Evaluation of the growth modulating effect of the River Chenab sediments on *Brassica napus* L., Pakistan

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BY

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DEPARTMENT OF ENVIRONMENTAL SCIENCES,

FACULTY OF BIOLOGICAL SCIENCES,

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APPROVAL CERTIFICATE

This is to certify that the dissertation entitled **"Evaluation of the growth modulating effect of the River Chenab sediments on *Brassica napus* L., Pakistan."** submitted by **Attarad Ali** is accepted in its present form by the Department of Environmental Sciences, Quaid-i-Azam University Islamabad, Pakistan, as satisfying the dissertation requirement for the degree of M.Phil in Environmental Phytotoxicity

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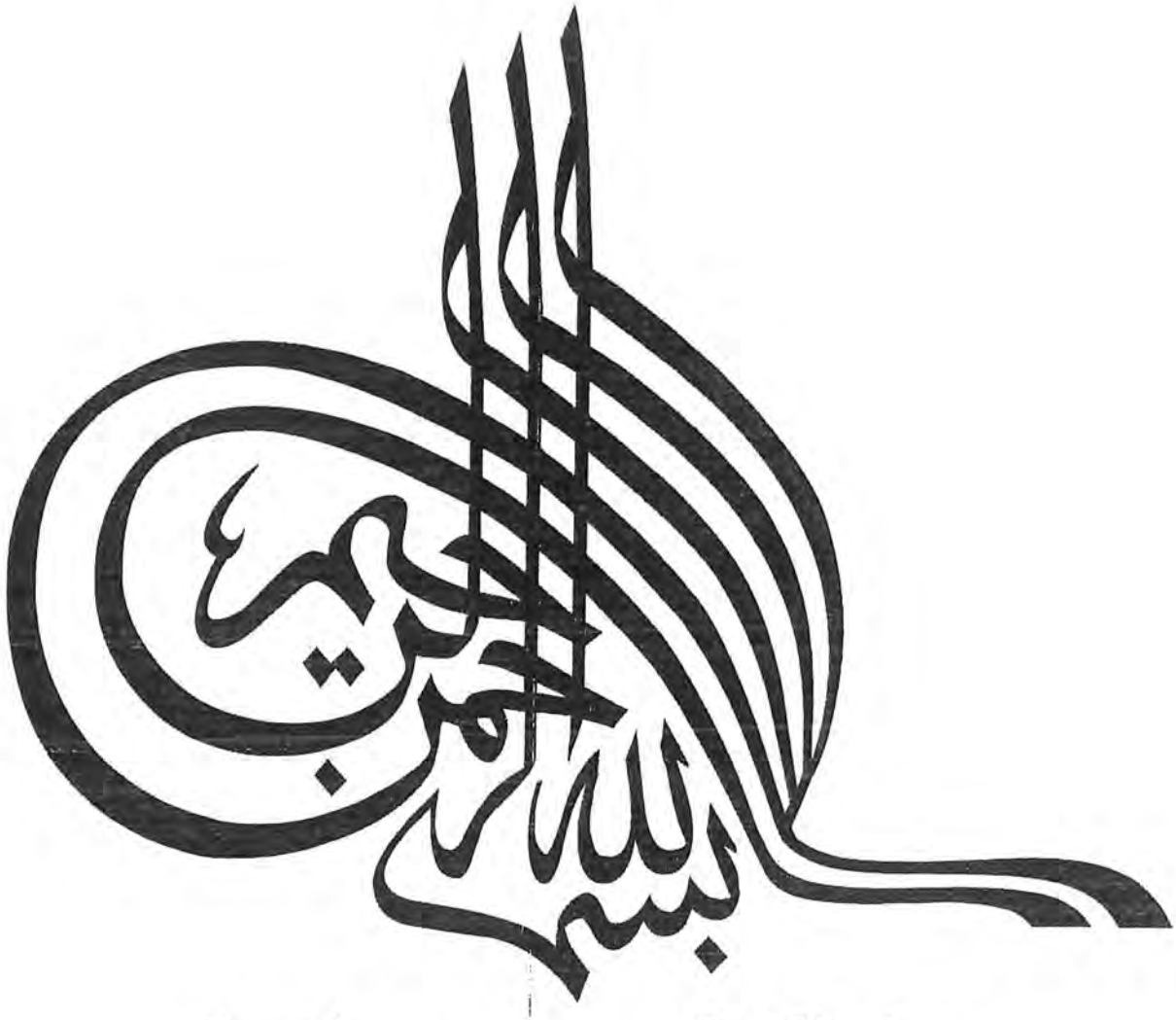

In the name of Allah,
the Most Beneficent,
the Most Merciful

Dedicated to

My Loving Mother

And

Father Late Haji Shaukat Ali

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Attarad Ali Atti~

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LIST OF ABBREVIATIONS

°C	Degree centigrade
µg	Microgram
µL	Micro liter
mg	Milligram
mg/mL	Milligram per milliliter
nM/min/mg	Nano mole per minute per milligram
U/g	Unit per gram
%	Percentage
% DPPH	Percent 2,2 diphenyl 1 picryl hydrazil
% RLI	Percent root length inhibition
% SLI	Percent shoot length inhibition
% ISL	Percent inhibition in seedling length
% WtL	Percent weight loss
µM/g	Micro mole per gram
AAE	Ascorbic acid equivalent
BSAE	Bovine serum albumen equivalent
D.H_2O	Distilled water
DW	Dry weight
DDT	Dichlorodiphenyltrichloroethane
FG%	Final germination percentage
FW	Fresh weight
GAE	Gallic acid equivalent
GI	Germination index
g	Gram
HCH	hexachlorocyclohexane
HCl	Hydro chloric acid
MDA	Malondialdehyde
MeOH	Methanol
MPFG	Mean period of final germination
OCPs	Organochlorine Pesticides

PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
pH	Hydrogen Potential
PISG	Percent inhibition in seed germination
PSVs	Protein storage vacuoles
POPs	Persistent Organic Pollutants
QE	Quercetin equivalent
RDW %	Relative dry weight percentage
RI	Relative induction
RG	Rate of germination
RWC	Relative water contents
RPA	Reducing power assay
S	Sample
SD	Standard deviation
SL	Shoot length
SOD	Superoxide dismutase
STS	Standard target species
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TFC	Total flavonoid contents
T-Chl	Total chlorophyll
TPC	Total phenolics contents
TPC	Total protein content
VI	Vigor index
WC	Water content
w/v	Weight per volume
Wt.	Weight

Abstract

This research was conducted to assess the effects of river Chenab sediments on growth modulation of a dicotyledonous plant. For this purpose 57 sediment samples collected from 25 selected sites of river Chenab were evaluated by using *Brassica napus* L. as a model plant. The physiological effects were determined by observing its different parameters. The process of germination was considerably delayed and growth was significantly inhibited by the samples obtained from various polluted sites. More than 80% growth inhibition was exhibited by seedlings of 49 samples. Seedlings of 37 samples showed above 30% weight loss. Relative dry weight (RDW) was decreased more than 50% in seedlings of 46 samples. Seedlings of all samples showed higher water contents (WC) while the seedlings of 39 samples showed higher relative water contents (RWC) as compared to the control. A set of multi-mode *in-vitro* assays were utilized to evaluate the changes in biochemical contents of target plant. Results showed the reduction of phenolics content in seedlings of 49 samples. The seedlings grown in all samples showed higher flavonoids content. Seedlings of 43 samples showed higher chlorophyll "a" content while the Seedlings of 27 samples showed higher chlorophyll "b" content. Seedlings of 41 samples showed total chlorophyll content higher than control. Seedlings of 45 samples showed the higher values of chlorophyll a ratio b as compared to control. Seedlings of 53 samples showed increase in carotenoids content. Seedlings of 52 samples showed increase in MDA content. Seedlings of 50 samples exhibited lower DPPH free radical scavenging potential. Seedlings of 44 samples exhibited decrease in total antioxidant capacity (TAC). Seedlings of 35 samples showed increase in reducing power. Seedlings of 32 samples showed increased total protein content and the remaining seedlings of 25 samples showed reduction in total protein content. Seedlings of 38 samples showed reduction in protease activity. Seedlings of 54 samples showed reduction in peroxidase (POD) activity while the seedlings of all 57 samples showed relative induction in their SOD activity as compared to the control. Overall, it was concluded that seedlings grown in samples of highly polluted urban and industrial sites showed negative effects on morphological, physiological and biochemical activities of target plant as compared to the samples taken from less contaminated, agricultural and rural areas.

Chapter No.1

Introduction and Literature Review

The dilemma of dreadful conditions of environment involving malicious changes in physical, chemical and biological characteristics of air, water and soil is one of the imperative matters for the world that have an effect on the lives of all organisms (Al-Othman *et al.*, 2012). Presently, the chemical pollution is considered as one of the greatest threat to living beings in the terrestrial environment (Carter *et al.* 1998). It affects agriculture, livestock production, their quality and ultimately human populations (Larson, 1989). The terrestrial risk assessment can be divided into ecological risk assessment (human resource) and human health risk assessment (Tarazona and Vega, 2002). The prime importance of the study of environmental risk assessment is the conservation of water availability system (Maxam *et al.*, 2000). Structural and functional evaluations, such as ecotoxicological bioassays involving plants, are performed to find the contamination of soil with toxic pollutants (CSTEE, 2000). Thus Bio-assessment can be used as a tool to evaluate the effects of mixtures, their mutual effects which can be expressed as antagonism, additivity and synergism. It can also be used for the analysis of the bioavailability of contaminants to different plant species (Carter *et al.* 1998). Varieties of organic, inorganic, gaseous and liquid substances are present in soil, which act as sink for various pollutants (Chan *et al.*, 2001). Higher concentrations of toxicants are present in crops which are grown on polluted soil (Dowdy and Larson, 1975). Plant bioassays used with natural soils have been derived from test procedures for evaluating the effects of single chemical in defined media (Karaguzel *et al.*, 2004). When samples of soil are to be tested for their quality or contamination, bioassays focus on survival of early seedling and plant growth (Gong *et al.*, 2001). However, the results found in such bioassays are due to biochemical changes after exposure to different chemicals (Karaguzel *et al.*, 2004). The stressful chemicals increase the generation of reactive oxygen species (ROS) within the cells (Blokhina *et al.*, 2003). ROS can cause peroxidation of membrane lipids, damage nucleic acids, amino acids and proteins (Stoeva and Bineva, 2003). Therefore, to prevent the oxidative damage of plant cells, it is necessary to scavenge the ROS. Partially, the enzymatic protection is carried out by peroxidase and catalase to degrade the ROS (Santos *et al.*, 2001). Adverse environmental conditions and the resulting production of ROS enhance gene expressions which are linked with activation or synthesis of enzymes involved in detoxification and removal of such toxic products (Prasad *et al.*, 1999). Hence the alteration in several enzymatic

activities and biochemicals may be observed, which are involved in the elimination and scavenging of ROS (Prasad *et al.*, 1999).

In this study, the quality or toxicity of sediments from river Chenab of Pakistan was evaluated by using plant bioassays with rapid cycle *Brassica napus* L. (Gong *et al.*, 2001). Series of *invitro* tests including determination of seed germination and growth tests (Debus and Hund 1997; Gong *et al.*, 2001) were performed to monitor the toxicity of contaminated sediments. Additionally, enzymatic bioassays for measuring biochemical adaptations and responses were carried out under controlled conditions (Milosevic *et al.*, 2010).

2.1. River Chenab

The major river of most densely populated province (Punjab) of Pakistan that fulfils the water necessities of regional, domestic, agricultural and industrial purposes is Chenab. (Bhatti and Latif, 2009). The current study area is very important as agriculture is the major source according to the country's per capita income, productivity basis and harvesting pattern in neighboring areas during Rabi and Kharif seasons. Cotton, sugarcane, rice, millet and maize are Kharif crops while wheat, mustered, toria and gram are the important Rabi crops. Fruits which are mainly grown in these areas are date palm, guava, mango, orange and lemon, while the leading vegetables are onions, chilies, turnips, brinjals, potatoes and lady fingers etc. For better quality and improvement in yielding crops, pesticides application is a common practice among the local farmers that leads to contaminate water throughout surface runoff from various non-point sources. Chenab keeps great importance in the economy, as the major crops of Pakistan like rice, wheat, cotton and sugarcane etc are irrigated by the river Chenab's water (Eqani *et al.*, 2012).

Due to several factors, Chenab's water quality has been degraded exhaustively, e.g. reduction of water inflow due to dams construction, effluents emancipation from industries, pesticides and fertilizers totaling through run-off from neighboring agricultural fields. This has threatened the aquatic resources and welfare of human beings to a great extent (Eqani *et al.*, 2012). River Chenab flows through the industrial cities and worldwide known for cotton and rice growing areas in Pakistan. The major cities linked with Chenab are Multan, Jhang, Gujranwala, Gujrat and Sialkot. Globally renowned habitats of fishes, amphibian species and migratory birds are also found in Chenab River. Chenab is also being polluted with heavy effluents from municipal and industrial sides exclusive of prior treatment through various

streams, drains together with river Jhelum and Ravi. In the previous study, the contamination status of persistent organic pollutants (POPs), poly-chlorinated biphenyls (PCBs), Organo-chlorine pesticides (OCPs) and polycyclic-aromatic hydrocarbons (PAHs) etc was evaluated in the sediments of Chenab. It was an important study on these pollutants in riverine sediments to assess probable pollutant sources and expected threats for the ecosystem of Chenab (Eqani *et al.*, 2011; Eqani *et al.*, 2012). It has also highlighted that, on daily basis, industrial and municipal effluents prior to treatment from seven districts of Punjab are being thrown into the Chenab River right through the fifteen point sources (1 each in Hafizabad and Sargodha districts, 2 in Chinyot, 2 in Jhang, 2 in Mandi Bahauddin, 3 in Multan and 4 in Gujrat district) posing grave risks to array of purposes like drinking, irrigation and nourishing riverine ecosystem. The Chenab's catchment area is also thought to be contaminated by adding plenty of dichloro-diphenyl-trichloroethane (DDTs) concentration discharging from pesticides manufacturing factories (Eqani *et al.*, 2012). In the present study *in-vitro* techniques are utilized to prove the correlation between the chemical contamination of water and their growth modulating effects on plant health.

2.2. Sediments

Sediments are the deposits of particulate substances having different form, size and composition. These can be soil originated and or transported by wind and runoff, fluvial processes, or decomposed living matter found at the bottom of aquatic environment (Bettinetti *et al.*, 2003). Sediments have an important function of buffering the concentrations of pollutants particularly by adsorption or precipitation. Surface sediments are specific elements of the natural environment, which act as natural sponges that adsorb all kinds of pollutants occurring in water. The chemical composition of sediments is much diversified as the different pollutants are added up along the length of river (Eqani *et al.*, 2012). Broadly the sediment can be classified on the basis of general characters of the pollutants, which are as under;

General groups	Sub-classes	References
1. Polycyclic aromatic hydrocarbons (PAHs)	-Naphthalene -Anthracene -Pyrene	Menzie *et al.*, 1992

General groups	Sub-classes	References
2. Polychlorinated Biphenyls (PCBs)	-Lower chlorinated (di-, tri- and tetra-chlorinated),	-Bano *et al.*, 1991
	-Moderately chlorinated (penta-, hexa- and hepta-chlorinated)	-Moysich *et al.*, 1999
	-Higher chlorinated (octa- and nona-chlorinated)	
3. Phthalates	Dimethyl-phthalate	Jacobs *et al.*, 1993
4. Organo Chlorinated Compounds	Penta-chlorophenol	Jabbar *et al.*, 1993
5. Metals	Mercury	-Bindler *et al.*, 2001
	Arsenic	-Long *et al.*, 1995
	Copper	-Saraee *et al.*, 2011

3.1.1. Riverine sediment and pollution

Riverine sediments are formed as a result of weathering processes and are considered as major carrier for impurities and pollutants (Horsfall and Spiff, 2002). Although, the anthropogenic and lithogenic sources are not only responsible, rather the organic matter contents, textural characteristics, mineralogical composition and depositional environment of the sediments are equally contributing to pollute the sediments (Lapaquellerie *et al.*, 1995). Through the processes of bioaccumulation and biomagnification, contaminated sediments may directly affect the aquatic media (consumed by plants and consequently diffused into the food chain) causing potential risk to human health (Larson, 1989). A variety of persistent bioaccumaulative toxins are present in the released contaminants like fossil fuels, metals, phenols, pesticides, phthalates, dioxins and furans (Keddy *et al.*, 1995).

The role of sediment is both sheltering for various organisms and are long-lasting source of pollutants showing variability in time and space which is the dynamic, essential and integral part of the river basins (Lopez *et al.*, 2005). For the estimation of risk to ecological receptors concerned with sediment contamination, various ecotoxicological assays are proposed (Loibner *et al.*, 2003). Consequently, there is a

dire need for the incorporation of toxicity tests to assess and identify the associated risks that has proved extremely useful in environmental and chemical hazard evaluation. Because it can be carried out fast and considered more economical than chemical analysis (Sibley *et al.*, 2001). Various researchers have utilized enzymatic assays for the evaluation of environmental impact of sediments and water (Chen *et al.*, 2000; Lopez *et al.*, 2005). These assays have a number of attributes that make them attractive choices including small sample requirement, high sample throughput, low cost, and ease of operation (Day *et al.*, 1995).

3.1.2. Mode of toxicity of Sediments

A variety of contaminants present in river water pollute the source sites from where samples were taken. Causes of contamination can be classified into various categories like industrial wastewater, domestic waste water, agricultural runoff, and storm-water runoff etc (Chan *et al.*, 2001; Malik and Nadeem, 2011). Mode of toxicity in sediment contamination is different for each group of pollutants like pesticides, chlorinated compounds and metals etc. Most of these pollutants show ecotoxicological effect as organic chlorinated compounds are dangerous to a broad range of living organisms (Lopez *et al.*, 2005) and these compounds are of international concern for our atmosphere (Zhang *et al.*, 2008). Various contaminants of sediment like Dichlorodiphenyl trichloroethane (DDT), Polychlorinated biphenyls (PCBs) and pesticides have negative impact on environment because they alter the normal functions of plants and animals (Kumi *et al.*, 2010; Malik and Nadeem, 2011).

Present study is designed to evaluate the modulating effect of sediment on enzymes, proteins, phytochemicals and chlorophyll contents in a model plant *Brassica napus*. In turn this will give us an idea about the positive or negative effect of river Chenab water on the dicotyledonous crops, it irrigates. Insufficient knowledge regarding the mode of action of sediments is available and it indicates that most of the reactions involved are yet to be discovered. Various researchers have found inhibitory effects of numerous chemical compounds on plants. Some of them interfere in physiological and biochemical processes in target plant, like interference with enzymes involved in seed germination, growth and in protein synthesis (Gniazdowska and Bogatek, 2005). Reactive oxygen species (ROS) have significant effects on plant response against biotic and abiotic stresses (Foyer, 2005).

3.2. Selection of model plant

Selection criteria for a model plant selection in scientific research is that, it should possess ideal characteristics i.e. rapid growth rate of seedling and high rate of seed germination. Various phytotoxicity assays are standardized by utilizing model plant or standard target species (Macias *et al.*, 2000). Weed cannot be used in research as model plant due to low germination rate (Dayan *et al.*, 2000). Collection of commercial crop seed is better option to be used as model for research (Macias *et al.*, 2000). In this study *Brassica napus* L. is selected as a model plant to carry out research and evaluate the effect of sediments. This plant has all the ideal scientific features to be chosen as model plant dicotyledonous representative plant.

3.2.1. Brassica *napus L.*

Taxonomy

Kingdom	Plantae
Family	Brassicaceae (Cruciferae)
Order	Brassicales
Genus	Brassica
Specie	napus
Name	*Brassica napus*

Figure 1. Aerial flowering top of *B. napus* (http://upload.wikimedia.org)

Brassica napus L. (rapeseed, oilseed rape or canola) as shown in figure 1 belongs to the Brassicaceae (Cruciferae), also known as the mustard family. The name crucifer comes from the shape of flowers, with four diagonally opposite petals in the form of a cross (Musil, 1950). *B. napus* is an ancient crop plant considered as the most important oilseed crop in Northern Europe, Canada, China, India, Pakistan and other several countries (Liu *et al.*, 2011). It has dark bluish green foliage, glaucous, smooth, or with a few scattered hairs near the margins, and partially clasping, well branched stems, elongated racemic branches and yellow flowers (Starner *et al.*, 1999). This plant helps in the prevention of development of different cancers, cardiovascular, degenerative and age related chronic diseases (Wang *et al.*, 2004). It is an important food crop of Pakistan which has a high economic impact (Brown *et al.*, 1999).

3.3. Seed germination and growth

3.3.1. Germination

Three partial processes constitute the germination process that includes imbibition, intra-seminal growth and activation process (Ranal and Santana, 2006). Germination is the sprouting of a seed usually after a period of dormancy on availability of favorable conditions like water absorption, time passage, warming, chilling, availability of oxygen, and exposure of light (Kucera *et al.*, 2005). In dicotyledonous species, the first emerging part of the plant from seed is the embryonic root, known as primary root or radicle. It allows the seedling to absorb water from soil and embryonic shoot emerges from the seed. This shoot mainly consists of three parts i.e. cotyledons (leaves of seed), hypocotyl (the section of shoot below the cotyledons) and epicotyl (the segment of shoot above the cotyledons) (Kucera *et al.*, 2005). Plants mobilize lipids and polysaccharide stored in seeds during germination (Murphy and Vance, 1999). Interference with the enzymes involved in these processes can affect the seed germination and growth to a great extent (Roach *et al.*, 2010).

3.3.2. Rate of germination

Rate of germination (RG) is the number of germinated seeds that germinate per unit time (Marin *et al.*, 2006). In many studies the rate is calculated by germinated seeds per day (Ellis and Roberts, 1980). The rate of germination and rapid start is dependent on environmental factors like temperature, light, soil moisture and pH etc (Koger *et al.*, 2004). Usually it is expressed as a percentage, e.g., an 80% RG shows that about 80 out of total 100 seeds will probably germinate under optimum conditions over the germination period given. The RG is helpful for calculating the requirements of seed in a particular area or desired number of plants. According to the plant scientists "RG" is the reciprocal of time taken for the process of germination to complete starting from time of sowing (Ranal and Santana, 2006).

