábregas. 2001. Analysis of a 30-year rainfall record (1967–1997) in semi–arid SE Spain for implications on vegetation. Journal of arid environments 48(3):373-395.
Lefcheck, J.S. 2016. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods in Ecology and Evolution 7(5):573-579.
Leonardi, V., V. Šašek, M. Petruccioli, A. D'Annibale, P. Erbanová, and T. Cajthaml. 2007. Bioavailability modification and fungal biodegradation of PAHs in aged industrial soils. International Biodeterioration & Biodegradation 60(3):165-170.
Lepš, J., and P. Šmilauer. 2003. Multivariate analysis of ecological data using CANOCO. Cambridge university press.
Li, P., Z. Feng, V. Catalayud, X. Yuan, Y. Xu, and E. Paoletti. 2017. A meta-analysis on growth, physiological, and biochemical responses of woody species to ground-level ozone highlights the role of plant functional types. Plant, Cell & Environment 40(10):2369-2380.
Likens, G.E., and F.H. Bormann. 1974. Acid rain: a serious regional environmental problem. Science 184(4142):1176-1179.
Lin, A.Y.-C., S.C. Panchangam, and H.-Y. Chen. 2010. Implications of human pharmaceutical occurrence in the Sindian river of Taiwan: a strategic study of risk assessment. Journal of Environmental Monitoring 12(1):261-270.
Lin, A.Y.-C., S.C. Panchangam, Y.-T. Tsai, and T.-H. Yu. 2014. Occurrence of perfluorinated compounds in the aquatic environment as found in science park effluent, river water, rainwater, sediments, and biotissues. Environmental monitoring and assessment 186(5):3265-3275.
Lin, M.-Z., and M.-F. Jin. 2018. Soil Cu contamination destroys the photosynthetic systems and hampers the growth of green vegetables. Photosynthetica 56(4):1336-1345.
Liu, D., W. Jiang, C. Liu, C. Xin, and W. Hou. 2000. Uptake and accumulation of lead by roots, hypocotyls and shoots of Indian mustard [Brassica juncea (L.)]. Bioresource Technology 71(3):273-277.
Liu, J., F. Cheng, W. Munger, P. Jiang, T.G. Whitby, S. Chen, W. Ji, and X. Man. 2020. Precipitation extremes influence patterns and partitioning of evapotranspiration and transpiration in a deciduous boreal larch forest. Agricultural and Forest Meteorology 287:107936.
Liu, L., J. Li, F. Yue, X. Yan, F. Wang, S. Bloszies, and Y. Wang. 2018a. Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth,

cadmium uptake and soil cadmium speciation in Cd-contaminated soil. Chemosphere 194:495-503.
Liu, L., W. Li, W. Song, and M. Guo. 2018b. Remediation techniques for heavy metal-contaminated soils: Principles and applicability. Science of The Total Environment 633:206-219.
Loidi, J. 2018. Plant eco-morphological traits as adaptations to environmental conditions: some comparisons between different biomes across the world. P. 59-71 in Geographical Changes in Vegetation and Plant Functional Types. Springer.
Mace, G.M., K. Norris, and A.H. Fitter. 2012. Biodiversity and ecosystem services: a multilayered relationship. Trends in ecology & evolution 27(1):19-26.
Madkour, S.A., and J. Laurence. 2002. Egyptian plant species as new ozone indicators. Environmental Pollution 120(2):339-353.
Maiti, S., D. Ray, D. Mitra, and A. Mukhopadhyay. 2013. Isolation and characterisation of starch/polyvinyl alcohol degrading fungi from aerobic compost environment. International Biodeterioration & Biodegradation 82:9-12.
Malik, R.N., S.Z. Husain, and I. Nazir. 2010. Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. Pak J Bot 42(1):291-301.
Malkani, M.S., M.H. Khosa, M.I. Alyani, K. Khan, N. Somro, T. Zafar, J. Arif, and M.A. Zahid. 2017. Mineral Deposits of Khyber Pakhtunkhwa and FATA, Pakistan. Lasbela University Journal of Science and Technology 6:23-46.
Mani, D., and C. Kumar. 2014. Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. International Journal of Environmental Science and Technology 11(3):843-872.
Manter, D.K., and J.M. Vivanco. 2007. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. Journal of Microbiological Methods 71(1):7-14.
Marini, L., M. Scotton, S. Klimek, J. Isselstein, and A. Pecile. 2007. Effects of local factors on plant species richness and composition of Alpine meadows. Agriculture, Ecosystems & Environment 119(3-4):281-288.
Massacci, A., S. Nabiev, L. Pietrosanti, S. Nematov, T. Chernikova, K. Thor, and J. Leipner. 2008. Response of the photosynthetic apparatus of cotton (Gossypium hirsutum) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. Plant physiology and biochemistry 46(2):189-195.
Mateos, B., C. Conrad-Billroth, M. Schiavina, A. Beier, G. Kontaxis, R. Konrat, I.C. Felli, and R. Pierattelli. 2020. The ambivalent role of proline residues in an intrinsically disordered protein: From disorder promoters to compaction facilitators. Journal of molecular biology 432(9):3093-3111.
Matkar, K., D. Chapla, J. Divecha, A. Nighojkar, and D. Madamwar. 2013. Production of cellulase by a newly isolated strain of Aspergillus sydowii and its optimization under submerged fermentation. International Biodeterioration & Biodegradation 78:24-33.
Mattioli, R., P. Costantino, and M. Trovato. 2009. Proline accumulation in plants: not only stress. Plant signaling & behavior 4(11):1016-1018.

McLean, E. 1982. Soil pH and lime requirement. Methods of soil analysis. Part 2. Chemical and microbiological properties(methodsofsoilan2):199-224.
Mendoza, J.-M.F., M. Feced, G. Feijoo, A. Josa, X. Gabarrell, and J. Rieradevall. 2014. Life cycle inventory analysis of granite production from cradle to gate. The International Journal of Life Cycle Assessment 19(1):153-165.
Miliša, M., V. Ţivković, and I. Habdija. 2010. Destructive effect of quarry effluent on life in a mountain stream. Biologia 65(3):520-526.
Moore, D. 2002. Fungal morphogenesis. Cambridge University Press.
Moreno-Jiménez, E., S. Vázquez, R.O. Carpena-Ruiz, E. Esteban, and J.M. Peñalosa. 2011. Using Mediterranean shrubs for the phytoremediation of a soil impacted by pyritic wastes in Southern Spain: a field experiment. Journal of environmental management 92(6):1584-1590.
Moscoso, F., I. Teijiz, F. Deive, and M. Sanromán. 2012. Efficient PAHs biodegradation by a bacterial consortium at flask and bioreactor scale. Bioresource technology 119:270-276.
Muhammad, I., and F. Umar. 2019. Factors Affecting Perceived Economic Growth of Khyber Pakhtunkhwa-Pakistan: A Comparative Study of Formal and Informal Users. Pakistan Business Review 21(2):140-155.
Mulk, S., A.L. Korai, A. Azizullah, L. Shahi, and M.N.K. Khattak. 2017. Marble industry effluents cause an increased bioaccumulation of heavy metals in Mahaseer (Tor putitora) in Barandu River, district Buner, Pakistan. Environmental Science and Pollution Research 24(29):23039-23056.
Mwegoha, W.J. 2008. The use of phytoremediation technology for abatement soil and groundwater pollution in Tanzania: opportunities and challenges. Journal of sustainable development in Africa 10(1):140-156.
Nakada, M., C. Tanaka, K. Tsunewaki, and M. Tsuda. 1994. RFLP analysis for species separation in the genera Bipolaris and Curvularia. Mycoscience 35(3):271-278.
Namgail, T., G.S. Rawat, C. Mishra, S.E. van Wieren, and H.H. Prins. 2012. Biomass and diversity of dry alpine plant communities along altitudinal gradients in the Himalayas. Journal of plant research 125(1):93-101.
Nascimento, C.W.A.d., and B. Xing. 2006. Phytoextraction: a review on enhanced metal availability and plant accumulation. Scientia agricola 63(3):299-311.
Nash III, T.H., and V. Wirth. 1988. Lichens, bryophytes and air quality.
Nasir, E., S. Ali, and R.R. Stewart. 1972. Flora of West Pakistan: an annotated catalogue of the vascular plants of West Pakistan and Kashmir. Fakhri.
Navarro-Cano, J.A., M. Verdú, and M. Goberna. 2018. Trait-based selection of nurse plants to restore ecosystem functions in mine tailings. Journal of Applied Ecology 55(3):1195-1206.
Nazir, A., R.N. Malik, M. Ajaib, N. Khan, and M.F. Siddiqui. 2011. Hyperaccumulators of heavy metals of industrial areas of Islamabad and Rawalpindi. Pak J Bot 43(4):1925-1933.
Nicolls, O., D. Provan, M. Cole, and J. Tooms. 1965. Geobotany and geochemistry in mineral exploration in the Dugald River Area, Cloncurry District, Australia. Transactions of the Institution of Mining and Metallurgy 74:695-799.
Nithamathi, C., and V. Indira. 2005. Impact of air pollution on Ceasalpinia sepiaria Linn. Tuticorin city. Indian Journal of Environment and Ecoplanning 10(2):449-452.
Noor, M.J., S. Sultana, S. Fatima, M. Ahmad, M. Zafar, M. Sarfraz, M.A. Balkhyour, S.Z. Safi, and M.A. Ashraf. 2015. Retracted Article: Estimation of anticipated

performance index and air pollution tolerance index and of vegetation around the marble industrial areas of Potwar region: bioindicators of plant pollution response. Environmental geochemistry and health 37(3):441-455.
Noreen, I., S.M. Khan, Z. Ahmad, I. Rahman, A. Tabassum, and E. Abd_Allah. 2019a. Response of different plant species to pollution emitted from oil and gas plant with special reference to heavy metals accumulation. Pak. J. Bot 51(4):1231-1240.
Noreen, U., Z. Ahmed, A. Khalid, A. Di Serafino, U. Habiba, F. Ali, and M. Hussain. 2019b. Water pollution and occupational health hazards caused by the marble industries in district Mardan, Pakistan. Environmental Technology & Innovation 16:100470.
Ojha, N., N. Pradhan, S. Singh, A. Barla, A. Shrivastava, P. Khatua, V. Rai, and S. Bose. 2017. Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization. Scientific Reports 7(1):1-13.
Okane, I., and Y. Ono. 2018. Phylogenetic study of indigenous grapevine leaf rust fungi in North America and biological identity of an invasive grapevine leaf rust fungus in Brazil. Mycoscience 59(2):99-104.
Økland, R.H., and O. Eilertsen. 1993. Vegetation-environment relationships of boreal coniferous forests in the Solhomfjell area, Gjerstad, S. Norway. Botanical Garden and Museum, University of Oslo.
Oosting, H.J. 1956. The study of plant communities. An introduction to plant ecology. The study of plant communities. An introduction to plant ecology.(2nd. Edn).
Oruru, J.A. 2014. Is the use of Brewery Spent Grain in Bioremediation of Diesel Contaminated Soil Sustainable?, University of Sunderland.
Osono, T., Y. Hagiwara, and H. Masuya. 2011. Effects of temperature and litter type on fungal growth and decomposition of leaf litter. Mycoscience 52(5):327-332.
Ottow, J., and H. Glathe. 1968. Rose bengal-malt extract-agar, a simple medium for the simultaneous isolation and enumeration of fungi and actinomycetes from soil. Applied microbiology 16(1):170.
Öztürk, M., V. Altay, and R. Efe. 2021. Biodiversity, Conservation and Sustainability in Asia.
Parmar, S., and V. Singh. 2015. Phytoremediation approaches for heavy metal pollution: a review. J Plant Sci Res 2(2):135.
Patel, M., and A.K. Parida. 2020. Salinity alleviates the arsenic toxicity in the facultative halophyte Salvadora persica L. by the modulations of physiological, biochemical, and ROS scavenging attributes. Journal of Hazardous Materials 401:123368.
Paz-Alberto, A.M., and G.C. Sigua. 2013. Phytoremediation: a green technology to remove environmental pollutants. American Journal of Climate Change 2(01):71.
Pel, H.J., J.H. de Winde, D.B. Archer, P.S. Dyer, G. Hofmann, P.J. Schaap, G. Turner, R.P. de Vries, R. Albang, and K. Albermann. 2007. Genome sequencing and analysis of the versatile cell factory Aspergillus niger CBS 513.88. Nature biotechnology 25(2):221-231.
Pellegrini, E., Y. Hoshika, N. Dusart, L. Cotrozzi, J. Gérard, C. Nali, M.-N. Vaultier, Y. Jolivet, G. Lorenzini, and E. Paoletti. 2019. Antioxidative responses of three oak species under ozone and water stress conditions. Science of the Total Environment 647:390-399.

Petersen, L.M., D.K. Holm, P.B. Knudsen, K.F. Nielsen, C.H. Gotfredsen, U.H. Mortensen, and T.O. Larsen. 2015. Characterization of four new antifungal yanuthones from Aspergillus niger. The Journal of antibiotics 68(3):201.

Pielke Sr, R.A. 2001. Influence of the spatial distribution of vegetation and soils on the prediction of cumulus convective rainfall. Reviews of Geophysics 39(2):151-177.

Pielou, E. 1977. The latitudinal spans of seaweed species and their patterns of overlap. Journal of Biogeography:299-311.

Pielou, E.C. 1966. The measurement of diversity in different types of biological collections. Journal of theoretical biology 13:131-144.

Pitt, M., and H. Heady. 1978. Responses of annual vegetation to temperature and rainfall patterns in northern California. Ecology 59(2):336-350.

Prajapati, S.K., and B. Tripathi. 2008. Seasonal variation of leaf dust accumulation and pigment content in plant species exposed to urban particulates pollution. Journal of environmental quality 37(3):865-870.

Prasad, M., H. Freitas, S. Fraenzle, S. Wuenschmann, and B. Markert. 2010. Knowledge explosion in phytotechnologies for environmental solutions. Environmental pollution 158(1):18-23.

Prescott, L., J. Harley, and D. Klein. 1993. Microbiology, William C. Brown, Dubuque:112-122.

Rahman, G., and M. Dawood. 2018. Spatial and temporal variation of rainfall and drought in Khyber Pakhtunkhwa Province of Pakistan during 1971–2015. Arabian Journal of Geosciences 11(3):1-13.

Ranjard, L., F. Poly, J.-C. Lata, C. Mougel, J. Thioulouse, and S. Nazaret. 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. Applied and environmental microbiology 67(10):4479-4487.

Rasheed, S., S.M. Khan, Z. Ahmad, G. Mustafa, Z.U. Haq, H. Shah, L. Ansari, and T. Jatt. 2021. Ecological Assessment and Indicator species analysis of the Cholistan Desert using Multivariate Statistical tools. Pak. J. Bot 54:2.

Raskin, I., P.N. Kumar, S. Dushenkov, and D.E. Salt. 1994. Bioconcentration of heavy metals by plants. Current Opinion in biotechnology 5(3):285-290.

Raunkiaer, C. 1934. The life forms of plants and statistical plant geography; being the collected papers of C. Raunkiaer. The life forms of plants and statistical plant geography; being the collected papers of C. Raunkiaer.

Rehman, F., F.A. Khan, and S.B. Anis. 2014. Assessment of aphid infestation levels in some cultivars of mustard with varying defensive traits. Archives of Phytopathology and Plant Protection 47(15):1866-1874.

Rodriguez, L., F. Lopez-Bellido, A. Carnicer, F. Recreo, A. Tallos, and J. Monteagudo. 2005. Mercury recovery from soils by phytoremediation. P. 197-204 in Environmental Chemistry. Springer.

Romero, M.C., E.H. Reinoso, M.I. Urrutia, and A. Moreno Kiernan. 2006. Biosorption of heavy metals by Talaromyces helicus: a trained fungus for copper and biphenyl detoxification. Electronic Journal of Biotechnology 9(3):0-0.

Rossi, S., C. Chapman, and B. Huang. 2020. Suppression of Heat-induced Leaf Senescence by γ-Aminobutyric Acid, Proline, and Ammonium Nitrate through Regulation of Chlorophyll Degradation in Creeping Bentgrass. Environmental and experimental botany:104116.

Roussos, S., M. De los Angeles Aquiahuatl, M. del Refugio Trejo-Hernández, I.G. Perraud, E. Favela, M. Ramakrishna, M. Raimbault, and G. Viniegra-González. 1995. Biotechnological management of coffee pulp—isolation, screening, characterization, selection of caffeine-degrading fungi and natural microflora present in coffee pulp and husk. Applied Microbiology and Biotechnology 42(5):756-762.

Roy, S., S. Labelle, P. Mehta, A. Mihoc, N. Fortin, C. Masson, R. Leblanc, G. Châteauneuf, C. Sura, and C. Gallipeau. 2005. Phytoremediation of heavy metal and PAH-contaminated brownfield sites. Plant and Soil 272(1/2):277-290.

Ruggiero, M.A., D.P. Gordon, T.M. Orrell, N. Bailly, T. Bourgoin, R.C. Brusca, T. Cavalier-Smith, M.D. Guiry, and P.M. Kirk. 2015. A higher level classification of all living organisms. PloS one 10(4):e0119248.

Rypien, K.L. 2008. The origins and spread of Aspergillus sydowii, an opportunistic pathogen of caribbean gorgonian corals.

Sairam, R., and A. Tyagi. 2004. Physiology and molecular biology of salinity stress tolerance in plants. Current science:407-421.

Salido, A.L., K.L. Hasty, J.-M. Lim, and D.J. Butcher. 2003. Phytoremediation of arsenic and lead in contaminated soil using Chinese brake ferns (Pteris vittata) and Indian mustard (Brassica juncea). International Journal of Phytoremediation 5(2):89-103.

Salt, D.E., R. Smith, and I. Raskin. 1998. Phytoremediation. Annual review of plant biology 49(1):643-668.

Samad, M., L. Badshah, and S.M. Khan. 2018. Biological spectra of Lala Kalay area distrist Peshawar Khyber Pakhtunkhwa Province Pakistan. Pakistan Journal of Weed Science Research 24(4).

Sampson, A.W. 1939. Plant indicators—concept and status. The Botanical Review 5(3):155-206.

Sasmaz, A., and M. Yaman. 2006. Distribution of chromium, nickel, and cobalt in different parts of plant species and soil in mining area of Keban, Turkey. Communications in soil science and plant analysis 37(13-14):1845-1857.

Schwacke, R., S. Grallath, K.E. Breitkreuz, E. Stransky, H. Stransky, W.B. Frommer, and D. Rentsch. 1999. LeProT1, a transporter for proline, glycine betaine, and γ-amino butyric acid in tomato pollen. The Plant Cell 11(3):377-391.

Schwitzguébel, J.-P. 2017. Phytoremediation of soils contaminated by organic compounds: hype, hope and facts. Journal of soils and sediments 17(5):1492-1502.

Seleiman, M.F., N. Al-Suhaibani, N. Ali, M. Akmal, M. Alotaibi, Y. Refay, T. Dindaroglu, H.H. Abdul-Wajid, and M.L. Battaglia. 2021. Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. Plants 10(2):259.

Sepehri, A., and M.-H. Sarrafzadeh. 2019. Activity enhancement of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in activated sludge process: metabolite reduction and CO 2 mitigation intensification process. Applied Water Science 9(5):1-12.

Sepehri, A., M.-H. Sarrafzadeh, and M. Avateffazeli. 2020. Interaction between Chlorella vulgaris and nitrifying-enriched activated sludge in the treatment of wastewater with low C/N ratio. Journal of Cleaner Production 247:119164.

Sethi, S., F.A. Shah, A. Khan, N. Shah, and M. Sohail. 2020. Assessing Management Effectiveness through Triple Bottom Line in the Context of National Park

Khyber Pakhtunkhwa: A Multi–Stakeholder Perspective for Sustainable Tourism Management. Ilkogretim Online 19(1):667-673.
Seuntjens, P., B. Nowack, and R. Schulin. 2004. Root-zone modeling of heavy metal uptake and leaching in the presence of organic ligands. Plant and Soil 265(1):61-73.
Shah, M. 2013. Phytosociological attributes and phytodiversity of Dheri baba hill and peer Taab graveyard, district Swabi, Khyber Pakhtunkhwa, Pakistan. Pakistan PJLS 1(01):1-16.
Shah, S.A.M., and S. Amjad. 2011. Cultural diversity in Pakistan: national vs provincial. Mediterranean Journal of Social Sciences 2(2):331-331.
Shahid, M., M. Srivastava, V. Kumar, A. Singh, A. Sharma, S. Pandey, S. Rastogi, N. Pathak, and A. Srivastava. 2014a. Phylogenetic diversity analysis of Trichoderma species based on internal transcribed spacer (ITS) marker. African Journal of Biotechnology 13(3).
Shahid, M.A., R.M. Balal, M.A. Pervez, T. Abbas, M.A. Aqeel, M.M. Javaid, and F. Garcia-Sanchez. 2014b. Exogenous proline and proline-enriched Lolium perenne leaf extract protects against phytotoxic effects of nickel and salinity in Pisum sativum by altering polyamine metabolism in leaves. Turkish Journal of Botany 38(5):914-926.
Sharma, S., S. Mukherjee, A. Kumar, and W.R. Dillon. 2005. A simulation study to investigate the use of cutoff values for assessing model fit in covariance structure models. Journal of Business Research 58(7):935-943.
Shimono, Y., T. Kasuya, and S. Takamatsu. 2018. Russula velenovskyi new to Japan, with phylogenetic implications of Russula species between Japanese subalpine forests and Northern Europe. Mycoscience.
Shoji, J.-Y., M. Arioka, and K. Kitamoto. 2006. Possible involvement of pleiomorphic vacuolar networks in nutrient recycling in filamentous fungi. Autophagy 2(3):226-227.
Shukla, K.P., N.K. Singh, and S. Sharma. 2010. Bioremediation: developments, current practices and perspectives. Genet. Eng. Biotechnol. J 3(8):1-20.
Siddiqui, M.H., S. Alamri, M.N. Khan, F.J. Corpas, A.A. Al-Amri, Q.D. Alsubaie, H.M. Ali, H.M. Kalaji, and P. Ahmad. 2020. Melatonin and calcium function synergistically to promote the resilience through ROS metabolism under arsenic-induced stress. Journal of Hazardous Materials:122882.
Simpson, E.H. 1949. Measurement of diversity. nature 163(4148):688-688.
Singh, J.S., P. Abhilash, H. Singh, R.P. Singh, and D. Singh. 2011. Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480(1):1-9.
Singh, M., V.P. Singh, G. Dubey, and S.M. Prasad. 2015a. Exogenous proline application ameliorates toxic effects of arsenate in Solanum melongena L. seedlings. Ecotoxicology and environmental safety 117:164-173.
Singh, M., P. Srivastava, P. Verma, R. Kharwar, N. Singh, and R. Tripathi. 2015b. Soil fungi for mycoremediation of arsenic pollution in agriculture soils. Journal of applied microbiology 119(5):1278-1290.
Smit, E., P. Leeflang, B. Glandorf, J.D. van Elsas, and K. Wernars. 1999. Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. Applied and environmental microbiology 65(6):2614-2621.
Smithson, P., K. Addison, and K. Atkinson. 2013. Fundamentals of the physical environment. Routledge.