3.3.3. Germination index

A measure of the capability of seed germination to become a seedling affected by various internal and externally generated factors (Kandil *et al.*, 2012). The speed of seed germination process can be termed as the germination index (GI). It is used to monitor the soil or sample phytotoxicity and can be calculated by percent germination of the target specie's seed dividing with percentage germination in control (Karim *et al.*, 1992). Matekaire and Maroyi, (2009) described it as the measure of seed vigor and can also be explained as the function of mean and total germination rate.

GI = (Total germination) x (Mean germination rate)

In general, the germination is quantitatively expressed to link the germination rate on daily basis to the highest germination value (Czabator, 1962). A number of these kinds of indices are generated mathematically to explain the parameters of the rate of germination (Kandil *et al.*, 2012).

3.3.4. Relative water content (RWC)

Relative water content is the most suitable measure of plant water status in accordance with the physiological consequence of cellular water deficit (Singh *et al.*, 2009). It is the ratio between capacities for holding maximum water by a specific tissue when fully turgid related to water contents of sample tissue. Weatherley (1950 and 1951) was the first who described this term of relative water content and currently it is widely used for plant water status. Similar word as water content (WC) used for the water content available in a particular tissue. Usual ranges of RWC in severely dried leaves and fully turgid leaves is 40% to 98% (Ullah *et al.*, 2013). Chemical stress can cause low percent of RWC and WC. Similar effect was observed by Yamasaki and Dillenburg, (1999) that increased concentration of allelopathic substances decrease the leaf RWC and WC. Various researchers have reported that plants exposed to allelochemicals have water stress and lower RWC (Singh *et al.*, 2009). As the sediments of River Chenab were observed to contain numerous pollutants like metals, pesticides, OCPs and PCBs etc in previous studies (Eqani *et al.*, 2011, 2012), which can affect the RWC and WC of target plant.

3.4. Biological assays

Biological assays are used to determine the potency or concentration of biological or physicochemical substances comparatively to standards in specific living system i.e. cell, tissue or any organism under suitable conditions. Bioassays are important tools in evaluating environmental pollutants and drug discovery (Carter *et al.* 1998). Effect of different chemicals like herbicide on the seed germination, seedling growth, fresh and dry weight can be observed on target plant (Hammann *et al.*, 1998).

The bioassays are rapid, easier, doesn't require expensive instrument to perform and reproducible results can be obtained. Sediment contaminants affect plants and these can be detected by using *in-vitro* bioassay techniques.

3.4.1. Phytotoxicity assay

Phytotoxicity is the interruption in seed germination and plant growth caused by chemical substances and environmental stresses (Haimi *et al.*, 2000). Bioassay is an

important way to check the effect of toxic substances on plants by monitoring early seedling, seed germination and growth (Sverdrup *et al.*, 2003). These kinds of studies are beneficial for the estimation of hazardous substances and contaminant in soil (Carter *et al.* 1998). Phytotoxic assays are usually carried out to determine the toxic substances in environment. For example, different toxic chemicals are poured into the rivers in close proximity to the industrial areas that accumulate in the form of sediments and ultimately posing risks to the nearby cultivated crops. Knowledge of chemical substances and their ecotoxicological properties could be used to control the impact of toxic compounds (Long *et al.*, 1995). Correlation between chemical substances, environmental components and biological response has been studied and well utilized to protect plant diversity from environmental pollutants (Hoke *et al.*, 1992).

3.4.2. Antioxidant Assays

Reactive species of typically nitrogen and oxygen are produced during various anabolic and catabolic reactions and physiological changes in living system against environmental stresses (Weir *et al.*, 2004). Biomolecules like RNA, DNA, lipids, proteins and peroxidation of cellular targets are affected by these reactive species that cause cellular injury, dysfunctioning and death (Stoeva and Bineva, 2003). Oxidation proliferation and initiation may be controlled by antioxidants (Velioglu *et al.*, 1998). Disturbance in cellular functions and damaging of biomolecules can result in the retardation of plant growth (Smirnoff, 1993). Plants in response to oxidative stress produce more antioxidant compounds (Keddy *et al.*, 1995). Elevated level of antioxidants in plants correlates with the oxidative stress (Smirnoff, 1993).

3.5. Phytochemical contents and chemical assays

3.5.1. Total phenolics content

Polyphenols are the organic compounds that contain multiple phenols as structural unit (Khoddami, 2013). These can be secondary plant metabolites that play important role in regulatory processes, act as antioxidants and perform vital functions in plants (Brandt *et al.*, 2001). Their production may increase during metabolic reactions against chemical or environmental stresses like pollutants (chemical stress) and temperature (Romero *et al.*, 2004). In oxidative stress conditions, reactive oxygen species (ROS) disturb the vital biological system which is counteracted by phenolic substances (Michalak, 2006). Plants are rich in phenolic substances with redox properties and act as hydrogen donators, singlet oxygen quenchers and reducing

agents (Justesen and Knuthsen, 2001). Phenolics actively scavenge the free radicals by donating hydrogen atom and break the chain reaction of lipid peroxidation and thus preventing oxidative deterioration (Erkan *et al.*, 2009). According to Manach *et al.*, (2004), plants grown with stress condition have the higher content of poly-phenols as compared to those grown in hydroponic or conventional conditions without stress. Biotic and abiotic stresses like pests pressure enhances the phenolic acids concentration which cures the wounded plant tissues by contributing in lignification (Manach *et al.*, 2004).

3.5.2. Total flavonoids content

Flavonoids are the poly-phenolic compounds of plants that have the strong antioxidant activity and efficiently act as free radical scavengers (Ou *et al.*, 2003). To predict the flavonoid's ability for the transference of hydrogen atoms into radicals, a well known method is used, based on DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical that reacts with flavonoids through transfer of hydrogen and the reactivity against peroxidation of lipid could be predicted (Moreno *et al.*, 1998). *Brassica* species contain a variety of polyphenolic compounds like hydorooxycinnamic acids and flavonoids. This chemical group is involved in potential pathways which are involved in plant defense against oxidative stress. Polyphenols and flavonoids are capable of breaking free radical chain reactions and act as free radical scavengers. Similar studies are carried out on transition metal oxidation, as iron or copper and leads to chelation of metal ion which occurs due to antioxidant action of flavonoids (Evans *et al.*, 1996).

3.5.3. Total chlorophyll content

Chlorophyll synthesis can be affected by different chemicals or environmental stresses. Adverse effects of allelochemicals on accumulation and biosynthesis of chlorophyll has been studied by various plant scientists, which results in lowering the total chlorophyll content, poor plant growth and photosynthetic activities (Colton and Enhellig, 1980). Organic substances in sediment may enhance the biosynthesis and total content of chlorophyll, while opposite results could also be obtained due to the presence of toxic substances.

3.5.4. Carotenoid content

Carotenoids are organic pigments found in plants, photosynthetic bacteria (Cyano-bacteria) and fungi. These pigments have various physiological functions and capable of free-radical scavenging, thus improving the immune system of vertebrates

(Armstrong *et al.*, 1996). These are terpenoids in nature and composed of isoprene units and give vital properties like colors and fragrances to vegetables, fruits, leave specifically yellow color due to xanthophyll and orange to red color in several plants (Armstrong *et al.*, 1996). These are present in chromoplast and chloroplast that protect chlorophyll from oxidative damage and is visible from color change in the organs of plant (Armstrong *et al.*, 1996). Total carotenoids in related to chlorophyll contents are measured in this study which will be used for understanding the mechanism of growth modulating effects of sediments.

3.5.5. Total malondialdehyde (MDA) content

Membrane lipid peroxidation can be assessed by using malondialdehyde as a marker because it is the end product of lipid peroxidation (Parvanova *et al.*, 2004). Malondialdehyde is an important indicator of oxidative damage (Sung, 1996). Membrane deterioration causes decrease in seed germination (Van *et al.*, 1987). Cellular membrane damage is caused by lipid peroxidation that is induced by ROS and damage to tissue lipids is estimated by measuring the malondialdehyde content. Bogatek and Gniazdowska, (2007) demonstrated the positive correlation between elevated malondialdehyde and ȮH free radicals.

3.5.6. DPPH free radical assay

For the measurement of free radical scavenging ability of samples, DPPH (2,2 diphenyl-1-picrylhydrazyl) is simple, inexpensive and accurate method (Singleton *et al.*, 1999). This assay is also utilized for beverage and foods to find out radical scavenging potential of the ingredients (Haq *et al.*, 2012). When plants are grown under oxidative and free radical stress, they produce more metabolites to cope up with the situation. This mechanism of induced free radical scavenger production provides indirect information about the oxidative stress (Kamkar *et al.*, 2010). DPPH assay can be utilized very easily to monitor such changes in the seedling (Sharma & Bhat, 2009). The ease of performance, accuracy and cost effectiveness of this assay has made it most widely used technology among researchers (Medini *et al.*, 2013). The same was utilized here to monitor the impact of different stress on the biochemistry of *B. napus*.

3.5.7. Total antioxidant capacity

As majority of the compounds in food plants have greater antioxidant potential, which are utilized by living organisms in their nutrition. Plants produce these antioxidant compounds as their defensive mechanism against oxidative stress, which they face

daily (Ou *et al.*, 2002). An increased oxidative stress compels the plant to produce more antioxidant compounds. So, to measure the increase in total antioxidant activity can be well correlated with the induced oxidative stress. (Sharififar *et al.*, 2007). Plants produce a wide range of phytochemicals which have different mechanisms to resolve the oxidative stress. Therefore measurement of antioxidant potential through a panel of multi-mode antioxidant assays has been considered more precise tool to monitor the oxidative stress in plants indirectly (Prieto *et al.*, 1999). Phosphomolybdenum method is usually used to find total antioxidant activities of various substances on the basis of reduction of Mo (VI) to green phosphate Mo (V) complex. This method is quantitative and total antioxidant activity is measured as equivalent number of ascorbic acid (Prieto *et al.*, 1999). Various other methods are also described in literature to measure the TAC of different substrates (Perez *et al.*, 2008).

3.5.8. Total reducing power

Redox properties of particular substances are due to their antioxidant properties by neutralizing free radicals, peroxide decomposition, and quenching singlet or triplet oxygen (Javanmardia *et al*, 2003). This assay determines the reduction potential of sample when it forms potassium ferrocyanide (Fe^{2+}) by reacting with potassium ferricyanide (Fe^{3+}). Ferric reducing antioxidant power (FRAP) is based on the electron transference during the reaction of antioxidants when they reduce the oxidants through transference of single electron (Singh and Rajini, 2004). It differs from other assays on the basis of no any involvement of free radicals, rather the monitoring is to be done on reduction of ferric iron (Fe^{3+}) to form ferrous (Fe^{2+}) (Gorinstein *et al.*, 2010). The same principle may be employed on most of the other assays where actively-redox compound or colored synthetic radical is produced and the scavenging capacity of biological sample against radical is measured by micro-plate reader or spectrophotometer with the application of a suitable standard for quantifying the antioxidant potential or reduction in redox-active compound (Dudonne *et al.*, 2009).

3.6. Protein contents and enzymatic assays

3.6.1. Total protein content

Protein is the vital component of cells, which is comprised of large molecules that are the long chains of sub-units known as amino acids (Ma *et al.*, 2003). Generally, environmental stresses are harmful to the growth of plants, which can negatively

affect the plants metabolism, and causing imbalance in the level of protein. It consequently leads to adversely affect the process of hydrolysis and synthesis of proteins (Shah and Dubey, 1998). Its content in growing seeds determines the germination and vegetative growth. Various studies are carried out to evaluate the correlation of protein content and stress conditions produced in plants after treating with toxic chemicals (Abu-Romman *et al.*, 2010). Vital processes of plant like respiration, transcription, translation, photosynthesis and mineral uptake can be interrupted by toxic substances as allelochemicals (Grodszinsky *et al.* 1989). Many toxic substances can interrupt the normal cellular processes and protein synthesis, which in turn suppress the plant growth (Yongqing, 2005). So, comparative analysis of protein contents in plants of the same species can be utilized to indirect measurement of chemical stress during their development (Dubey, 1994).

3.7. Enzymatic assays

Enzymes are the vital constituents of living organisms that play an important role in different reactions (Oracz *et al.*, 2007). Reactive oxidative species (ROS) are present in balanced form and their disturbance or over production may lead to oxidative stress involved in affecting cellular metabolism. It damages the components of plasma membrane and causes membrane leakage (Mittler *et al.*, 2004) consequently disturb the cellular permeability (Apel and Hirt, 2004). There is a well developed defense mechanism in plants to cope with such oxidative stresses comprised of peroxidases (POD), catalase (CAT) and superoxide dismutase (SOD) (Blokhina *et al.*, 2003). Involvement of these enzymes in seed germination and stresses are studied and characterized by Cai *et al.* (2011).

Generally in plants, antioxidant mechanism against oxidative stress is comprised of peroxidases (POD), superoxide dismutases (SOD) and catalase (CAT). A variety of assays have been brought in to measure antioxidant capacity of target plant and other biological samples. The concept of antioxidant capacity was first originated from chemistry and later adapted in biology, medicine, epidemiology and nutrition (Floegel *et al.*, 2010). It illustrates the ability of redox molecules in biological systems to scavenge free radicals (Cao and Prior, 1998). Following are given some brief introduction of the enzymes of target plant which were evaluated in the current study.

3.7.1. Protease activity

Plant seeds are reservoir of proteins and some proteases in the form of protein storage vacuoles (PSVs) (Gruis *et al.*, 2004). Proteases are required for the mobilization of stored proteins during seed germination (Muntz *et al.*, 2001). Inhibition or damaging of proteases could result in the failure of seed germination and suppression of seedling growth (Schaller, 2004). As sediments contain different contaminants in the form of organic and inorganic chemicals that may act as protease inhibitors or inhibiting *de novo* synthesis. So measuring of protease activity can give us information about the mechanism of inhibition of seed germination and seedling growth.

3.7.2. Peroxidases (POD)

Peroxidases are very important group of enzymes that catalyze the reactions of hydrogen peroxide and lipids. Role of cytochrome c peroxidase is involved in defense against pathogens and oxidative stress in plants (Gaspar *et al.*, 2006). These enzymes are the part of antioxidant system of plant and are expressed in high concentration during oxidative stress (Mazorra *et al.*, 2002).

3.7.3. Superoxide dismutase (SOD)

Superoxide dismutase are essential antioxidant enzymes in plants and formerly known as group of metalloproteins. On the basis of metal cofactor these enzymes are classified into three groups as Fe/Mn, Cu/Zn and Ni containing SODs (Brewer, 1967). These enzymes are involved in the conversion of Superoxide reactive oxygen to oxygen and peroxide. Fe/Mn SOD is found in mitochondria of prokaryotes and eukaryotes, Cu/Zn SOD is usually found in eukaryotes and Ni SOD is found in prokaryotes (Barondeau *et al.*, 2004). When plants are under oxidative stress, expression of these enzymes is increased as a major part of defense mechanism.

3.8. Aims and objectives

- Evaluation of the growth modulating effect of sediments on model plant (*Brassica napus* L.)
- To find out the mechanism of action of growth modulating activity of sediment by utilizing phytochemical and biological assays.

Chapter No.2

Materials and Methods

This study was carried out in Quaid-i-Azam University, Islamabad, Pakistan. A brief detail of procedures and specification of chemical and instruments are given below.

2.1 Study area

The River Chenab is one of the greater branches of the Indus River in Pakistan that enters from India at the upstream of rim station Marala, which has the total catchment of almost 38,000 Km^2. In Punjab, this river transverse 560 km in the course of highly contaminated and industrial cities like Faisalabad, Gujranwala, Sialkot, Gujarat, Jhang, Khanewal and Multan.

2.2 Sampling

In support of the preceding study Eqani *et al.* (2012), surface sediments of 25 selected sites were collected from river stretch of about 500 kilometers beginning at Marala to Punjnad station in between the period of may 2007 to November 2009. Each site selection was according to the anthropogenic activities in the catchment and were marked using a GPS (Global Positioning System, Garmin). As of all twenty five samples, one each was collected from industrial drain near district Jhang where it discharges into Chenab and Sutlej River, 2 sites each on Jhelum and Ravi River and the remaining 19 sites were selected on the mainstream of River Chenab. While collecting samples in the field, each was taken as composite of 5 sub-samples from the focused area of 15 to 30 cm^2 with in the distance of hundred meters using a ladle of stainless steel. Samples were then kept in pre-washed containers of glass and after the careful labeling; all samples were transferred to the laboratory and set aside at -20 °C in refrigerator (Mai *et al.*, 2002). For further analysis, samples were dried in air, sieved and kept in pre-washed glass bottles. Weight of each of the collected sample was almost 2 kilograms. The minimum space of almost 500 meters was covered for each site during sample collection. For the current study we collected total fifty-seven (57) sediment samples representing the predefined 25 sites.

2.3 Sites distribution on River Chenab

Generally, the river Chenab stretch is disintegrated into the three main regions with respect to its land utilization that starts from headwork Marala to the Punjnad headwork. These regions are devised as Urban, Industrial and Agricultural. The conceptual map of the proposed 25 sites of sampling area (River Chenab) is shown in Figure. 2, their details, latitudes and longitudes along with sediment codes are given as under in the table. 1;

Table 1. The details of sampling sites, their latitudes and longitudes, sediment codes and description of sites.

Site	Lat-Long	Sample (S)	Site description
Site 1	31°25′02.3″ N, 72°11′37.3″ E	S-18, S-38	Agricultural area at upstream of Chenab, 10 km above Trimmun headwork which is the meeting point of river Chenab and river Jhelum in district Jhang.
Site 2	31°25′81.2″ N, 72°20′71.8″ E	S-20, S-28	Urban area at upstream of Chenab, 10 km above Trimmun Headwork in Jhang city.
Site 3	31°18′7.5″ N, 72°16′46″ E	S-10, S-24	Sub-urban area at downstream of Trimmun headworks where river Chenab joins river Jhelum.
Site 4	31°08′27.5″ N, 72°14′25.3″ E	S-6, S-31	Agricultural area, 10 km below Trimmun Headwork in Nikokara town.
Site 5	30°99′20.7″ N, 72°08′65.3″ E	S-16, S-56	Agricultural area of the district Jhang.
Site 6	30° 87′90.3″ N, 71°97′70″ E	S-9	Agricultural area of the district Jhang.
Site 7	30° 76′19.3″ N, 71°88′23.1″ E	S-26, S-43	Agricultural area at upstream on the boundary region of district Jhang and Khanewal.
Site 8	30°62′14.8″ N, 71°82′72.3″ E	S-41, S-42	Area on the river Ravi upstream, 10 km above meeting point of river Chenab and Ravi in district Khanewal.
Site 9	30°62′61.3″ N, 71°80′76.6″ E	S-30, S-32	Agricultural area at downstream of Chenab, 10 km below the joining point between river Ravi and Chenab.
Site 10	30°57′63.3″ N, 71°65′99.7″ E	S-1, S-46	Sub-urban and agricultural area at upstream of Chenab in district Khanewal.
Site 11	30°52′61″ N, 71°59′50.1″ E	S-25, S-33	Agricultural area of district Khanewal.
Site 12	30°45′01″ N, 71°50′70.1″ E	S-19, S-49	Agricultural region of district Multan.

Site	Lat-Long	Sediment	Site description
Site 13	30°35′91.8″ N, 71°42′32.1″ E	S-7, S-23	Sub-urban and agricultural area at the joining point between Chenab and Indus link canal situated in Muzaffarghar district.
Site 14	30°44′33″ N, 71°50′68″ E	S-5, S-27	Sub-urban area at the mainstream of river Chenab.
Site 15	30°152′06″ N, 71°29′03.1″ E	S-36, S-47	Sub-urban area of district Multan.
Site 16	30°07′48.2″ N, 71°28′42.7″ E	S-35, S-44	Urban area near at Shershah Bridge located in district Multan.
Site 17	30°62′14.8″ N, 71°82′72.3″ E	S-17, S-39	Urban area on the river Ravi upstream, located in PulBaghar of district Khanewal.
Site 18	31°54′43.3″ N, 72°33′12.3″ E	S-34, S-45	Urban area at the mainstream of Chenab, An industrial drain from Faisalabad city joins this site in district Jhang.
Site 19	31°70′61″ N, 72°93′53″ E	S-2, S-29	Agricultural area at the mainstream of Chenab in Chinyot district.
Site 20	31°56′45″ N, 72°52′52″ E	S-21	Industrial drainage area receiving huge amount of pollutants, located in district Jhang.
Site 21	32°95′58″ N, 73°75′22″ E	S-48, S-51, 52	Sub-urban and agricultural area on the River Jhelum.
Site 22	32°67′09.9″ N, 74°47′09.2″ E	S-15, S-40	Upstream of Marala rim station near at the Sialkot city where Chenab enters Pakistan.
Site 23	32°40′29.2″ N, 73°97′25.2″ E	S-12, S-22, S-53, S-54	Sub-urban and agricultural area at the upstream of Chenab near Gujranwala district in Khanki Barrage.
Site 24	29°79′68″ N, 72°28′52.8″ E	S-11, S-50, S-55, S-57	Agricultural area at the downstream of Head Islam on Sutlej river.
Site 25	29°35′04″ N, 71°02′27.8″ E	S-3, S-4, S-8, S-13, S-14, S-37	Area at the downstream of river Chenab near at Punjnad Headworks.