Sreedevi, S., K.N. Remani, and S. Benjamin. 2013. Biotic stress induced biochemical and isozyme variations in ginger and tomato by Ralstonia solanacearum. American Journal of Plant Sciences 2013.
Srivastava, D.S., and M. Vellend. 2005. Biodiversity-ecosystem function research: is it relevant to conservation? Annu. Rev. Ecol. Evol. Syst. 36:267-294.
Steiger, J.H. 2007. Understanding the limitations of global fit assessment in structural equation modeling. Personality and Individual differences 42(5):893-898.
Szabados, L., and A. Savoure. 2010. Proline: a multifunctional amino acid. Trends in plant science 15(2):89-97.
Tahkokorpi, M., K. Taulavuori, K. Laine, and E. Taulavuori. 2007. After-effects of drought-related winter stress in previous and current year stems of Vaccinium myrtillus L. Environmental and experimental botany 61(1):85-93.
Takamatsu, S., S. Katsuyama, and T. Shinoda. 2018. First record of Erysiphe quercicola (Ascomycota: Erysiphales) on species of Quercus subgenus Cyclobalanopsis (evergreen oaks, Fagaceae). Mycoscience 59(2):105-109.
Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular biology and evolution 28(10):2731-2739.
Taylor, J.W., E. Turner, J.P. Townsend, J.R. Dettman, and D. Jacobson. 2006. Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. Philosophical Transactions of the Royal Society of London B: Biological Sciences 361(1475):1947-1963.
Ter Braak, C.J., and I.C. Prentice. 1988. A theory of gradient analysis. P. 271-317 in Advances in ecological research. Elsevier.
Ter Braak, C.J., and P. Smilauer. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). www. canoco. com.
Tfaily, M.M., R.K. Chu, J. Toyoda, N. Tolić, E.W. Robinson, L. Paša-Tolić, and N.J. Hess. 2017. Sequential extraction protocol for organic matter from soils and sediments using high resolution mass spectrometry. Analytica chimica acta 972:54-61.
Thornthwaite, C.W. 1948. An approach toward a rational classification of climate. Geographical review 38(1):55-94.
Toledo-Hernández, C., A. Zuluaga-Montero, A. Bones-González, J. Rodriguez, A. Sabat, and P. Bayman. 2008. Fungi in healthy and diseased sea fans (Gorgonia ventalina): is Aspergillus sydowii always the pathogen? Coral Reefs 27(3):707-714.
Török, Á., T. Licha, K. Simon, and S. Siegesmund. 2011. Urban and rural limestone weathering; the contribution of dust to black crust formation. Environmental Earth Sciences 63(4):675-693.
Treesubsuntorn, C., and P. Thiravetyan. 2018. Botanical biofilter for indoor toluene removal and reduction of carbon dioxide emission under low light intensity by using mixed C3 and CAM plants. Journal of Cleaner Production 194:94-100.
Trovato, M., R. Mattioli, and P. Costantino. 2008. Multiple roles of proline in plant stress tolerance and development. Rendiconti Lincei 19(4):325-346.
Tucker, C.J., H.E. Dregne, and W.W. Newcomb. 1991. Expansion and contraction of the Sahara Desert from 1980 to 1990. Science 253(5017):299-300.
Tucker, C.J., J.R. Townshend, and T.E. Goff. 1985. African land-cover classification using satellite data. Science 227(4685):369-375.

Tudzynski, B., A. Sharon, and H. OSIEWACZ. 2002. The Mycota X: industrial applications. The mycota X: industrial applications.
Ullah, M., A.B. Rasli, F.A. Shah, and M.A. Orakzai. 2019. An Exploratory Study of the Factors that Promote, or Impede Sustainable Eco-Tourism Development in Saiful Muluk National Park Khyber Pakhtunkhwa. Journal of Political Studies 26(1).
Ullah, Z., M. Ahmad, H. Sher, H. Shaheen, and S.M. Khan. 2015. Phytogeographic analysis and diversity of grasses and sedges (Poales) of northern Pakistan. Pak. J. Bot 47:93-104.
Van Breusegem, F., and J.F. Dat. 2006. Reactive oxygen species in plant cell death. Plant physiology 141(2):384-390.
Van der Walt, L., S. Cilliers, K. Kellner, D. Tongway, and L. Van Rensburg. 2012. Landscape functionality of plant communities in the Impala Platinum mining area, Rustenburg. Journal of environmental management 113:103-116.
Van Ginneken, L., E. Meers, R. Guisson, A. Ruttens, K. Elst, F.M. Tack, J. Vangronsveld, L. Diels, and W. Dejonghe. 2007. Phytoremediation for heavy metal-contaminated soils combined with bioenergy production. Journal of Environmental Engineering and Landscape Management 15(4):227-236.
Vara Prasad, M.N., and H.M. de Oliveira Freitas. 2003. Metal hyperaccumulation in plants: biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology 6(3):285-321.
Varga, J., S. Kocsube, B. Tóth, J.C. Frisvad, G. Perrone, A. Susca, M. Meijer, and R.A. Samson. 2007. Aspergillus brasiliensis sp. nov., a biseriate black Aspergillus species with world-wide distribution. International Journal of Systematic and Evolutionary Microbiology 57(8):1925-1932.
Verslues, P.E., and S. Sharma. 2010. Proline metabolism and its implications for plant-environment interaction. The Arabidopsis Book/American Society of Plant Biologists 8.
Verta, M., K. Tolonen, and H. Simola. 1989. History of heavy metal pollution in Finland as recorded by lake sediments. Science of the Total Environment 87:1-18.
Vickers, N.J. 2017. Animal communication: when i'm calling you, will you answer too? Current biology 27(14):R713-R715.
Vidali, M. 2001. Bioremediation. an overview. Pure and Applied Chemistry 73(7):1163-1172.
Vijayalakshmi, V., S. Singh, and D. Bhatnagar. 2003. Developmental efforts in R & D for gainful utilization of marble slurry in India. Centre for Development of Stones.
Vile, D., B. Shipley, and E. Garnier. 2006. A structural equation model to integrate changes in functional strategies during old-field succession. Ecology 87(2):504-517.
Vitousek, P.M., H.A. Mooney, J. Lubchenco, and J.M. Melillo. 1997. Human domination of Earth's ecosystems. Science 277(5325):494-499.
Wahid, A., S. Gelani, M. Ashraf, and M.R. Foolad. 2007. Heat tolerance in plants: an overview. Environmental and experimental botany 61(3):199-223.
Waltner-Toews, D. 2001. An ecosystem approach to health and its applications to tropical and emerging diseases. Cadernos de Saúde Pública 17:S07-S36.
Wang, J., K. Price, and P. Rich. 2001. Spatial patterns of NDVI in response to precipitation and temperature in the central Great Plains. International journal of remote sensing 22(18):3827-3844.

Webster, R., and M.A. Oliver. 2007. Geostatistics for environmental scientists. John Wiley & Sons.
Wei, Z., Q. Van Le, W. Peng, Y. Yang, H. Yang, H. Gu, S.S. Lam, and C. Sonne. 2020. A review on phytoremediation of contaminants in air, water and soil. Journal of Hazardous Materials 403:123658.
Wen, Z., W. Liao, and S. Chen. 2005. Production of cellulase/β-glucosidase by the mixed fungi culture Trichoderma reesei and Aspergillus phoenicis on dairy manure. Process Biochemistry 40(9):3087-3094.
Wheeler, Q.D. 2004. Taxonomic triage and the poverty of phylogeny. Philosophical Transactions of the Royal Society of London B: Biological Sciences 359(1444):571-583.
White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1):315-322.
Whittaker, R.H. 1967. Gradient analysis of vegetation. Biological reviews 42(2):207-264.
Whittaker, R.H. 1978. Direct gradient analysis. P. 7-50 in Ordination of plant communities. Springer.
Whittaker, R.H., and W.A. Niering. 1965. Vegetation of the Santa Catalina Mountains, Arizona: a gradient analysis of the south slope. Ecology 46(4):429-452.
Wiegand, I., K. Hilpert, and R.E. Hancock. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols 3(2):163-175.
Worrall, R., D. Neil, D. Brereton, and D. Mulligan. 2009. Towards a sustainability criteria and indicators framework for legacy mine land. Journal of cleaner production 17(16):1426-1434.
Wu, H., Q. Guan, X. Lu, and D.P. Batzer. 2017. Snail (Mollusca: Gastropoda) assemblages as indicators of ecological condition in freshwater wetlands of Northeastern China. Ecological Indicators 75:203-209.
Wu, M., Y. Xu, W. Ding, Y. Li, and H. Xu. 2016. Mycoremediation of manganese and phenanthrene by Pleurotus eryngii mycelium enhanced by Tween 80 and saponin. Applied microbiology and biotechnology 100(16):7249-7261.
Xu, J., H. Yin, and X. Li. 2009. Protective effects of proline against cadmium toxicity in micropropagated hyperaccumulator, Solanum nigrum L. Plant cell reports 28(2):325-333.
Xu, L., D.D. Baldocchi, and J. Tang. 2004. How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature. Global Biogeochemical Cycles 18(4).
Yan, L., Q. Van Le, C. Sonne, Y. Yang, H. Yang, H. Gu, N.L. Ma, S.S. Lam, and W. Peng. 2020. Phytoremediation of radionuclides in soil, sediments and water. Journal of Hazardous Materials:124771.
Yu, J., S. Yao, R. Chen, K. Zhu, and L. Yu. 2005. A quantitative integrated evaluation of sustainable development of mineral resources of a mining city: a case study of Huangshi, Eastern China. Resources Policy 30(1):7-19.
Yuanan, H., K. He, Z. Sun, G. Chen, and H. Cheng. 2020. Quantitative source apportionment of heavy metal (loid) s in the agricultural soils of an industrializing region and associated model uncertainty. Journal of Hazardous Materials 391:122244.

Zeb, A., Z. Iqbal, S.M. Khan, I.U. Rahman, F. Haq, A. Afzal, G. Qadir, and F. Ijaz. 2020. Species diversity, biological spectrum and phenological behaviour of vegetation of Biha Valley (Swat), Pakistan. Acta Ecologica Sinica 40(3):190-196.

Zhang, D., D. Spadaro, S. Valente, A. Garibaldi, and M.L. Gullino. 2012. Cloning, characterization, expression and antifungal activity of an alkaline serine protease of Aureobasidium pullulans PL5 involved in the biological control of postharvest pathogens. International journal of food microbiology 153(3):453-464.

Zhao, J., T. Luo, H. Wei, Z. Deng, X. Li, R. Li, and Y. Tang. 2019. Increased precipitation offsets the negative effect of warming on plant biomass and ecosystem respiration in a Tibetan alpine steppe. Agricultural and Forest Meteorology 279:107761.

Zhou, Q., J. Zhang, J. Fu, J. Shi, and G. Jiang. 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica chimica acta 606(2):135-150.

Zhou, X., H. Zhu, L. Liu, J. Lin, and K. Tang. 2010. A review: recent advances and future prospects of taxol-producing endophytic fungi. Applied microbiology and biotechnology 86(6):1707-1717.

Zornoza, R., Á. Faz, D.M. Carmona, J.A. Acosta, S. Martínez-Martínez, and A. de Vreng. 2013. Carbon mineralization, microbial activity and metal dynamics in tailing ponds amended with pig slurry and marble waste. Chemosphere 90(10):2606-2613.

Zouari, M., C.B. Ahmed, W. Zorrig, N. Elloumi, M. Rabhi, D. Delmail, B.B. Rouina, P. Labrousse, and F.B. Abdallah. 2016. Exogenous proline mediates alleviation of cadmium stress by promoting photosynthetic activity, water status and antioxidative enzymes activities of young date palm (Phoenix dactylifera L.). Ecotoxicology and environmental safety 128:100-108.

Appendices

Appendix table 1 Plant families along with species number and percentage recorded from the studied area.

S. No.	Families	No of Spp.	Percentage	S. No.	Families	No of Spp.	Percentage
1	Poaceae	28	12.73	34	Zygophyllaceae	2	0.91
2	Asteraceae	16	7.27	35	Alismataceae	1	0.45
3	Fabaceae	11	5.00	36	Anacardiaceae	1	0.45
4	Amaranthaceae	10	4.55	37	Araucariaceae	1	0.45
5	Polygonaceae	10	4.55	38	Asparagaceae	1	0.45
6	Rosaceae	10	4.55	39	Bignoniaceae	1	0.45
7	Solanaceae	9	4.09	40	Cactaceae	1	0.45
8	Moraceae	8	3.64	41	Cannaceae	1	0.45
9	Brassicaceae	7	3.18	42	Cleomaceae	1	0.45
10	Euphorbiaceae	6	2.73	43	Combretaceae	1	0.45
11	Cucurbitaceae	5	2.27	44	Cupressaceae	1	0.45
12	Myrtaceae	5	2.27	45	Dryopteridaceae	1	0.45
13	Nyctaginaceae	5	2.27	46	Ebenaceae	1	0.45
14	Pteridaceae	5	2.27	47	Equisetaceae	1	0.45
15	Rutaceae	5	2.27	48	Iridaceae	1	0.45
16	Verbenaceae	5	2.27	49	Juglandaceae	1	0.45
17	Lamiaceae	4	1.82	50	Juncaceae	1	0.45
18	Malvaceae	4	1.82	51	Lythraceae	1	0.45
19	Boraginaceae	3	1.36	52	Meliaceae	1	0.45
20	Salicaceae	3	1.36	53	Musaceae	1	0.45
21	Vitaceae	3	1.36	54	Oleaceae	1	0.45
22	Acanthaceae	2	0.91	55	Onagraceae	1	0.45
23	Apocynaceae	2	0.91	56	Oxalidaceae	1	0.45
24	Araceae	2	0.91	57	Pinaceae	1	0.45
25	Arecaceae	2	0.91	58	Platanaceae	1	0.45
26	Cannabaceae	2	0.91	59	Proteaceae	1	0.45
27	Commelinaceae	2	0.91	60	Scrophulariaceae	1	0.45
28	Convolvulaceae	2	0.91	61	Simaroubaceae	1	0.45
29	Cyperaceae	2	0.91	62	Tamaricaceae	1	0.45
30	Plantaginaceae	2	0.91	63	Typhaceae	1	0.45
31	Portulacaceae	2	0.91	64	Urticaceae	1	0.45
32	Rhamnaceae	2	0.91	65	Xanthorrhoeaceae	1	0.45
33	Sapindaceae	2	0.91				

Appendix table 2 List of plant species reported from Marble waste polluted ecosystem, Khyber Pakhtunkhwa, Pakistan.

S. No.	Botanical Names	TIVI	S. No.	Botanical Names	TIVI
1	Acacia modesta Wall	114.52	111	Cleome viscosa L	57.42
2	Acacia nilotica (L.) Delile	345.77	112	Colocasia esculenta (L.) Schott	38.07
3	Ailanthus altissima (Mill.) Swingle	1647.28	113	Commelina albescens Hassk.	13.02
4	Albizia lebbeck (L.) Benth.	284.25	114	Commelina benghalensis L.	15.02
5	Araucaria heterophylla (Salisb.) Franco	8.47	115	Convolvulus arvensis L	266.16
6	Azadirachta indica A.Juss.	898.69	116	Corchorus olitorius L.	10.82
7	Bombax ceiba L.	151.57	117	Cortaderia selloana (Schult. & Schult.f.) Asch. & Graebn.	80.95
8	Broussonetia papyrifera (L.) L'Hér. ex Vent	1339.62	118	Cucumis melo var agrestis	98.42
9	Callistemon lanceolatus (Sm.) Sweet	52.26	119	Cucurbita maxima Duchesne	25.81
10	Celtis australis L.	153.34	120	Cymbopogon citratus (DC.) Stapf	42.40
11	Citrus aurantium L.	55.03	121	Cynodon dactylon (L.) Pers.	2982.38
12	Citrus limon (L.) Osbeck	16.67	122	Cynoglossum lanceolatum Forssk.	36.22
13	Citrus medica L.	35.60	123	Cyperus difformis L.	15.51
14	Citrus reticulata Blanco	30.00	124	Cyperus rotundus L.	463.29
15	Citrus sinensis (L.) Osbeck	42.59	125	Dactyloctenium aegyptium (L.) Willd	323.76
16	Cupressus sempervirens L.	8.47	126	Desmostachya bipinnata (L.) Stapf	296.85
17	Dalbergia sissoo DC.	970.83	127	Dichanthium annulatum (Forssk.) Stapf	4.54
18	Diospyros lotus L.	271.09	128	Dicliptera bupleuroides Nees	34.91
19	Eriobotrya japonica (Thunb.) Lindl.	140.31	129	Digera muricata (L.) Mart.	109.24
20	Eucalyptus camaldulensis Dehnh.	88.89	130	Digitaria ciliaris (Retz.) Koeler	10.46
21	Eucalyptus globulus Labill.	1308.94	131	Dryopteris stewartii Fraser-Jenk.	10.11
22	Ficus benjamina L.	27.50	132	Dysphania ambrosioides (L.) Mosyakin & Clemants	49.03
23	Ficus carica L.	2441.66	133	Dysphania nepalensis (Link ex Colla) Mosyakin & Clemants	104.44
24	Ficus macrophylla Desf. ex Pers.	17.57	134	Echinochloa colona (L.) Link	166.06
25	Ficus palmata Forssk	83.33	135	Eleusine indica (L.) Gaertn.	619.73
26	Grevillea robusta A.Cunn. ex R.Br.	33.33	136	Emex spinosa (L.) Campd	13.33
27	Juglans regia L.	13.10	137	Epipremnum aureum (Linden & André) G.S.Bunting	10.65
28	Litchi chinensis Sonn.	12.22	138	Equisetum arvense L.	110.51
29	Mangifera indica L.	236.88	139	Erigeron bonariensis L.	216.72
30	Morus alba L.	1699.01	140	Erigeron canadensis L.	993.84

31	Morus macroura Miq.	33.33	141	Euphorbia helioscopia L.	40.18
32	Morus nigra L.	1655.66	142	Euphorbia hirta L.	115.81
33	Phoenix dactylifera L.	239.47	143	Euphorbia prostrata Aiton	137.71
34	Pinus wallichiana A.B.Jacks.	34.64	144	Fragaria nubicola (Lindl. ex Hook.f.) Lacaita	13.13
35	Platanus orientalis L.	52.78	145	Fragaria vesca L.	6.80
36	Populus alba L.	1624.92	146	Helianthus annuus L.	132.61
37	Populus ciliata Wall. ex Royle	34.44	147	Heliotropium europaeum L.	71.40
38	Prosopis juliflora (Sw.) DC.	529.49	148	Heliotropium strigosum Willd	16.92
39	Prunus armeniaca L.	73.25	149	Ipomoea purpurea (L.) Roth	98.23
40	Prunus domestica L.	48.70	150	Iris hookeriana Foster	33.07
41	Prunus persica (L.) Batsch	61.43	151	Jasminum sambac (L.) Aiton	15.31
42	Psidium guajava L	215.25	152	Juncus maritimus Lam.	135.70
43	Punica granatum L.	107.22	153	Lepidium didymum L.	19.76
44	Pyrus communis L.	6.78	154	Lepidium sativum L.	7.14
45	Robinia pseudoacacia L.	38.68	155	Luffa cylindrica (L.) M.Roem.	37.77
46	Salix tetrasperma Roxb.	724.16	156	Malva sylvestris L.	5.09
47	Sapium sebiferum (L.) Roxb.	17.42	157	Malvastrum coromandelianum (L.) Garcke	52.16
48	Syzygium cumini (L.) Skeels	90.01	158	Medicago polymorpha L.	16.08
49	Tamarix aphylla (L.) H.Karst.	428.98	159	Mentha arvensis L.	105.13
50	Tribulus pentandrus Forssk.	146.30	160	Mentha longifolia (L.) L.	141.46
51	Ziziphus jujuba Mill.	259.48	161	Mentha royleana Wall. ex Benth.	114.90
52	Bougainvillea spectabilis Willd.	17.78	162	Mirabilis jalapa L.	9.74
53	Calotropis procera (Aiton) Dryand	1799.81	163	Momordica charantia L.	7.87
54	Catharanthus roseus (L.) G.Don	257.59	164	Musa paradisiaca L	7.33
55	Cestrum nocturnum L.	133.33	165	Nasturtium officinale R.Br.	15.46
56	Combretum indicum (L.) DeFilipps	13.89	166	Nepeta laevigata (D.Don) Hand.-Mazz.	97.77
57	Datura innoxia Mill.	1050.28	167	Oenothera rosea L'Hér. ex Aiton	77.92
58	Datura metel L.	33.33	168	Opuntia dillenii (Ker Gawl.) Haw	11.11
59	Debregeasia saeneb (Forssk.) Hepper & J.R.I.Wood	66.67	169	Oxalis corniculata L	525.15
60	Dodonaea viscosa (L.) Jacq	320.56	170	Parthenium hysterophorus L.	1122.48
61	Duranta stenostachya Tod	22.73	171	Parthenocissus quinquefolia (L.) Planch.	28.36
62	Duranta erecta L	66.67	172	Paspalum distichum L.	185.25
63	Indigofera heterantha Brandis	66.67	173	Persicaria barbata (L.) H.Hara	249.76
64	Lantana camara L	444.72	174	Persicaria glabra (Willd.) M.Gómez	552.37
65	Nannorrhops ritchieana (Griff.) Aitch.	66.67	175	Persicaria hydropiper (L.) Delarbre	11.78
66	Parthenocissus inserta (A.Kern.) Fritsch	120.74	176	Persicaria maculosa Gray	291.90
67	Ricinus communis L.	797.04	177	Phalaris minor Retz.	365.65