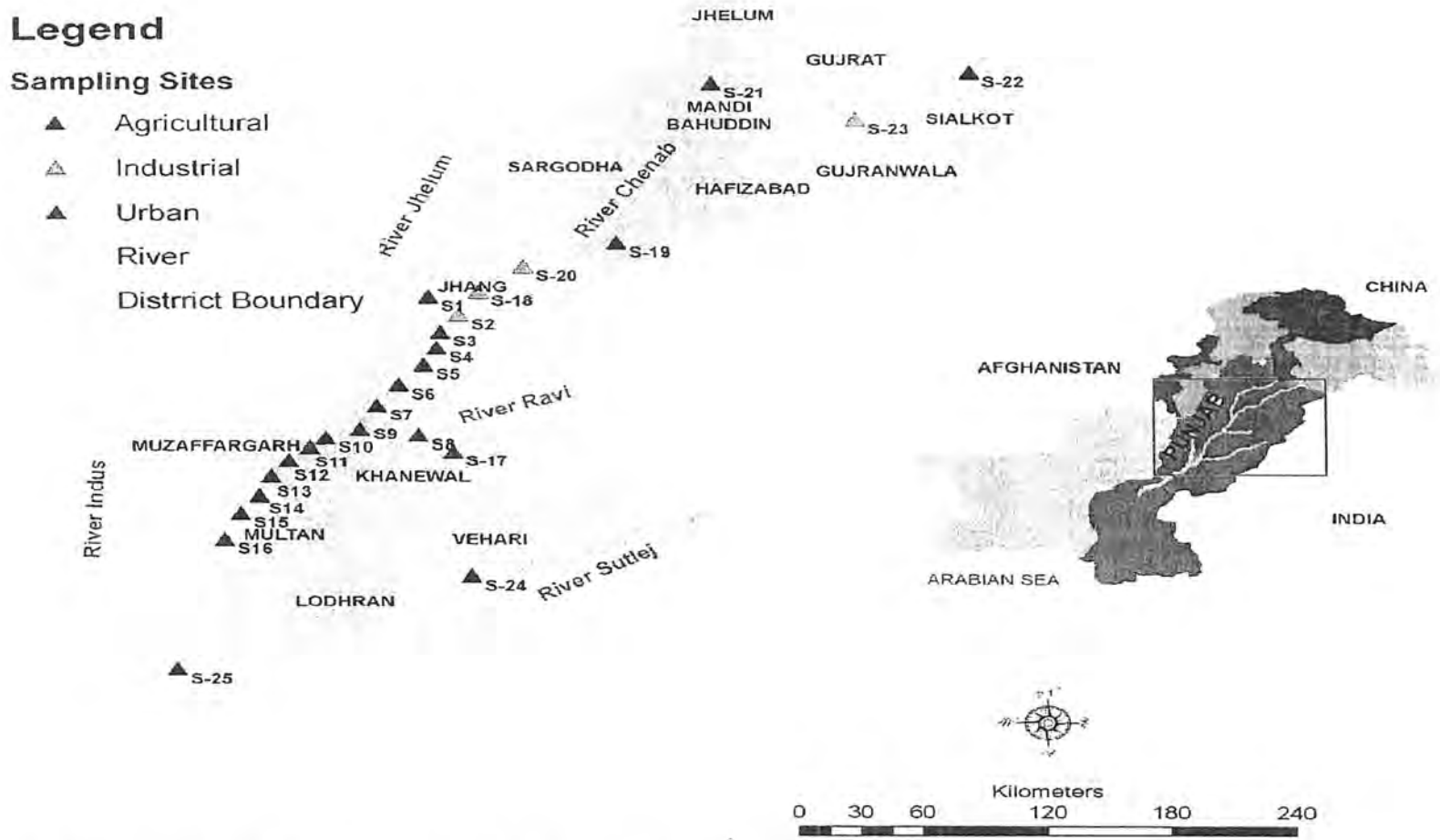

Figure. 2 Conceptual map of the proposed sites in sampling area (River Chenab).

△ Points S1-S25 represent sites of collected samples utilized in the study.

2.4 Model Plant selection

Brassica napus L. was selected for this study as a model plant. Seeds of this plant were purchased from local market of Barakahu, Islamabad.

2.5 Extraction from sediments

Requirements

Sediment samples, Digital balance, Sonicator, Glass vials, Aluminium foil, Autoclave, Foreseps, Pipettes, Parafilm, Incubator, Permanent marker, Paper tape, Plastic tape, Digital camera.

Procedure

To evaluate the effect of sediment on target plant (*B. napus*), the experiment were carried out according to Turker and Camper, (2002). The samples were prepared by adding two grams of sediment in 16 milli-liter of autoclaved distilled water. Glass vials with open-able tight lids having the capacity to fill 25 ml of fluid were used to contain sediment and distilled water for extract preparation. Initially the glass vials and respective petri-plates were labeled with sample names according to their site codes with the help of permanent marker on paper tape. These were then wrapped with the transparent plastic sticky tape to avoid tag removal during sonication. The empty glass vials were taken for weighing first and then again weight of each vial was taken after filling with 2 grams of sediment samples. The weighing process was carried out by using digital balance (Shimadzu ELB600). The vials were then filled with autoclaved distilled water. After manually shaking for few seconds, the vials were placed in sonicator (E 30 H Elmasonic) to sonicate for approximately 1 hour repeatedly by taking three intervals after each 20 minutes. The vials were then removed from sonicator and placed aside to settle down large particles. With the help of a suitable pipette, a particular amount of supernatant with respect to the continuing assay protocol was taken as sediment extracts and poured in respective petri-plate. In this way the target plant (*B. napus*) was treated with sediment extract at the concentration of 125 mg/ml to find out their modulating effects while the concentrations of 0.5 mg/ml and 1.0 mg/ml of 2, 4 di-chloro acetic acid was used as a positive control in these assays.

2.6 Evaluation of growth modulating effect

All viable and dormant seeds have the capacity to germinate and become plants. The process of germination needs optimum conditions like water (during imbibition and growth stages), oxygen (for respiration) and temperature (suitable for metabolism and

growth) (Baskin *et al.*, 1998). The detailed procedure for evaluation of sediment effects on the germination and growth of *B. napus* are given as below:-

2.6.1. Seed germination

Requirements

Sediment extracts, Target plant (*B. napus*) seeds, Chloroform, 2,4-dichloro acetic acid, Sodium hypochlorite aqueous solution, Methanol, Digital balance, Glass vials, Pasteur pipettes, Aluminium foil, Autoclave, Foreseps, Oven, Pipettes, Parafilm, Incubator, Whatman No.1 filter paper, Petri-plates (9 cm), Digital camera.

Procedure

This assay was performed as previously reported by Ullah *et al.* (2013) with slight modification according to the system suitability. Briefly 5 ml of the sediment extract was poured in respective petri-plate containing whatman No.1 filter paper. Seeds were sterilized for two minutes in 10 % aqueous solution of sodium hypochlorite, followed by 10 washings with sterilized distilled water. Total 20 seeds were placed in each petri-plate containing the extract solution adsorbed on whatman no. 1 filter paper. Then plates were incubated at 25 ± 1 °C in dark. Germinated seeds were counted and observed daily upto five days. More sterilized distilled water was added where required to prevent water shortage during incubation. Seed is considered germinated only when the radical size becomes two millimeter in length. Whole experiment performed under sterilized environment and triplicate set of plate were used for each sediment sample.

Calculation to evaluate the effect of sediment extract on germination of *B. napus* was performed as given below;

i) Final germination percentage (FG %) is the maximum average percentage of germinated seeds

FG % = No of germinated seeds/No of total planted seeds x 100

i) Formula for the Rate of Germination RG = $\sum$ Ni/Di

ii) Formula for the Mean period of final germination MPFG = $\sum$ (NiDi)/S

Here

N = daily increase in seedling number

D = number of days from seed placement

S = Total number of seeds germinated

iii) Germination index (GI) = (% Germination against sediment sample) ÷ (% Germination in the control)

While inhibition percentage was calculated according to given formula;

iv) Percentage inhibition (PI) = [1 – FG % in extract ÷ FG % in control] x 100

2.6.2. Seedling growth and seedling weight loss

Requirements

Ruler (± 1.0 mm), Digital balance, Note book, Pen, Magnifying glass, Foreseps and Digital camera.

Procedure

Method described by Ullah *et al.* (2013) was followed for evaluation of the effect of sediments on seedling weight loss and seedling growth. Briefly in continuation of the above experiment, at the fifth day of experiment seedling growth, shoot length and root length was measured. Shoot length was measured from seed base to shoot tip while root length was measured from base to root tip with the help of ruler (± 1.0 mm). Digital balance (Ohaus, ± 0.0001 g) was used to measure seedling fresh weight, and the dry weight was also measured after drying at 70°C in oven for 28 hours.

Percent inhibition of seedling growth (% iSG) was calculated by the given formula;

% iSG = [(N-S)/N] x100

Here

iSG is inhibition in seedling growth

N is negative control [N=RL/SL]

S is sample [S=RL/SL]

Seedling growth is the emergence of the plantlet from soil. It can be measured by different ways like number of seedlings per unit area, time required for the emergence of plantlet from soil, top of seedlings mass and seedlings height as described by Kandil *et al.* (2012).

i) Seedling length =

[RL+SL (control) - RL+SL (sample)/ RL+SL (control)] x 100

ii) Percentage of weight loss in seedling was found according to given formula:-

% WL = [(Wn-Ws)/Wn] x100

Here

WL is weight loss

Wn is weight of negative control

Ws is weight of sample.

iii) Relative dry weight (RDW) % = [DW of sample/ DW of control] x 100

2.6.3. Water content and relative water content

Requirements

Fresh leaves of seedlings, Distilled water, Oven, Digital balance (Ohaus, ± 0.0001 g), Tissue paper.

Procedure

Seedling fresh leaves weighing 200 mg were taken for the determination of water content and relative water content. Leaves were placed in distilled water and removed after 12 hours. These leaves were then dried using tissue paper. These were fully turgid leaves. Weight of these leaves were taken and designed as fully turgid weight. Then these leaves were placed in oven at 70°C till completely dry and then weighted to get dry weight.

Equation as described by Turner, (1986) was used to calculate percent of relative water content.

RWC (%) = [(FW-DW)/ (TW-DW)] x 100

Here

RWC is relative water content, FW is fresh weight, DW is dry weight and TW is total weight.

Determination of water content was found by weighing 200 mg as fresh weight. Then these were dried in oven at 70°C weighted for dry weight. Finally water content percentage was obtained using given formula

WC (%) = (FW-DW/FW) x 100

Here

WC is water content, FW is fresh weight, and DW is dry weight.

2.7. Mechanism of growth modulating activity of sediments

Total protein content and variation in different enzymes like POD, SOD and Protease activity were evaluated against the sediment samples and the given procedures were followed to perform assays of these enzymes.

2.7.1. Phytochemical contents and chemical assays

2.7.1 (a). Total flavonoids content

Requirements

Plant extracts, Methanol, Aluminum chloride, Distilled water, Agilent spectrophotometer- DAD, Potassium acetate, Quercetine, Transparent 96-well plates

Procedure

Aluminum chloride colorimetric method as described by Haq *et al.* (2012) and Chang *et al.* (2002) was used for total flavonoids determination. Plant extracts 100 mg DW/ml of methanol was prepared as stock solution and 25 µl from stock solution, 100 µl of 10% aluminum chloride, 1975 µl of methanol, and 100 µl (1M) potassium acetate were mixed then 2.8 ml of distilled water was added. These samples solution were kept for 30 minutes at room temperature and the absorbance was checked at 415 nm (Agilent spectrophotometer- DAD, 8453). Quercetine was used as a standard and its equivalent in 10 mg DW of the sample was used for the determination of total flavonoids.

2.6.1 (b). Total phenolics determination

Requirements

Plant extracts, DMSO, Folin-Ciocalteu reagent, Test tube, Spectrophotometer (DAD 8453 Agilent), Sodium carbonate, Gallic acid Incubator, Transparent 96-well plates.

Procedure

Determination of total phenolics in samples was carried out as described by Haq *et al.* (2012). Stock solution of sample was made at the concentration of 100 mg DW/ml of methanol. Then 40 µl of this solution and 0.75 ml of ten time diluted Folin-Ciocalteu reagent were added in test tube. Waited for 5 minutes at room temperature and then added 0.75 ml of 6% (w/v) of sodium carbonate and mix this solution gently. This mixture was incubated for 90 minutes at room temperature (25°C) and absorbance was recorded by using spectrophotometer (DAD 8453 Agilent) at 725nm. Gallic acid was used as standard and its equivalent in 10 mg DW was used for the expression of total phenolics content.

2.7.1 (c). Total chlorophyll content (TCC) and a ratio b

Requirements

Fresh leaves of target plant, Glass vials, DMSO, Spectrophotometer (Shimadzu, UV-120-01)

Procedure

To evaluate the impact of sediments on chlorophyll contents of *B. napus* seed, already described procedure by Ullah *et al.* (2013) was utilized. For this purpose 100 mg of leaves in 5 ml of DMSO were extracted over night at room temperature, then spectrophotometer (Shimadzu, UV-120-01) was used to record the color intensity of extracts at 645 nm and 663 nm for the estimation of chlorophyll a, b contents.

Formula proposed by Ellis *et al.* (1980) was used for the calculation of chlorophyll content as mg/g fresh weight of leaves.

→ Formula for chlorophyll a

$$\text{Chl } a = [(12.7(OD663) - 2.69(OD645)] \times v/a \times 1000 \times w$$

→ Formula for chlorophyll b

$$\text{Chl } b = [(22.9(OD645) - 4.68(OD663)] \times v/a \times 1000 \times w$$

→ Sum of chlorophyll a and chlorophyll b will be total chlorophyll content.

→ Ratio of chlorophyll a to b = Chl *a*/Chl *b*

2.7.1 (d). Total carotenoids content

Sediment effect on total carotenoids content in *B. napus* leaves were measured by checking OD of sample extracts at 480 nm.

Given formula was used for calculation of total carotenoids

Carotenoids = absorbance at 480 x w

Here

a = length of light path in cell (usually 1 cm)

w = fresh weight of leaves in grams

v = Extract volume (5 ml)

2.7.1 (e). Malondialdehyde (MDA) content

Requirements

Thio-barbituric acid (TBA), *B. napus* seeds, Tri-chloro acetic acid (TCA), Mortar and pestle, Centrifuge, Incubator, Micro-pate reader or Spectrophotometer (Shimadzu, UV-120-01).

Procedure

Thio-barbituric acid (TBA) test was used for the determination of melondialdehyde content described by Bailly *et al.* (1996) and Velikova *et al.* (2000). One gram of *B. napus* seeds was homogenized with 0.1% TCA (1ml) in mortar and pestle. It is centrifuged for 30 minutes at 15000 g and supernatant was preceded for assay performance. Mixture of 0.1 ml supernatant and 0.3 ml of 0.5% TBA in 5% TCA was incubated for 30 minutes at 95°C. It was cooled immediately in ice bath in order to stop the reaction. Absorbance of supernatant was observed at 532 nm and 600 nm after the centrifugation of reaction mixture. Absorbance at 532 nm was specific while at 600 nm that was nonspecific. OD values at 600 nm were subtracted from OD

values of 532 nm. Extinction coefficient of 155 mM^{-1} cm^{-1} was used for melondialdehyde content (lipid per oxidation) expressed as µM/g fresh weight.

2.7.1 (f). DPPH free radical scavenging assay

Requirements

DMSO, Methanol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid ($C_6H_8O_6$), Test samples, Transparent 96-well plates, Incubator, Micro plate reader.

Procedure

In this study the radical scavenging potential of the samples was observed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) described by Lee *et al.* (1998) method. DPPH (316 µM) was dissolved (0.104 mg/ml w/v) in methanol. Samples were also extracted in methanol 100 mg/ml and 20 µl samples extract was taken in respective well of 96 well plate. Then 180 µl of DPPH was added to make 200 µl. Absorbance was noted by using micro-plate reader at 515 nm after incubating at 37°C for 1 h. Ascorbic acid was used as positive control and methanol solvent as negative control. The experiment was carried out in triplicate and percentage scavenging was calculated as under;

% of Scavenging effect = [1-As/Ac] x100

Where "Ac" means OD of control and "As" means OD of the test sample.

2.7.1 (g). Total antioxidant capacity

Requirements

Samples, 28 mM sodium phosphate, 4 mM solution of ammonium molybdate, DMSO, 0.6 M H_2SO_4, Ascorbic acid, Micro-plate reader.

Procedure

Total antioxidant potential of samples was measured by phosphomolybdenum method as described by Ullah *et al.* (2013). One ml of reagent solutions (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid) and 0.1 ml of plant sample (100 mg DW/ml methanol) was mixed. Then it was incubated at 95°C for 90 minutes. Finally the absorbance was checked after cooling the reaction mixture at 695 nm. Blank was prepared by dissolving 0.1 ml of methanol instead of sample. Total antioxidant capacity was measured as equivalent of ascorbic acid. Final concentrations of ascorbic acid were tested at 125 µg/ml, 100 µg/ml, 75 µg/ml, 50 µg/ml, and 25 µg/ml to prepare the calibration curve.

2.7.1 (h). Reducing power assay

Requirements

Samples, 0.1% ferric chloride ($FeCl_3$), 0.2 M phosphate buffer (pH 6.6), DMSO, 10% trichloroacetic acid, 1% potassium ferricyanide K_3 Fe $(CN)_6$, Micro-plate reader.

Procedure

The previously described method of Ullah *et al.* (2013) was followed for this assay. Reaction mixture was prepared by mixing 1% potassium ferricyanide, 0.2 M phosphate buffer and 100 µl of sample solution (100 mg DW/ml methanol). It was incubated for 20 minutes at 50°C. Mixture was centrifuged at 3000 rpm for ten minutes after the addition of 500 µl of 10% trichloroacetic. Then 100 µl from upper layer, 20 µl of 0.1% ferric cyanide and 80 µl of distilled water was mixed. OD was checked at 700 nm and blank was prepared by using methanol instead of sample. Ascorbic acid was used as standard at 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml concentrations to make the calibration curve.

2.7.2. Protein contents and enzymatic assays

2.7.2 (a). Total protein content (TPC)

Requirement

Samples, Bovine serum albumin (BSA), Test tube, Incubator, Spectrophotometer (Shimadzu, UV-120-01) or Micro plate reader and Reagents.

The composition of reagents used (A, B and C) is given below;

Reagent A: 7 mM Na-K tartrate, 0.5 N NaOH and 0.81 M Na_2CO_3

It was prepared by the addition of 100 g Na_2CO_3, 2 g Na-K tartrate x 4 H_2O, 500 ml of 1N NaOH and further distilled water was added to raise the volume upto one liter.

Reagent B: 70 nM Na-K tartrate and 40 mM CuSO4.

It was prepared by 1g CuSO4 .5 H_2O, 10 ml 1N NaOH, 2 g Na-K tartrate x $4H_2O$ and 90 ml of distilled water

Reagent C: Folin-Ciocalteau reagent was diluted ten times with distilled water.

Protein extract

Slightly modified method of Nayyar and Gupta, (2006) was followed for preparation of enzyme extract. One gram of seeds were taken and soaked in 8 ml of sediment extract for two days. Then these were crushed and homogenized with 10 ml of 50 mM potassium phosphate buffer containing 1% PVPP at pH 7. Then it was centrifuged at 15000 g for 30 minutes on 4°C after which the supernatant (protein extract, PE) was taken and utilized for protein contents and all enzymatic assays.

Procedure

Lowry *et al.* (1951) method was followed for the estimation of protein content. The enzyme extract used for the estimation of protein was used for all chemical and antioxidant enzyme assays. Determination of the total protein content was carried out by using 96 well plate. The reaction mixture was prepared by taking 40 µl of protein extract as described above in respective well. Then 36 µl of reagent A was added and incubated for 10 minutes at 50°C. Reaction mixture was then allowed to cool at room temperature (25°C) and 4 µl of reagent B was added. Again the reaction mixture was subjected to incubate for 10 minutes at 50°C. After cooling again at room temperature 120 µl of reagent C was added and incubated for 10 minutes at 50°C. Absorbance was recorded at 650 nm by using the micro-plate reader. Bovine serum albumen (BSA) was used as standard and calibration curve was made at the series of concentrations 25, 50, 75, 100, 125 and 150 µg/ml used.

2.7.2 (b). Protease activity assay

Requirements

Five percent Tri-chloro acetic acid (TCA), Whatman No. 40 filter paper, Folin Ciocalteu reagent, Citrate phosphate buffer (pH 7), 1 N NaOH, 0.2 M Na_2HPO_4

Alkaline reagent was prepared by the addition of 100 ml of A + 1 ml of B + 2 ml of C (A = 2% Na_2CO_3, B = 2.7% Na-K-tartrate, C = 1% $CuSO_4$)

Citrate phosphate buffer (pH 7) was prepared by following the method of Gomori, (2004, Appendix).

Procedure

This assay was performed according to the procedure reported previously Ullah *et al.* (2013). Protein extract of 100 ml was mixed with 1 ml of 1% casein and was incubated at 30°C for one hour. Casein was used as substrate dissolved in citrate phosphate buffer of pH 7. Reaction was stopped by adding 1 ml of 5% TCA and placed for half an hour. After filtering this mixture, one ml was picked and mixed with 1 ml of alkaline reagent. For making reagent more basic 2 ml of 1N NaOH was added. This mixture was incubated for ten minutes at room temperature then 0.5 ml Folin Ciocalteu reagent was added and incubated at 37 °C for 30 minutes. Optical density (OD) was measured at 660 nm by using micro-plate reader. Enzyme activity was described as increase 0.1 in absorbance of reaction mixture per unit time under given condition of assay.

Activity of proteases was calculated according to formula;

Protease Activity = (OD/0.1) U/gFW

U/gFW= units/g fresh weight

2.7.2 (c). Peroxidases (POD) activity assay

Requirements

Enzyme extract, 27.5mM H_2O_2 (10x), 100 mM Guaiacol (10X), Distilled water, 1% polyvinyl-pyrrolidone (PVPP), 50 mM K-phosphate buffer of pH 7, Spectrophotometer Shimadzu, UV-120-01) or Micro-plate reader.

Procedure

Modified method of Lagrimini, (1991) was used for the determination of peroxidases (POD) activity. Reaction mixture of one milliliter was prepared by taking 200 µl of K-phosphate buffer, 100 µl of 100 mM Guaiacol, 100 µl of enzyme extract, 500 µl of distilled water, 100 µl of 27.5 mM H_2O_2. Blank was prepared with 300 µl of K-phosphate buffer, 100 µl of Guaiacol, 500 µl of distilled water and 100 µl of H_2O_2. POD is involved in catalytic conversion of guaiacol to tetra quaiacol in the presence of H_2O_2. Immediately after the addition of H_2O_2 increase in absorbance was checked 10 times with the gap of 20 seconds at 470nm.