68	Rosa indica L.	382.78	178	Phyla nodiflora (L.) Greene	10.52
69	Rosa webbiana Wall. ex Royle	210.61	179	Physalis divaricata D. Don	82.03
70	Rubus fruticosus L.	133.33	180	Plantago minor	29.47
71	Rumex hastatus D. Don	133.33	181	Plantago major L.	71.35
72	Senna occidentalis (L.) Link	332.87	182	Poa annua L.	49.01
73	Vitis vinifera L.	317.78	183	Poa bulbosa L.	39.76
74	Withania somnifera (L.) Dunal	781.20	184	Polygonum aviculare L.	30.05
75	Ziziphus nummularia (Burm.f.) Wight & Arn.	392.15	185	Polygonum plebeium R.Br.	108.61
76	Achyranthes aspera L	279.27	186	Polypogon monspeliensis (L.) Desf.	8.02
77	Acrachne racemosa (B.Heyne ex Roth) Ohwi	21.30	187	Portulaca grandifloraL.	15.44
78	Adiantum capillus-veneris L	739.55	188	Portulaca oleracea L.	77.53
79	Adiantum incisum Forssk.	215.71	189	Pteris cretica L.	3.68
80	Adiantum venustum D. Don	150.45	190	Ruellia simplex C.Wright	18.95
81	Aerva javanica (Burm.f.) Juss. ex Schult.	5.28	191	Rumex nepalensis Spreng.,	46.02
82	Aloe vera (L.) Burm.f	4.19	192	Rumex dentatus L.	61.81
83	Amaranthus retroflexus L	39.23	193	Saccharum bengalense Retz.	515.70
84	Amaranthus spinosus L	224.22	194	Saccharum spontaneum L.	44.88
85	Amaranthus viridis L	621.18	195	Sagittaria sagittifolia L.	24.95
86	Apluda mutica L	33.54	196	Sesbania sesban (L.) Merr.	15.19
87	Aristida adscensionis L.	6.10	197	Setaria pumila (Poir.) Roem. & Schult.	42.52
88	Artemisia vulgaris L.	14.44	198	Setaria verticillata (L.) P.Beauv.	19.44
89	Artemisia persica Boiss	118.00	199	Setaria viridis (L.) P.Beauv.	90.94
90	Artemisia scoparia Waldst. & Kitam.	5.79	200	Sisymbrium irio L.	6.00
91	Arundo donax L.	795.23	201	Solanum americanum Mill.	268.22
92	Asparagus racemosus Willd.	9.26	202	Solanum lycopersicum L.	7.64
93	Bidens bipinnata L.	3.67	203	Solanum surattense Burm. f.	83.11
94	Bidens pilosa L.	9.10	204	Sonchus asper (L.) Hill	27.58
95	Boerhavia diandra L.	10.00	205	Sonchus oleraceus (L.) L.	157.71
96	Boerhavia diffusa L.	116.46	206	Sorghum bicolor (L.) Moench	6.10
97	Boerhavia procumbens Banks ex Roxb	108.01	207	Sorghum halepense (L.) Pers.	128.84
98	Brachiaria ramosa (L.) Stapf	459.86	208	Tagetes erecta L.	96.17
99	Brassica campestris	5.46	209	Taraxacum officinale L.	627.87
100	Brassica nigra (L.) K.Koch	15.45	210	Tribulus terrestris L.	40.08
101	Campsis radicans (L.) Seem.	45.08	211	Trifolium repens L.	13.33
102	Canna indica L.	45.99	212	Triticum aestivum L.	40.14
103	Cannabis sativa L.	664.84	213	Typha angustifolia L.	187.86
104	Capsella bursa-pastoris (L.) Medik	5.04	214	Verbascum thapsus L.	22.95
105	Capsicum annuum L.	21.36	215	Verbena officinalis L.	58.01

106	Cheilanthes acrostica (Balb.) Tod.	57.01	216	Verbesina encelioides (Cav.) Benth. & Hook.f. ex A.Gray	50.99
107	Chenopodium album L.	476.19	217	Xanthium strumarium L.	633.65
108	Chenopodium murale L.	90.67	218	Zea mays L.	17.12
109	Chrozophora tinctoria (L.) A.Juss.	65.03	219	Tithonia diversifolia (Hemsl.) A.Gray	28.66
110	Citrullus lanatus (Thunb.) Matsum. & Nakai	102.55	220	F9 (1) Ab	11.67

Appendix table 3 Plant families along with species number and percentage recorded from the Humid, Semi Humid and Dry subtropical Marble Waste Polluted Ecosystem (MWPE).

S. No	Family	No of Plants	%age	S. No	Family	No of Plants	%age
Humid Subtropical Vegetation Zone of MWPE							
	Poaceae	14	11.47541	25.	Anacardiaceae	1	0.819672
	Asteraceae	9	7.377049	26.	Araceae	1	0.819672
	Solanaceae	7	5.737705	27.	Arecaceae	1	0.819672
	Polygonaceae	6	4.918033	28.	Bignoniaceae	1	0.819672
	Amaranthaceae	5	4.098361	29.	Boraginaceae	1	0.819672
	Brassicaceae	5	4.098361	30.	Commelinaceae	1	0.819672
	Fabaceae	5	4.098361	31.	Cyperaceae	1	0.819672
	Rosaceae	5	4.098361	32.	Ebenaceae	1	0.819672
	Cucurbitaceae	4	3.278689	33.	Equisetaceae	1	0.819672
	Lamiaceae	4	3.278689	34.	Juglandaceae	1	0.819672
	Malvaceae	4	3.278689	35.	Juncaceae	1	0.819672
	Moraceae	4	3.278689	36.	Lythraceae	1	0.819672
	Myrtaceae	4	3.278689	37.	Meliaceae	1	0.819672
	Euphorbiaceae	3	2.459016	38.	Oleaceae	1	0.819672
	Pteridaceae	3	2.459016	39.	Onagraceae	1	0.819672
	Apocynaceae	2	1.639344	40.	Oxalidaceae	1	0.819672
	Cannabaceae	2	1.639344	41.	Pinaceae	1	0.819672
	Convolvulaceae	2	1.639344	42.	Platanaceae	1	0.819672
	Nyctaginaceae	2	1.639344	43.	Portulacaceae	1	0.819672
	Plantaginaceae	2	1.639344	44.	Proteaceae	1	0.819672
	Rutaceae	2	1.639344	45.	Rhamnaceae	1	0.819672
	Salicaceae	2	1.639344	46.	Simaroubaceae	1	0.819672
	Verbenaceae	2	1.639344	47.	Urticaceae	1	0.819672
	Vitaceae	2	1.639344				
Semi Humid							
1	Poaceae	24	15.09	27	Rutaceae	2	1.25
2	Asteraceae	11	6.91	28	Alismataceae	1	0.62
3	Fabaceae	9	5.66	29	Anacardiaceae	1	0.62
4	Rosaceae	9	5.66	30	Araceae	1	0.62
5	Amaranthaceae	8	5.03	31	Arecaceae	1	0.62
6	Polygonaceae	8	5.03	32	Bignoniaceae	1	0.62
7	Solanaceae	7	4.40	33	Boraginaceae	1	0.62

8	Moraceae	6	3.77	34	Cactaceae	1	0.62
9	Euphorbiaceae	5	3.14	35	Cannaceae	1	0.62
10	Myrtaceae	5	3.14	36	Cleomaceae	1	0.62
11	Pteridaceae	5	3.14	37	Dryopteridaceae	1	0.62
12	Brassicaceae	3	1.88	38	Ebenaceae	1	0.62
13	Cucurbitaceae	3	1.88	39	Equisetaceae	1	0.62
14	Lamiaceae	3	1.88	40	Juncaceae	1	0.62
15	Salicaceae	3	1.88	41	Lythraceae	1	0.62
16	Verbenaceae	3	1.88	42	Meliaceae	1	0.62
17	Vitaceae	3	1.88	43	Onagraceae	1	0.62
18	Apocynaceae	2	1.25	44	Oxalidaceae	1	0.62
19	Cannabaceae	2	1.25	45	Pinaceae	1	0.62
20	Commelinaceae	2	1.25	46	Sapindaceae	1	0.62
21	Convolvulaceae	2	1.25	47	Scrophulariaceae	1	0.62
22	Cyperaceae	2	1.25	48	Simaroubaceae	1	0.62
23	Malvaceae	2	1.25	49	Tamaricaceae	1	0.62
24	Nyctaginaceae	2	1.25	50	Typhaceae	1	0.62
25	Plantaginaceae	2	1.25	51	Zygophyllaceae	1	0.62
26	Rhamnaceae	2	1.25				
Dry Subtropical							
1	Poaceae	18	12.33	28	Anacardiaceae	1	0.68
2	Asteraceae	11	7.53	29	Araceae	1	0.68
3	Amaranthaceae	9	6.16	30	Araucariaceae	1	0.68
4	Solanaceae	8	5.48	31	Asparagaceae	1	0.68
5	Fabaceae	6	4.11	32	Brassicaceae	1	0.68
6	Moraceae	6	4.11	33	Cannaceae	1	0.68
7	Polygonaceae	6	4.11	34	Cleomaceae	1	0.68
8	Rosaceae	5	3.42	35	Combretaceae	1	0.68
9	Cucurbitaceae	4	2.74	36	Cupressaceae	1	0.68
10	Lamiaceae	4	2.74	37	Cyperaceae	1	0.68
11	Myrtaceae	4	2.74	38	Equisetaceae	1	0.68

12	Nyctaginaceae	4	2.74	39	Iridaceae	1	0.68
13	Rutaceae	4	2.74	40	Lythraceae	1	0.68
14	Euphorbiaceae	3	2.05	41	Meliaceae	1	0.68
15	Pteridaceae	3	2.05	42	Musaceae	1	0.68
16	Verbenaceae	3	2.05	43	Oleaceae	1	0.68
17	Acanthaceae	2	1.37	44	Onagraceae	1	0.68
18	Apocynaceae	2	1.37	45	Oxalidaceae	1	0.68
19	Arecaceae	2	1.37	46	Pinaceae	1	0.68
20	Boraginaceae	2	1.37	47	Plantaginaceae	1	0.68
21	Cannabaceae	2	1.37	48	Scrophulariaceae	1	0.68
22	Convolvulaceae	2	1.37	49	Simaroubaceae	1	0.68
23	Malvaceae	2	1.37	50	Tamaricaceae	1	0.68
24	Portulacaceae	2	1.37	51	Typhaceae	1	0.68
25	Rhamnaceae	2	1.37	52	Vitaceae	1	0.68
26	Salicaceae	2	1.37	53	Zygophyllaceae	1	0.68
27	Sapindaceae	2	1.37				

Appendix table 4 Summary of Species-area Curves of all the studied quadrats/stations of the MWPE, Khyber Pakhtunkhwa, Pakistan.

Number Plots	Number of Species		Distance		Number Plots	Number of Species		Distance	
	Average	SD	Average	SD		Average	SD	Average	SD
1	8.12	3.2514	0.8681	0.04404	165	183.36	5.6602	0.0899	0.00576
2	14.85	4.1353	0.7676	0.04769	166	183.63	5.5035	0.0891	0.00575
3	21.1	4.7289	0.6915	0.04355	167	183.62	5.5059	0.0885	0.00568
4	26.99	5.3176	0.6266	0.04413	168	184.44	5.8077	0.0878	0.00575
5	31.72	5.4962	0.5856	0.0409	169	184.3	5.5513	0.0877	0.00574
6	37.01	5.5975	0.5399	0.04067	170	185.01	5.0724	0.0874	0.00548
7	41.19	6.0955	0.5085	0.03621	171	185.37	5.8481	0.0869	0.0057
8	44.4	6.2552	0.4859	0.03499	172	185.38	5.4924	0.0868	0.00554
9	48.09	6.6055	0.4703	0.03212	173	185.49	5.9076	0.0864	0.00536
10	51.79	6.0964	0.4539	0.03047	174	186.5	5.2415	0.0854	0.00561
11	55.2	6.4874	0.4351	0.03003	175	185.72	5.8658	0.0851	0.00561
12	58.61	6.73	0.4199	0.02727	176	186.84	5.4636	0.0844	0.00523
13	61.08	6.6817	0.4067	0.02769	177	186.54	5.1265	0.0839	0.00523
14	64.02	6.7512	0.3924	0.02548	178	187.53	5.4118	0.0829	0.00525
15	65.88	6.9014	0.3791	0.02581	179	187.9	5.4904	0.0828	0.00559
16	68.83	7.0926	0.3695	0.02468	180	187.8	5.3491	0.0822	0.00527
17	71.19	7.1678	0.3607	0.02477	181	188.05	5.6061	0.0819	0.00504
18	73.46	7.2965	0.3514	0.02355	182	188.11	5.098	0.0814	0.00527
19	75.76	7.1658	0.3429	0.02225	183	188.89	5.3575	0.0807	0.00524
20	77.58	7.3408	0.3357	0.02101	184	189.12	4.8054	0.0804	0.00499
21	79.66	7.5905	0.3269	0.02105	185	189.41	5.2207	0.0796	0.00489
22	82.11	7.524	0.3209	0.02009	186	189.77	5.2668	0.0794	0.00521
23	84.47	7.5831	0.3126	0.01949	187	190	5.3613	0.0785	0.00499
24	85.88	7.7352	0.3058	0.02015	188	190.07	5.0147	0.0778	0.00511
25	87.04	7.1011	0.299	0.01993	189	190.62	5.1474	0.0778	0.00494
26	89.04	7.0036	0.2948	0.0185	190	190.55	5.2374	0.0777	0.00502
27	90.54	7.5152	0.2892	0.01956	191	191.01	5.0667	0.0766	0.00513
28	92.3	7.276	0.2839	0.01869	192	191.31	5.2279	0.0761	0.00505
29	93.78	7.7106	0.2784	0.01796	193	191.58	5.1334	0.0759	0.00498
30	95.25	7.3278	0.272	0.01719	194	192.04	5.0294	0.0756	0.00476
31	97.05	7.4948	0.2686	0.01791	195	192.57	4.8457	0.0747	0.00475
32	98.92	7.5712	0.2647	0.0169	196	192.5	4.877	0.0738	0.00457
33	100.56	7.1675	0.2618	0.01767	197	192.91	4.9673	0.0736	0.00477
34	102.36	7.3284	0.2573	0.01697	198	192.75	4.7992	0.0732	0.0046
35	103	7.6998	0.2542	0.01531	199	193.4	4.9633	0.0728	0.00471
36	103.91	7.5107	0.2503	0.01636	200	193.63	4.9499	0.0725	0.00475
37	105.49	7.9261	0.2467	0.016	201	194.08	4.9045	0.0722	0.00455
38	107.02	7.6524	0.2434	0.01586	202	194.05	4.7325	0.0717	0.0047

39	108.31	7.7	0.2392	0.01444	203	194.15	5.0648	0.0712	0.00464
40	109.3	7.5834	0.2367	0.01504	204	194.66	5.2416	0.0704	0.00443
41	110.33	7.609	0.2335	0.01505	205	194.56	4.683	0.07	0.00438
42	111.39	7.7884	0.231	0.01481	206	195.09	4.7098	0.0698	0.0048
43	112.68	7.7147	0.2283	0.01402	207	195.35	5.0189	0.0695	0.00448
44	113.67	7.4981	0.2264	0.01503	208	195.87	4.7105	0.0688	0.00446
45	114.72	7.2251	0.2223	0.01406	209	195.71	4.5867	0.0684	0.00466
46	115.99	7.272	0.2188	0.01404	210	196.42	4.7663	0.0681	0.00451
47	117.28	7.5174	0.2186	0.01394	211	196.62	4.8073	0.0677	0.00444
48	118.27	7.2545	0.2145	0.0135	212	197.03	4.6627	0.0667	0.00452
49	119.59	7.5424	0.2129	0.01376	213	196.78	4.6867	0.0664	0.00403
50	120.21	7.2997	0.2114	0.01395	214	197.59	4.576	0.0655	0.00406
51	121.45	7.2147	0.2084	0.01343	215	197.55	4.6865	0.0654	0.00395
52	122.45	7.8106	0.206	0.01281	216	197.74	4.815	0.0652	0.00461
53	123.29	7.7625	0.205	0.0128	217	197.88	4.4814	0.0646	0.00411
54	124.25	7.0627	0.2006	0.01289	218	198.09	4.8621	0.0642	0.0042
55	124.67	7.4912	0.1997	0.01313	219	198.43	4.3698	0.0635	0.00422
56	126.42	7.6428	0.1975	0.01273	220	198.77	4.639	0.0632	0.00432
57	127.37	7.2478	0.195	0.01251	221	199.24	4.4802	0.0628	0.00416
58	127.73	7.696	0.1937	0.01274	222	199.06	4.7263	0.0622	0.00412
59	128.75	7.4536	0.1904	0.01221	223	199.34	4.5527	0.0619	0.00415
60	129.75	7.3222	0.1902	0.01325	224	199.85	4.5444	0.0615	0.00387
61	131.11	7.3176	0.1876	0.01173	225	199.75	4.5566	0.0607	0.004
62	131.57	7.4394	0.1866	0.01141	226	200.2	4.3562	0.0603	0.00404
63	131.93	7.3077	0.1845	0.01199	227	200.48	4.4427	0.0602	0.00396
64	132.98	7.498	0.1829	0.01149	228	200.73	4.3565	0.0596	0.00406
65	133.89	7.3099	0.1812	0.01114	229	200.82	4.5266	0.0594	0.00396
66	134.75	7.5421	0.178	0.0112	230	200.75	4.3616	0.059	0.00379
67	135.02	7.1497	0.1776	0.01149	231	201.51	4.2973	0.0585	0.00388
68	136.27	7.0884	0.1753	0.01103	232	201.9	4.2548	0.0578	0.0037
69	136.96	6.8513	0.1745	0.01076	233	202.07	4.2136	0.0576	0.00362
70	138.01	7.2232	0.1731	0.01107	234	201.94	4.3319	0.0571	0.00364
71	138.71	7.0567	0.1714	0.01094	235	202.24	4.1074	0.0568	0.00368
72	138.79	6.9252	0.1709	0.01072	236	202.68	4.446	0.0562	0.00386
73	140.16	6.8545	0.1685	0.01051	237	202.71	4.3607	0.0559	0.00366
74	140.8	7.373	0.1665	0.01083	238	202.9	4.1102	0.0552	0.00368
75	141.66	6.7773	0.1664	0.01097	239	202.89	4.1656	0.0548	0.00369
76	141.71	7.0698	0.1644	0.01051	240	203.41	4.2996	0.0547	0.00358
77	142.55	6.9426	0.1625	0.01024	241	203.57	4.1278	0.0541	0.00352
78	144.03	7.0969	0.1617	0.01024	242	204.1	4.3915	0.0535	0.00359
79	144.15	6.9613	0.1607	0.01011	243	203.75	4.1599	0.0532	0.00335
80	144.66	6.9902	0.1586	0.01019	244	204.31	3.9785	0.0528	0.00332
81	144.86	7.0559	0.1574	0.01055	245	204.78	4.0996	0.0522	0.00356
82	145.82	7.2547	0.1561	0.01029	246	204.74	3.9884	0.0517	0.00355