Activity of this enzyme was calculated according to formula;

A = ELC

Where

A = Absorbance

E = Extinction coefficient (6.39 mM^{-1} cm^{-1})

L = Length of each well was 0.25 cm

C = Concentration of enzyme (value of C was measured as nM/min/mg FW)

FW = Fresh weight of the sample

2.7.2 (d). Superoxide dismutases (SOD) activity assay

Requirement

1 mM EDTA, 130 mM methionine, 0.75 mM NBT, 0.02 mM Riboflavin, Enzyme extract, 0.05 M phosphate buffer (pH 7), Spectrophotometer (Shimadzu, UV-120-01) or Micro-plate reader.

Procedure

This assay was performed as reported previously by Ullah *et al.* (2013) with slight modification. Photochemical reduction of Nitro blue tetrazolium (NBT) is inhibited by SOD, which is the principle of this assay. One ml of reaction mixture was prepared by addition of 100 µl of 1 mM EDTA, 100 µl 0.75 mM NBT, 390 µl 0.05 M

phosphate buffer, 10 µl 0.02 mM Riboflavin and 300 µl protein extract. Blank was prepared as, only instead of protein extract, buffer was added then reaction mixture was incubated under florescent light for 7 minutes. Then OD of both blank and mixture was measured at 560 nm.

Activity of this enzyme was calculated according to formula:-

$A = ELC$

Where

A = Absorbance

E = Extinction coefficient (6.39 mM^{-1} cm^{-1})

L = Length of each well was 0.25 cm

C = Concentration of enzyme (value of C was measured as nM/min/mg FW)

FW = Fresh weight of the sample

Chapter No.3

Results

In this study, total 57 samples of river Chenab sediments were collected from different areas of Punjab, Pakistan. These samples were investigated *in vitro* for their growth modulating effect on *Brassica napus*. The detailed results of the investigation are as follow:-

3.1. Effect of different sediment samples on seed germination of *B. napus*

Effects of various sediments on germination, rate and mean period of final germination were evaluated by using the seeds of *Brassica napus*. Extracts of sediment (125mg DW/ml of DW were used to carry out this study. Germinating potential of control seeds grown in distilled water was considered as their normal and natural potential to express different parameters like final germination percentage, rate of germination and mean period of final germination etc in the standard environment.

3.1.1. Effect on final germination percentage (FG %)

Modulating potential of various sediment samples on germination of *B. napus* was observed. Among all, 40 samples (70% of total) were found to have no effect on percent final germination. The remaining 17 sediment samples (30% of total) were observed to have slight inhibitory effects on germination. The highest inhibitory effect (20%) on germination was produced by sediment samples S-3 and S-41. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml concentration showed 15% FG while the seeds treated with 1.0 mg/ml concentration of 2, 4 dichloro-acetic acid showed null germination.

3.1.2. Effect on rate of germination (RG)

The effect of sediment extracts on the rate of germination was also studied. The seeds grown in distilled water under standard experimental conditions were used as control to calculate the rate of germination. Lesser rate of germination (RG) was recorded in the seeds of all 57 samples (100% of total) with respect to the control. However the highest germinating rate among the seeds of all samples was recorded against the sample S-15 (47% less than control) as 8.67 while the lowest were exhibited by the seeds of sample S-1 (59% less than control) as 6.67. Comparatively to control the seeds of sample S-55 showed 50% lesser RG and seeds of five samples i.e. S-13, S-15, S-28, S-31 and S-40 were observed to show more RG inhibition than 50% while in the remaining seeds of 52 samples RG inhibition was lower than 50%. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml concentration

showed very low RG as 0.77 while the seeds treated with 1.0 mg/ml concentration of 2, 4 dichloro-acetic acid showed zero RG.

3.1.3. Effect on mean period of final germination (MPFG)

Likewise the effect of samples on mean period of final germination (MPFG) was also evaluated. MPFG of seedlings in all samples were found to be significantly high comparative to the control. The maximum MPFG value of 2.95 was shown by the seeds of sample S-5 while the minimum was measured against sample S-23 as 2.40. Overall results showed a wide range of sediments have more than 70% higher values of MPFG as compared to the control (1.45). The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml concentration showed MPFG as 4.33 while the seeds treated with 1.0 mg/ml concentration of 2, 4 dichloro-acetic acid showed no germination to calculate MPFG.

3.1.4. Effect on germination index (GI)

Effect of sediment extracts on germination index was also determined. Germination index (GI) of seeds in all samples was found to be less affected. The seeds of 38 samples (67% of total) remained unaffected having GI value as 1.0, same as that of control. The seeds of 19 (33% of total) were observed to have lesser GI comparative to the control with minimum index was shown by samples S-3 and S-41 as 0.8. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml concentration showed very low GI as 0.15 and at 1.0 mg/ml as zero GI.

3.1.5. Effect on percent inhibition of seed germination

Percent inhibition in seed germination was found to be slightly affected by the seeds of 19 samples (33% of total). The rupturing of *Brassica napus* seeds observed during germination test in petriplate are shown in the figure 3.The two samples S-3 and S-41 were found to have the highest percent inhibition (20%) in seed germination. Remaining 38 samples (67% of total) showed no germination inhibition. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 85% and 100% inhibition in seed germination respectively.

Figure 3. Radicle protrusion and seedling development of *Brassica napus* seeds.

The results of all parameters involved in seed germination (Final germination percentage (FG%), rate of germination (RG), mean period of final germination (MPFG) and the percentage inhibition) are shown in table 2.

3.2. Effect of sediment samples on seedling growth of *B. napus*

Effect of various sediment samples were observed on seedling growth of *B. napus*. An aqueous extract of sediments at 125 mg/ml distilled water was used to evaluate the effects. The effect on root length (RL) and shoot length (SL) was determined by measuring lengths on daily basis since the day of seeds placement up to the end of 5th day. A control set of seedlings was prepared by growing it in distilled water and taken as standard for all measurements through comparing their different parameters like seedling growth, root and shoot lengths. The pictures of grown seedlings in sample S-28 and controls on day 3rd and final day 5th are shown in the figure 4 and figure 5 respectively. Effects of various parameters calculated *in-vitro* assay are given below:-

3.2.1. Effect on root length (RL)

Seedlings of all 57 samples (100%) showed percent root length inhibition activity more than 80% comparative to the control with a maximum of 92% shown by seedlings of sample S-42. Among seedlings of all samples, inhibitory effect exceeding 90% was shown by 8 samples i.e. S-15, S-17, S-21, S-28, S-34, S-41, S-42 and S-54. The minimum percent RLI (81.2%) was shown by the sample S-55. The overall result is given in table 2. These results show that all of the sediments have a wide range of inhibitory effect on the root length of seedlings. The *Brassica napus* seeds treated

with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 91% and 100% root length inhibition respectively.

3.2.2. Effect on shoot length (SL)

The seedlings of all 57 samples (100%) showed percent shoot length (SL) inhibition activity. Seedlings of 31 samples exhibited above 80% SL inhibition with a maximum of 87% shown by three samples i.e. S-28, S-34 and S-41. The remaining 26 samples showed less than 80% SL inhibition with a minimum of 62% exhibited by the sample S-37. The overall result is given in table 2. These results show that all of the sediments have a wide range of inhibitory effect on the shoot length of seedlings. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 85% and 98% shoot length inhibition respectively.

3.2.3. Effect on percent inhibition in seedling length

The lengths of all seedlings from their shoot tips to the end points of roots were measured with reference to the control. The seedlings grown in distilled water under standard experimental conditions were used as control to calculate the percent inhibition in seedling length of *B. napus*. The seedlings of control showed their maximum root and shoot lengths in an optimal growth environment while the sediment extracts were observed to oppose the natural growth of seedlings by inhibiting their root and shoot lengths. The seedlings of 3 samples i.e. S-28, S-41 and S-42 were observed to show highest (89%) inhibition in their seedling length while seedlings grown in sample S-55 exhibited lowest (73%) inhibition in seedling lengths comparatively to the control. The seedlings of 49 samples (86% of total) showed more than 80% of inhibition in their seedling lengths while the remaining 8 samples (14% of total) i.e. S-33, S-37, S-43, S-46, S-49, S-50, S-51, S-52 and S-55 were observed to exhibit less than 80% of inhibition in their seedling lengths. The overall result is given in table 2. These results show that all of the sediments have a wide range of negative effect on the seedling lengths of target specie. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 88% and 100% inhibition in the lengths of seedlings respectively.

3.2.4. Effect of sediment samples on weight loss of *B. napus*

Effect of all sediment samples on seedling weight loss of *B. napus* was observed. Seedlings grown in distilled water under standard environment were considered as control for all calculations. The seedlings of 3 samples i.e. S-3, S-5 and S-12 showed

more than 50% of weight loss with a maximum of 62.9% exhibited by the sample S-5. Seedlings grown in 37 samples (65% of total) showed less than 50% weight loss while 17 samples (30% of total) exhibited less than 30% weight loss of the seedlings grown. The seedlings grown in sample S-37 showed lowest percent weight loss (15.03%). The overall result is given in table 2. These results show that a considerable number of sediments have a negative effects on weight loss of seedlings. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 74% and 39% weight losses respectively.

3.2.5. Effect on relative dry weight (RDW)

Relative dry weight of seedlings grown in extracts of all the sediments was calculated with reference to control. Seedlings grown in distilled water under standard experimental conditions was considered as control. Overall the decrease in relative dry weight (RDW) of seedlings grown in the extracts of all samples was observed. The control seedlings showed 17.67% RDW of plant material. Comparative to the control the lowest decrease in RDW was shown by the seedlings of sample S-37 as 13.76% (22% less than control) while highest decrease was recorded against sample S-47 as 8.13% (54% less than control). Seedlings of sample S-35 showed 50% decrease in RDW as compared to control and 46 samples (81% of total) exhibited decrease in RDW more than 50% as compared to control. Remaining 11 samples (19% of total) showed less than 50% decrease in RDW compared to control. The overall result is given in table 2 which showed that seedlings in most of the sediments were found to decrease their relative dry weight percentages more than 50% but less than 80% comparative to the control. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 8.84% and 10.74% decrease in RDW of plant material respectively.

3.2.6. Effects on water content (WC)

Effect of all sediment samples on seedling water contents (WC) of *B. napus* was observed. A standard environment was provided to prepare control by growing target specie in distilled water and was used in all calculations for the determination of water contents (WC). Generally the seedlings of all samples showed elevated level of water contents with maximum of 93.7% shown by sample S-40 and the minimum content of 70.1% was shown by sample S-21. Overall the seedlings of 30 samples (53% of total) showed WC above 90%, seedlings of 10 samples (18% of total) showed WC above

80% and the remaining seedlings of 17 samples (30% of total) showed above 70% of water content. The seedlings of 8 samples i.e. S-2, S-27, S-32, S-33, S-40, S-46, S-50 and S-51 exhibited the maximum water content (> 93%) while seedlings grown in 4 samples i.e. S-3, S-21, S-35 and S-41 showed minimum water content (< 71%) as compared to the control. Overall results displayed in table 2 showed the seedlings grown in 40 samples (71% of total) showed WC above 80% and the remaining seedlings of 17 samples (30% of total) exhibited WC between 70 - 80%. Thus seedlings of majority of the samples were found stimulating towards their water contents (WC). *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 61.6% and 52.3% water contents (WC) respectively.

3.2.7. Effects on the relative water content (RWC)

Effect of all sediment samples on seedling RWC of *B. napus* was observed. A standard environment was provided to prepare control by growing target specie in distilled water and was used for all calculations for the determination of RWC. The seedlings of 39 samples (68% of total) showed higher relative water contents (RWC) comparatively to the control with a maximum of 64.3% exhibited by the sample S-2 (11% higher than control). Seedlings grown in 5 samples i.e. S-1, S-15, S-22, S-24 and S-37 showed no effect on RWC as they exhibited the same RWC as compared to the control. The remaining 13 (23% of total) samples showed lesser RWC as compared to the control with a minimum of 49.2% exhibited by sample S-3 (15% lower than control). Overall results displayed in table 2 show most of all samples produce positive effect on RWC of seedlings. *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 56.7% and 47.3% RWC respectively.

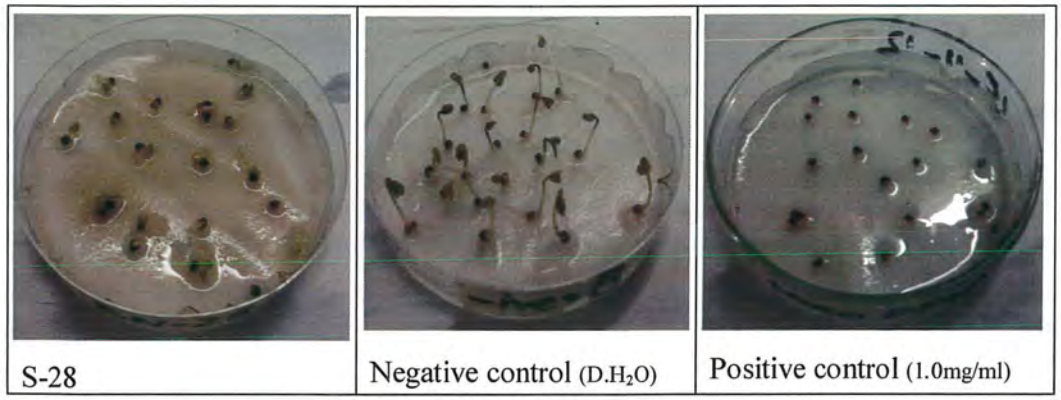

Figure 4. Seedlings grown in sample S-28 and controls on 3rd day of incubation.

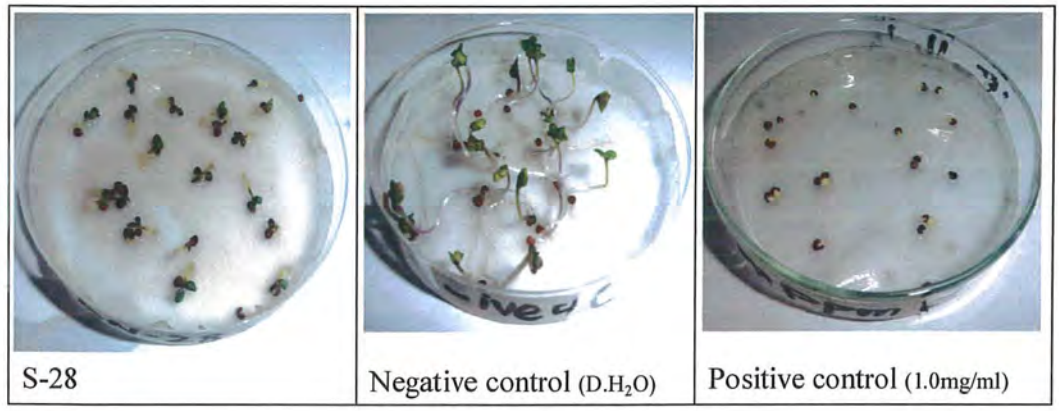

Figure 5. Seedlings grown in sample S-28 and controls on 5th day of incubation.

Table 2. Effect of sediments extracts on germination and growth of *Brassica napus*.

Sample	FG%	RG	MPFG	GI	PISG	% RLI	% SLI	% ISL	% WtL	RDW %	WC %	RWC
1	95	6.67±0.59	2.74±0.06	0.95	5	88.9	75.5	83	44.69	8.73	91.0	57.9
2	100	7.22±1.63	2.60±0.05	1.00	0	88.9	80.6	85	18.86	9.14	93.6	64.3
3	80	7.17±1.05	2.83±0.46	0.8	20	88.0	79.6	84	60.86	10.14	70.5	49.2
4	100	7.75±1.07	2.80±0.38	0.9	10	88.9	83.7	87	42.51	10.44	74.7	58.6
5	95	7.56±1.06	2.95±0.70	0.95	5	88.9	80.6	85	62.86	8.94	86.3	49.7
6	95	7.78±1.04	2.68±0.06	0.95	5	88.9	81.6	86	41.31	9.14	91.1	56.4
7	100	7.25±1.06	2.65±0.65	1.00	0	88.9	77.6	84	36.17	8.94	92.0	61.7
8	100	7.56±1.04	2.75±0.48	1.00	0	88.9	80.6	85	24.29	11.24	88.2	59.1
9	95	7.23±1.05	2.74±0.09	0.95	5	88.9	80.6	85	31.20	9.64	83.1	60.2
10	100	7.33±1.07	2.75±0.19	1.00	0	88.0	79.6	84	18.63	9.54	75.7	67.2
11	100	7.00±1.06	2.80±0.05	1.00	0	88.9	79.6	85	39.14	9.04	91.5	62.0
12	100	8.03±2.09	2.70±0.10	1.00	0	88.9	76.5	83	58.40	9.54	80.2	51.7
13	100	8.50±2.07	2.80±0.08	1.00	0	88.9	83.7	87	45.09	10.64	75.6	56.8
14	100	7.67±1.03	2.75±0.20	1.00	0	86.3	78.6	83	26.34	9.64	92.6	60.9
15	100	8.67±2.06	2.55±0.30	1.00	0	89.7	80.6	86	38.06	9.14	86.2	57.8
16	100	7.50±1.04	2.85±0.69	1.00	0	88.0	78.6	84	33.71	8.94	81.6	59.6
17	95	7.75±1.05	2.74±0.08	0.95	5	91.5	81.6	87	40.80	9.64	73.1	55.7
18	100	7.42±1.06	2.80±0.16	1.00	0	88.0	79.6	84	31.89	12.05	89.9	55.2
19	95	7.94±1.05	2.74±0.09	0.95	5	88.9	76.5	83	35.83	9.94	91.2	57.3
20	95	8.06±2.07	2.58±0.13	0.95	5	88.0	83.7	86	45.54	9.24	90.3	59.2
21	100	7.46±1.06	2.75±0.14	1.00	0	89.7	80.6	86	35.14	11.35	70.1	55.0
22	100	7.87±1.11	2.60±0.08	1.00	0	88.9	76.5	83	36.86	9.34	91.6	57.9
23	100	7.40±1.06	2.40±0.06	1.00	0	88.0	79.6	84	44.63	10.14	89.6	57.8
24	95	7.86±1.05	2.63±0.07	0.95	5	88.0	79.6	84	47.20	8.53	90.8	59.7
25	100	7.33±1.11	2.60±0.10	1.00	0	87.2	79.6	84	42.97	8.94	77.5	61.0

Sample	FG%	RG	MPFG	GI	PISG	% RLI	% SLI	% ISL	% WtL	RDW %	WC %	RWC
26	100	7.89±.102	2.65±0.08	1.00	0	87.2	72.5	80	30.63	9.74	92.0	59.5
27	100	7.36±1.06	2.45±0.15	1.00	0	88.9	73.5	82	23.89	8.73	93.5	63.0
28	95	8.25±2.06	2.70±0.41	0.85	15	90.6	86.7	89	32.69	9.64	74.5	58.9
29	100	6.97±0.82	2.70±0.30	1.00	0	86.3	75.5	81	30.46	9.24	92.4	60.0
30	100	7.67±1.05	2.75±0.09	1.00	0	88.9	81.6	86	32.51	9.14	92.3	59.5
31	100	8.35±2.11	2.70±0.11	1.00	0	88.9	82.7	86	40.86	9.24	78.4	56.2
32	100	7.83±1.06	2.50±0.07	1.00	0	85.5	81.6	84	24.74	9.24	93.0	62.1
33	100	7.67±1.17	2.60±0.16	1.00	0	88.0	63.3	77	23.43	8.63	93.6	63.4
34	95	7.83±2.11	2.47±0.06	0.95	5	90.6	86.7	89	33.77	10.54	75.9	57.1
35	100	7.92±2.06	2.60±0.12	1.00	0	88.0	72.5	81	24.11	8.84	70.8	62.7
36	95	7.42±1.32	2.74±0.14	0.95	5	87.2	72.5	80	19.14	10.44	92.7	61.8
37	100	7.44±1.05	2.75±0.60	0.90	10	88.9	62.2	77	15.03	13.76	77.3	57.6
38	95	7.44±1.05	2.74±0.13	0.95	5	88.0	75.5	82	38.97	9.14	91.5	57.2
39	100	7.75±1.05	2.45±0.15	1.00	0	87.2	78.6	83	27.83	9.44	92.6	60.5
40	100	8.22±2.06	2.75±0.25	1.00	0	87.2	77.6	83	36.51	8.94	93.7	58.9
41	80	7.28±1.04	2.65±0.16	0.80	20	90.6	86.7	89	31.77	8.23	70.6	61.4
42	95	7.36±1.13	2.74±0.08	0.95	5	92.3	84.7	89	39.20	8.33	71.2	58.7
43	100	7.94±2.06	2.75±0.03	1.00	0	82.1	68.4	76	42.46	8.43	91.7	57.4
44	100	7.67±1.07	2.80±0.14	1.00	0	88.0	78.6	84	36.29	8.73	76.3	59.7
45	100	7.31±1.03	2.65±0.13	1.00	0	87.2	79.6	84	22.69	9.04	74.9	63.8
46	100	7.89±1.28	2.80±0.17	1.00	0	82.9	68.4	76	29.20	8.33	93.3	63.7
47	100	6.69±0.91	2.60±0.10	1.00	0	85.5	79.6	83	39.37	8.13	92.4	60.2
48	95	7.69±1.06	2.84±0.18	0.95	5	88.9	83.7	87	36.23	8.73	79.2	60.2
49	100	7.00±1.05	2.75±0.09	1.00	0	84.6	69.4	78	34.97	8.94	92.2	59.3
50	100	7.50±1.05	2.45±0.05	1.00	0	84.6	66.3	76	24.91	9.24	93.0	63.7
51	100	7.33±1.12	2.60±0.053	1.00	0	86.3	69.4	79	25.31	8.94	93.2	63.5
52	100	7.11±1.11	2.70±0.12	1.00	0	88.9	78.6	84	34.00	9.74	91.6	59.9

Sample	FG%	RG	MPFG	GI	PISG	% RLI	% SLI	% ISL	% WtL	RDW %	WC %	RWC
53	100	7.83±1.15	2.65±0.15	1.00	0	88.9	78.6	84	31.83	9.04	92.5	61.1
54	100	7.40±1.13	2.60±0.13	1.00	0	89.7	79.6	85	20.06	9.44	81.8	63.8
55	100	8.17±2.11	2.75±0.18	0.90	10	81.2	63.3	73	33.77	10.04	91.4	58.9
56	100	7.29±1.14	2.80±0.09	1.00	0	87.2	77.6	83	27.37	10.64	91.7	60.1
57	100	7.33±1.11	2.75±0.11	1.00	0	88.0	81.6	85	35.60	9.74	84.1	58.0
N	100	16.22±5.01	1.45±0.05	1.00	0	0.0	0.0	0	0.00	17.67	87.5	57.9
p500	15	0.77±0.04	4.33±0.70	0.15	85	91.5	84.7	88	74.06	8.84	61.6	56.7
p100	0	0.00±0.000	0.00±0.000	0.00	100	100.0	98.0	99	39.09	10.74	52.3	47.3

FG% = final germination percentage, RG = rate of germination, MPFG = mean period of final germination, GI = germination index, PISG = percentage inhibition in seed germination, %RLI = percent root length inhibition, %SLI = percent shoot length inhibition, %ISL = percent inhibition in seedling length, % WtL = percent weight loss, RDW % = relative dry weight percentage, WC % = water content percentage, RWC % = relative water content percentage

3.3. Phytochemical contents and chemical assays

The altering effect of sediment extracts on the chemical metabolite production in *B. napus* seedlings and their antioxidant potential was evaluated by using a panel of multimode assays. The analysis utilized include determination of total phenolic contents, total flavanoid contents, total chlorophyll contents, MDA contents, DPPH free radical scavenging potential, total antioxidant capacity, and total reducing power. The detailed result of each analysis is discussed below:-

3.3.1. Effect on total phenolics content

The total phenolics content was determined in seedlings grown in extracts of all sediment samples. The control seedlings showed 19.13 µg GAE (Gallic acid equivalent) phenolics content for each 10 mg DW of plant material. Seedlings of 8 samples i.e. S-8, S-40, S-46, S-47, S-48, S-50, S-52 and S-55 (14% of total) showed phenolics content higher than the control with a maximum of 23.27 µg GAE/10 mg DW shown by seedlings of sample S-55 (21% increase comparative to the control). The remaining seedlings of 49 (86% of total) samples showed reduction in total phenolics content as compared to the control with a minimum of 9.85 µg GAE/10 mg DW by the sample S-3, which was found 49% less as compared to the control. The overall result is given in table 3. These results show that most of the sediments have a wide range of inhibitory effect on the total phenolics content of seedlings. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed total phenolics 12.42 and 11.86 µg GAE at 10 mg/ml DW of plant material respectively.

3.3.2. Effect on total flavonoids content

Evaluation of total flavonoids content was carried out in seedlings grown in all extracts of sediment samples. The control seedlings showed 29.4 µg QE (Quercetin equivalent) flavonoids content for each 10 mg DW of plant material. Seedlings of all 57 samples (100%) showed flavonoids content higher than the control with a maximum of 124.4 µg QE/10 mg DW shown by seedlings of sample S-42 (3.12 times increase in the total content comparative to the control). The least stimulatory effect among seedlings of all samples was shown by sample S-3 which exhibited 43.57 µg QE/10 mg DW of plant material which was found to produce 42% increase in total flavonoids content as compared to the control. The overall result is given in table 3. These results show that all of the sediments have a wide range of inductory effect on the total flavonoids content of seedlings. The *Brassica napus* seeds treated with 2, 4

dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 16.07 and 29.88 µg QE at 10 mg/ml DW.

3.3.3. Effects on chlorophyll contents

The effect of all sediment extracts was determined on chlorophyll "a", chlorophyll "b", their ratio and total chlorophyll content of grown seedlings.

The control seedlings showed 0.549 mg chlorophyll "a" per gram FW of plant material. Seedlings of 43 (75% of total) samples showed chlorophyll "a" higher than the control with a maximum of 2.26 mg/g FW showed by seedlings of sample S-9 (3.8 times high as compared to the control). The remaining seedlings of 14 (24% of total) sediment samples showed reduction in chlorophyll "a" comparatively to the control with a minimum of 0.206 mg/g FW by the seedlings of sample S-41, which exhibited 65% less chlorophyll "a" as compared to the control. These results show that most of the sediments have a wide range of inductory effect on the chlorophyll "a" of seedlings as displayed in table 3. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 0.049 and 0.012 mg chlorophyll "a" per gram FW of the plant material respectively.

For the evaluation of chlorophyll "b", the control seedlings showed 0.287 mg chlorophyll "b" per gram FW of plant material. The seedlings grown in sample S-6 exhibited the same content of chlorophyll "b" (0.287 mg/g FW) as were observed in the seedlings of control. Over all the seedlings of 27 (47% of total) samples showed chlorophyll "b" higher than the control with a maximum of 2.172 mg/g FW showed by seedlings of sample S-4 (6.5 times high as compared to the control). The remaining seedlings of 24 (42% of total) sediment samples showed reduction in chlorophyll "b" comparatively to the control with a minimum of 0.002 mg/g FW by the sample S-31. These results show that most of the sediments have a wide range of inductory (47%) as well as inhibitory (42%) effect on the chlorophyll "b" of seedlings as displayed in table 3. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 0.012 and 0.002 mg chlorophyll "b" per gram FW of the plant material respectively.

The total chlorophyll contents of the grown *B. napus* seedlings were determined and the control seedlings showed 0.673 mg total chlorophyll per gram FW of plant material. Seedlings of 41 (72% of total) samples showed total chlorophyll higher than the control with a maximum of 4.463 mg/g FW showed by seedlings of sample S-4 (6.5 times high as compared to the control). The remaining seedlings of 16 (28% of

total) sediment samples showed reduction in total chlorophyll contents with a minimum of 0.040 mg/g FW by the sample S-31, which was found 94% less comparative to the control. These results show that most of the sediments have a wide range of inductory effect on the total chlorophyll of seedlings as displayed in table 3. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 0.061 and 0.014 mg total chlorophyll per gram FW of the plant material respectively.

To determine the chlorophyll a ratio b, the control seedlings were observed to show Chlorophyll a : b as 0.207. Over all the seedlings of 45 (79% of total) samples showed chlorophyll a : b higher than the control with a maximum value of 163.0 shown by seedlings of sample S-31 (700 times high as compared to the control). The remaining seedlings of 12 (21% of total) sediment samples showed reduction in chlorophyll a : b comparatively to the control with a minimum value of 0.12 by the sample S-3. These results show that most of sediments exhibited the increased level of chlorophyll a : b of seedlings as given in table 3. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml concentration showed the value of chlorophyll a : b as 4.08.

3.3.4. Effects on carotenoid contents

Effect of all sediment extracts on carotenoid contents of seedlings was determined. The control seedlings showed 0.073 mg carotenoid per gram FW of plant material. Seedlings of 53 (93% of total) samples showed carotenoids higher than the control with a maximum of 0.222 mg/g FW showed by seedlings of sample S-4 (2 times high as compared to the control). The remaining seedlings of 4 (7% of total) sediment samples i.e. S-21, S-22, S-31 and S-49 showed reduction in total carotenoids comparative to the control with a minimum of 0.032 mg g^{-1} FW by the sample S-21, which was found 57% less comparatively to the control. These results show that most of the sediments have a wide range of inductory effect on the carotenoid contents of seedlings as displayed in table 3. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 0.053 and 0.038 mg carotenoid contents per gram FW of the plant material respectively.

3.3.5. Effect on malondialdehyde (MDA) content

The melondialdehyde (MDA) content was measured for seedlings grown in the extracts of all sediment samples. The control seedlings showed 38.5 µM of MDA content per gram FW of plant material. Seedlings of 52 samples showed MDA content higher than the control with a maximum of 145.4 µM/g FW exhibited by

seedlings of sample S-33 (2.3 times high as compared to the control). The remaining seedlings of 5 (9% of total) sediment samples i.e. S-1, S-2, S-3, S-7 and S-19 showed reduction in MDA content as compared to the control with a minimum of 27.7 µM/g FW by the sample S-2, which was found 28% less as compared to the control. The activity of MDA content estimation is shown in the figure 6 and the overall result is displayed in table 3. These results show that most of the sediments (91% of total) have a wide range of inductory effect on the MDA content of seedlings. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentrations showed 6.8 and 5.6 µM MDA per gram FW of plant material respectively.

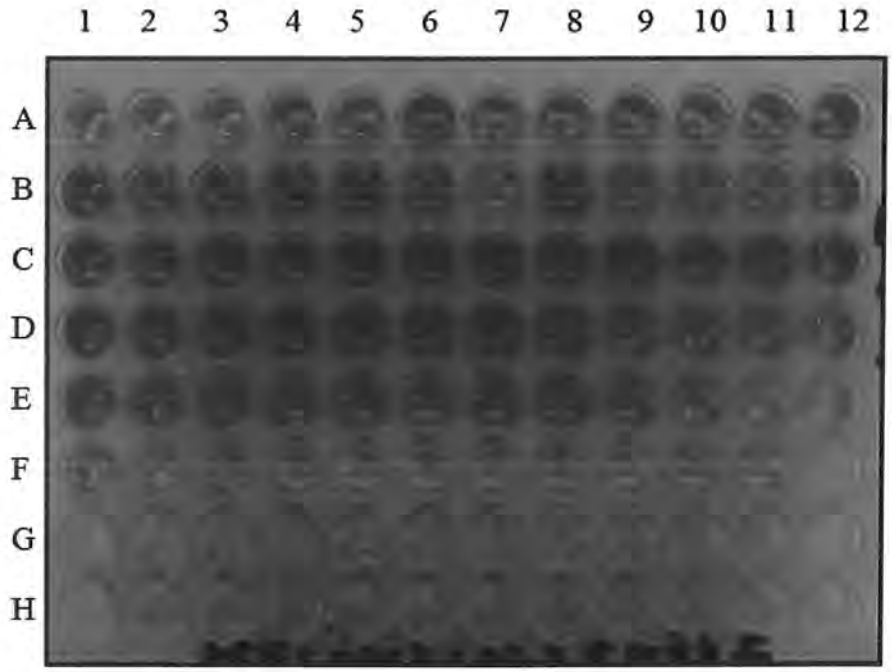

Figure 6. Estimation of melondialdehyde contents in 96-well plate format.

3.3.6. Effect on DPPH free radical scavenging activity

Seedlings grown in the extracts of all sediment samples were evaluated for the variation in their free radical scavenging potential. Free radical scavenging potential of control seedlings grown in distilled water was considered as their natural, inborn potential to combat oxidative stress in standard environment. The control seedlings showed 79.4% free radical scavenging at 10 mg/ml DW of plant material. Seedlings of 7 samples i.e. S-8, S-11, S-48, S-52, S-53, S-55 and S-56 exhibited higher free

radical scavenging activity than the control with a maximum of 87.4%. The remaining seedlings of all the 50 samples showed less free radical scavenging potential compared to control with a minimum of 19.3% by sample S-19. Overall result of free radical scavenging potential is given in table 3. Briefly seedlings of three sediment samples showed less than 30% free radical scavenging while seedlings of 4 samples showed more than 30% and less than 50% scavenging potential. Remaining seedlings of 43 samples showed more than 50% and less than 79.4% (control's activity) scavenging potential. These results show that sediments have a wide range of inductory as well as inhibitory effect on the seedlings ability to scavenge free radicals. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 75% and 78% scavenging of free radical at 10 mg/ml DW.

3.3.7. Effect on total antioxidant capacity (TAC)

TAC assay was carried out to determine the variation shown by seedlings grown in all sediment extracts. TAC of control seedlings grown in distilled water was considered as their inborn and natural potential to respond oxidative stress in standard environment. The control seedlings showed 787.3 µg AAE (ascorbic acid equivalent) total antioxidant capacity for each 10 mg DW of plant material. Seedlings of 13 samples (23% of total) exhibited total antioxidant capacity higher than the control with a maximum of 880.0 µg AAE/10 mg DW shown by seedlings of sample S-31. The remaining seedlings of all 44 (77% of total) samples showed less TAC comparatively to the control with a minimum of 490.7 µg AAE/10 mg DW by sample S-3, which was found to produce 40% reduction as compared to the control. The activity of TAC in 96 well plate is shown by the figure 7 and overall result of TAC is given in table 3. These results show that most of the sediments have a wide range of inductory as well as inhibitory effect on TAC of the seedlings. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed TAC 444.7 and 475.0 µg AAE at 10 mg/ml DW respectively.

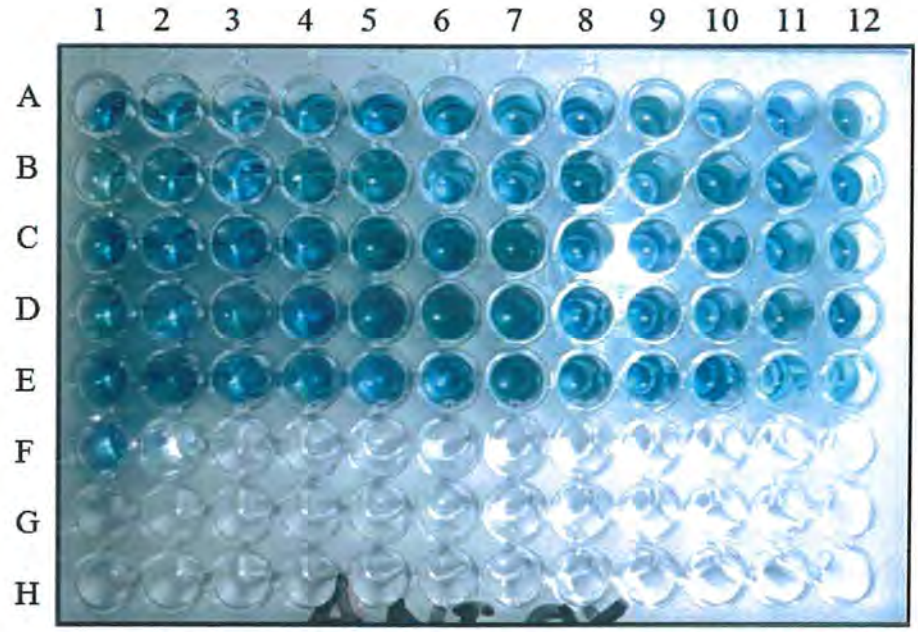

Figure 7. Estimation of total antioxidant capacity in 96-well plate format.

3.3.8. Effect on reducing power

Seedlings grown in all extracts of sediment samples were carried out for the evaluation of variation in their reducing power. Reducing potential of control seedlings grown in distilled water was considered as their ordinary, natural potential to respond oxidative stress in ideal environment. The control seedlings exhibited 400.4 µg AAE reducing power for each 10 mg DW of plant material. Seedlings of 35 samples (61% of total) showed reducing power higher than the control with a maximum of 559.3 µg AAE/10 mg DW shown by seedlings of sample S-50. The remaining seedlings of 22 (39% of total) samples showed less reducing power as compared to the control with a minimum of 239.3 µg AAE/10 mg DW by sample S-8, which was found to produce 40% lessening as compared to the control. The activity of reducing power assay in 96 well plate is shown by the figure 8 and overall result of reducing power is given in table 3. These results show that most of the sediments have a wide range of inductory and inhibitory effect on reducing potential of the seedlings. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed the reducing potential of 322.2 and 343.4 µg AAE at 10 mg/ml DW respectively.

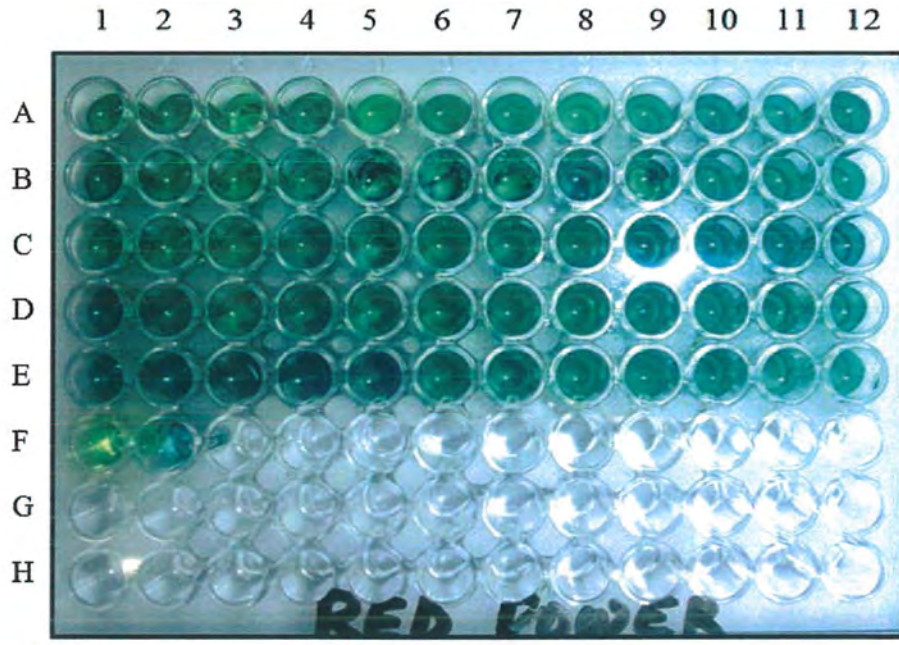

Figure 8. Estimation of reducing power in 96-well plate format.

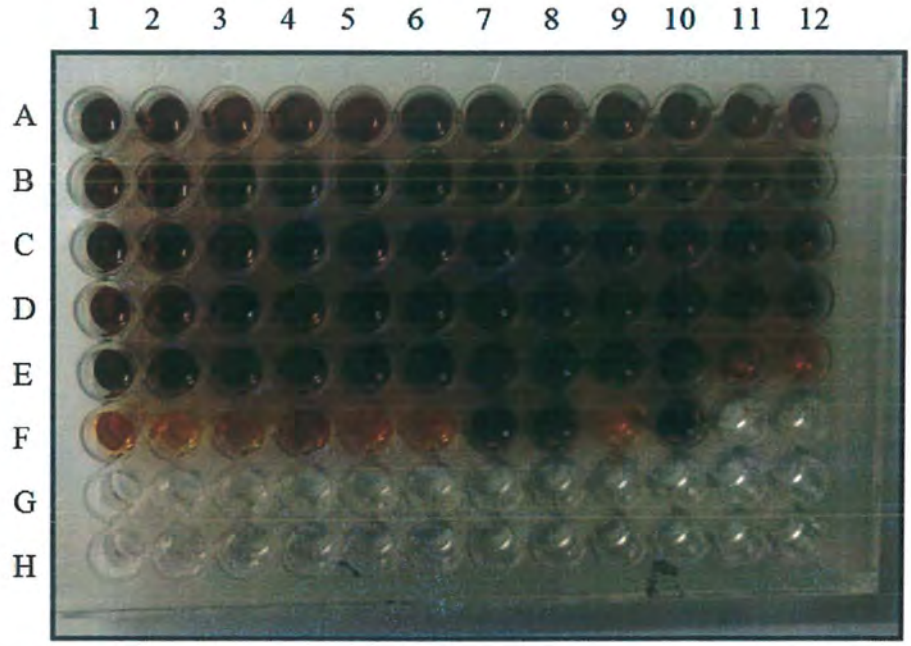

Figure 9. Estimation of peroxidases in 96-well plate format.