83	146.57	6.597	0.1551	0.01023	247	204.62	4.0191	0.0514	0.00322
84	147.37	6.8024	0.1534	0.00962	248	205.18	3.9869	0.0511	0.00335
85	148.05	6.9398	0.1528	0.00958	249	205.1	4.1815	0.051	0.0034
86	148.45	6.7938	0.1518	0.00971	250	205.66	3.9087	0.0501	0.00324
87	149.11	6.8853	0.1503	0.00979	251	206.01	3.8467	0.0496	0.00323
88	149.36	6.5553	0.1487	0.00894	252	206.29	4.0698	0.0492	0.00325
89	150.7	6.9059	0.148	0.00957	253	206.04	3.8634	0.0486	0.00305
90	151.29	6.799	0.1481	0.00989	254	206.5	4.0332	0.0484	0.0033
91	151.79	6.8229	0.1464	0.00953	255	206.83	3.6515	0.048	0.00321
92	152.19	7.1907	0.1443	0.00953	256	206.6	3.8385	0.0476	0.00306
93	151.98	6.5838	0.1441	0.00973	257	207.05	3.8038	0.0472	0.00309
94	153.02	7.1515	0.1435	0.00914	258	207.44	3.8741	0.0463	0.00314
95	153.4	7.1202	0.142	0.009	259	207.6	3.869	0.046	0.00276
96	154.72	6.6667	0.1405	0.00924	260	207.61	3.7451	0.0457	0.00302
97	154.67	6.9745	0.1393	0.00852	261	207.92	3.736	0.0454	0.00311
98	155.34	6.6674	0.1388	0.00852	262	208.22	3.6656	0.0448	0.00298
99	155.98	6.5452	0.1373	0.00882	263	208.46	3.6137	0.0443	0.0028
100	156.52	6.8454	0.1368	0.00891	264	208.37	3.684	0.0441	0.0029
101	157.36	6.651	0.1353	0.00865	265	208.83	3.6189	0.0435	0.00296
102	157.45	6.3287	0.1344	0.00874	266	208.91	3.6077	0.0431	0.00286
103	158.05	6.434	0.1337	0.00914	267	209.09	3.3936	0.0426	0.00289
104	158.71	6.7891	0.1328	0.00826	268	209.5	3.3419	0.0422	0.00287
105	158.97	6.8433	0.1324	0.00843	269	209.49	3.3574	0.0418	0.00291
106	159.47	6.1616	0.1311	0.00825	270	209.66	3.4268	0.0414	0.00286
107	160.09	6.3062	0.1295	0.00897	271	210.13	3.3161	0.0406	0.00258
108	160.83	6.5585	0.1283	0.00835	272	210.03	3.5534	0.0402	0.00259
109	161.19	6.5238	0.1279	0.00847	273	210.25	3.4862	0.0399	0.00267
110	162.23	6.526	0.1271	0.0081	274	210.62	3.3013	0.0394	0.0026
111	161.67	6.5949	0.1263	0.00781	275	210.81	3.1681	0.039	0.0026
112	162.64	6.4399	0.1262	0.00795	276	210.96	3.3766	0.0387	0.00258
113	162.29	6.617	0.125	0.00807	277	211.19	3.1164	0.038	0.00245
114	163.7	6.6989	0.1243	0.00812	278	211.13	3.0911	0.0377	0.00268
115	163.76	6.9167	0.1232	0.00788	279	211.71	3.0735	0.037	0.00252
116	164.44	6.3139	0.1234	0.00768	280	211.71	3.0176	0.0365	0.00237
117	164.78	6.4685	0.1217	0.00744	281	211.87	3.1122	0.0362	0.00239
118	165.5	6.2144	0.1206	0.00748	282	211.8	2.9753	0.0357	0.00244
119	165.2	6.5024	0.1196	0.00751	283	212.3	2.9033	0.0351	0.00249
120	166.36	6.1769	0.1196	0.00758	284	212.52	2.8918	0.0345	0.00235
121	166.72	6.0748	0.1188	0.00744	285	212.54	2.9198	0.0339	0.00222
122	166.92	6.2181	0.1181	0.00761	286	212.78	2.9359	0.0334	0.00213
123	167.47	6.1505	0.1171	0.00731	287	212.94	2.941	0.033	0.00213
124	168	6.3684	0.1171	0.00757	288	213.16	2.8568	0.0325	0.00226
125	168.65	6.1638	0.1155	0.00699	289	213.17	2.8769	0.0319	0.00217
126	168.86	5.9388	0.1149	0.00738	290	213.37	2.971	0.0315	0.00213

127	169.27	6.0193	0.1141	0.00732	291	213.56	2.8061	0.031	0.00207
128	169.66	6.4515	0.1136	0.00718	292	213.87	2.8601	0.0305	0.00222
129	170.18	6.1918	0.1128	0.00728	293	214.19	2.6866	0.0299	0.00212
130	170.5	5.9316	0.1118	0.00714	294	214.46	2.5269	0.0292	0.00206
131	170.7	5.7676	0.1105	0.00674	295	214.48	2.7794	0.0288	0.00202
132	171.09	5.9911	0.1106	0.00709	296	214.68	2.5868	0.0282	0.00207
133	171.67	6.2876	0.1097	0.00693	297	214.59	2.6337	0.0276	0.00197
134	172.43	5.7689	0.1085	0.00723	298	215.03	2.4469	0.0272	0.0018
135	172.56	6.0494	0.1086	0.00688	299	214.95	2.3985	0.0266	0.00189
136	172.74	6.0953	0.1079	0.00678	300	215.31	2.4677	0.0259	0.00191
137	173.8	5.9928	0.1072	0.00662	301	215.53	2.4586	0.0253	0.00183
138	173.65	6.2104	0.1061	0.00686	302	215.82	2.1973	0.0247	0.00178
139	174.29	6.3374	0.1055	0.00692	303	215.92	2.232	0.0242	0.00172
140	174.54	5.9846	0.1052	0.00644	304	216.1	2.116	0.0235	0.00174
141	175.01	6.1205	0.1051	0.00698	305	216.24	2.173	0.0232	0.00181
142	175.51	6.0301	0.104	0.00623	306	216.49	2.1742	0.0226	0.00168
143	175.5	5.8273	0.1033	0.00651	307	216.58	2.1821	0.0219	0.00159
144	175.7	6.048	0.103	0.00669	308	216.85	1.9858	0.0211	0.00158
145	176.5	5.8715	0.1024	0.00665	309	216.97	1.9567	0.0205	0.00154
146	176.21	6.1422	0.1015	0.00659	310	217.21	1.9626	0.0198	0.00152
147	177.05	5.7192	0.101	0.00632	311	217.44	1.8883	0.0191	0.00153
148	177.36	5.846	0.1001	0.00663	312	217.44	1.8057	0.0183	0.00141
149	177.71	5.9886	0.0996	0.0064	313	217.73	1.697	0.0175	0.00133
150	178.24	5.6291	0.0988	0.00638	314	217.86	1.6327	0.0168	0.00133
151	178.51	5.8445	0.098	0.00638	315	218.03	1.6353	0.016	0.00128
152	178.22	5.693	0.0983	0.00626	316	218.11	1.5924	0.0152	0.00122
153	179.46	6.081	0.0973	0.00588	317	218.39	1.4512	0.0143	0.00119
154	179.24	5.6664	0.0966	0.00613	318	218.54	1.4215	0.0132	0.00113
155	179.88	5.8443	0.096	0.00629	319	218.63	1.2917	0.0123	0.00107
156	180.83	5.6512	0.0953	0.00603	320	218.91	1.2446	0.0111	0.00107
157	180.63	5.7308	0.0952	0.00564	321	218.99	1.1604	0.0101	0.00096
158	181.27	5.6479	0.0943	0.0061	322	219.13	1.0403	0.009	0.00096
159	180.96	5.6305	0.0933	0.00623	323	219.34	0.9918	0.0077	0.0008
160	181.52	5.5569	0.0928	0.00605	324	219.52	0.7791	0.0064	0.00077
161	181.79	5.7479	0.092	0.00577	325	219.66	0.6991	0.0047	0.00066
162	182.73	5.6456	0.0916	0.0059	326	219.84	0.5231	0.0027	0.00056
163	182.28	5.7194	0.0908	0.00606	327	220		0	
164	182.94	5.7292	0.0899	0.00559					

Appendix table 5 Indicator species Analysis indicating the topmost indicator species (with bold font) of each subtropical vegetation zone (1-3) in relation with various environmental factors at 25% threshold level of indicators founded on Monte Carlo Test of significance for the observed maximum IV (percentage of perfect indication established on combining values for the relative abundance and frequency for plant species along with probability value ≤ 0.05. [Max grp= Maximum group (group identifier for maximum observed IV), IV=Observed indicator values, p^*= Probability value (1+number of runs>=observed)/(1+number of randomized runs)].

S.No.	Botanical Names	Humid subtropical vegetation zone defined based on soil pH			Semi-Humid subtropical vegetation zone defined based on Precipitation			Dry subtropical vegetation zone defined based on temperature		
		Maxgrp	**IV**	**p ***	**Maxgrp**	**IV**	**p ***	**Maxgrp**	**IV**	**p ***
1	*Acacia modesta Wall*	8	1.9	1	55	3.6	0.4721	-	-	-
2	*Acacia nilotica (L.) Delile*	-	-	-	31	4.8	0.2567	30	12.9	0.1846
3	*Achyranthes aspera L*	8	5.8	1	55	12	0.0658	25	24.1	0.0754
4	*Acrachne racemosa (B.Heyne ex Roth) Ohwi*	-	-	-	55	1.9	1	33	16.7	0.3655
5	*Adiantum capillus-veneris L*	9	11.3	0.5613	45	8.7	0.4759	24	15.2	0.1564
6	*Adiantum incisum Forssk.*	9	15.5	0.2386	64	4.3	0.7473	29	10.8	0.2693
7	*Adiantum venustum D. Don*	8	7.7	0.7201	64	12	0.047	35	4.2	1
8	*Aerva javanica (Burm.f.) Juss. ex Schult.*	-	-	-	-	-	-	32	16.7	0.3673
9	*Ailanthus altissima (Mill.) Swingle*	7	25.6	0.2911	64	12.6	0.2368	20	61.5	0.0012
10	*Albizia lebbeck (L.) Benth.*	-	-	-	45	4.4	0.7467	30	26.7	0.2681
11	*Aloe vera (L.) Burm.f*	-	-	-	45	2.1	0.6065	-	-	-
12	*Amaranthus retroflexus L*	-	-	-	45	4.4	0.3733	-	-	-

13	*Amaranthus spinosus L*	-	-	-	55	14.6	0.0266	35	12.5	0.6413
14	*Amaranthus viridis L*	7	13.8	0.884	55	12.3	0.1638	32	7.7	0.6805
15	*Apluda mutica L*	-	-	-	45	4.2	0.3751			
16	*Araucaria heterophylla (Salisb.) Franco*	-	-	-	-	-	-	39	33.3	0.2605
17	*Aristida adscensionis L.*	-	-	-	45	2.1	0.6101	-	-	-
18	*Artemisia vulgaris L.*	8	3.8	1	-	-	-	-	-	-
19	*Artemisia persica Boiss*	-	-	-	-	-	-	19	33.3	0.0236
20	*Artemisia scoparia Waldst. & Kitam.*	-	-	-	-	-	-	34	8.3	0.8088
21	*Arundo donax L.*	9	24.8	0.1348	55	4.6	0.5159	38	13	0.1354
22	*Asparagus racemosus Willd.*	-	-	-	-	-	-	39	33.3	0.2605
23	*Azadirachta indica A.Juss.*	8	5.9	1	55	8.7	0.4555	27	7.5	0.5327
24	*Bidens bipinnata L.*	-	-	-	45	2.1	0.6171	-	-	-
25	*Bidens pilosa L.*	8	1.9	1	-	-	-	-	-	-
26	*Boerhavia diandra L.*	-	-	-	-	-	-	35	4.2	1
27	*Boerhavia diffusa L.*	-	-	-	55	3.6	0.4733	26	43.2	0.006
28	*Boerhavia procumbens Banks ex Roxb*	8	3.8	1	55	13	0.0256	24	66.7	0.0036
29	*Bombax ceiba L.*	8	1.9	1	31	9.5	0.0338	39	14.3	0.4071
30	*Bougainvillea spectabilis Willd.*	-	-	-	-	-	-	27	11.1	0.5141
31	*Brassica campestris*				45	2.1	0.6103			
32	*Brassica nigra (L.) K.Koch*	8	3.8	1						
33	*Brachiaria ramosa (L.) Stapf*	7	12.5	0.5077	55	10.2	0.233	23	7.1	0.864
34	*Broussonetia papyrifera (L.) L'Hér. ex Vent*	7	15.5	0.2392	55	21.1	0.034	34	5.1	0.6603

35	*Callistemon lanceolatus (Sm.) Sweet*	8	1.9	1				32	10	0.6269
36	***[2]Calotropis procera (Aiton) Dryand***	8	1.9	1	**55**	**15.4**	**0.0374**	25	17.6	0.1174
37	*Campsis radicans (L.) Seem.*	9	20	0.0794	45	4.2	0.3609			
38	*Canna indica L.*				45	6.2	0.13	32	16.7	0.3737
39	*Cannabis sativa L.*	8	12.5	0.8614	55	9.8	0.5209	28	31.4	0.0526
40	*Capsicum annuum L.*	8	1.9	1	55	1.9	1	34	8.3	0.8088
41	*Capsella bursa-pastoris (L.) Medik*				45	2.1	0.6083			
42	***[1]Catharanthus roseus (L.) G.Don***	**7**	**20.5**	**0.0566**	55	5.6	0.192	31	8.3	0.8102
43	*Celtis australis L.*	8	3.8	1	45	7.8	0.136	34	8.3	0.7994
44	*Cestrum nocturnum L.*	8	3.8	1				34	8.3	0.8088
45	*Cheilanthes acrostica (Balb.) Tod.*				45	8.3	0.0732			
46	*Chenopodium album L.*	9	18.2	0.1444	31	13.9	0.1132	38	19.5	0.0996
47	*Chenopodium murale L.*	8	3.8	1	55	1.9	1	30	53.3	0.0006
48	*Chrozophora tinctoria (L.) A.Juss.*				31	2.5	0.6649	39	22.9	0.1954
49	*Citrus aurantium L.*	8	1.9	1	31	2.1	0.6847			
50	*Citrullus lanatus (Thunb.) Matsum. & Nakai*	9	16.8	0.2138	55	3.7	0.5095	25	17	0.3763
51	*Citrus medica L.*	9	20	0.0794	31	4.8	0.2643	31	8.3	0.8102
52	*Citrus reticulata Blanco*							27	22.2	0.4127
53	*Citrus sinensis (L.) Osbeck*							27	6.3	0.815
54	*Citrus limon (L.) Osbeck*							27	11.1	0.5127
55	*Cleome viscosa L*				31	2.3	0.9002	20	21.1	0.3383
56	*Colocasia esculenta (L.) Schott*				55	3.7	0.4943	31	8.3	0.8066

57	*Commelina albescens Hassk.*				55	1.9	1			
58	*Commelina benghalensis L.*	9	20	0.0794	45	2.1	0.5983			
59	*Combretum indicum (L.) DeFilipps*							33	16.7	0.3655
60	*Convolvulus arvensis L*	8	13.5	0.4217	31	4.6	0.4603	19	10.4	0.3757
61	*Corchorus olitorius L.*	8	1.9	1						
62	*Cortaderia selloana (Schult. & Schult.f.) Asch. & Graebn.*				31	5.5	0.2865	34	8.3	0.804
63	*Cucumis melo var agrestis*	9	18.2	0.1522	31	2.2	0.8706	24	24.8	0.1038
64	*Cucurbita maxima Duchesne*	8	1.9	1	31	1.9	0.9302			
65	*Cupressus sempervirens L.*							39	33.3	0.2605
66	*Cymbopogon citratus (DC.) Stapf*				31	4.8	0.2643	33	8.3	0.6777
67	*Cynodon dactylon (L.) Pers.*	7	33.6	0.2226	64	50.9	0.0002	33	7.8	0.8912
68	*Cynoglossum lanceolatum Forssk.*	8	1.9	1	31	9.5	0.0342			
69	*Cyperus difformis L.*				45	4.2	0.2535			
70	*Cyperus rotundus L.*	8	15.6	0.5695	55	5.5	0.3807	24	25.8	0.0314
71	*Dactyloctenium aegyptium (L.) Willd*	9	32.2	0.0388	55	6.8	0.5011	39	9.8	0.7211
72	***[3]Dalbergia sissoo DC.***	7	6.6	1	55	8.6	0.19	**25**	**24**	**0.048**
73	*Datura innoxia Mill.*	8	1.9	1	31	18.8	0.0118	33	6.2	0.9048
74	*Datura metel L.*							35	4.2	1
75	*Debregeasia saeneb (Forssk.) Hepper & J.R.I.Wood*	9	20	0.0754						
76	*Desmostachya bipinnata (L.) Stapf*	8	7.7	0.7335	31	4.8	0.2655	26	21.1	0.0518
77	*Dichanthium annulatum (Forssk.) Stapf*							31	8.3	0.8022

78	*Dicliptera bupleuroides Nees*							31	25	0.0542
79	*Digitaria ciliaris (Retz.) Koeler*	7	9.5	0.3773						
80	*Digera muricata (L.) Mart.*	8	5.8	1	31	4.3	0.4985	34	16.7	0.5085
81	*Diospyros lotus L.*	9	9	0.8142	45	2.1	0.5983			
82	*Dodonaea viscosa (L.) Jacq*				45	6.2	0.1306	26	19.5	0.188
83	*Dryopteris stewartii Fraser-Jenk.*				55	1.9	1			
84	*Duranta stenostachya Tod*				45	2.1	0.6065			
85	*Duranta erecta L*	8	1.9	1						
86	*Dysphania ambrosioides (L.) Mosyakin & Clemants*							35	25	0.0648
87	*Dysphania nepalensis (Link ex Colla) Mosyakin & Clemants*							19	44.4	0.0146
88	*Echinochloa colona (L.) Link*	7	6	1	55	5.8	0.2392	27	6.3	0.8164
89	*Eleusine indica (L.) Gaertn.*	7	38.3	0.0492	31	6.8	0.6901	39	12.5	0.6521
90	*Emex spinosa (L.) Campd*				31	4.8	0.2679			
91	*Epipremnum aureum (Linden & André) G.S.Bunting*	8	3.8	1						
92	*Equisetum arvense L.*	8	5.8	1	55	3.7	0.6003	34	8.3	0.8012
93	***[1]Erigeron canadensis L.***	**9**	**48.7**	**0.016**	64	12.5	0.3629	24	29.1	0.02
94	*Erigeron bonariensis L.*	8	13.5	0.4253	45	14.6	0.0332			
95	*Eriobotrya japonica (Thunb.) Lindl.*	9	15.5	0.2591	31	3	0.5291	39	33.3	0.2605
96	*Eucalyptus camaldulensis Dehnh.*				64	13.3	0.0118			
97	*Eucalyptus globulus Labill.*	9	16.8	0.1968	55	20.7	0.0176	24	36	0.0074
98	*Euphorbia helioscopia L.*				64	33.3	0.0002			
99	*Euphorbia hirta L.*	8	1.9	1	31	6.1	0.4349			

100	*Euphorbia prostrata Aiton*				31	7.7	0.0826	36	8.3	0.6795
101	*F9 (1) Ab*	8	1.9	1						
102	*Ficus benjamina L.*							32	16.7	0.3569
103	***Ficus carica L.***	**9**	**23.1**	**0.0591**	45	22.7	0.0264	23	11.2	0.5345
104	*Ficus macrophylla Desf. ex Pers.*				45	4.2	0.2833			
105	*Ficus palmata Forssk*				64	3	0.6055			
106	*Fragaria nubicola (Lindl. ex Hook.f.) Lacaita*	8	1.9	1						
107	*Fragaria vesca L.*				45	2.1	0.6083			
108	*Grevillea robusta A.Cunn. ex R.Br.*	8	1.9	1						
109	*Helianthus annuus L.*	8	3.8	1	31	9.5	0.0324	27	22.9	0.069
110	*Heliotropium europaeum L.*							20	33.3	0.0244
111	*Heliotropium strigosum Willd*							23	33.3	0.2675
112	*Indigofera heterantha Brandis*							38	33.3	0.2741
113	*Ipomoea purpurea (L.) Roth*	7	6.6	1	45	6.4	0.176	33	9.5	0.7377
114	*Iris hookeriana Foster*							33	11.1	0.5999
115	*Jasminum sambac (L.) Aiton*	8	1.9	1				27	11.1	0.5119
116	*Juglans regia L.*	8	1.9	1						
117	*Juncus maritimus Lam.*	7	8.3	0.5187	64	42.9	0.0002			
118	*Lantana camara L*				55	3.7	0.5857	30	11.4	0.2741
119	*Lepidium didymum L.*	8	1.9	1	55	1.9	1	33	16.7	0.3707
120	*Lepidium sativum L.*	8	1.9	1						
121	*Litchi chinensis Sonn.*							27	11.1	0.5119
122	*Luffa cylindrica (L.) M.Roem.*	7	8.3	0.5131				31	8.3	0.8066
123	*Malvastrum coromandelianum*	7	11.1	0.2064	45	2.8	0.6011	31	16.7	0.5159

	(L.) Garcke									
124	*Malva sylvestris L.*	8	1.9	1						
125	*Mangifera indica L.*	7	11.1	0.2158	55	3.6	0.7137	32	11.1	0.5947
126	*Medicago polymorpha L.*	8	1.9	1						
127	*Mentha arvensis L.*	9	18.2	0.15	31	2.4	0.7129	38	26.7	0.2793
128	*Mentha longifolia (L.) L.*	7	6	1	31	1.8	1	32	11.1	0.6041
129	*Mentha royleana Wall. ex Benth.*	8	1.9	1				31	8.3	0.8088
130	*Mirabilis jalapa L.*	8	3.8	1						
131	*Momordica charantia L.*							34	8.3	0.8088
132	*Morus alba L.*	8	11.8	0.7469	31	16.1	0.094	33	8.1	0.6837
133	*Morus macroura Miq.*							36	8.3	0.7976
134	***[2]Morus nigra L.***	9	20.6	0.3329	**31**	**17.8**	**0.0744**	26	11.7	0.2328
135	*Musa paradisiaca L*							34	8.3	0.8022
136	*Nannorrhops ritchieana (Griff.) Aitch.*							36	8.3	0.7976
137	*Nasturtium officinale R.Br.*	8	1.9	1						
138	*Nepeta laevigata (D.Don) Hand.-Mazz.*	9	20	0.0844	45	8.3	0.0784	29	19	0.4199
139	*Oenothera rosea L'Hér. ex Aiton*	7	6	1	45	6.2	0.12	36	8.3	0.8078
140	*Opuntia dillenii (Ker Gawl.) Haw*				45	2.1	0.6051			
141	*Oxalis corniculata L*	9	24.1	0.1582	31	6.2	0.4809	27	10	0.4671
142	*Parthenium hysterophorus L.*	8	13.2	0.7544	31	21.5	0.014	25	14.8	0.144
143	*Parthenocissus inserta (A.Kern.) Fritsch*	8	1.9	1	55	5.6	0.2509			
144	*Parthenocissus quinquefolia (L.) Planch.*				31	9.5	0.0322	27	11.1	0.5119

145	*Paspalum distichum L.*	8	7.7	0.7203	64	17.2	0.0048	29	19	0.4299
146	*Persicaria maculosa Gray*	7	8.3	0.5323	45	7.3	0.3099	24	22.2	0.1296
147	*Persicaria barbata (L.) H.Hara*	8	7.4	1	31	7.4	0.1938	23	10.4	0.5045
148	***[2]Persicaria glabra (Willd.) M.Gómez***	8	19.2	0.3211	**64**	**24.7**	**0.009**	29	31.4	0.0508
149	*Persicaria hydropiper (L.) Delarbre*				45	2.1	0.6123			
150	*Phalaris minor Retz.*	9	21.6	0.2745	45	10.8	0.0682	29	26	0.0202
151	*Phoenix dactylifera L.*	8	7.7	0.7237	55	3.7	0.5991	30	8.2	0.8704
152	*Physalis divaricata D. Don*	8	1.9	1	55	8.1	0.1194	39	44.4	0.013
153	*Phyla nodiflora (L.) Greene*							35	4.2	1
154	*Pinus wallichiana A.B.Jacks.*	8	3.8	1	55	1.9	1	27	11.1	0.5141
155	*Plantago minor*	9	20	0.0754	45	6.2	0.1066			
156	*Plantago major L.*	9	33.5	0.0336	45	4.2	0.3403	27	22.2	0.4019
157	*Platanus orientalis L.*	8	3.8	1						
158	*Poa annua L.*				64	20	0.0012			
159	*Poa bulbosa L.*				64	33.3	0.0002			
160	*Polygonum aviculare L.*							35	12.5	0.6599
161	*Polypogon monspeliensis (L.) Desf.*				55	1.9	1			
162	*Polygonum plebeium R.Br.*	8	13.5	0.4191	45	8.3	0.0772	20	22.2	0.4193
163	*Populus alba L.*	8	13.5	0.4279	45	43.9	0.0002	28	20.8	0.0556
164	*Populus ciliata Wall. ex Royle*				64	13.3	0.013			
165	*Portulaca grandifloraL.*							37	11.1	0.5181
166	*Portulaca oleracea L.*	8	3.8	1	55	2.4	0.7842	39	29.6	0.1188
167	*Prosopis juliflora (Sw.) DC.*	8	1.9	1	31	3.4	0.4379	36	12.3	0.3865

168	*Prunus armeniaca L.*	8	3.8	1	45	4.2	0.3263	31	8.3	0.8102
169	*Prunus domestica L.*				31	4.8	0.2643	28	21.1	0.3361
170	*Prunus persica (L.) Batsch*	8	1.9	1	45	4.2	0.3663			
171	*Psidium guajava L*	8	1.9	1	31	11.2	0.0214	39	16.7	0.243
172	*Pteris cretica L.*				45	2.1	0.5983			
173	*Punica granatum L.*	9	18.2	0.1422	55	1.9	1	19	25	0.3507
174	*Pyrus communis L.*				45	2.1	0.6065			
175	*Ricinus communis L.*	8	1.9	1	55	7.4	0.106	30	6.5	0.8038
176	*Robinia pseudoacacia L.*				45	2.1	0.6143	36	8.3	0.8022
177	*Rosa indica L.*	9	18.2	0.1504	55	1.9	1	39	16.7	0.2386
178	*Rosa webbiana Wall. ex Royle*				45	8.3	0.0756	33	16.7	0.3655
179	*Rubus fruticosus L.*				45	4.2	0.3263			
180	*Ruellia simplex C.Wright*							37	11.1	0.5181
181	*Rumex hastatus D. Don*							23	16.7	0.5079
182	*Rumex nepalensis Spreng.,*	8	5.8	1	55	3.7	0.5917			
183	*Rumex dentatus L.*	7	11.1	0.2092	64	6.6	0.1982			
184	***[3]Saccharum bengalense Retz.***				55	11.3	0.062	**38**	**21.2**	**0.0272**
185	*Saccharum spontaneum L.*				64	13.3	0.0126	33	16.7	0.3597
186	*Sagittaria sagittifolia L.*				45	4.2	0.3171			
187	*Salix tetrasperma Roxb.*	7	15.5	0.2573	45	10.3	0.0666	28	31.4	0.0404
188	*Sapium sebiferum (L.) Roxb.*	9	20	0.0754						
189	*Setaria viridis (L.) P.Beauv.*	9	14.4	0.3101	55	1.9	1	31	8.3	0.8094
190	*Senna occidentalis (L.) Link*	8	1.9	1	55	10.3	0.0662			
191	*Sesbania sesban (L.) Merr.*				45	1.3	0.8698			
192	*Setaria pumila (Poir.) Roem. &*	9	18.2	0.1438						

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193	*Setaria verticillata (L.) P.Beauv.*				55	1.9	1			
194	*Sisymbrium irio L.*	9	20	0.0754						
195	*Solanum lycopersicum L.*				55	1.9	1			
196	*Solanum americanum Mill.*	9	20.6	0.3361	45	3.1	0.8632	24	24.1	0.1074
197	*Solanum surattense Burm. f.*	8	1.9	1	31	9.5	0.0358	35	8.3	0.8604
198	*Sonchus asper (L.) Hill*				64	20	0.0008			
199	*Sonchus oleraceus (L.) L.*				31	7.5	0.1908	36	11.1	0.3207
200	*Sorghum bicolor (L.) Moench*				55	1.9	1			
201	*Sorghum halepense (L.) Pers.*	8	7.7	0.7237	31	20.2	0.0024	28	33.3	0.2731
202	*Syzygium cumini (L.) Skeels*	8	1.9	1	31	3.3	0.6827	39	17.4	0.3657
203	*Tagetes erecta L.*	7	10.3	0.7546	31	2.8	0.7213	39	33.3	0.2605
204	*Tamarix aphylla (L.) H.Karst.*				31	4.5	0.1882	39	8.2	0.3749
205	*Taraxacum officinale L.*	9	26.3	0.3607	55	10	0.3185	23	4.5	0.9866
206	*Tithonia diversifolia (Hemsl.) A.Gray*							39	21.1	0.3337
207	*Triticum aestivum L.*							35	4.2	0.9488
208	*Tribulus pentandrus Forssk.*							29	44.4	0.0134
209	*Trifolium repens L.*				64	13.3	0.0108			
210	*Tribulus terrestris L.*				31	23.8	0.001			
211	*Typha angustifolia L.*				45	2.9	0.6009	24	42.7	0.0038
212	*Verbesina encelioides (Cav.) Benth. & Hook.f. ex A.Gray*				31	3.9	0.4359	23	29.6	0.119
213	*Verbena officinalis L.*	8	1.9	1	45	4.7	0.4055	27	11.1	0.5119
214	*Verbascum thapsus L.*				45	2.1	0.6023	32	33.3	0.0082
215	*Vitis vinifera L.*	8	3.8	1	31	4.1	0.3713			

216	[3]***Withania somnifera (L.) Dunal***	8	1.9	1	31	4.8	0.2679	**31**	**8.3**	**0.3971**
217	*Xanthium strumarium L.*	8	9.6	0.7367	64	11.5	0.183	25	12.4	0.161
218	*Zea mays L.*	9	20	0.0844	45	4.2	0.3135			
219	*Ziziphus jujuba Mill.*				55	6.6	0.2555	24	21.1	0.3471
220	*Ziziphus nummularia (Burm.f.) Wight & Arn.*	7	9.5	0.3783	31	4.8	0.2505	25	28.6	0.0636

Appendix table 6 Soil characteristics at zone wise

	pH	**EC**	**TDS**	**OM**	**CaCO3**	Ni	Cr	Cd	Zn	Fe	Mg	Ca
Humid Subtropical MWPE												
AB-F10Q1	8.50	111.00	206.00	0.65	12.98	17.25	26.98	49.09	81.02	125.59	26.86	15.82
AB-F10Q2	8.74	128.00	147.00	0.58	2.34	15.57	21.75	52.87	38.84	198.25	325.00	95.35
AB-F10Q3	8.68	187.00	118.00	0.63	8.43	7.82	59.27	51.08	36.99	74.34	51.80	239.01
AB-F1Q1	8.97	98.40	108.00	0.55	11.40	22.95	22.56	49.18	67.65	171.80	138.54	29.58
AB-F1Q2	8.80	52.30	52.00	0.62	12.61	16.19	20.03	49.01	110.03	243.17	29.79	271.78
AB-F1Q3	7.88	151.90	169.00	0.83	9.20	3.83	56.86	51.15	73.51	72.48	178.99	41.10
AB-F2Q1	8.05	91.20	144.00	0.75	2.19	25.80	19.96	50.38	76.87	170.24	156.87	23.27
AB-F2Q2	8.56	153.00	110.00	0.58	8.66	19.71	18.61	52.90	40.33	248.01	265.41	370.92
AB-F2Q3	7.73	47.70	253.00	0.76	8.06	6.82	64.63	51.23	39.80	59.34	116.01	15.81
AB-F3Q1	9.03	123.00	102.00	0.57	13.89	25.78	26.81	48.66	56.55	154.78	19.83	5.18
AB-F3Q2	8.98	185.00	161.00	0.69	8.63	15.88	33.45	49.87	129.89	180.32	61.06	216.48
AB-F3Q3	9.07	233.30	47.00	0.53	8.71	3.93	63.95	51.30	70.48	55.88	1.54	258.75
AB-F4Q1	9.02	88.30	104.00	0.56	0.84	17.13	18.52	52.38	75.43	126.36	125.07	18.24
AB-F4Q2	8.69	124.00	174.00	0.79	3.66	20.34	17.46	52.08	130.66	258.38	251.56	161.96
AB-F4Q3	8.86	56.90	67.00	0.82	8.20	7.74	57.14	50.87	64.85	55.18	14.07	205.39
AB-F5Q1	8.77	136.00	184.00	0.68	2.02	18.47	24.89	49.37	74.25	178.17	111.20	3.46
AB-F5Q2	8.40	66.20	83.00	0.55	10.86	18.89	11.26	50.38	142.25	139.77	32.64	138.52
AB-F5Q3	8.84	46.00	47.00	0.82	9.31	7.34	54.44	50.95	32.88	60.21	172.01	115.46
AB-F6Q1	8.96	55.00	72.00	0.64	0.83	21.70	25.81	53.08	68.94	180.59	149.09	28.08
AB-F6Q2	9.06	50.50	65.00	0.56	6.84	18.60	22.81	51.52	95.30	214.11	94.92	330.53
AB-F6Q3	9.30	31.00	38.00	0.70	8.50	6.64	56.95	51.06	42.20	64.87	83.03	122.40
AB-F7Q1	8.95	82.10	110.00	0.48	6.31	17.93	24.62	49.54	74.71	97.87	154.03	12.50

AB-F7Q2	8.97	57.40	68.00	0.56	4.90	17.30	16.20	52.91	146.48	174.76	211.22	198.16
AB-F7Q3	8.25	164.00	222.00	0.53	9.02	6.26	62.27	50.85	50.69	71.67	23.15	186.94
AB-F8Q1	8.45	81.40	174.00	0.58	5.95	21.98	18.17	49.12	58.77	156.62	126.17	12.21
AB-F8Q2	8.71	100.80	105.00	0.54	5.30	12.75	29.99	49.98	38.43	139.96	22.67	83.98
AB-F8Q3	7.95	103.70	266.00	0.73	8.66	6.46	59.98	50.97	66.23	55.21	126.56	113.09
AB-F9Q1	8.53	202.00	208.00	0.75	15.50	24.41	19.95	52.73	85.71	157.70	44.61	17.38
AB-F9Q2	8.73	163.00	116.00	0.69	2.00	16.67	22.78	51.42	38.59	211.79	333.43	194.72
AB-F9Q3	8.60	150.00	155.00	0.57	9.22	9.05	61.39	51.03	52.88	80.07	114.53	20.35
HR-F1Q1	7.95	109.00	82.00	0.59	14.91	21.71	21.59	51.16	86.00	111.50	67.50	20.42
HR-F1Q2	8.99	87.50	50.00	0.58	11.96	15.99	19.96	50.26	55.06	155.07	219.16	164.14
HR-F1Q3	8.68	68.50	32.00	0.61	7.94	8.44	54.06	50.73	57.70	54.05	24.91	195.72
HR-F2Q1	8.24	118.00	63.00	0.73	10.88	25.98	24.61	50.35	53.42	109.66	70.10	19.11
HR-F2Q2	8.49	144.00	78.00	0.67	9.84	14.18	30.57	49.34	123.92	195.75	81.52	77.65
HR-F2Q3	8.59	108.00	65.00	0.63	8.26	7.05	67.59	50.78	46.73	60.60	33.21	223.37
MN-F10Q1	7.38	87.00	110.00	0.70	14.02	18.81	20.82	51.97	72.61	139.04	114.16	21.31
MN-F10Q2	8.73	73.00	99.00	0.54	10.97	17.19	17.13	53.59	36.16	229.80	310.15	37.37
MN-F10Q3	8.49	58.00	83.00	0.71	8.66	9.71	55.04	51.00	54.64	59.12	61.57	31.55
MN-F1Q1	8.79	82.00	61.00	0.68	16.39	17.34	19.25	49.24	81.30	136.33	148.23	2.76
MN-F1Q2	8.59	90.00	114.00	0.78	5.66	19.63	35.04	49.58	111.54	123.99	103.30	275.16
MN-F1Q3	8.91	34.00	112.00	0.65	7.94	7.32	71.66	51.15	66.59	71.48	21.98	143.88
MN-F2Q1	8.46	201.00	325.00	0.66	0.48	17.90	24.22	48.79	74.58	139.39	63.66	28.42
MN-F2Q2	8.24	120.00	107.00	0.54	7.52	19.72	28.48	54.15	109.76	163.71	61.72	273.79
MN-F2Q3	8.24	79.00	140.00	0.56	8.42	6.90	57.06	51.27	49.72	73.11	186.93	13.51
MN-F3Q1	8.11	149.00	191.00	0.75	10.26	22.21	21.97	48.80	60.43	138.70	46.91	22.05
MN-F3Q2	8.46	64.00	95.00	0.72	1.55	16.59	10.87	51.01	133.40	141.50	40.13	264.39
MN-F3Q3	8.37	47.00	60.00	0.67	8.32	5.87	55.38	50.80	52.75	66.35	165.24	218.73

MN-F4Q1	8.47	82.00	100.00	0.65	9.85	21.60	27.13	49.77	71.45	167.69	88.91	19.27
MN-F4Q2	8.52	79.00	108.00	0.54	1.23	11.74	31.16	51.39	79.26	109.79	290.22	196.20
MN-F4Q3	8.68	43.00	56.00	0.65	9.10	8.93	70.59	50.90	36.72	64.70	117.06	226.73
MN-F5Q1	7.82	170.00	206.00	0.58	17.00	25.38	19.66	49.54	74.69	114.54	96.63	28.94
MN-F5Q2	8.02	134.00	194.00	0.73	2.64	14.23	32.32	48.95	130.99	213.58	37.88	89.90
MN-F5Q3	7.72	229.33	352.00	0.75	8.14	2.32	56.62	50.91	36.73	64.66	98.86	195.61
MN-F6Q1	7.95	108.00	134.00	0.55	4.37	23.75	27.54	51.49	55.29	145.88	81.73	25.10
MN-F6Q2	8.08	194.00	175.00	0.54	10.44	11.63	25.43	50.48	81.09	207.20	333.32	117.90
MN-F6Q3	7.66	140.00	356.00	0.76	7.77	1.85	71.58	50.87	55.93	69.20	98.42	38.31
MN-F7Q1	8.79	230.00	116.00	0.71	16.82	24.69	20.67	52.75	70.74	166.68	144.84	28.47
MN-F7Q2	8.40	160.00	87.00	0.67	3.91	18.64	32.34	52.65	44.45	217.11	162.61	15.29
MN-F7Q3	8.36	86.00	58.00	0.58	7.57	8.71	68.16	51.05	70.57	71.04	90.93	200.61
MN-F8Q1	8.81	119.00	139.00	0.48	10.85	23.60	23.69	50.49	68.75	130.56	71.37	17.77
MN-F8Q2	8.82	39.00	86.00	0.77	12.27	18.96	32.01	49.66	121.43	154.11	28.57	210.78
MN-F8Q3	8.86	94.00	69.00	0.58	8.44	6.92	54.48	50.84	58.96	70.88	11.08	80.14
MN-F9Q1	8.29	182.00	190.00	0.63	16.76	20.14	18.54	48.19	86.03	148.39	27.73	21.76
MN-F9Q2	8.30	86.00	278.00	0.53	9.09	19.51	21.15	52.94	132.41	113.90	111.19	245.76
MN-F9Q3	8.48	96.00	93.00	0.56	9.12	9.33	64.04	51.13	34.51	59.95	115.21	39.17
Semi Humid Subtropical Zone												
BU-F1Q1	8.94	26.40	27.00	0.56	7.18	45.73	5.96	19.02	50.48	36.80	247.00	589.59
BU-F1Q2	9.45	46.60	48.00	0.79	11.21	22.00	9.10	52.08	111.52	100.28	319.49	313.77
BU-F1Q3	9.33	36.50	43.00	0.75	7.86	16.84	28.80	49.04	30.60	121.73	198.16	360.88
BU-F2Q1	9.57	42.50	50.00	0.54	17.10	47.10	12.60	20.93	30.33	19.53	293.23	554.77
BU-F2Q2	9.49	26.90	32.00	0.71	4.50	22.32	17.35	51.02	77.62	144.95	108.49	475.62
BU-F2Q3	9.33	34.60	41.00	0.69	9.62	16.65	21.70	50.95	52.83	73.08	371.77	568.28
BU-F3Q1	9.54	21.20	24.00	0.56	12.58	41.91	8.22	20.65	50.48	45.42	244.99	551.27

BU-F3Q2	9.41	30.00	36.00	0.77	5.29	22.37	13.15	52.61	66.42	70.61	341.88	317.40
BU-F3Q3	9.36	37.70	44.00	0.55	9.37	12.89	18.45	48.71	29.80	142.39	288.22	321.36
BU-F4Q1	8.93	28.20	27.00	0.62	3.14	42.52	10.23	17.54	49.86	46.84	241.98	576.46
BU-F4Q2	9.53	23.00	33.00	0.56	10.64	23.65	13.51	52.27	68.24	75.51	391.46	492.21
BU-F4Q3	9.34	35.00	41.00	0.80	7.66	13.72	27.19	50.99	64.80	69.05	353.38	447.27
BU-F5Q1	9.38	35.20	42.00	0.72	16.83	45.18	7.87	19.03	55.52	28.26	260.15	634.96
BU-F5Q2	8.91	102.00	130.00	0.67	12.09	25.29	12.41	52.64	81.94	143.78	410.65	557.07
BU-F5Q3	9.36	35.20	38.00	0.79	9.48	11.88	18.42	49.28	110.19	146.54	188.92	579.47
BU-F6Q1	9.18	55.20	59.00	0.57	4.28	43.74	4.48	15.09	37.49	41.48	247.35	537.84
BU-F6Q2	9.14	55.90	63.00	0.76	10.01	22.57	12.46	50.64	47.40	117.25	356.40	245.38
BU-F6Q3	8.93	60.03	147.00	0.54	8.45	18.67	21.66	49.15	112.47	159.99	202.23	555.77
BU-F7Q1	9.38	112.00	37.00	0.53	3.51	45.90	5.28	15.20	42.84	57.20	305.56	626.48
BU-F7Q2	9.34	35.40	39.00	0.79	12.54	24.69	7.77	50.62	88.16	163.67	391.54	554.84
BU-F7Q3	9.39	40.00	41.00	0.66	8.08	15.80	27.59	50.45	101.17	159.10	428.23	266.50
BU-F8Q1	9.53	40.30	33.00	0.63	11.46	45.00	2.12	18.72	59.11	53.18	249.00	643.86
BU-F8Q2	9.24	33.70	67.00	0.71	3.58	22.51	4.84	49.41	74.34	73.99	321.31	462.56
BU-F8Q3	9.64	65.50	71.00	0.77	9.38	16.84	19.03	49.80	100.96	89.15	281.76	286.85
CH-F1Q1	9.30	26.00	29.00	0.53	3.49	49.59	4.80	15.38	44.14	129.12	91.20	472.41
CH-F1Q2	9.32	25.00	27.00	0.78	9.16	24.52	19.27	46.10	38.35	78.56	225.84	540.16
CH-F1Q3	9.10	52.00	67.00	0.76	9.33	13.15	29.49	46.87	125.16	128.03	328.25	331.30
CH-F2Q1	9.24	32.00	37.00	0.49	6.85	50.55	3.81	7.14	51.56	71.21	279.38	414.55
CH-F2Q2	9.27	26.00	30.00	0.54	11.91	24.84	8.91	51.73	61.17	153.38	243.52	502.72
CH-F2Q3	9.09	35.00	44.00	0.76	8.14	16.58	26.35	48.60	147.19	183.16	632.47	179.85
CH-F3Q1	9.05	113.00	140.00	0.72	11.44	41.26	2.08	14.96	49.24	96.08	69.03	416.73
CH-F3Q2	9.09	45.00	51.00	0.60	10.61	21.09	19.81	50.27	67.94	85.40	221.23	544.58
CH-F3Q3	9.27	29.00	34.00	0.68	8.93	12.62	20.70	44.84	27.96	218.43	235.09	419.66