Table 3. Effect of sediments on biochemicals synthesis and antioxidant potential of *Brassica napus.*

Sample	Phen GAE (µg/10mgDW)	Flav QE (µg/10mgDW)	Chll a (mg/g FW)	Chll b (mg/g FW)	Tot Chll (mg/g FW)	Chl a:b	Carotenoid (mg/g FW)	MDA (µM/g FW)	% DPPH (10 mg/ml DW)	TAC (µg AAE/10mg DW)	RPA (µg AAE/10 mg DW)
1	17.33±3.02	58.10±5.07	0.642 ±0.12	0.151 ±0.07	0.752 ±0.042	4.25±1.05	0.122 ±0.062	29.5 ±4.9	67.30	709.0±46.4	356.0±15.5
2	14.74±2.03	64.05±6.52	0.384 ±0.04	0.111 ±0.05	0.376 ±0.025	3.46±0.15	0.112 ±0.041	27.7 ±3.0	65.80	577.0±11.5	368.9±6.2
3	9.85±1.01	43.57±4.69	0.677 ±0.17	0.003±0.001	0.655 ±0.031	0.12±0.01	0.106 ±0.023	33.6 ±5.5	44.00	490.70±9.6	272.4±10.1
4	12.50±1.02	50.95±5.01	2.238 ±0.27	2.172 ±0.28	4.463 ±0.142	1.03±0.06	0.222 ±0.063	47.4 ±4.3	48.30	658.0±15.8	413.8±37.7
5	17.24±2.03	100.83±7.98	1.203 ±0.15	0.382 ±0.10	1.551 ±0.035	3.15±0.44	0.112 ±0.021	45.9 ±6.5	70.70	728.0±17.0	309.8±11.5
6	13.56±1.58	95.00±6.94	1.132 ±0.26	0.287 ±0.10	1.249 ±0.026	3.94±0.58	0.146 ±0.037	86.4 ±5.9	50.60	718.70±5.9	376.6±11.7
7	15.00±2.14	66.67±4.02	0.937 ±0.15	0.494 ±0.08	1.251 ±0.040	1.90±0.05	0.150 ±0.031	37.5 ±2.5	57.20	718.30±11.0	506.1±5.8
8	21.61±3.15	92.26±5.03	0.848± 0.16	0.252 ±0.06	0.850 ±0.040	3.36±0.09	0.149 ±0.035	52.3 ±3.9	82.80	752.30±11.4	239.3±31.4
9	14.55±2.59	71.90±5.54	2.260 ±0.90	0.603 ±0.10	2.532 ±0.023	3.75±0.86	0.162 ±0.036	61.4 ±5.5	69.10	811.70±12.1	393.6±17.5
10	13.03±2.17	87.86±7.67	0.677 ±0.16	0.067 ±0.02	0.546 ±0.032	10.16±2.15	0.138 ±0.033	53.1 ±4.9	52.30	550.70±12.3	454.8±23.1
11	18.24±2.71	100.10±6.68	0.760 ±0.20	0.290 ±0.05	0.857 ±0.021	2.62±0.11	0.146 ±0.042	52.4 ±3.8	84.20	599.70±11.1	462.5±17.3
12	14.52±1.73	89.50±5.61	1.463 ±0.43	0.376 ±0.09	1.563 ±0.038	3.89±0.76	0.148 ±0.061	73.2 ±6.9	72.20	772.0±13.1	413.0±24.4
13	18.46±2.72	89.30±3.57	0.880 ±0.05	0.275 ±0.05	1.076 ±0.021	3.19±0.61	0.146 ±0.036	100.1 ±5.1	73.90	427.3±28.5	384.5±23.1
14	18.51±3.73	121.40±5.88	0.557 ±0.06	0.477 ±0.07	1.130 ±0.012	1.17±0.18	0.136 ±0.033	75.8 ±3.8	34.20	703.3±14.0	351.7±23.1
15	14.38±2.12	109.17±8.79	1.630 ±0.35	0.495 ±0.08	1.631 ±0.165	3.29±0.19	0.125 ±0.024	80.2 ±7.0	63.40	520.30±11.4	313.9±20.4
16	13.42±2.15	100.48±7.22	0.664 ±0.05	0.291 ±0.05	0.824 ±0.105	2.28±0.07	0.134 ±0.031	93.1 ±5.1	68.00	733.70±12.9	393.0±11.1
17	14.26±1.59	73.57±5.99	0.431 ±0.16	0.060 ±0.02	0.379 ±0.061	7.19±1.15	0.116 ±0.020	109.1 ±8.0	53.70	707.0±11.0	532.5±34.6
18	14.53±2.14	84.88±4.61	1.457 ±0.33	0.196 ±0.05	1.350 ±0.053	7.42±1.27	0.136 ±0.021	83.2 ±5.1	54.40	539.0±14.4	477.9±11.2

Sample	Phen GAE (µg/10mgDW)	Flav QE (µg/10mgDW)	Chll a (mg/g FW)	Chll b (mg/g FW)	Tot Chll (mg/g FW)	Chl a:b	Carotenoid (mg/g FW)	MDA (µM/g FW)	% DPPH (10 mg/ml DW)	TAC (µg AAE/10mg DW)	RPA (µg AAE/10 mg DW)
19	13.02±1.58	78.10±5.85	0.625 ±0.10	0.198 ±0.08	0.696 ±0.150	3.17±0.71	0.115 ±0.034	31.2 ±2.9	19.70	645.70±12.3	478.2±23.1
20	17.29±2.15	96.31±4.56	0.755 ±0.09	0.188 ±0.07	0.730 ±0.017	4.02±0.30	0.106 ±0.025	113.1 ±9.7	23.30	877.70±11.9	490.1±24.1
21	10.33±1.93	70.60±3.55	0.426 ±0.03	0.087 ±0.02	0.144 ±0.032	4.87±1.32	0.032 ±0.004	73.1 ±6.1	25.20	599.30±12.7	466.2±17.4
22	15.52±2.15	85.00±6.14	0.780 ±0.05	1.477 ±0.72	1.950 ±0.036	0.53±0.19	0.072 ±0.015	61.8 ±5.8	65.50	855.70±12.7	472.0±28.9
23	16.07±2.16	92.50±7.66	0.481 ±0.08	0.087 ±0.02	0.352 ±0.010	5.50±1.07	0.087 ±0.015	48.5 ±3.8	70.10	797.0±11.3	306.2±46.2
24	15.20±2.17	73.21±6.51	0.796 ±0.18	0.291 ±0.09	1.144 ±0.061	2.73±0.14	0.112 ±0.018	67.3 ±3.9	61.30	762.70±13.1	405.4±29.0
25	14.27±2.73	61.19±5.53	1.838 ±0.19	0.478 ±0.04	2.139 ±0.920	3.84±0.09	0.137 ±0.020	77.6 ±4.6	72.30	649.30±12.7	385.9±23.1
26	15.68±2.14	60.12±4.52	0.542 ±0.10	0.115 ±0.07	0.560 ±0.070	4.71±0.82	0.122 ±0.021	90.7 ±6.6	64.20	650.30±13.1	385.1±22.9
27	18.45±3.73	90.83±7.61	0.784 ±0.24	0.786 ±0.07	1.776 ±0.118	1.00±0.10	0.148 ±0.015	96.2 ±5.1	75.30	685.0±12.6	401.8±11.4
28	14.31±2.13	74.76±5.54	1.269 ±0.28	0.216 ±0.07	1.348 ±0.132	5.87±1.10	0.146 ±0.025	85.4 ±7.8	66.30	667.70±11.8	443.7±17.4
29	18.78±3.15	84.88±6.58	0.799 ±0.05	1.056 ±0.059	1.561 ±0.163	0.76±0.07	0.076 ±0.020	108.0 ±6.5	61.80	872.30±13.7	390.8±17.3
30	17.56±2.61	92.62±8.59	1.611 ±0.20	0.390 ±0.058	1.695 ±0.104	4.13±1.11	0.124 ±0.067	111.3 ±9.0	51.20	824.70±12.2	392.5±23.2
31	14.24±2.15	86.07±6.57	0.326 ±0.06	0.002 ±0.001	0.040 ±0.008	163±9.28	0.071 ±0.011	121.2 ±8.2	64.10	880.0±15.4	405.7±32.0
32	16.71±2.74	92.50±5.63	1.561 ±0.39	0.397 ±0.07	2.083 ±0.076	3.93±0.18	0.127 ±0.021	108.1 ±6.1	66.40	714.70±12.6	425.4±34.4
33	15.71±2.15	76.90±5.61	0.674 ±0.11	0.403 ±0.09	0.903 ±0.081	1.67±0.10	0.134 ±0.030	145.4 ±4.8	58.10	732.70±13.1	459.7±17.1
34	17.11±3.82	92.62±4.60	1.232 ±0.17	0.410 ±0.15	1.352 ±0.050	3.01±0.77	0.138 ±0.021	88.3 ±6.3	73.30	795.00±12.3	499.6±34.5
35	13.80±2.16	76.07±3.53	1.185 ±0.18	0.597 ±0.12	1.409 ±0.273	1.98±0.01	0.114 ±0.020	109.2 ±7.1	57.50	671.70±14.2	517.3±30.4
36	15.58±2.71	66.79±3.58	0.403 ±0.10	0.206 ±0.10	0.407 ±0.051	1.95±0.02	0.128 ±0.015	89.6 ±5.9	60.30	713.30±12.7	423.3±34.8
37	13.60±2.15	83.10±5.54	0.781 ±0.30	0.339 ±0.11	1.260 ±0.050	2.30±0.19	0.080 ±0.021	88.7 ±4.7	51.90	532.0±13.6	465.5±17.2

Sample	Phen GAE (µg/10mgDW)	Flav QE (µg/10mgDW)	Chll a (mg/g FW)	Chll b (mg/g FW)	Tot Chll (mg/g FW)	Chl a:b	Carotenoid (mg/g FW)	MDA (µM/g FW)	% DPPH (10 mg/ml DW)	TAC (µg AAE/10mg DW)	RPA (µg AAE/10 mg DW)
38	14.85±2.13	76.07±4.56	1.498 ±0.28	0.386 ±0.13	1.850 ±0.050	3.88±0.81	0.140 ±0.025	89.4 ±3.1	39.60	528.70±12.3	409.3±29.6
39	14.77±2.79	93.10±8.55	0.688 ±0.09	0.294 ±0.08	0.725 ±0.105	2.34±0.65	0.123 ±0.031	92.2 ±7.1	50.20	643.70±13.6	409.3±27.3
40	21.63±4.33	79.52±4.60	1.912 ±0.16	0.725 ±0.13	2.460 ±0.301	2.64±0.52	0.146 ±0.026	93.2 ±6.2	66.10	695.0±12.7	418.7±34.7
41	17.96±2.93	106.79±7.88	0.206 ±0.08	0.003 ±0.001	0.057 ±0.009	68.59±5.14	0.064 ±0.015	91.4 ±3.1	70.90	848.30±10.8	325.7±23.0
42	16.68±2.82	124.40±6.93	0.765 ±0.13	0.323 ±0.08	1.287 ±0.243	2.37±0.08	0.112 ±0.010	95.2 ±4.5	68.20	816.70±11.9	402.4±22.9
43	15.05±2.12	77.26±5.55	1.642 ±0.21	0.707 ±0.05	2.588 ±0.240	2.32±0.12	0.148 ±0.020	102.1 ±4.6	65.00	789.7±13.2	390.3±11.5
44	18.28±3.89	120.60±6.87	0.838 ±0.10	0.184 ±0.07	1.161 ±0.064	4.56±1.03	0.093 ±0.015	91.5 ±6.2	75.00	640.7±14.1	398.5±17.6
45	17.19±2.18	74.05±4.54	1.616 ±0.18	0.486 ±0.08	2.182 ±0.122	3.32±0.95	0.138 ±0.020	62.6 ±5.7	66.80	706.7±13.6	423.1±23.1
46	19.15±3.17	85.71±4.72	0.505 ±0.04	0.381 ±0.05	0.843 ±0.10	1.32±0.03	0.092 ±0.024	54.4 ±3.3	74.00	613.3±14.8	450.2±23.1
47	19.37±3.13	97.02±5.79	1.290 ±0.28	0.204 ±0.08	1.376 ±0.118	6.33±1.75	0.076 ±0.010	46.6 ±5.2	75.00	741.7±13.5	336.1±34.6
48	20.28±4.03	85.71±7.54	1.749 ±0.10	0.388 ±0.05	1.867 ±0.161	4.51±1.04	0.140 ±0.025	46.5 ±4.2	87.30	825.± 12.5	363.7±11.6
49	17.17±3.21	84.88±4.59	0.863 ±0.06	0.285 ±0.07	1.101 ±0.06	3.03±0.17	0.056 ±0.013	68.4 ±5.1	63.30	683.7±15.4	514.7±45.8
50	20.00±4.17	97.26±5.52	0.639 ±0.10	0.251 ±0.05	0.699 ±0.08	2.55±0.64	0.082 ±0.026	62.3 ±4.9	70.00	812.7±13.2	559.3±17.5
51	18.24±2.12	111.67±6.25	0.582 ±0.15	0.070 ±0.03	0.611 ±0.06	8.28±2.07	0.090 ±0.014	62.5 ±6.9	63.30	596.0±14.7	522.3±28.8
52	20.04±3.15	96.79±7.17	0.723 ±0.17	0.142 ±0.05	0.608 ±0.05	5.09±1.02	0.086 ±0.016	56.3 ±4.4	80.60	644.7±14.9	527.7±34.8
53	17.79±3.73	70.71±6.55	0.561 ±0.32	0.133 ±0.04	0.345 ±0.11	4.23±0.81	0.092 ±0.013	54.4 ±3.8	79.60	636.7±11.4	473.5±11.5
54	18.56±3.14	80.95±5.91	1.450 ±0.20	0.248 ±0.05	1.532 ±0.09	5.84±1.11	0.081 ±0.015	45.8 ±5.8	71.80	575.3±12.3	442.0±23.0
55	23.27±4.19	95.00±4.86	0.489 ±0.25	0.002 ±0.001	0.310 ±0.08	0.14±0.02	0.082 ±0.017	60.2 ±5.1	80.70	787.0±11.8	398.6±17.1
56	17.75±2.73	78.45±5.60	1.291 ±0.15	0.259 ±0.04	1.333 ±0.053	4.98±1.08	0.128 ±0.020	65.7 ±4.8	87.40	641.0±14.1	423.0±17.4

Sample	Phen GAE (µg/10mgDW)	Flav QE (µg/10mgDW)	Chll a (mg/g FW)	Chll b (mg/g FW)	Tot Chll (mg/g FW)	Chl a:b	Carotenoid (mg/g FW)	MDA (µM/g FW)	% DPPH (10 mg/ml DW)	TAC (µg AAE/10mg DW)	RPA (µg AAE/10 mg DW)
57	17.55±2.02	73.10±4.68	0.282 ±0.10	0.048 ±0.01	0.204 ±0.09	5.84±1.04	0.083 ±0.015	69.5 ±6.4	64.80	604.70±12.6	450.3±23.0
N	19.13±2.77	29.40±2.19	0.593 ±0.20	0.291 ±0.08	0.674 ±0.050	2.07±0.82	0.073 ±0.010	38.5 ±2.9	79.40	787.30±14.9	400.4±5.6
P500	12.42±2.11	16.07±1.32	0.049 ±0.01	0.012 ±0.002	0.026 ±0.007	4.08±1.02	0.053 ±0.005	6.8 ±1.08	75.00	444.70±18.0	322.2±10.1
P1000	11.86±1.98	29.88±3.96	0.012 ±0.00	0.002 ±0.001	0.020 ±0.006	5.87±1.04	0.038 ±0.006	5.6 ±1.88	78.80	475.0±13.2	343.4±9.9

Phen = total phenolics content, Flav = total flavonoids content, Chll = chlorophyll contents, Tot chll = total chlorophyll contents, MDA = melondialdehyde content, % DPPH= percent 2,2 di-phenyl 1picryl hydrazil, TAC = total antioxidant capacity, RP = reducing power

3.4. Effect on total protein content (TPC)

The total protein content was calculated for seedlings grown in the extracts of all sediment samples. The control seedlings showed 696.5 µg BSAE (Bovine serum albumen equivalent) of total protein content per 10 mg DW of plant material. Seedlings of 32 samples (56% of total) showed protein content higher than the control with a maximum of 807.5 µg BSAE/10 mg DW exhibited by seedlings of sample S-21 (14% high as compared to the control). The remaining seedlings of 25 (44% of total) sediment samples showed reduction in total protein content as compared to the control with a minimum of 566.5 µg BSAE/10 mg DW by the samples S-4 and S-54, which was found 19% less as compared to the control. The overall result is given in table 4. These results show that most of the sediments have a wide range of inductory as well as inhibitory effect on the total protein content of seedlings. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed total protein content of 581.0 and 591.0 µg BSAE at 10 mg/ml DW of plant material.

3.5. Effects on antioxidant enzymes activity

Superoxide dismutase (SOD) and Peroxidases (POD) enzymes are important parts of plant antioxidant system. Activities of both of these enzymes were checked for the evaluation of oxidative stress in germinating seeds of *B. napus* produced due to sediment samples. Proteases are involved in every cellular mechanism that play major role in regulating the biological processes like detecting stress conditions, pests and pathogens etc. They maintain quality control of proteins and degrade some proteins of specific sets with respect to different environmental conditions. They are also involved in the hydrolysis of stored protein to utilize as energy sources and consumption in anabolism of other proteins. The results of studied enzymatic activities POD, SOD (Superoxide dismutase) and Proteases in *Brassica napus*) are discussed below in detail.

3.5.1. Effects on activity of proteases

Protease activity was measured in the seedlings grown in extracts of all sediment samples. A control was prepared by growing the seed in distilled water in a standard environment to determine natural or inborn protease activity. The control seedlings showed 2.535 unit of protease activity per gram fresh weight of plant material. Seedlings of 19 (33% of total) samples showed protease activity higher than the control with a maximum of 3.49 U/g FW exhibited by sample S-8. The remaining

seedlings of 38 (67% of total) sediment samples showed reduction in protease activity as compared to the control with a minimum of 2.075 U/g FW by the sample S-37, which was found to reduce 18% of protease activity as compared to the control. Overall result for protease activity is displayed in table 4. These results show sediments having a wide range of inhibitory and inductory effect on the protease activity of seedlings. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 2.33 and 2.095 U protease activity per gram FW of plant material respectively.

3.5.2. Effect on peroxidases (POD) activity

Seedlings grown for two days in the extracts of sediment samples were evaluated for the variation in their peroxidases activity. A control was prepared by growing the seeds in distilled water in a standard environment to determine their natural or inborn peroxidases activity. The control seedlings showed POD activity as 0.76 nM/min/mg of FW. Seedlings of sample S-33 showed neither inductory nor inhibitory effect on POD activity by exhibiting the same activity as showed by the control given in table 4. Seedlings of only 2 samples i.e. S-55 and S-57 showed POD activity higher than the control with a maximum of 0.78 nM/min/mg of FW (table 4). The remaining seedlings of 54 samples showed reduction in POD activity as compared to the control with a minimum of 0.15 nM/min/mg of FW by the sample S-19. The overall result is given in table 4 and shown in figure 9. These results show that most of the sediments have a wide range of inhibitory effect on POD activity of seedlings. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed POD activity as 0.26 and 0.17 nM/min/mg FW of plant material respectively.

3.5.3. Effect on superoxide dismutase (SOD) activity

The effect of sediments on the SOD activity of seedlings of *B. napus* was evaluated. Over all SOD activity was inductive in *B. napus* seedlings grown in the extracts of all sediment samples for two days as compared to the control. A control was prepared by growing the seed in distilled water in a standard environment to determine natural or inborn SOD activity. The highest percent relative induction i.e. 80% as compared to control was measured in seedlings of sample S-19. The seedlings of 47 samples (82% of total) showed less than 80% of SOD relative induction activity. Seedlings of 6 (11% of total) samples exhibited the % relative induction of SOD activity below 50% and the remaining seedlings of 2 samples i.e. S-51 and S-57 showed less than 30% of

relative induction in SOD activity with a minimum of 24% by the sample S-57. The overall result of SOD activity is given in table 4. These results showed most of the samples were observed to have significant capability for induction of SOD activity in target plant. The *Brassica napus* seeds treated with 2, 4 dichloro-acetic acid at 0.5 mg/ml and 1.0 mg/ml concentration showed 14% and 0% SOD induction as compared to the control respectively.

Table 4. Effect of sediments on total protein contents and different enzymes production in *Brassica napus* seedlings.

Sample	TPC (µg BSAE/10mg FW)	Protease (U/g FW)	POD (nM/min/mg)	SOD (% RI)
1	655.5±40.10	3.44± 0.72	0.62 ±0.05	60
2	713.5±45.14	2.66± 0.13	0.55 ±0.04	75
3	659±35.66	2.825± 0.16	0.51 ±0.03	69
4	566.5±22.58	2.59± 0.19	0.40 ±0.05	77
5	618±51.02	2.115± 0.15	0.54 ±0.02	76
6	680.5±19.67	2.765± 0.19	0.70 ±0.04	69
7	598.5±33.28	2.36± 0.14	0.58 ±0.02	66
8	604.5±42.09	3.49± 0.83	0.51 ±0.06	69
9	620.5±44.88	2.435± 0.17	0.57 ±0.07	69
10	653±39.52	2.68± 0.19	0.55 ±0.03	58
11	620±35.80	2.51± 0.16	0.44 ±0.02	72
12	646±41.02	2.63± 0.19	0.63 ±0.01	75
13	665±29.36	2.33± 0.14	0.66 ±0.03	75
14	693.5±25.74	2.52± 0.17	0.56 ±0.01	67
15	706±35.99	2.455± 0.15	0.60 ±0.05	67
16	743±49.06	2.56± 0.16	0.55 ±0.04	66
17	690.5±40.21	2.46± 0.08	0.53 ±0.03	56
18	683.5±47.22	2.485± 0.11	0.62 ±0.04	66
19	739±21.56	2.53± 0.18	0.15 ±0.02	80
20	703.5±30.58	2.42± 0.16	0.57 ±0.06	73
21	807.5±58.29	2.94± 0.10	0.45 ±0.03	76
22	758.5±51.47	2.53± 0.14	0.49 ±0.01	74
23	678.5±53.08	2.41± 0.13	0.36 ±0.02	78
24	728.5±19.87	2.585± 0.19	0.58 ±0.03	62
25	691±50.39	2.32± 0.10	0.46 ±0.02	72
26	669.5±38.26	2.505± 0.16	0.61 ±0.05	69
27	727.5±24.38	2.48± 0.13	0.56 ±0.07	74
28	718±28.55	2.65± 0.16	0.51 ±0.05	73
29	766±43.12	2.69± 0.19	0.69 ±0.07	73
30	766±46.91	2.365± 0.13	0.54 ±0.03	74
31	750.5±36.16	2.115± 0.10	0.65 ±0.08	72

Sample	TPC (µg BSAE/10mg FW)	Protease (U/g FW)	POD (nM/min/mg)	SOD (% RI)
32	741.5±52.26	2.465± 0.16	0.49 ±0.03	73
33	741.5±18.28	2.915± 0.19	0.76 ±0.07	67
34	733±37.19	2.375± 0.15	0.49 ±0.03	77
35	611±32.58	2.62± 0.17	0.55 ±0.04	66
36	696±34.61	2.12± 0.14	0.55 ±0.03	60
37	612.5±39.80	2.075± 0.11	0.55 ±0.02	57
38	678.5±41.25	2.6± 0.12	0.58 ±0.04	58
39	747.5±48.37	2.685± 0.13	0.55 ±0.03	42
40	737±38.21	2.35± 0.11	0.60 ±0.04	48
41	673±35.12	2.325± 0.17	0.61 ±0.05	55
42	694±26.14	2.225± 0.16	0.57 ±0.06	70
43	789.5±61.08	2.54± 0.13	0.59 ±0.03	54
44	738.5±53.02	2.69± 0.17	0.60 ±0.02	43
45	762±42.87	2.15± 0.16	0.67 ±0.03	57
46	733±37.29	2.2± 0.14	0.60 ±0.06	57
47	726±15.93	2.15± 0.10	0.55 ±0.04	52
48	754.5±37.10	2.445± 0.15	0.62 ±0.03	34
49	745.5±27.15	2.36± 0.11	0.67 ±0.02	42
50	724±23.55	2.575± 0.18	0.56 ±0.05	34
51	753.5±46.21	2.36± 0.13	0.65 ±0.07	26
52	753.5±34.51	2.225± 0.12	0.66 ±0.05	61
53	759.5±31.08	2.085± 0.15	0.60 ±0.04	70
54	566.5±19.85	2.14± 0.17	0.62 ±0.03	49
55	758.5±24.72	2.395± 0.19	0.77 ±0.06	54
56	735±42.11	2.825± 0.16	0.67 ±0.02	57
57	801.5±66.02	2.72± 0.17	0.78 ±0.05	24
N	696.5±22.84	2.535± 0.15	0.76 ±0.06	0
P500	581±21.02	2.33± 0.10	0.26 ±0.02	14
P1000	591±12.63	2.095± 0.12	0.17 ±0.01	0

TPC = Total protein content, POD = Protease activity, Peroxidases and SOD = Superoxide dismutase

Chapter No.4

Discussion

A bioassay technique was utilized in this study by using petri-plates for ecotoxicity evaluation of sediments, which might contain different organic and inorganic contaminants of agricultural and industrial waste of Punjab, Pakistan. This assay was useful in the sense that direct exposure of target plant to sediment extracts can produce measureable effect on germination. Phytotoxicity assays are frequently used to evaluate the adverse effects of polluted sediment of hazardous riverine ecosystem on morphology and phytochemistry of plants (Frische, 2003). In this methodology, a known quantity of seeds is planted in contaminated sediment and after a preset incubation period, the emerged seedlings are counted. This requires sensitive monitoring and data collection on daily basis for calculating different parameters of germination test like final germination percentage, germination rate, germination index, mean period of final germination and percent inhibition in seed germination. The results of seedlings grown in contaminated sediment are compared to the seedlings grown in non-contaminated control (Maila and Cloete, 2002). Organic and inorganic pollutant's stress of river Chenab sediments (Eqani *et al.*, 2012) might be the cause of reducing the germination and growth which could lead to various molecular, physiological and biochemical changes in the target plant (*B. napus*).