CH-F4Q1	9.37	27.00	32.00	0.52	10.17	39.65	7.76	9.58	49.45	58.86	23.11	526.22
CH-F4Q2	9.20	31.00	37.00	0.70	5.05	24.49	10.51	50.99	32.92	205.16	479.69	571.55
CH-F4Q3	9.36	25.10	30.00	0.71	8.47	11.54	22.30	45.43	64.57	184.33	458.71	291.49
CH-F5Q1	9.29	29.20	35.00	0.67	6.38	33.32	6.50	10.18	51.89	104.80	125.10	496.94
CH-F5Q2	9.47	31.80	37.00	0.68	9.95	18.74	6.46	51.65	42.61	195.00	427.34	530.20
CH-F5Q3	8.87	42.00	50.00	0.79	8.17	11.82	20.37	45.88	130.10	202.27	360.91	311.79
CH-F6Q1	8.96	170.00	180.00	0.56	3.67	34.87	2.89	9.94	43.51	79.05	154.59	300.77
CH-F6Q2	9.27	84.00	93.00	0.58	1.22	21.35	27.35	50.62	15.86	123.64	42.47	559.88
CH-F6Q3	9.05	52.00	52.00	0.70	9.65	8.81	21.89	50.36	60.49	98.02	243.84	253.54
CH-F7Q1	7.80	50.00	47.00	0.71	14.21	38.42	1.63	6.16	43.87	125.81	89.22	119.50
CH-F7Q2	8.72	255.00	352.00	0.78	6.85	24.81	8.57	48.15	33.48	204.57	111.19	492.26
CH-F7Q3	9.11	186.00	110.00	0.54	8.26	10.94	22.49	47.72	55.75	165.50	112.18	200.40
DL-F1Q1	8.06	256.00	190.00	0.60	2.97	31.28	12.02	56.58	46.16	46.86	284.68	402.70
DL-F1Q2	8.44	254.00	233.00	0.72	8.94	15.14	10.54	51.74	20.10	23.59	96.22	419.88
DL-F1Q3	8.44	254.00	233.00	0.75	8.60	14.11	45.76	50.27	56.72	204.38	541.92	210.74
DL-F2Q1	9.03	114.00	130.00	0.51	12.74	25.25	22.10	54.98	84.22	31.88	130.05	5.67
DL-F2Q2	8.89	63.00	67.00	0.72	5.08	17.37	10.87	51.93	22.15	23.05	95.61	624.60
DL-F2Q3	8.89	63.00	67.00	0.76	8.66	13.94	44.92	50.14	50.26	196.43	539.23	212.79
DL-F3Q1	8.38	115.00	144.00	0.52	17.17	29.66	17.95	56.93	80.40	20.78	75.66	12.10
DL-F3Q2	8.48	91.00	100.00	0.67	10.19	18.81	12.35	51.72	55.07	17.48	98.24	476.09
DL-F3Q3	8.48	91.00	100.00	0.80	9.25	14.33	44.80	51.19	45.52	205.34	544.62	211.72
DL-F4Q1	8.08	115.00	134.00	0.50	0.80	32.24	24.43	51.28	64.59	36.99	147.53	44.57
DL-F4Q2	8.45	74.00	75.00	0.78	1.43	20.18	12.34	52.05	22.06	28.79	92.61	486.49
DL-F4Q3	8.45	74.00	75.00	0.60	9.26	14.35	45.94	51.12	52.42	195.96	538.21	208.43
DL-F5Q1	8.51	91.00	94.00	0.53	4.92	30.20	28.10	54.28	75.51	22.34	355.50	74.44
DL-F5Q2	8.58	142.00	158.00	0.76	9.79	17.32	13.87	51.87	65.22	14.53	100.52	574.04

DL-F5Q3	8.58	142.00	158.00	0.84	8.11	14.19	46.78	50.17	51.18	205.37	542.42	208.34
MR-F1Q1	8.07	225.00	343.00	0.67	4.91	33.56	6.03	2.54	55.83	91.17	394.86	318.46
MR-F1Q2	8.28	187.00	220.00	0.70	8.26	15.15	15.39	45.76	16.34	60.93	346.24	222.50
MR-F1Q3	8.49	210.00	261.00	0.69	7.66	5.59	15.37	51.10	38.85	118.09	694.31	559.71
MR-F2Q1	8.35	229.00	290.00	0.71	2.60	32.10	15.31	2.24	55.26	186.42	366.78	23.46
MR-F2Q2	8.49	187.00	232.00	0.70	12.40	19.26	14.66	48.65	66.28	54.50	423.18	417.48
MR-F2Q3	8.53	97.00	121.00	0.60	8.57	8.32	19.40	50.69	37.68	101.70	354.28	427.86
MR-F3Q1	8.52	102.00	124.00	0.48	8.61	32.44	12.59	0.59	65.44	186.36	434.83	333.98
MR-F3Q2	8.61	262.00	330.00	0.78	8.51	15.18	14.17	45.70	27.94	103.01	390.06	285.48
MR-F3Q3	8.11	262.00	341.00	0.58	9.22	6.34	15.10	51.28	36.20	110.42	662.55	578.21
MR-F4Q1	8.22	260.00	332.00	0.48	17.39	33.11	2.72	2.72	71.67	103.78	274.54	305.20
MR-F4Q2	8.61	73.00	91.00	0.76	5.38	19.28	12.72	48.83	30.65	89.76	350.04	476.66
MR-F4Q3	8.29	93.00	184.00	0.67	9.23	8.68	4.50	50.82	46.00	108.31	622.18	498.40
MR-F5Q1	8.56	124.00	157.00	0.62	11.76	32.28	22.57	1.95	54.39	130.16	247.62	402.14
MR-F5Q2	8.33	219.00	272.00	0.54	9.80	16.47	13.68	45.14	29.16	124.64	296.27	341.41
MR-F5Q3	8.62	121.00	147.00	0.74	9.73	9.71	4.98	50.70	33.71	80.51	380.72	476.38
MR-F6Q1	8.05	217.00	372.00	0.63	5.81	34.35	17.39	1.97	52.31	89.87	178.11	431.30
MR-F6Q2	8.42	217.00	371.00	0.58	4.85	20.18	20.09	44.06	47.07	86.71	292.54	610.42
MR-F6Q3	8.56	61.00	81.00	0.75	9.54	5.07	5.84	51.20	45.49	59.66	280.37	493.92
MR-F7Q1	8.32	72.00	178.00	0.63	1.89	32.74	7.10	2.91	69.73	167.10	326.11	97.00
MR-F7Q2	8.42	71.00	149.00	0.77	9.69	19.07	19.30	42.71	47.28	103.61	410.52	277.97
MR-F7Q3	8.08	132.00	319.00	0.79	8.01	10.63	20.77	50.82	25.54	108.42	702.47	483.33
MR-F8Q1	8.49	95.00	120.00	0.49	17.09	33.97	19.71	1.68	58.52	195.63	187.05	100.66
MR-F8Q2	8.49	132.00	170.00	0.72	11.47	21.46	12.10	44.45	0.70	131.40	305.40	582.16
MR-F8Q3	8.52	192.00	290.00	0.71	7.63	7.73	9.78	50.73	47.20	110.25	386.25	419.64
SA-F10Q1	8.93	128.00	131.00	0.57	2.55	33.96	12.43	3.90	57.64	151.31	251.34	152.88

SA-F10Q2	8.78	83.00	117.00	0.77	1.69	21.83	17.20	47.16	16.42	102.51	277.72	253.97
SA-F10Q3	8.92	83.00	51.00	0.62	9.54	8.58	18.40	51.18	31.68	99.22	511.25	460.24
SA-F1Q1	8.93	73.00	151.00	0.66	1.29	35.15	17.55	1.11	66.40	128.30	168.85	363.04
SA-F1Q2	8.91	155.00	175.00	0.75	4.94	21.87	12.74	42.83	34.42	132.52	400.70	222.14
SA-F1Q3	8.45	165.00	77.00	0.71	9.22	6.82	15.54	50.70	41.73	105.21	696.77	416.25
SA-F2Q1	8.86	109.00	111.00	0.62	14.66	32.84	10.07	4.39	48.18	133.80	323.73	330.38
SA-F2Q2	8.70	100.00	136.00	0.65	10.67	20.44	12.76	46.63	5.69	83.19	433.38	225.95
SA-F2Q3	8.62	93.00	103.00	0.76	8.80	6.28	7.34	51.08	30.38	47.23	622.08	406.01
SA-F3Q1	8.73	127.00	137.00	0.58	11.41	34.37	22.08	2.63	69.63	139.26	325.81	64.00
SA-F3Q2	8.63	152.00	160.00	0.73	5.64	17.61	14.94	44.07	32.20	42.24	404.62	384.40
SA-F3Q3	8.88	52.00	48.00	0.64	9.49	10.06	2.19	51.35	30.72	84.22	442.92	492.44
SA-F4Q1	8.23	170.00	189.00	0.65	16.44	32.75	11.07	2.80	65.56	124.50	215.18	296.20
SA-F4Q2	8.89	64.00	55.00	0.60	10.64	22.03	16.80	44.97	65.39	104.18	293.40	565.76
SA-F4Q3	8.95	42.00	37.00	0.83	8.36	9.07	0.90	51.23	25.58	83.69	365.59	517.25
SA-F5Q1	8.94	64.00	50.00	0.55	1.20	32.65	19.80	0.74	57.44	149.61	353.89	245.71
SA-F5Q2	8.62	91.00	112.00	0.70	7.81	19.30	19.48	44.67	37.95	119.92	387.07	515.04
SA-F5Q3	8.85	47.00	30.00	0.70	9.59	9.92	3.12	51.29	37.16	86.52	655.17	430.72
SA-F6Q1	7.84	267.00	329.00	0.57	17.63	32.55	21.41	3.12	61.02	175.88	176.73	421.51
SA-F6Q2	8.71	268.00	306.00	0.54	4.82	16.76	20.10	48.17	13.27	66.96	327.94	241.83
SA-F6Q3	8.96	132.00	86.00	0.53	8.53	5.01	11.56	50.79	26.19	66.82	640.37	445.17
SA-F7Q1	7.94	163.00	126.00	0.65	6.12	33.70	16.63	1.35	51.52	195.33	164.71	476.62
SA-F7Q2	8.58	154.00	117.00	0.78	9.37	16.81	15.61	49.01	28.31	47.50	375.66	477.79
SA-F7Q3	8.57	182.00	126.00	0.60	9.54	5.52	16.98	50.78	41.76	84.58	380.10	487.88
SA-F8Q1	8.93	128.00	131.00	0.47	1.65	32.56	6.87	4.04	69.55	183.34	458.24	395.05
SA-F8Q2	8.78	83.00	117.00	0.56	9.08	16.37	17.29	47.48	32.82	128.50	456.97	556.05
SA-F8Q3	8.92	83.00	51.00	0.76	8.28	11.27	5.81	51.08	29.54	96.70	302.38	418.24

SA-F9Q1	8.93	73.00	151.00	0.55	0.41	33.74	21.09	2.25	64.41	225.28	161.91	471.00
SA-F9Q2	8.91	155.00	175.00	0.61	11.63	21.86	14.53	44.41	25.72	58.28	313.34	441.13
SA-F9Q3	8.45	165.00	77.00	0.58	8.35	9.29	1.80	51.15	42.29	77.33	453.48	423.37
ST-F1Q1	9.08	58.30	55.00	0.59	5.03	46.44	2.14	18.25	35.61	37.79	246.79	593.08
ST-F1Q2	8.68	174.00	339.00	0.61	5.83	22.77	0.52	50.56	39.22	60.19	292.31	131.24
ST-F1Q3	8.88	168.00	194.00	0.53	7.69	13.42	25.37	49.58	103.52	95.85	318.85	406.31
ST-F2Q1	8.92	91.50	97.00	0.71	6.67	45.83	3.33	18.51	55.46	14.02	258.95	601.20
ST-F2Q2	9.15	35.00	37.00	0.75	5.01	23.65	13.78	53.23	82.24	155.43	96.41	136.34
ST-F2Q3	8.18	265.00	311.00	0.54	9.12	12.09	23.19	50.33	25.95	94.33	419.78	510.67
ST-F3Q1	8.73	101.00	77.00	0.67	10.12	42.64	0.11	17.16	43.76	38.15	278.95	625.99
ST-F3Q2	9.06	172.00	188.00	0.74	8.95	23.57	14.64	52.75	90.26	102.58	142.76	403.19
ST-F3Q3	9.10	79.00	34.00	0.78	8.27	14.22	28.40	49.24	64.01	112.70	389.17	506.14
ST-F4Q1	9.05	79.60	73.00	0.57	0.31	45.90	4.78	20.90	57.28	15.36	306.09	584.25
ST-F4Q2	8.86	70.90	71.00	0.58	10.10	25.14	13.36	51.07	112.02	117.76	163.10	2.40
ST-F4Q3	9.63	129.00	150.00	0.79	9.13	16.71	20.39	49.00	91.35	147.63	202.23	273.81
ST-F5Q1	8.77	170.00	95.00	0.68	15.06	45.31	8.16	18.56	49.71	50.31	278.64	609.89
ST-F5Q2	7.89	96.00	130.00	0.59	2.27	25.14	7.72	52.12	69.92	88.75	98.53	163.56
ST-F5Q3	8.43	203.00	148.00	0.61	8.46	14.19	23.81	49.58	41.33	144.66	403.93	382.79
ST-F6Q1	9.07	63.00	57.00	0.58	1.00	42.44	1.63	20.26	55.85	54.52	306.24	596.84
ST-F6Q2	8.49	237.00	191.00	0.65	11.94	25.01	0.45	53.55	113.98	166.79	55.35	124.77
ST-F6Q3	8.97	214.00	125.00	0.65	9.69	17.89	25.47	48.99	105.87	71.40	387.02	474.22
ST-F7Q1	9.06	91.00	51.00	0.66	15.48	43.85	4.46	15.43	31.17	43.98	278.47	610.12
ST-F7Q2	9.23	66.00	67.00	0.64	10.41	23.64	13.24	49.34	108.33	120.50	218.09	586.76
ST-F7Q3	9.47	64.00	61.00	0.75	8.93	14.98	28.47	49.89	89.96	130.04	192.35	474.07
ST-F8Q1	9.06	77.80	81.00	0.74	9.07	46.21	8.03	17.44	42.33	52.38	279.39	595.07
ST-F8Q2	9.49	186.00	223.00	0.57	10.37	24.04	4.36	51.81	112.75	93.33	287.04	172.25

ST-F8Q3	9.07	160.00	180.00	0.79	8.96	18.63	25.30	50.93	60.31	98.50	297.47	515.22
Dry subtropical Zone of MWPE												
BA-F1Q1	9.09	39.60	32.00	0.71	6.29	32.13	5.58	12.04	45.71	135.46	102.47	444.49
BA-F1Q2	9.22	37.50	33.00	0.76	7.71	23.93	10.53	47.89	39.93	129.28	78.75	628.21
BA-F1Q3	9.57	50.00	37.00	0.58	8.24	15.83	16.04	44.48	132.51	151.15	590.67	401.14
BA-F2Q1	9.04	36.30	57.00	0.53	9.03	36.76	2.19	13.50	48.66	79.24	65.11	257.53
BA-F2Q2	8.97	25.90	38.00	0.55	5.58	22.94	23.15	49.51	23.21	188.63	387.39	509.74
BA-F2Q3	9.02	32.70	40.00	0.70	8.95	16.70	5.90	44.86	34.91	247.90	404.77	161.15
BA-F3Q1	9.09	15.60	36.00	0.65	9.96	39.53	2.16	5.45	51.92	136.97	240.02	106.76
BA-F3Q2	9.13	34.70	44.00	0.68	2.04	20.68	17.86	50.41	45.30	109.73	109.92	574.43
BA-F3Q3	9.12	31.80	38.00	0.81	8.00	9.26	19.29	44.88	104.98	141.61	616.23	476.11
BA-F4Q1	8.91	50.60	58.00	0.57	14.15	45.30	0.74	8.09	55.91	105.52	69.54	262.56
BA-F4Q2	8.97	38.70	41.00	0.60	4.03	24.99	8.62	53.91	31.13	42.57	488.00	492.41
BA-F4Q3	9.02	14.50	36.00	0.64	9.44	14.43	36.20	45.23	74.42	217.02	345.13	474.51
BA-F5Q1	8.97	37.40	56.00	0.48	7.47	45.10	6.17	10.01	44.68	0.38	254.51	273.14
BA-F5Q2	8.89	29.20	49.00	0.77	1.41	22.55	17.63	51.94	39.32	70.76	445.18	608.07
BA-F5Q3	9.12	38.10	39.00	0.77	8.70	11.50	18.09	50.05	137.69	170.00	630.80	477.01
BN-F1Q1	8.49	317.00	331.00	0.64	0.57	31.92	30.92	2.76	49.87	42.18	81.52	269.37
BN-F1Q2	8.36	90.10	119.00	0.68	4.74	14.57	4.49	51.98	34.56	158.54	373.80	470.72
BN-F1Q3	8.04	369.00	331.00	0.73	9.55	11.73	54.61	50.88	32.74	68.48	310.87	145.34
BN-F2Q1	9.02	42.60	66.00	0.74	9.87	32.16	32.08	2.69	50.39	42.72	80.59	381.59
BN-F2Q2	8.46	86.50	222.00	0.65	12.05	13.90	4.65	52.71	34.68	158.48	372.25	472.92
BN-F2Q3	8.67	104.00	118.00	0.81	8.83	11.88	54.58	49.93	32.35	69.87	691.53	151.65
BN-F3Q1	8.96	164.00	230.00	0.60	2.04	32.31	32.02	2.95	49.16	42.92	79.93	413.77
BN-F3Q2	8.99	37.80	46.00	0.77	4.22	14.63	4.77	51.84	33.89	160.16	371.96	471.79
BN-F3Q3	9.06	255.00	356.00	0.60	9.18	11.90	54.91	49.82	32.61	69.18	604.27	151.51

Chi-F1Q1	8.57	246.00	144.00	0.62	4.01	25.92	13.90	54.96	48.51	38.50	145.89	352.00
Chi-F1Q2	9.14	119.00	69.00	0.58	9.11	18.21	8.91	51.81	48.68	17.40	92.26	606.10
Chi-F1Q3	8.58	137.00	84.00	0.62	7.66	14.12	46.68	51.28	56.44	212.36	539.23	208.44
Chi-F2Q1	8.91	62.30	23.00	0.55	6.09	24.65	19.71	55.17	74.38	33.88	250.13	408.25
Chi-F2Q2	8.98	52.40	34.00	0.66	11.95	16.74	19.27	51.75	37.01	13.66	90.17	387.78
Chi-F2Q3	8.91	46.60	24.00	0.58	8.88	14.16	46.65	51.26	56.72	212.31	539.33	209.43
Chi-F3Q1	8.03	108.00	74.00	0.51	1.04	31.50	13.13	55.27	82.67	77.53	432.19	155.70
Chi-F3Q2	9.33	86.70	40.00	0.55	9.28	18.26	18.33	51.65	71.51	12.84	100.90	511.60
Chi-F3Q3	8.87	75.10	39.00	0.60	8.38	14.19	46.56	51.33	56.52	212.39	542.45	208.62
KB-F1Q1	8.87	38.80	43.00	0.62	5.67	36.43	0.99	6.52	48.47	72.06	39.85	413.75
KB-F1Q2	7.83	268.00	135.00	0.72	6.86	23.30	16.36	47.38	9.40	5.64	211.88	510.68
KB-F1Q3	7.82	269.00	167.00	0.72	8.62	11.50	19.18	50.31	127.67	109.67	257.44	427.28
KB-F2Q1	8.95	40.90	47.00	0.53	1.25	31.86	5.53	7.05	49.04	84.16	249.87	404.33
KB-F2Q2	8.48	128.00	171.00	0.56	6.86	22.26	21.18	48.80	60.94	145.26	543.74	494.88
KB-F2Q3	8.66	232.00	297.00	0.64	9.08	10.95	19.28	46.29	123.57	154.51	491.25	207.96
KB-F3Q1	8.75	153.00	360.00	0.68	7.18	33.07	5.43	7.18	41.60	4.14	191.53	159.21
KB-F3Q2	8.77	95.50	114.00	0.74	12.05	21.16	8.56	50.04	26.13	45.02	449.45	628.18
KB-F3Q3	8.87	57.11	68.00	0.76	8.11	9.92	12.25	48.48	38.51	99.96	468.18	250.60
KB-F4Q1	8.84	64.70	83.00	0.74	13.02	50.24	0.81	7.79	54.14	41.66	272.54	570.31
KB-F4Q2	8.86	184.00	232.00	0.66	5.97	19.40	22.76	51.00	13.10	165.64	278.05	529.17
KB-F4Q3	8.69	139.00	181.00	0.70	9.26	11.29	26.41	45.63	73.41	130.78	347.30	292.45
KB-F5Q1	8.82	237.00	73.00	0.63	11.30	41.38	5.47	13.55	54.32	40.03	249.90	330.83
KB-F5Q2	8.74	58.20	78.00	0.76	10.39	18.60	12.35	53.17	53.44	52.41	525.86	598.75
KB-F5Q3	8.72	65.00	85.00	0.68	8.36	13.56	28.42	44.95	141.17	139.68	341.23	385.03
KB-F6Q1	8.90	223.00	373.00	0.68	4.35	38.24	1.81	5.38	51.10	34.26	204.44	17.55
KB-F6Q2	8.82	244.00	373.00	0.69	12.06	21.78	15.94	50.64	9.35	144.44	174.93	625.90

KB-F6Q3	8.45	305.00	350.00	0.82	7.73	15.11	13.82	47.69	21.92	253.46	468.31	300.50
KB-F7Q1	9.52	327.00	317.00	0.64	7.36	41.37	0.38	7.67	51.20	110.39	13.50	329.79
KB-F7Q2	8.77	371.00	317.00	0.57	3.67	21.97	25.64	53.75	34.35	98.46	85.70	619.57
KB-F7Q3	8.22	357.00	317.00	0.68	9.24	13.72	38.58	49.59	70.94	235.34	701.14	188.06
KB-F8Q1	8.87	72.70	72.00	0.48	3.91	41.11	8.72	4.72	55.78	55.67	269.31	48.46
KB-F8Q2	9.77	357.00	365.00	0.67	6.23	24.85	5.94	54.00	8.26	167.61	327.83	598.85
KB-F8Q3	8.49	72.70	364.00	0.78	8.17	16.23	12.71	46.57	148.55	238.73	453.12	213.65
KO-F1Q1	8.35	340.00	380.00	0.50	11.19	31.92	32.08	2.88	49.90	43.12	80.55	299.14
KO-F1Q2	8.27	278.00	344.00	0.70	4.11	14.56	4.68	52.96	34.86	160.16	372.24	471.04
KO-F1Q3	8.17	168.00	356.00	0.65	8.21	11.88	54.76	51.20	32.68	69.84	626.00	150.26
KO-F2Q1	8.52	104.00	193.00	0.51	7.43	32.16	32.07	2.91	49.87	43.17	80.52	472.30
KO-F2Q2	8.44	78.00	104.00	0.75	4.78	14.57	4.53	52.87	34.82	160.10	371.59	471.01
KO-F2Q3	8.21	97.00	214.00	0.71	8.86	11.90	54.79	51.27	32.71	69.54	491.26	147.74
KO-F3Q1	8.16	250.00	387.00	0.64	11.40	32.31	32.11	2.86	49.38	43.02	70.39	243.49
KO-F3Q2	8.48	144.00	387.00	0.65	1.89	14.52	4.67	52.93	34.85	159.87	372.09	470.82
KO-F3Q3	8.60	167.00	235.00	0.79	9.42	11.83	54.83	51.24	32.69	69.58	551.05	151.37
MM-F1Q1	9.01	166.00	195.00	0.71	12.74	48.08	7.86	8.66	51.70	55.38	132.96	236.65
MM-F1Q2	9.23	58.00	60.00	0.67	11.40	22.79	9.24	53.95	20.68	86.53	417.97	511.77
MM-F1Q3	8.62	269.00	325.00	0.82	8.84	10.23	24.84	47.57	109.43	214.63	578.44	357.84
MM-F2Q1	9.37	50.00	34.00	0.61	15.43	44.71	15.30	5.22	54.93	21.77	242.82	39.94
MM-F2Q2	9.10	75.70	74.00	0.55	1.79	23.00	15.02	50.97	7.92	114.87	262.28	532.45
MM-F2Q3	9.18	54.00	51.00	0.62	7.57	8.88	14.95	50.08	22.75	85.82	531.82	501.46
MM-F3Q1	9.26	48.00	46.00	0.51	10.82	46.95	11.60	15.43	47.56	68.28	121.96	247.84
MM-F3Q2	9.36	89.00	88.00	0.70	8.40	24.51	19.26	46.78	21.06	16.50	94.74	576.10
MM-F3Q3	9.43	120.00	118.00	0.63	8.29	9.69	19.94	45.12	133.15	263.90	560.44	360.16
MM-F4Q1	9.18	59.00	33.00	0.53	6.48	49.51	5.76	13.57	56.33	55.64	223.64	517.72

MM-F4Q2	9.23	200.00	212.00	0.56	9.11	21.34	6.92	48.87	2.61	172.11	113.39	625.89
MM-F4Q3	8.99	95.00	69.00	0.65	8.36	13.13	23.28	46.35	111.80	122.52	593.39	501.69
MM-F5Q1	9.12	72.50	18.00	0.71	3.16	33.68	2.06	5.98	57.62	58.18	119.94	141.77
MM-F5Q2	9.17	292.00	165.00	0.67	12.57	24.36	14.43	51.70	59.15	168.97	397.80	583.22
MM-F5Q3	9.18	90.00	89.00	0.62	8.51	8.90	26.73	49.46	142.20	250.55	625.11	195.08
MM-F6Q1	8.96	161.00	140.00	0.68	12.65	48.92	2.86	13.42	48.03	31.14	119.04	358.03
MM-F6Q2	8.85	122.00	110.00	0.65	12.82	21.84	12.58	49.71	33.90	181.39	254.76	541.31
MM-F6Q3	8.89	71.50	72.00	0.78	7.78	14.16	18.05	50.18	137.82	95.58	514.87	303.13
MM-F7Q1	9.05	79.00	46.00	0.61	0.35	34.39	7.02	11.96	48.65	125.73	146.03	310.54
MM-F7Q2	9.04	143.00	84.00	0.78	6.93	20.60	16.00	49.37	35.55	138.69	499.71	621.33
MM-F7Q3	9.13	50.00	107.00	0.57	7.75	16.25	35.15	48.79	86.32	148.92	274.94	264.05
MM-F8Q1	9.17	92.00	57.00	0.64	5.32	36.05	1.87	12.48	56.74	34.53	280.29	529.29
MM-F8Q2	9.16	337.00	73.00	0.72	5.19	24.98	25.72	47.99	26.38	118.61	165.28	608.90
MM-F8Q3	8.40	79.00	209.00	0.62	9.53	14.84	12.96	49.73	84.92	112.78	217.14	228.07
NW-F1Q1	9.50	33.40	34.00	0.69	2.59	34.84	3.42	0.86	57.52	225.90	268.93	221.24
NW-F1Q2	9.35	56.00	67.00	0.62	3.35	16.17	17.34	43.36	32.97	114.01	388.60	564.77
NW-F1Q3	9.04	229.00	280.00	0.68	7.85	11.32	15.43	50.73	31.35	64.15	521.22	609.58
NW-F2Q1	8.87	261.00	315.00	0.66	13.56	33.06	9.42	2.81	68.92	147.89	423.01	216.92
NW-F2Q2	9.19	225.00	287.00	0.76	11.40	16.72	16.11	46.38	20.92	45.83	430.57	246.59
NW-F2Q3	8.92	110.00	304.00	0.58	9.14	9.69	13.04	51.15	46.89	82.94	533.27	450.15
NW-F3Q1	9.05	76.70	95.00	0.59	11.25	35.41	13.12	1.91	67.23	90.08	207.97	346.12
NW-F3Q2	9.35	37.80	32.00	0.62	8.84	15.30	15.97	48.64	6.03	124.40	441.03	370.18
NW-F3Q3	8.24	1.84	365.00	0.83	7.76	6.88	0.97	50.74	39.88	79.88	322.32	453.12
NW-F4Q1	9.01	178.00	144.00	0.71	10.72	32.18	12.16	2.07	65.18	137.13	471.73	259.70
NW-F4Q2	8.94	135.00	162.00	0.79	11.12	19.96	20.35	47.18	5.03	79.26	352.19	433.27
NW-F4Q3	9.24	106.00	124.00	0.70	8.41	6.62	14.53	51.18	33.97	64.51	578.29	616.00

NW-F5Q1	9.01	167.00	234.00	0.73	17.49	34.91	17.96	2.96	49.93	171.31	260.83	412.46
NW-F5Q2	8.54	174.00	220.00	0.78	5.63	17.81	12.41	49.27	32.07	132.71	356.25	355.34
NW-F5Q3	8.58	267.00	361.00	0.76	7.54	11.76	2.26	50.70	27.79	110.66	256.40	495.08
NW-F6Q1	8.84	23.00	139.00	0.51	1.93	32.90	5.61	2.64	57.07	174.81	211.69	113.02
NW-F6Q2	8.71	173.00	213.00	0.65	9.49	16.36	13.17	43.94	35.33	42.66	344.16	438.70
NW-F6Q3	9.17	109.00	129.00	0.83	8.41	6.11	13.01	50.96	28.62	44.14	618.33	566.60
P-F1Q1	8.97	108.00	66.00	0.58	4.46	43.05	0.34	10.96	41.04	87.30	290.04	150.21
P-F1Q2	9.02	67.00	58.00	0.57	4.87	22.72	24.81	47.06	67.01	54.15	197.43	532.01
P-F1Q3	8.60	94.00	32.00	0.73	9.21	12.64	9.53	49.87	136.29	162.30	236.56	190.32
P-F2Q1	8.94	73.00	149.00	0.50	3.55	34.42	11.42	9.53	40.69	22.85	59.80	350.27
P-F2Q2	9.02	43.00	41.00	0.59	11.99	23.47	21.88	52.22	12.77	203.06	319.21	629.97
P-F2Q3	8.73	100.00	73.00	0.70	8.32	15.16	10.26	49.12	117.22	237.21	391.02	461.95
P-F3Q1	8.78	102.00	80.00	0.66	1.27	35.09	11.52	14.52	55.80	63.81	257.59	329.68
P-F3Q2	8.68	226.00	174.00	0.60	8.83	23.18	23.87	48.22	37.10	111.35	117.45	544.07
P-F3Q3	8.98	140.00	133.00	0.57	8.36	15.55	38.78	50.29	88.03	90.72	98.01	263.16
P-F4Q1	8.78	61.00	57.00	0.66	1.89	47.00	11.40	10.27	42.14	3.94	48.62	141.54
P-F4Q2	8.72	73.00	63.00	0.60	8.64	22.23	17.68	50.24	39.07	47.15	482.01	606.73
P-F4Q3	8.86	76.00	63.00	0.77	8.84	8.83	33.30	45.70	117.68	146.07	194.44	230.22
P-F5Q1	8.66	58.00	56.00	0.53	8.90	31.84	10.58	9.38	53.92	46.63	200.42	435.28
P-F5Q2	8.77	88.00	93.00	0.77	1.50	21.36	18.19	53.39	52.65	122.10	489.49	502.51
P-F5Q3	8.71	96.00	99.00	0.62	7.77	15.33	7.77	50.28	32.23	276.05	246.36	449.90

Appendix table 7 NDVI at factory /quadrat level

Station	Zone	1990	1995	2000	2005	2010	2015	2020
AB-F1	C	0.338787	0.382742	0.297059	0.229888	0.175292	0.160973	0.140235
AB-F10	C	0.212892	0.252695	0.255366	0.24592	0.217249	0.203834	0.215789
AB-F10-C	NC	0.433257	0.4999	0.516055	0.512443	0.423821	0.523533	0.513308
AB-F1-C	NC	0.354611	0.408789	0.471501	0.501764	0.467262	0.529415	0.546109
AB-F2-C	NC	0.365792	0.419838	0.490784	0.504207	0.46775	0.535695	0.541436
AB-F3-C	NC	0.449592	0.530636	0.563112	0.581283	0.528201	0.590805	0.593419
AB-F4-C	NC	0.369664	0.474889	0.515569	0.528632	0.473879	0.554045	0.575807
AB-F5-C	NC	0.3295	0.459489	0.511546	0.610753	0.531072	0.651858	0.667865
AB-F6-C	NC	0.240298	0.376998	0.417709	0.526262	0.451688	0.572113	0.596297
AB-F7-C	NC	0.245006	0.442769	0.42518	0.544253	0.511	0.629647	0.653164
AB-F8-C	NC	0.303815	0.469882	0.552256	0.541152	0.583491	0.679373	0.692477
AB-F9-C	NC	0.420193	0.499472	0.555955	0.572976	0.542868	0.657499	0.681676
BA-F1-C	NC	0.167474	0.167739	0.233798	0.359205	0.368431	0.473472	0.492633
BA-F2-C	NC	0.190566	0.208035	0.29088	0.407401	0.463395	0.490023	0.462606
BA-F3-C	NC	0.346474	0.239902	0.383004	0.434602	0.35003	0.445989	0.433764
BA-F4-C	NC	0.23804	0.252465	0.289755	0.356449	0.31715	0.382735	0.383021
BA-F5-C	NC	0.149374	0.134442	0.128316	0.147894	0.170799	0.203855	0.226473
BN-F1-C	NC	0.480339	0.482755	0.443715	0.476699	0.452981	0.530572	0.573246
BN-F2-C	NC	0.491295	0.532814	0.488219	0.505838	0.499385	0.592757	0.572399
BN-F3-C	NC	0.523841	0.560082	0.51041	0.525425	0.490323	0.581864	0.571158
BU-F1-C	NC	0.446398	0.355923	0.378022	0.385103	0.508825	0.585108	0.584541
BU-F2-C	NC	0.354205	0.355562	0.433479	0.472534	0.443237	0.596185	0.623046
BU-F3-C	NC	0.31938	0.322388	0.369454	0.381326	0.380664	0.528123	0.517365
BU-F4-C	NC	0.325867	0.487258	0.455777	0.522239	0.487783	0.657975	0.647348
BU-F5-C	NC	0.31183	0.364756	0.365148	0.401684	0.345846	0.516347	0.494135
BU-F6-C	NC	0.280708	0.331178	0.335195	0.37483	0.339497	0.482626	0.460147
BU-F7-C	NC	0.349917	0.385556	0.422784	0.460633	0.451918	0.600135	0.587866
BU-F8-C	NC	0.260506	0.272401	0.283627	0.316873	0.337514	0.455857	0.446488
CH-F1-C	NC	0.386093	0.385102	0.319991	0.395301	0.331947	0.435139	0.350273
CH-F2-C	NC	0.102181	0.126095	0.104835	0.123931	0.139779	0.195623	0.231104
CH-F3-C	NC	0.104694	0.119827	0.095727	0.124389	0.17637	0.308479	0.355298
CH-F4-C	NC	0.107867	0.117606	0.084818	0.115014	0.144054	0.220715	0.199314
CH-F5-C	NC	0.11391	0.141885	0.099504	0.163956	0.217502	0.313515	0.268318
CH-F6-C	NC	0.199613	0.218727	0.169309	0.203674	0.208006	0.317637	0.282799
CH-F7-C	NC	0.21775	0.225365	0.173554	0.193697	0.217874	0.300895	0.299027
Chi-F1-C	NC	0.424939	0.338122	0.402201	0.461548	0.534251	0.58994	0.585728
Chi-F2-C	NC	0.370807	0.191083	0.29564	0.393789	0.427821	0.531852	0.493532
DL-F1-C	NC	0.294101	0.330023	0.344357	0.372741	0.351403	0.46766	0.508021
DL-F2-C	NC	0.300944	0.297076	0.301096	0.359104	0.426187	0.537868	0.564609
DL-F3-C	NC	0.306024	0.300221	0.306864	0.348011	0.425496	0.546027	0.548802
DL-F4-C	NC	0.393463	0.379297	0.359523	0.393347	0.396105	0.524124	0.550165
DL-F5-C	NC	0.310746	0.327614	0.360084	0.40643	0.424098	0.507831	0.517194

HR-F1-C	NC	0.309579	0.347328	0.289951	0.276024	0.258719	0.428666	0.428415
HR-F2-C	NC	0.303879	0.344537	0.287163	0.287746	0.265503	0.413696	0.419826
KB-F1-C	NC	0.179784	0.220053	0.158863	0.247116	0.253155	0.346906	0.33802
KB-F2-C	NC	0.124556	0.147466	0.098024	0.141148	0.167673	0.238603	0.242422
KB-F3-C	NC	0.114842	0.140751	0.102361	0.140865	0.172757	0.245058	0.245431
KB-F4-C	NC	0.126094	0.152795	0.1513	0.209438	0.210686	0.317077	0.311201
KB-F5-C	NC	0.123764	0.148087	0.140991	0.202534	0.209689	0.317721	0.293925
KB-F6-C	NC	0.102729	0.118403	0.08675	0.112213	0.138504	0.201841	0.186104
KB-F7-C	NC	0.078232	0.101574	0.065771	0.083115	0.105315	0.154947	0.15899
KB-F8-C	NC	0.271276	0.263248	0.216177	0.276817	0.258221	0.315758	0.35838
KO-F1-C	NC	0.286005	0.398848	0.479818	0.541307	0.525025	0.666423	0.622712
KO-F2-C	NC	0.37116	0.352813	0.271029	0.334718	0.345593	0.507302	0.46646
MM-F1-C	NC	0.135133	0.170511	0.10797	0.153676	0.175822	0.213501	0.192241
MM-F2-C	NC	0.148716	0.187687	0.104543	0.149499	0.185333	0.20955	0.199858
MM-F3-C	NC	0.117005	0.155221	0.090051	0.120062	0.155212	0.195262	0.179824
MM-F4-C	NC	0.110996	0.253777	0.262904	0.264042	0.294955	0.372178	0.318343
MM-F5-C	NC	0.342509	0.333097	0.32947	0.467824	0.404222	0.49107	0.53743
MM-F6-C	NC	0.183437	0.217208	0.191121	0.273516	0.239605	0.294529	0.309165
MM-F7-C	NC	0.125078	0.131179	0.113331	0.155398	0.183485	0.262886	0.241369
MM-F8-C	NC	0.07062	0.08567	0.104947	0.134478	0.142235	0.21351	0.19653
Mn-F10-C	NC	0.426374	0.431595	0.452445	0.474306	0.463368	0.562919	0.551089
Mn-F1-C	NC	0.298764	0.4409	0.409199	0.478097	0.469008	0.601428	0.574698
Mn-F2-C	NC	0.315474	0.499968	0.439916	0.439098	0.469817	0.597116	0.600163
Mn-F3-C	NC	0.333588	0.382486	0.447397	0.402323	0.447353	0.514217	0.543551
Mn-F4-C	NC	0.320978	0.38737	0.438567	0.464041	0.442005	0.57266	0.580061
Mn-F5-C	NC	0.372743	0.459302	0.477777	0.473562	0.479296	0.57356	0.584385
Mn-F6-C	NC	0.329698	0.423381	0.475311	0.501546	0.472551	0.593177	0.598681
Mn-F7-C	NC	0.333835	0.411574	0.464327	0.45612	0.466346	0.572167	0.573561
Mn-F8-C	NC	0.330429	0.466712	0.474517	0.499366	0.486714	0.638628	0.608411
Mn-F9-C	NC	0.361877	0.401113	0.396362	0.431151	0.418408	0.490449	0.510893
MR-F1-C	NC	0.211257	0.210512	0.22023	0.240961	0.248002	0.381875	0.378196
MR-F2-C	NC	0.216595	0.242623	0.277642	0.280078	0.310443	0.488698	0.505915
MR-F3-C	NC	0.232876	0.271419	0.286916	0.292286	0.28875	0.418075	0.444375
MR-F4-C	NC	0.297454	0.318075	0.271691	0.292683	0.303424	0.452409	0.484407
MR-F5-C	NC	0.377687	0.386584	0.396336	0.436653	0.428577	0.584024	0.526883
MR-F6-C	NC	0.321379	0.4074	0.454767	0.522871	0.490851	0.652127	0.655986
MR-F7-C	NC	0.377958	0.428545	0.468556	0.532256	0.493068	0.649053	0.662977
MR-F8-C	NC	0.39655	0.43865	0.460228	0.532851	0.507947	0.686037	0.676788
NW-F1-C	NC	-0.08382	-0.02787	0.053061	-0.0252	0.168353	0.527167	0.55286
NW-F2-C	NC	0.305772	-0.09078	0.079925	0.01114	0.258491	0.468105	0.476154
NW-F3-C	NC	0.381434	0.365572	0.411686	0.482124	0.462522	0.653052	0.643566
NW-F4-C	NC	0.359432	0.353934	0.375129	0.46708	0.450219	0.604297	0.647168
NW-F5-C	NC	0.293807	0.321066	0.329999	0.35979	0.360323	0.544314	0.579805
NW-F6-C	NC	0.342484	0.350807	0.320112	0.422699	0.406333	0.597834	0.63165