A total of 57 sediment samples used in current study were taken from 25 different sites of river Chenab. Evaluation of the growth modulating effects of sediments was determined by using a number of morphological parameters, secondary metabolites monitoring and proteinous activity including enzymes in *Brassica napus* (Macias *et al.*, 2000). Various researchers have utilized this specie to observe the alteration in biochemical, physiological and enzymatic activities pertinent to the toxicity stress (Weir *et al.*, 2004). Such effects of the sediments of river Chenab are expected by the presence of toxic compounds, as this river receives intensive pollutants from various industries, agricultural run-off, municipal waste disposal and atmospheric deposition (Eqani *et al*, 2012). Consequently this study will be helpful in identification of the possible mode of action of stresses produced by the sediments. A panel of multimode assays was carried out to evaluate the varying effects of sediments on the metabolic processes and antioxidant power of *B. napus* seedlings. The assays utilized and their results are discussed below in detail:-

4.1. Modulation of seed germination of *B. napus*

One part of current study is comprised of the determination of modulation effects of sediment samples on *B.napus* germination. In order to analyze it in detail, various parameters are taken in account including rate of germination, germination index, mean period of final germination and percent inhibition in seed germination (Jefferson and Pennacchio, 2003).

4.1.1. Final germination percentage (FG %)

FG% was calculated on 5th day of the experiment. Most of the samples (68%) showed no effect on germination. However, a delayed germination process was observed in all samples and only few sediment samples were observed to be the inhibitors for germination of a limited number of seeds. The highest inhibitory effect (20%) on germination was produced by sediment samples S-3 and S-41 i.e. taken from downstream of river Chenab near at Punjnad Headworks and district Khanewal at river Ravi upstream respectively. As demonstrated previous study (Eqani *et al.*, 2012) the inhibitory effects produced may be due to the location of respective polluted sites affected by discharged chemicals like DDTs, heptachlor and endosulfan etc that reflected the widespread use of pesticides in surrounding agricultural lands, while the other site on River Ravi that receives heavy municipal and industrial waste (organic and inorganic contaminants) from Qasoor and Lahore cities. However, the germination of *Brassica* seeds (dicots) may not be virtually effected by the presence of any kind of contaminant in sediments due to their own nutrition obtain from hypertrophic cotyledons instead of being dependant on soil or sample nutrients at the time of germination (Bona *et al.*, 2011).

4.1.2. Rate of germination (RG)

Results showed that nearly all the samples were affected adversely when compared by the rate of germination with control. A delayed germination process was observed in seeds of all samples but no significant inhibition was noticed evidently. The seedlings of sample S-15 showed highest RG that is taken from agricultural land on upstream of Chenab where relatively low organic contaminants i.e. PCBs and OCPs, were found due to high water flow and velocity (Eqani *et al.*, 2012). While the lowest RG was exhibited by the seeds of sample S-1 i.e. taken from mainstream of river Chenab in district Khanewal where large scale application of pesticides like DDT, dieldrin, aldrin and endrin etc was observed. These may contaminate the river environment and

can be the significant source of toxic chemicals in this area (Eqani *et al.*, 2012; Ahad *et al.*, 2010). The variation in the rate of germination represents the degree of contamination of that site and is in accordance with Eqani *et al.* (2012).

4.1.3. Mean period of final germination (MPFG)

As indicated previously the sites, which were highly contaminated by various chemicals that significantly affect the physiological parameters of experimental plant. The seedlings of all samples showed inhibitory effects as their mean period of final germination was calculated higher than the seedlings grown in control. The maximum MPFG was shown by sample S-5 i.e. from river Chenab mainstream where store of obsolete pesticides are reported in this region (Eqani *et al.*, 2012). Other pollutants from nearby electric power plant waste, low dissolved oxygen, heavier chlorinated biphenyls and high OCPs can be the significant source of toxic chemicals in this area (Ahad *et al.*, 2010; Eqani *et al.*, 2012). The lowest increase in MPFG that showed mild effects was measured against the seeds of sample S-23 that was taken from cotton growing areas of Muzaffarghar district, where pesticide application takes place, but they had no direct impact on the river water pollution (Eqani *et al.*, 2012). A wide range of sediments have more than 70% higher values of MPFG as compared to the control which are in accordance with Eqani *et al.* (2012). Higher the degree of contamination, higher will be the MPFG.

4.1.4. Germination index (GI)

The effects of sediments on Germination index (GI) of the test seeds were similar to the already explained parameter of final germination percentage (FG %).

4.1.5. Percent inhibition of seed germination

According to Besalatpour *et al.* (2008), the seed's resistance to phytotoxic properties of organic pollutants (oils and other hydrocarbons) present in sediment is due to their cell wall structures. However, the seed germination is significantly inhibited by hazardous chemicals (amines, phenols, benzenes etc) and metallic pollutants (heavy metals) in soil or other samples (Maila and Cloete, 2002; Adam and Duncan, 2002). Effects of sediments on final germination percentage (FG%), germination index (GI) and percentage inhibition of the test seeds were almost similar to those previously reported by Sera, (2012) for weed plant and Banks and Schultz, (2005) for Lettuce, millet, and red clover seeds. Banks and Schultz, (2005) found that the contaminated soil decreases the germinability of seeds as we also have observed in our five days

experiment. In previous study, Eqani *et al.* (2012) has indicated the presence of different levels of organic contaminants spread over in the entire catchment of river Chenab. The two samples S-3 and S-41 were found to have relatively highest percent inhibition in seed germination while the seeds of all other samples showed no considerable inhibition on fifth day of experiment. Sample S-3 may contain high DDTs residues level and reflected the wide scale use of DDTs in the neighboring area of the collected site (Site-25) as reported by Eqani *et al.* (2012). The sample S-41 was taken from most polluted site of the river Chenab of Pakistan that receives pollutants from industries of Qasoor, Shiekhupura and Lahore districts and also from the surrounding agricultural fields due to intensive farming activities.

4.2. Modulation of the growth of *B. napus*

To evaluate the growth modulating effect, various parameters are taken into account and are described below in detail.

4.2.1. Root length (RL)

Seedlings of all samples showed % RL inhibition above 80% as compared to the control. Results of this study correlate the findings of previous study (Eqani *et al.*, 2012). The maximum inhibition in RLs of the grown seedlings was shown by S-42 i.e. taken from district Khanewal at river Ravi upstream which receives pollutants from municipal, urban, industrial discharges (organic and metallic pollutants) and agricultural runoff (pesticides and other toxic chemicals) from various cities of Punjab i.e. Lahore, Qasoor and Shiekhupura Eqani *et al.*, 2012). RLs of plants are significantly affected by metallic toxicity (Araujo and Monteiro, 2005). These pollutants initially inhibit the root lengths before acting on shoot lengths of the newly grown seedlings (Chen *et al.*, 2000). The root tissues have greater potential to accumulate metallic pollutants in any condition (excess or deficiency) rather than to transport these towards shoots (Fuentes *et al.*, 2007). So the RL of seedlings may be affected by all such kind of metallic and non-metallic pollutants released by industrial cities of Punjab in a greater extent.

4.2.2. Shoot length (SL)

The maximum inhibitory effect on SLs of seedlings were observed against the samples S-28, S-41 and S-34 collected from those sites (Site-2, Site-8 and Site-18) which were closed to the urban and industrial areas. These sites receive largest amount of the toxic waste from surrounding agricultural fields, industrial drains and

urban run-off from industrial cities like Faisalabad (Eqani *et al.*, 2012). This hazardous waste contains a lot of harmful chemicals and heavy metals that may increase the toxicity of sediments towards the SLs and growth of experimental plant (Jidere *et al.*, 2012). Metals in low concentration are more essential to the growth of shoots as micronutrients however, high concentration of these metals are phytotoxic (Xiong and Wang, 2005). Song *et al.* (2006) showed that seedling growth in soil contaminated with petroleum oil showed the sensitivity of three phytotoxicity parameters as RL > SL > RG. Our results are also in accordance with Xiong and Wang, (2005) and Song *et al.* (2006).

4.2.3. Percent inhibition in seedling length

Seedlings grown in all samples showed significant decrease in their lengths as compared to the control. The samples showing inhibitory effects in seedling growth of experimental plant were reported as hazardous in previous study by Eqani *et al.* (2012). These samples were taken from the areas having elevated levels of organic (Pesticides, POPs, OCPs and PCBs etc) and inorganic (mainly metallic) (Jidere *et al.*, 2012) contaminants. Organic pollutants, accumulated in sediment may induce physical constraints which in turn inhibit seed germination and subsequent seedling growth (Adam and Duncan, 2002). In present study, the highest inhibition (89%) was exhibited by three samples i.e. S-28 taken from a site close to the industrial and urban areas, which receives largest amount of the noxious waste from urban, agricultural and industrial areas, Samples S-41 and S-42 were both taken from an area close to river Ravi upstream that receives toxic pollutants from different industrial discharges of Lahore, Qasoor and Shiekhupura districts. It seems that organic contaminants indirectly affecting the sediment's capability to provide favorable environment, nutrients and water to seeds for their proper germination and growth (Besalatpour *et al.*, 2008). The lowest inhibition (73%) of seedling length was shown by sample S-55 i.e. taken from Site-2 at downstream of river Chenab considered as an urban and industrial site. Considerably a higher level of DDTs was observed in this site (Eqani *et al.*, 2012). Results showed that the seedlings of 86% samples exhibiting more than 80% inhibition in their lengths. Thus most of the samples are considered to have a wide range of negative effect on the seedling lengths of target specie. This study shows significant inhibitory effects of sediments on seedling growth of *B. napus*. Such kind of relevant studies were also carried out by Escudero *et al.* (2000) and

Macias *et al.* (2000) by working on other target plant species treated with the extracts of plant and soil samples. They found the sample extracts produce inhibitory effects in target species. Our results are in agreement with their findings and show a wide range of inhibitory effect on seedling growth.

4.2.4. Percent weight loss in seedling

Among all samples tested, the highest weight loss was observed against the seedlings of sediment sample S-5 i.e. taken from moderately polluted site (Site-14) of Chenab mainstream which has reduced % weight up to 62.9% comparative to the control. Here the store of obsolete pesticides is reported in this region that showed high values of DDT, endrin, dieldrin and aldrin, in collected water samples (Eqani *et al.*, 2012). The seedlings of 2 other samples showed more than 50% of weight loss i.e. S-3 taken from an agricultural area (Site-25) receiving pollution load from surrounding agricultural lands, however, pollutants load from upstream flow cannot be ignored and S-12 taken from a site (Site-23) near the urban and industrial areas of high pollution threats and posing potential risks to the entire Chenab ecosystem (Eqani *et al.*, 2012). Seedlings grown in 65% of total samples showed less than 50% weight loss while 30% of total samples exhibited less than 30% weight loss of seedlings. Results showed that sediments have a wide range of negative effects on weight loss of seedlings. Hazardous chemical compounds present in sediment, like polycyclic aromatic hydrocarbons (PAHs), indirectly disrupt the water-air relationships of plants (Renault *et al.*, 2000). Different seeds respond separately against same sample and it depends on structure, size (Hodgson and Mackey, 1986) and coat penetrability of seed (Hanley and Whiting, 2005). Such kind of studies was carried out by various plant scientists like Prati and Bossdorf, (2004) and Sodaeizadeh *et al.* (2009). These scientists found that the sample extracts producing inhibitory effects on weights of the target species is concentration dependent. Higher concentration of sample extracts produce significant inhibitory effects and our results are in agreement with their findings. The samples taken from hazardous sites (Eqani *et al.*, 2012) showed significantly negative effects in experimental plant as compared to other non-hazardous sites.

4.2.5. Relative dry weight (RDW)

The seedlings grown in all samples showed decrease in their RDW comparative to the control. The lowest decrease in RDW (Table 3.1) was shown by the seedlings of

samples that were taken from agricultural areas containing relatively lower concentrations of chemical contaminants like POPs, OCPs, Cyclodiens and PCBs (Eqani *et al.*, 2012). While the highest decrease in RDW was recorded against those samples which were considered the most contaminated due to heavy load of organic and inorganic pollutants discharged from urban and industrial as well as agricultural wastes (Eqani *et al.*, 2012). As illustrated in results (Table 3.1), the seedlings in most of the samples (81%) showed above 50% decrease in their RDW comparative to the control. Under stressful conditions growth rate and size of leaf are first affected, followed by a decrease in rates of transpiration due to partial closure of stomata potentially (Kirnak *et al.*, 2001). Results showed, there were significant reductions in dry weight of seedlings grown in samples under stressful conditions as compared to the control. These results are in accordance with the findings of Sun *et al.* 1995 and Chartzoulakis *et al.* (1993). Our results showed that toxic substances in the sediments might have produced significant reduction in relative dry weight of the plants. Chartzoulakis *et al.* (1993) and Kirnak *et al.*,(2001) reported the same adverse effects of stressful conditions on dry weight in *Kiwifruit* and *Egg plants* respectively.

4.2.6. Water content (WC) of seedling leaves

Water is a very crucial and vital constituent of life. Its presence in leaves is used to assess water status in plants (Mittler, 2002). In present study, all samples increased the water contents of plant. Although majority of the samples collected from polluted sites (Eqani *et al.*, 2012) produced negative effects on growth of target plant. The significant increase in WC was found in seedlings of samples S-2, S-33 and S-40 that were taken from an agriculture area where pesticides are extensively used. Due to stressful conditions of the polluted sites the basic metabolic processes of seedlings may be disturbed, which consequently leads to inhibition of the synthesis of plant metabolites like proteins (Dubey, 1994). Water is the major medium for transporting nutrients and metabolites in plants (Ejieji and Adeniran, 2010). The depressed metabolic processes decreases the biochemical contents like proteins, consequently the water contents become greater in proportion (Olaniyi and Ajibola, 2008). When these plants are allowed to dry, their net weight becomes very low comparative to their fresh normal weight (Olaniyi and Ajibola, 2008; Ejieji and Adeniran, 2010). So, the seedlings grown in samples of polluted sites showed higher water contents due to stressful conditions. The lowest stimulating effect was shown by seedlings grown in

samples S-3, S-21, S-35 and S-41 that were taken from significantly polluted areas having high levels of heavier chlorinated biphenyles, low dissolved oxygen due to electric waste received from a nearby electric power plant (Eqani *et al.*, 2012). Metallic pollution also inhibits the water uptake and transport in plants (Becerril *et al.*, 1989). Similar studies has been reported by Barkosky and Einhellig, (2003) who described the effects of various chemicals on water content of target plant, which are in accordance with our findings.

4.2.7. Relative water content (RWC) of seedling leaves

Relative water content can be used to assess the status of water in target plants in response to a number of stress conditions (Singh *et al.*, 2009). Results showed that RWC of *B. napus* leaves was not significantly affected by most of the sediment samples. The samples taken from least contaminated areas showed high contents of RWC while the samples of highly contaminated areas with organo chlorine pesticides, urban and industrial wastes (Eqani *et al.*, 2012) exhibited the low contents of RWC in experimental plant as shown in the results on table 3.1. Total 13 samples have reduced RWC and its lowest value (15% less) was observed due to the sample S-3 that contains high DDT residues, contaminated from neighboring agricultural fields (Eqani *et al.*, 2012). Most of samples (68%) have positive effect on RWC of seedlings with a maximum of 11% increase (comparative to the control) was produced by the sample S-2 i.e. taken from the least polluted site (Site-19) having lowest values of organic contaminants like POPs, OCPs and PCBs (Eqani *et al.*, 2012). Therefore, the results of RWC vary broadly ranging from 15% decrease to 11% increase.

4.3. Phytochemical contents and chemical assays

A panel of multimode assays was carried out to evaluate the varying effects of sediments on the phytochemical contents of *B. napus* seedlings. The assays utilized and their results are discussed below in detail:-

4.3.1. Total phenolics content

Phenolics are the group of organic aromatic compounds in which -OH is linked with aromatic hydrocarbon (Khoddami, 2013). Total phenolics are one of the markers for the evaluation of oxidative stress (Inderjit and Nilsen, 2003). Poly-phenols and other oxygenated phyto-constituents are considered to have significant free radical scavenging potential and antioxidant power (Devasagayam *et al.*, 2004). Results of this study showed that most of sediment samples were exhibiting inhibitory effects

towards phenolics production because 86% samples reduced the total phenolics content in target plant comparative to the control. Only 14% samples have stimulating effect on total phenolics content with maximum of 21% more than control. This highest increase was shown by seedlings of sample S-55 i.e. taken from an agricultural site (Site-24), but receives pollutants from surrounding rural, urban and industrial areas (Eqani *et al.*, 2012). Considerably higher level of DDTs was observed in this site (Eqani *et al.*, 2012). Results indicate that elevated level of phenolic compounds is present in seedlings of those samples that were taken from the downstream areas of river Chenab i.e. Site-21, Site-22, Site-24 and Site-25. This may be due to the oxidative stress produced by samples of highly polluted sites. In response to this oxidative stress target specie has produced the high contents of poly-phenols (Romero *et al.*, 2004b). The lowest phenolics content was observed in seedlings of sample S-3 i.e. taken from a least polluted site of Chenab having lowest concentrations of POPs, OCPs and PCBs as reported in previous study (Eqani *et al.*, 2012). It illustrates that some pollutants still do not have enhancing effect on phenolic compounds in target plants.

4.3.2. Total flavonoids content

Flavonoids are the secondary plant metabolites that are subjected to various stressful conditions like low nutrient situations, oxidative stress, low temperatures, injury or any infection (Ruiz *et al.*, 2003). They may act as antioxidants through different ways, most likely by free radical scavenging during which the flavonoid type phenolic substances can break the chain reaction of free radicals (Croft, 1998). In current study, results showed that seedlings of all samples had flavonoids content higher than the control. The maximum content was exhibited by sample S-42 i.e. taken from district Khanewal at river Ravi upstream. This sample might have produced the significant oxidative stress (Romero *et al.*, 2004b) due to its location on most polluted river of Pakistan, receiving heavy municipal and industrial waste from Qasoor and Lahore cities (Eqani *et al.*, 2012). It indicates the stress generated by sediments may enhance the production of flavonoids in target plant (Khan *et al.*, 2012). Stressful conditions enhance the total flavonoids content in plants to combat such environmental and oxidative stresses (Agati and Tattini, 2010). The least stimulatory effect among seedlings of all samples was shown by sample S-3 i.e. taken from a least polluted site

of Chenab having lowest concentrations of POPs, OCPs and PCBs as reported in previous study of Eqani *et al.* (2012).

4.3.3. Chlorophyll contents

Generally the chlorophyll contents in plants are measured with the aim of evaluating the impact of environmental stress and variation in the pigment content are linked to visual indications of plant diseases and photosynthetic productivity (Parekh, 1990). Being photosynthetic, plants have the greater potential to tolerate environmental and oxidative stresses (Valladares and Niinemets, 2008). However, various characteristics of plants like their physiological and morphological traits may be affected by disturbance in chlorophyll content, growth rate, photosynthetic rate, food storage and respiration rate etc (Valladares and Niinemets, 2008). Results of the current study are in accordance with previous work (Eqani *et al.*, 2012), which reported the levels of some important organic pollutants along the stretch of river Chenab. The samples of highly polluted sites can disturb the physiological and morphological processes of experimental plant and induce oxidative stress (Mittler, 2002). The effect of sediments was evaluated for chlorophyll a, chlorophyll b, their ratios and total chlorophyll of target plant. Seedlings of 75% samples showed chlorophyll "a" higher than the control with a maximum content showed by the seedlings of sample S-9 i.e. taken from a least polluted site (Site-6) situated after the joining of Jhelum River with Chenab. It is an agriculture area of the Jhang district which is not affected by urban or industrial wastes. This might be the reason of higher chlorophyll contents of seedlings which were grown in non-contaminated sediment samples (Lamb, 1997). The remaining seedlings of 24% sediment samples showed reduction in chlorophyll "a" comparatively to the control with a minimum showed by the sample S-41 i.e. taken from the most polluted site (Site-8) located on River Ravi before joining with River Chenab that receives pollutants from intensive farming activities and industries of Qasoor, Lahore and Shiekhupura cities (Eqani *et al.*, 2012). These intensively toxic chemicals and metallic pollutants may reduce the production of chlorophyll by creating stressful conditions in target plant (Mahhou, 2005).