P-F1-C	NC	0.298803	0.221455	0.257636	0.391487	0.361097	0.509145	0.530356
P-F2-C	NC	0.328179	0.196617	0.199386	0.323479	0.278787	0.425312	0.429036
P-F3-C	NC	0.320532	0.33605	0.350101	0.399064	0.320305	0.443843	0.499578
P-F4-C	NC	0.308297	0.202051	0.19802	0.364862	0.30747	0.451723	0.457468
P-F5-C	NC	0.30867	0.195073	0.193097	0.333178	0.297495	0.447973	0.446272
SA-F10-C	NC	0.216835	0.241344	0.215921	0.224869	0.22833	0.357347	0.409356
SA-F1-C	NC	0.311568	0.330223	0.265226	0.29541	0.23395	0.347283	0.388101
SA-F2-C	NC	0.224268	0.237312	0.228134	0.229305	0.205597	0.312313	0.38722
SA-F3-C	NC	0.350412	0.342369	0.337854	0.368052	0.223758	0.376129	0.404978
SA-F4-C	NC	0.323884	0.260732	0.221284	0.254545	0.204942	0.292672	0.277586
SA-F5-C	NC	0.417023	0.388589	0.380043	0.399995	0.348492	0.415063	0.408918
SA-F6-C	NC	0.425724	0.408117	0.38441	0.403826	0.358526	0.403735	0.411713
SA-F7-C	NC	0.256171	0.31493	0.243944	0.290657	0.313487	0.440335	0.408247
SA-F8-C	NC	0.271352	0.317321	0.264539	0.31019	0.335668	0.457627	0.444508
SA-F9-C	NC	0.248265	0.267885	0.233083	0.258342	0.253905	0.389309	0.439883
ST-F1-C	NC	0.251134	0.282341	0.330379	0.388933	0.4248	0.530824	0.539638
ST-F2-C	NC	0.310159	0.375571	0.394268	0.426938	0.438082	0.56137	0.663996
ST-F3-C	NC	0.459501	0.463544	0.4782	0.481556	0.458667	0.516204	0.526127
ST-F4-C	NC	0.393052	0.431977	0.447736	0.484123	0.498085	0.578908	0.636467
ST-F5-C	NC	0.475326	0.446257	0.481215	0.510751	0.359643	0.493237	0.52598
ST-F6-C	NC	0.451376	0.457593	0.500356	0.526563	0.329957	0.465376	0.536716
ST-F7-C	NC	0.45888	0.431387	0.470306	0.487224	0.3844	0.484563	0.482147
ST-F8-C	NC	0.438631	0.397236	0.477586	0.493799	0.35471	0.515186	0.593872
AB-F2	C	0.352887	0.413688	0.339959	0.283316	0.21818	0.19171	0.165046
AB-F3	C	0.301032	0.374709	0.272503	0.233174	0.170524	0.179918	0.161666
AB-F4	C	0.281305	0.320532	0.294648	0.277426	0.219239	0.191495	0.175121
AB-F5	C	0.287013	0.30405	0.226803	0.174709	0.138803	0.149182	0.132056
AB-F6	C	0.300846	0.268868	0.274936	0.262006	0.197301	0.213762	0.228569
AB-F7	C	0.205456	0.215855	0.193225	0.19535	0.139994	0.142614	0.115832
AB-F8	C	0.290755	0.267316	0.262274	0.257073	0.208886	0.235438	0.269608
AB-F9	C	0.212061	0.250619	0.236953	0.222815	0.18689	0.182622	0.182871
BA-F1	C	0.259729	0.243319	0.329915	0.295493	0.222646	0.285993	0.25105
BA-F2	C	0.193649	0.190637	0.301899	0.233927	0.195402	0.287406	0.269446
BA-F3	C	0.310332	0.248043	0.381748	0.338507	0.295502	0.362023	0.352391
BA-F4	C	0.156086	0.159491	0.180193	0.150895	0.154648	0.224577	0.224126
BA-F5	C	0.137953	0.142345	0.14499	0.155459	0.163077	0.21275	0.220841
BN-F1	C	0.224193	0.240489	0.207322	0.210215	0.216178	0.192075	0.155168
BN-F2	C	0.197147	0.22044	0.172552	0.191699	0.204661	0.194119	0.171287
BN-F3	C	0.20626	0.210872	0.160137	0.18474	0.179061	0.192811	0.182181
BU-F1	C	0.377646	0.304289	0.339286	0.390295	0.319988	0.420805	0.410499
BU-F2	C	0.428634	0.350524	0.356537	0.322702	0.287266	0.35597	0.382838
BU-F3	C	0.429203	0.319747	0.391163	0.331568	0.297874	0.387835	0.387817
BU-F4	C	0.378544	0.345004	0.391943	0.404975	0.346087	0.468962	0.470666
BU-F5	C	0.27501	0.375258	0.344793	0.291938	0.288348	0.387645	0.371319

BU-F6	C	0.302223	0.290417	0.332082	0.36461	0.330703	0.409747	0.395064
BU-F7	C	0.297603	0.27183	0.348457	0.346508	0.360729	0.439547	0.409378
BU-F8	C	0.176089	0.16415	0.168905	0.158916	0.155205	0.24556	0.243258
CH-F1	C	0.413075	0.361222	0.403014	0.312484	0.215848	0.263788	0.192608
CH-F2	C	0.368456	0.350585	0.374893	0.300888	0.185503	0.210887	0.148895
CH-F3	C	0.402814	0.385656	0.403075	0.371423	0.174247	0.24895	0.174713
CH-F4	C	0.418676	0.396007	0.406811	0.350169	0.217125	0.278456	0.194332
CH-F5	C	0.444248	0.402431	0.44163	0.300896	0.210846	0.278513	0.199116
CH-F6	C	0.463559	0.438255	0.395197	0.365683	0.302863	0.257342	0.157177
CH-F7	C	0.408626	0.362242	0.393947	0.306736	0.234826	0.325094	0.241244
Chi-F1	C	0.054364	0.036037	0.041956	0.08687	0.073733	0.086906	0.101943
Chi-F2	C	0.03166	0.047312	0.046365	0.101676	0.077839	0.084402	0.093202
Chi-F3	C	0.111321	0.152052	0.159329	0.254004	0.217494	0.287116	0.301181
DL-F1	C	0.437507	0.356226	0.38973	0.364071	0.357551	0.483112	0.429107
DL-F2	C	0.331675	0.324161	0.296182	0.2835	0.267731	0.336828	0.267339
DL-F3	C	0.399619	0.360941	0.346776	0.333396	0.30896	0.313625	0.340208
DL-F4	C	0.472075	0.388356	0.407902	0.325469	0.167291	0.16053	0.186996
DL-F5	C	0.452411	0.348529	0.456562	0.321043	0.225273	0.155764	0.135888
HR-F1	C	0.347161	0.380172	0.346342	0.31142	0.285846	0.413563	0.334596
HR-F2	C	0.274597	0.308471	0.28103	0.271909	0.248771	0.367712	0.34016
KB-F1	C	0.25782	0.272062	0.213637	0.240036	0.229382	0.299579	0.292583
KB-F2	C	0.312595	0.332099	0.257879	0.296522	0.140147	0.132482	0.121509
KB-F3	C	0.312155	0.322744	0.341089	0.429181	0.191049	0.090793	0.13822
KB-F4	C	0.35913	0.346506	0.240887	0.260377	0.193905	0.244414	0.21552
KB-F5	C	0.130951	0.151419	0.138339	0.162861	0.212193	0.282992	0.270832
KB-F6	C	0.085373	0.11889	0.081274	0.11285	0.119254	0.214005	0.222184
KB-F7	C	0.06356	0.080272	0.062784	0.074388	0.115408	0.16744	0.158149
KB-F8	C	0.285856	0.286084	0.299853	0.335409	0.308026	0.454241	0.435583
KO-F1	C	0.351132	0.338561	0.295908	0.29226	0.287903	0.361828	0.289851
KO-F2	C	0.348228	0.351356	0.306489	0.298814	0.299425	0.333696	0.314219
KO-F3	C	0.329816	0.350723	0.330246	0.309759	0.297197	0.343153	0.290662
MM-F1	C	0.33879	0.313414	0.368157	0.3508	0.338969	0.403817	0.316671
MM-F2	C	0.270775	0.29458	0.325281	0.360409	0.334686	0.356598	0.280242
MM-F3	C	0.173609	0.16788	0.170712	0.189566	0.203299	0.321707	0.304079
MM-F4	C	0.40132	0.457633	0.394419	0.494353	0.409621	0.449224	0.374331
MM-F5	C	0.303944	0.362066	0.242026	0.189416	0.163142	0.212716	0.176758
MM-F6	C	0.240233	0.290088	0.230635	0.187026	0.197749	0.296582	0.185143
MM-F7	C	0.137639	0.123637	0.193732	0.195228	0.19176	0.35251	0.381687
MM-F8	C	0.141181	0.114597	0.117825	0.130845	0.110443	0.239878	0.261643
MN-F1	C	0.235029	0.246159	0.26155	0.257014	0.218078	0.263914	0.260914
MN-F10	C	0.310992	0.33362	0.390827	0.398502	0.262614	0.278732	0.250507
MN-F2	C	0.370716	0.354348	0.375218	0.37068	0.348016	0.411179	0.37747
MN-F3	C	0.371211	0.359175	0.397729	0.395873	0.351226	0.417426	0.361256
MN-F4	C	0.328473	0.303016	0.348873	0.346606	0.318392	0.376951	0.31719

MN-F5	C	0.314151	0.304077	0.303519	0.308478	0.27589	0.263245	0.204167
MN-F6	C	0.422333	0.354484	0.398928	0.388232	0.372032	0.438903	0.407085
MN-F7	C	0.330944	0.326662	0.350369	0.351283	0.257435	0.323488	0.290938
MN-F8	C	0.36303	0.349786	0.35111	0.317067	0.256079	0.289729	0.253678
MN-F9	C	0.216063	0.235817	0.249506	0.243736	0.195499	0.240988	0.23297
MR-F1	C	0.147735	0.19064	0.165353	0.196627	0.162837	0.194213	0.160906
MR-F2	C	0.148166	0.189905	0.147506	0.181767	0.156084	0.155842	0.126828
MR-F3	C	0.163199	0.186634	0.136859	0.153142	0.14473	0.163239	0.126296
MR-F4	C	0.238939	0.254449	0.222596	0.278734	0.198402	0.228838	0.184622
MR-F5	C	0.220281	0.247823	0.183782	0.21969	0.179812	0.165629	0.129329
MR-F6	C	0.2602	0.237172	0.220202	0.303386	0.239936	0.329188	0.283842
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MR-F8	C	0.225048	0.251832	0.244429	0.289198	0.250625	0.345984	0.242534
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P-F3	C	0.372522	0.368598	0.365835	0.399894	0.2978	0.423573	0.30549
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SA-F3	C	0.235009	0.222023	0.24643	0.281458	0.209719	0.329283	0.263167
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ST-F4	C	0.377079	0.359426	0.360888	0.373741	0.356333	0.386246	0.412658
ST-F5	C	0.424744	0.40286	0.39796	0.340604	0.245809	0.333742	0.322782
ST-F6	C	0.396443	0.359715	0.417447	0.351838	0.190676	0.236691	0.292105
ST-F7	C	0.414776	0.406465	0.401165	0.336209	0.234171	0.285696	0.2792
ST-F8	C	0.397443	0.408077	0.432695	0.365107	0.225664	0.25809	0.261162
Grand Total		0.298611	0.295304	0.298055	0.323443	0.291595	0.379017	0.371198

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Research Paper

Politics of the natural vegetation to balance the hazardous level of elements in marble polluted ecosystem through phytoremediation and physiological responses

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ABSTRACT

The current paper evaluates the phytoremediation ability and physiological responses of selected resistant plant species to the hazardous levels of elements in the marble waste polluted ecosystem. Preliminary results demonstrate that all the indicator/resistant plant species i.e., *Ailanthus altissima, Arundo donax, Cynodon dactylon, Erigeron canadensis, Cannabis sativa, Ficus carica, Lathyrus aphaca, Morus alba, Populus alba, Robinia pseudoacacia* and *Vitex negundo* were the best Phyto-extractors and Phyto-stabilizers for most of the heavy metals in general and Mg, Ca, Fe, Cu and Na in particular (at $p < 0.05$). Structural Equation Modeling confirmed that marble waste pollution has a direct and significant ($R^2 = 0.80$) impact on proline synthesis and hence a role in combating the pollution. Chlorophyll content decreased by 4% in studied plant species when the concentration of pollutants increased. It is concluded that the studied bio-indicators - the abundant plant species of the Marble Waste Polluted Systems (MWPS) have a significant role in its remediation. Increasing proline accumulation and decreasing chlorophyll contents with an increase in pollution in the studied plants show resilience of the ecosystem in response to the external lithospheric toxicities. It is recommended that the recognized plant species could be planted abundantly to remediate the MWPS around the marble processing and other such industries and their catchments.

1. Introduction

Lithosphere pollution has been one of the basic detrimental sources of biosphere pollution affects at landscape as well as species level. An increase in human population coupled with rapid industrialization has worsened pollution problems (Hu et al., 2020; Lai et al., 2018; Lin et al., 2019; Yuan et al., 2020). One example of such industrial pollution is the extraction of marbles and their processing. Marbles consist of recrystallized carbonate minerals i.e., Calcite and Dolomite (Kechagia et al., 2020; Tozsin et al., 2011), which generally give rise to oxides of Ca, Mg, Fe, Si, Ti, P, Al and Na etc. (Knezlie et al., 1995). Mining, processing and polishing of marbles results in about 70% waste materials (Gazi et al., 2012). The quarrying operation also causes approximately 40% of marble waste, mostly in the form of rock fragments. Such wastes are discarded in empty pits, roads, agricultural lands and rivers that lead to sedimentation and a wide range of lithospheric, hydrospheric and atmospheric pollution (Aukour and Al-Qinna, 2008; Gamma et al., 2017; Liu and Tsai, 2009; Mondovi et al., 2014). Huge amounts of water are used during marble processing, which directly affects all sorts of water channels. Lack of proper treatments has negative impacts on habitats and aquatic flora and fauna, in particular. Marble wastewater adds heavy metals to neighboring ecosystems due to the lack of primary treatments and detoxification procedures (El-Moghazy et al., 2013) and consequently levels of pollutants increase in water and soil that in turn adversely affect the natural biotic and abiotic environments. This affects the flora and fauna composition through physical and chemical alterations in the environment (Yu et al., 2005).

Environmental deterioration is not only dangerous for flora and fauna; it affects humans both directly and indirectly as well. To the contrary, some organisms, especially plants, can survive in such toxic conditions (Navarro-Cano et al., 2018). Such organisms can be identified as pollution indicators, phytoremediators, phytostabilizers and phytoextractors of these special habitats or biomes (Freeman et al., 2004; Li

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Pollution indicandum and marble waste polluted ecosystem; role of selected indicator plants in phytoremediation and determination of pollution zones

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ABSTRACT

The absence or presence of particular plant species indicates specific level of pollution. It was hypothesized that the marble waste polluted ecosystem also host specific plant indicators that can grow, survive and tolerate more successfully than others and can be utilized in better managements of such systems. The current research work was therefore, conducted to determine the indicators of marble polluted region of Buner, Pakistan. Ecological techniques using varying sized quadrats i.e., 1×1 m^2, 5×2 m^2 and 10×2 m^2 for herbs, shrubs and tree species were used respectively. Standard protocols were used to prepare soil samples and plant parts (root, leaf and shoot) for Atomic Absorption Spectrophotometry. Indicator i.e., *Populus alba* L., *Arundo donax* L., *Erigeron canadensis* L and *Morus alba* L. were identified via Indicator Species Analyses (ISA) in various polluted zones. All the collected data were put in MS Excel for analyses in PCORD through Cluster Analysis (CA), Two Way Cluster Analysis (TWCA) and ISA. CANOCO software was used to examine the impact of miscellaneous environmental variables in zonation via both direct and indirect gradient techniques i.e., Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (CCA). A total of 102 plant species belonging to 95 genera and 48 families were recorded in the marble waste polluted ecosystem. CA and TWCA through Jaccard Distance measurements and Wards Linkage methods gave rise to 3 major polluted zones. These zones were i) Heavily Polluted Zone (HPZ), ii) Moderate Polluted Zone (MPZ) and iii) Less Polluted Zone (LPZ). The recorded values of Biological Concentration Factor (BCF), Translocation Factor (TF) and Bioaccumulation Coefficient (BAC) for analyzed heavy metals i.e. Fe, Mg, Ca, Na and Cu in root and shoot of *P. alba* L., *A. donax* L., and *M. alba* L. showed that these species were significant phytostabilizers and *E. canadensis* L. was phytoextracters and hence best indicandum of marble waste polluted ecosystems. It was concluded that among all the measured environmental variables, higher phosphorus level, higher pH, moderate potassium and lowest electrical conductivity (EC) had significant effects ($p < 0.05$) on the functions of these indicators as phytoremediaters. It is recommended to develop green belts of these indicators around the marble industrial areas for the better management and hazardous free environment.

1. Introduction

Environmental pollution is a substantial issue of the modern world, causing huge damages to the natural ecosystems. Approximately, 2.01 billion metric tons waste per annum is produced worldwide (Bilgen et al.,2008). According to the World Bank's estimates, waste generation will increase up to 3.4 billion metric tons by 2050. Only 13.5% of the today's waste is recycled and 5.5% is

Abbreviations: CA, Cluster Analysis; ISA, Indicator Species Analysis; TWCA, Two Way Cluster Analysis; DCA, Detrended Correspondence Analysis; CCA, Canonical Correspondence Analysis; TF, Translocation Factor; BAC, Bioaccumulation coefficient; BCF, Biological Concentration Factor; KP, Khyber Pakhtunkhwa; EC, Electrical Conductivity; ST, Soil Texture; OM, Organic Matter; K, Potassium; P, Phosphorus.

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Plants predict the mineral mines – A methodological approach to use indicator plant species for the discovery of mining sites

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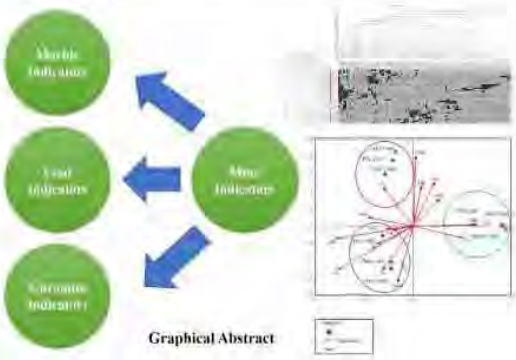
HIGHLIGHTS

- Plant species predict presence of specific mineral reserves.
- These plants can be used as indicators for economically important mineral reserves.
- Indicator Species and modelling approaches were used for indicators of mineral mines.
- Coal indicators were Olea ferruginea, Gymnosporia royleana and few more.
- These approaches could potentially be applied for exploration of mineral reserves.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: There has been limited research conducted on the identifications/methodological approaches of using plant species as indicators of the presence of economically, important mineral resources.
Objectives: This study set out to answer the following questions (1) Do specific plant species and species assemblages indicate the presence of mineral deposits? and (2) if yes, then what sort of ecological, experimental, and statistical procedures could be employed to identify such indicators?
Methods: Keeping in mind these questions, the vegetation of subtropical mineral mines sites in northern Pakistan were evaluated using Indicator Species Analysis (ISA), Canonical Correspondence Analysis (CCA) and Structural Equation Modeling (SEM).
Results: A total of 105 plant species belonging to 95 genera and 43 families were recorded from the three mining regions. CA and TWCA classified all the stations and plants into three major mining zones, corresponding to the presence of marble, coal, and chromite, based on Jaccard distance and Ward's linkage

Abbreviations: ISA, Indicator Species Analysis; CCA, Canonical Correspondence Analysis; SEM, Structural Equation Model; KPK, Khyber Pakhtunkhwa; CA, Cluster Analysis; TWCA, Two-way Cluster Analysis; EC, electrical conductivity; TDS, total dissolved solids; K, potassium; P, phosphorus; Mn, Manganese; Ni, Nickle; Cu, Copper; Cd, Cadmium; Fe, Iron; Cr, Chromium; Co, Cobalt; Na, Sodium; Mg, Magnesium; Ca, Calcium.

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1

Microbial Flora of Marble Waste-Polluted Environment in the Phylogenetic Perspectives

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Quaid-i-Azam University

CONTENTS

1.1 Introduction 1
1.1.1 Isolation and Characterization of Microbes 2
1.1.2 Marble Waste-Polluted System 3
1.1.3 Introduction to Study Area 4
1.1.4 Justification 5
1.2 Materials and Methods 5
1.2.1 Collection of Samples 5
1.2.2 Isolation, Morphological and Microscopic Identification of Fungal Flora 5
1.2.2.1 Molecular and Phylogenetic Analyses 6
1.2.3 Isolation, Morphological and Microscopic Identification of Bacterial Flora 6
1.3 Results 7
1.3.1 Characterization and Phylogenetic Studies of Fungal Flora 7
1.3.1.1 Phylogenetic Analysis of the Selected Microfungi 8
1.3.2 Characterization of Bacterial Strains 9
1.3.2.1 Gram Staining Microscopic Characterization 9
1.3.2.2 Biochemical Characteristics 10
1.4 Discussion 15
1.5 Conclusion 17
Acknowledgments 17
References 17

1.1 Introduction

Microbial flora is one of the most diverse and abundant forms of life on the earth (Dawson et al. 2017; Lu et al. 2013; Reshi et al. 2015). Microbes are present in every type of natural habitat, i.e., water, soil, air, hot springs, rocks, deep in the ocean and up to high in the atmosphere (Brock 2012; Sterflinger et al. 2012). According to a study, 10 million microorganisms were present in 1 mm of ocean water and 40 million in 1 g of soil (Richardson and Jones 1958). Microbes make up 10% of human dry weight. Microbes are the first living organisms on the earth's surface. Majority of them are beneficial. They keep natural ecosystems clean by removing toxins from soil, water, and degraded organic remnants of animals and plants. Microbes are crucial in the nutrient recycling of an ecosystem as they act as decomposers. They are also used as an indicator of landmines and build radioactive metals inert. Microorganisms help in the digestion process and prevent humans from harmful effects. In short, one can say that life could not be possible without microbes. Biogeographic patterns of an ecosystem provide evidences of

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