In the case of chlorophyll "b", seedlings of 47 % samples showed higher chlorophyll content, 42% exhibited the reduced content of chlorophyll "b" and the remaining 11% showed the same content of chlorophyll "b" comparative to the control. This observation is in accordance with the above discussed figures of Chlorophyll "a". The

whole of our results show that stressful conditions lead to a reduction of the average chlorophyll contents in experimental plant (Ghozlene *et al.*, 2013) while the seedlings grown in samples of productive areas with respect to agriculture view point showed higher contents of average chlorophyll. The amount of Chl "a" was detected as higher than that of Chl "b" i.e. in accordance with the past relevant studies (Aguero *et al.*, 2008). Likewise the results showed seedlings of majority (72%) of the samples contain higher contents of total chlorophyll comparative to the control, while the remaining 28% exhibited lower contents of total chlorophyll as compared to the control. Under the favorable environmental conditions, chlorophyll contents are increased (Boonpragob and Nash, 1991). While the decrease in total chlorophyll contents is due to the presence of higher concentrations of toxic substances (Xia and Tian, 2009). Our results indicate that the chlorophyll content was influenced by toxic emissions like pesticides, PCBs, POPs and metallic pollutants (agricultural run-off, municipal, urban and industrial discharges). As the concentration of pollutants in urban and industrial areas are higher than agricultural lands of rural areas where nitrogen and sulphur compounds can serve as nutrients. It was suggested that chlorophyll synthesis could be stimulated at the agricultural sites due to nitrogen compounds (NO_3- NH_4+), which are beneficial to plants (Boonpragob and Nash, 1991). In our study, a higher content of chlorophyll a and b in the rural areas could be explained by a fertilizing influence of collected sediment samples. Previous study (Eqani *et al.*, 2012) has reported the occurrence of various organic contaminant levels in the sediments of river Chenab like POPs, OCPs and PCBs. Joshi, *et al.* (2009) has also observed the significant reduction in chlorophyll a, b and their total content against various extracts of secondary plant metabolites that are released into the environment. Decomposition of chloroplast also reduces the chlorophyll content of plants (Cornoy *et al.*, 1988). Various other researchers have observed the reduction in chlorophyll content in different plants under stressful conditions (Abu-Romman *et al.*, 2010; Bagavathy and Xavier, 2007).

Chlorophyll a ratio b, which can be used as a stress indicator, was measured higher in seedlings of majority of the sediment treatments. Results showed the seedlings of 79% samples had chlorophyll a ratio b higher than the control with a maximum shown by seedlings of sample S-31 i.e. taken from agricultural area of Nikokara, a town suspected to organochlorine contamination (Eqani *et al.*, 2012). High chlorophyll a

ratio b is due to the environmental stresses in plant leaves (Delfine *et al.*, 1999). The remaining seedlings of 21% samples showed reduction in chlorophyll a ratio b comparatively to the control with a minimum exhibited by seedlings of sample S-3 i.e. taken from downstream of Chenab i.e. suspected to contaminate with agricultural run-off from surrounding fields. The decreased level of chlorophyll a ratio b in target plant under stressful conditions indicated that various organic and inorganic pollutants like pesticides and heavy metals may change the amount of chlorophyll in plant (Tewari *et al.*, 2002; Parekh, 1990).

4.3.4. Carotenoids content

The main role of carotenoid is to prevent the generation of singlet oxygen and protects from oxidative damage through direct quenching of triplet chlorophyll (Ramel *et al.*, 2012). Here the transfer of excitation energy is involved which is followed by thermal deactivation (Ramel *et al.*, 2012). Carotenoids are the set of lipophilic antioxidants that inhibit oxidative damage and promote plant development in response to biotic or abiotic stresses (Armstrong *et al.*, 1996). In current study, seedlings of 93% samples showed carotenoids content higher than the control with a maximum showed by seedlings of sample S-4 i.e. taken from downstream of Chenab near at Punjnad Headworks. The higher contents of carotenoids in seedlings of majority of the samples are in accordance with the work of Wang *et al.* (2001), who reported that the carotenoids content increase under stressful conditions. As most of our sediments are affected with organic contaminants like POPs, OCPs and PCBs from the surrounding agricultural fields where an intensive amount of pesticides are used (Eqani *et al.*, 2012). Elevated levels of carotenoids are also observed in seedlings of those samples which were collected from agricultural areas where a huge quantity of pesticides are commonly used. While the remaining seedlings of only 7% samples i.e. S-21, S-22, S-31 and S-49 which were taken from urban and industrial areas showed reduction in their total carotenoids comparative to the control. The minimum carotenoids content was shown by the sample S-21 i.e. taken from industrial drain joining with Chenab in district Jhang. Such samples may contain various organic and inorganic pollutants like hazardous chemicals of waste effluents released from factories and heavy metals etc which can damage the entire synthetic machinery of plant (Mishra *et al.*, 2011). The enhancement of carotenoids content may also correlated with the improved tolerance of target plant against various environmental stresses (Zaefyzadeh *et al.*, 2009).

4.3.5. Malondialdehyde (MDA) content

End product of lipid peroxidation is the malondialdehyde (MDA) which could be used to predict the cellular damage and is considered as an important marker for observation of membrane lipid peroxidation (Cai *et al.*, 2011). Reactive oxidative species are produced against various internal and external stresses that lead to induce the lipid peroxidation. Greater amount of MDA is an indication of more lipid peroxidation so its content is quantified to study the cellular membrane injury or detrimental effects. Seeds of *B.napus* are more sensitive towards sediment samples which could be the basis for oxidative damage and are assessed by using Malondialdehyde as an important marker (Sung, 1996). In the present study, seedlings of 91% samples showed MDA content higher than the control with a maximum value exhibited by seedlings of sample S-33 i.e. taken from agriculture area of the Khanewal district. Here a huge quantity of pesticides is used and the run-off from surrounding agricultural fields is suspected to contaminate the sediments and water of river Chenab (Eqani *et al.*, 2012). Those contaminated samples may damage the cells of target plant owing to contain the elevated levels of MDA. The remaining seedlings of only 5 samples i.e. S-1, S-2, S-3, S-7 and S-19 showed reduction in MDA content as compared to the control with a minimum exhibited by the sample S-2 i.e. taken from an agricultural area of Chinyot district. This site is famous for the cultivation of various important crops like rice, sugarcane and wheat etc (Eqani *et al.*, 2012). The sample may produce least cellular injury in target plant by exhibiting the low MDA content (Cai *et al.*, 2011).

4.3.6. DPPH free radical scavenging activity

The degree to which discoloration of DPPH radical occurs is mainly attributed to the donation ability of hydrogen in tested compounds (Sharma & Bhat, 2009). Diphenyl-picryl-hydrazine forms a stable free radical in methanol which gives violet color. Upon addition of samples, DPPH turns out to be reduced, which can be observed by the change of color i.e. from violet to yellow (Kamkar *et al.*, 2010). This assay is performed to test the reducing capability of sample by using DPPH (Singleton *et al.*, 1999). In current study, the results of DPPH radical scavenging activity were related to those of the total phenolic content as phenolic compounds have been demonstrated to exhibit a scavenging effect for free radicals (Shahidi & Wanasundara, 1992). The stressful conditions of urban and industrial toxic waste caused a significant decrease

in the DPPH radical scavenging activity of 88% samples. The minimum DPPH radical scavenging activity was exhibited by the seedlings of sample S-19 i.e. taken from a renowned agricultural area of Multan district. Previous study showed presence of elevated levels of various contaminants of sediments like POPs, OCPs and PCBs (Eqani *et al.*, 2012). These sediments can induce oxidative stress as well as metabolic stress which can lead to lessening the DPPH activity as compared to control (Bressano *et al.*, 2010). Seedlings of only 7 samples exhibited the increase in DPPH radical scavenging activity. These samples were taken from agricultural sites of rural areas but are linked with a number of small tributaries that receive heavy load of industrial waste from renowned industrial cities of Punjab (Eqani *et al.*, 2012). Such pollutants may contain various organic and inorganic contaminants like pesticides and heavy metals that can produce stressful conditions for the growth, development and biochemical processes of plants (Gniazdowska *et al.*, 2005). Sediments contain chemical and metallic pollutants of urban and industrial wastes that can induce stress in plants leading to the production of more antioxidant compounds (Keddy *et al.*, 1995; Eqani *et al.*, 2012).

4.3.7. Total antioxidant capacity (TAC)

This method is cost effective and simple. Ascorbic acid equivalent is used for the measurement of antioxidant potential of samples (Prieto *et al.*, 1999). Here sample itself oxidizes and reduces the Mo (VI) to Mo (V) in reaction mixture. Mo (V) complex is a green in color, which is formed in acidic environment. The total antioxidant capacity of the seedlings grown in 13 samples was found to be higher when compared to the control, showing their greater antioxidant potential. According to the previous study (Eqani *et al.*, 2012), majority of these samples were taken from agricultural areas, not affected from urban and industrial pollutants. These sediment samples could be considered as the most important contributor in the production of phenolic compounds like flavonoids, which can enhance the overall antioxidant effectiveness of plants (Sharififar *et al.*, 2007). The remaining seedlings of 77% samples showed low TAC as compared to the control. Majority of the samples were found to inhibit TAC in seedlings, which may be due to contaminations of moderate to high levels of organic and inorganic pollutants as reported in previous studies (Eqani *et al.*, 2012). Organic and inorganic pollutants of industrial and urban sites trigger oxidative and metabolic stresses commonly exhibited by decreased TAC as

compared to the control (Bressano *et al.*, 2010). The results of present study are in agreement with the observations of Halvorsen *et al.*, (2002) who found significant decrease in TAC of tested samples.

4.3.8. Reducing power

The determination of reducing power of plants has been used as one of the significant parameters to observe the antioxidant ability of plants (Tanaka *et al.*, 1988). Their redox properties are involved in neutralizing, decomposing and quenching free radicals (Osawa, 1994). To determine the reducibility of sample, method described by Sarikurkcu, (2011) was followed, in which transformation of Fe^{3+} to Fe^{2+} was observed. The higher electron donating capacity of biologically active compounds i.e. phenolics and flavonoids of plant extract may be involved in increased reducing potential (Justesen and Knuthsen, 2001). Reducing ability of samples depend on their antioxidant activity (Tanaka *et al.*, 1988). In present study, seedlings of 61% samples showed higher reducing power comparative to the control. The maximum value was shown by the seedlings of sample S-50 i.e. taken from an agricultural site (Site-24), receiving pollutants from surrounding rural, urban and industrial areas. Considerably a higher level of DDTs was observed in this site (Eqani *et al.*, 2012). Such samples can induce stresses in plants due to intensive pesticides, chemicals and metallic contamination of agriculture, urban and industrial discharges, as reported in the previous study (Eqani *et al.*, 2012). Such stressful conditions may lead to the production of more antioxidant compounds like flavonoid and phenolic compounds that can enhance the reducing abilities of target plant (Keddy *et al.*, 1995). The reducing property of test plant reflects the presence of reductones (Duh, 1998). These are not only responsible to break the free radical sequence by donating single hydrogen, but also react with precursors of peroxide with quenching capability of peroxide formation (Gordon, 1990). The remaining seedlings of 39% samples showed less reducing power as compared to the control. Minimum activity was exhibited by sample S-8 which was taken from downstream of river Chenab near Punjnad Headworks, which is least polluted area as reported in the previous study (Eqani *et al.*, 2012). These less polluted samples may not be able to induce considerable stress in target plant which leads to the less production of antioxidant compounds and secondary metabolites to cope with oxidative stress (Keddy *et al.*, 1995).

4.4. Total protein content (TPC)

Different impacts on plant biochemistry such as the changes in protein synthesis, production of new sets of stress induced proteins, alteration in the behavior of various enzymes, genetic modifications and depletion of certain metabolites occur when they are subjected to stressful environmental conditions (Dubey, 1994). In present study, seedlings of 56% samples showed higher protein content comparative to the control. Maximum content was exhibited by seedlings of sample S-21, taken from the most polluted site on an industrial drain joining with Chenab in district Jhang. Sample of this site contains the highest values of POPs, OCPs and PCBs contamination, due to the heavy load of industrial effluents receiving from Faisalabad city (Eqani *et al.*, 2012). Plants may synthesize fresh proteins on getting exposure to adverse environmental situations (salinity, metallic pollutants particularly cadmium, osmotic and oxidative stresses and organic pollutants etc) (Dubey, 1994; Shah and Dubey, 1998). These newly synthesized proteins under stressful conditions enhance the survival of plants (Dubey, 1994). Our results are corroborated with findings of Dubey, (1994) and Shah and Dubey, (1998) who found that, protein content of target plant may be enhanced under stressful environmental conditions. However, various researchers had also found the decrease in total protein content of target species under stress conditions which are contrary to our findings (Costa and Spitz, 1997; Palma *et al.*, 2002). In another report, abiotic stress inhibited the protein synthesis while promoted some other stress-induced proteins in plants (Ericson & Alfinito, 1984) with a trend of decline in overall content. As observed in current study, the remaining seedlings of 44% samples showed reduction in total protein content as compared to the control. The minimum content was exhibited by the samples S-4 and S-54, taken from agricultural area of river's downstream side, where pesticides are extensively used in surrounding fields for better crop yield (Eqani *et al.*, 2012). The decrease in protein contents may be due to the various types of toxic chemicals having potential to degrade (Mersie and Singh, 1993) or inhibit the synthesis of protein in plants (Einhelling, 1996). The decrease in total content of protein may be due to the adverse environmental conditions and presence of toxic chemicals (Mersie and Singh, 1993). Stressful conditions, chemical contaminants and heavy metals may have induced fragmentation of proteins and lipid peroxidation in target plant due to the toxic effects of ROS (Davies *et al.*, 1987). Although the specific proteins due to stress-induction

can be identified, but still the plant scientists are investigating to measure whole metabolic status of soluble and total protein contents of different metabolic pathways in plants grown under stress conditions. It can lead to the simple evaluation of impact of stresses on different parameters of biochemistry and morphology of plant (Dubey, 1994; Shah and Dubey, 1998).

4.5. Antioxidant enzymes activity

In nature, plants are provided with numerous biochemical mechanisms to combat with oxidative and environmental stresses. Superoxide dismutases, catalases and peroxidases are the vital antioxidant enzymes in the metabolism of ROS, generated under stressful conditions (Blokhina *et al.*, 2003). These enzymes are the integral parts of plant antioxidant system (Milosevic *et al.*, 2010). In present study, activities of protease, peroxidases (POD) and superoxide dismutase (SOD) were evaluated by using *B. napus* as experimental plant against 57 sediment samples. The results of studied enzymatic activities are discussed below in detail.

4.5.1. Effects on activity of proteases

Proteases are involved in each aspects of the life cycle of plant ranging from the stored protein mobilization during germination of seed to the disruption of metabolism and initiation of cell death (Muntz *et al.*, 2001). This enzyme plays a vital role during protein mobilization throughout the chemical or environmental stresses (Domash *et al.*, 2008). However, such stresses can lead to inhibition or damaging of proteases which possibly may result in failure in seed germination (Schaller, 2004). In current study, basal level of protease activity was remained significantly high in germinating seeds, treated with approximately all sediment samples. Overall the seedlings of 19 samples showed higher protease activity as compared to the control. The samples producing stimulatory effects towards protease activity of seedlings were all taken from pure agricultural areas with high levels of organic contaminants (PCBs and OCBs), as revealed in the previous study (Eqani *et al.*, 2012). The remaining seedlings of 38 samples showed reduction in their protease activity as compared to the control. Majority of such samples were taken from organo-chemically contaminated (high levels of POPs, PCBs and OCPs) areas reported in the previous study (Eqani *et al.*, 2012). Possibly, these pollutants may have inhibitory and damaging effect on the metabolic processes or they could interfere with the process of enzymatic activity in plant (Oracz *et al.*, 2007).

4.5.2. Peroxidases (POD) activity

Peroxidases decompose hydrogen peroxide by their co-substrate's oxidation such as antioxidants and or phenolic compounds. They may provide hydrogen peroxide to other PODs (Gaspar *et al.*, 2006). Peroxidases are the isozymes that perform various functions in cell, such as association in cell wall synthesis and the formation of lignin (Van *et al.*, 1987). Various stressful conditions like environmental changes, toxic chemicals and oxidative stress induce the production of reactive oxygen species that leads to damage to the oxidative system and suppress the activities of the antioxidant enzymes like superoxide dismutase, peroxidase and catalase (Ortega *et al.*, 2007; Bogatek and Gniazdowska, 2007). Seedlings of only two samples S-55 and S-57 showed higher POD activities which were taken from an agricultural area (Site-24) on Sutlej River. Higher levels of DDTs residues were observed in these areas, reflecting the wide scale use of pesticides in the neighboring areas (Eqani *et al.*, 2012). As reported in the previous study the samples showing higher POD activities in seedlings were not affected with the heavy pollution load of urban and industrial discharges rather slightly affected by some agricultural processes. Although these results are in accordance with the study of Mazorra *et al.*, (2002), who found the stimulatory effects of abiotic stresses towards production of antioxidant enzymes like peroxidase in plants and enhancement of their tolerance level. While remaining seedlings of 95% samples showed reduction in POD activity comparative to the control. As discussed earlier the samples of polluted areas reported in the previous study (Eqani *et al.*, 2012) showed significant reduction of POD activities in their grown seedlings. Those areas contain high levels of agricultural, industrial, urban and municipal wastes that may cause a wide range of inhibitory effect on POD activity of seedlings. Hence our results are in accordance with the findings of Ortega *et al.* (2007) and Oracz *et al.* (2007) who found the suppression of antioxidant enzymatic activities under stressful conditions.

4.5.3. Superoxide dismutase (SOD) activity

Superoxide dismutase converts superoxide ion generated during adverse (stressful) environmental conditions to molecular oxygen and H_2O_2, so plays a crucial role in antioxidative defense mechanisms (Harinasut *et al.*, 2003). An increase in SOD activity of seedlings in response to all sediment samples was observed during the current study. Our results support previous findings as increased SOD activity was

observed in plants exposed to different environmental stresses (Cai *et al.*, 2011; Harinasut *et al.*, 2003). Relatively higher induction of SOD was shown by seedlings of those samples that contain the elevated levels of chemical contaminants and were exposed to various environmental stresses (Mishra *et al.*, 2011) as revealed in the previous study (Eqani *et al.*, 2012). The highest (80%) relative induction was measured in seedlings of sample S-19, taken from an agricultural area of Multan district where pesticides are extensively used. A significant difference in relative induction of SOD was also observed. Seedlings in 82% of samples showed relative induction activity between 50 – 80%. Seedlings of only 11% samples exhibited the SOD induction below 50% and the remaining seedlings of only 2 samples i.e. S-51 and S-57 showed less than 30% induction of SOD activity. It could be proposed that SOD can be utilized for screening oxidative resistant materials (phenolics and flavanoids etc.) of plant as an indirect selection criterion (Zaefyzadeh *et al.*, 2009). The excessive induction of SOD in plant has been considered as enhancement in its tolerance against oxidative stresses (Gupta *et al.*, 1993). The current study and other similar studies suggest that superoxide dismutase and peroxidase isozymes can act as useful markers in the analysis of metabolic regulations and gene functions, including stress-tolerance characteristics (Dubey, 1994).

Results of the current study suggest that samples of contaminated areas affect the antioxidant system, enzymes and substrates of target plant through different mechanisms. The production of oxidative stress caused by adverse conditions depends on the type of target tissue, species, concentration and type of contamination (Avci *et al.*, 2005; Rosa *et al.*, 2005).

4.6. Conclusion

- In this study, the effect of river Chenab sediments on morphological processes and biochemical activities of *Brassica napus* were studied. The changes in chemical contents and antioxidant properties such as phenolics, flavonoids, chlorophyll, carotenoid, MDA contents DPPH scavenging activity, total antioxidant capacity, reducing power, protein, protease, peroxidase and superoxide dismutase were evaluated by using multimode *invitro* assays.
- Sediments of least contaminated areas i.e. upstream and mainstream of river did not show significant inhibition of the growth of model plant as compared to the samples of highly polluted areas i.e. downstream of river that receives

pollution load from world known industrial cities e.g. Lahore, Qasoor, Sialkot and Faisalabad etc.

- The sediments that produced significantly suppressing effects in morphological and biochemical processes of target plant were taken from urban and industrial sites i.e. Site-2, Site-5, Site-8, Site-16, Site-18 and S-20.
- Majority of the sediments that produced significantly stimulatory effects in morphological and biochemical processes of target plant were taken from rural and agricultural sites i.e. Site-1, Site-6, Site-10, Site-12 and Site-19.
- The sediments of urban and industrial sites that produced significantly stimulatory effects in biochemical processes of target plant were taken from the sites i.e. Site-3, Site-9, Site-13, Site-14, Site-17, S-22 and S-25.
- Some of the sediments of rural and agricultural areas that showed significantly suppressing effects in target plant were taken from sites i.e. Site-4, Site-7, Site-11, Site-15, Site-23 and S-24.
- The sediments of agricultural areas producing negative effects in morphology and physiology of target plant might be affected by anthropogenic activities and extensive utilization of pesticides. Such sediments can induce oxidative stress as well as metabolic stress which could lead to the low antioxidant and biochemical activities of target plant.
- Results of present study are in accordance with the previously reported findings (Eqani *et al.*, 2012) that showed a vast variability of elevated levels of pesticides and other hazardous chemicals among the selected sites of river Chenab.
- Sediments of industrial, urban and farming areas with greater utilization of pesticides showed suppressing effects in the physiology and biochemistry of experimental plant. Majority of those samples contain high levels of hazardous chemicals like OCPs, PCBs, DDTs and cyclodiens etc (Eqani *et al.*, 2012) that might be responsible for adverse affects on model plant.

4.7. Future prospects

- This research will be advantageous for the evaluation of toxic substances, water pollutants and their effect on plant physiology and biochemistry.
- Toxicity issues in plants and riverine sediments demand for proper understanding of the relationships between bioavailable contamination

fractions in the sediment and plant responses to different levels of organic and inorganic contaminants and their proper management.

- A well designed, quantitative research should be undertaken on this issue, which would enable the rehabilitation of contaminated areas by growing appropriate Pakistani species.
- It will be helpful for the proper implementation of laws and regulations against waste emissions as well as will support the identification of toxicants having deteriorating effects on plants.

References

Abu-Romman S, Shatnawi M, Shibli R. 2010. Allelopathic effects of spurge (*Euphorbia hierosolymitana*) on wheat (*Triticum durum*). *American-Eurasian J. Agric.& Environ. Sci.* 7, (3): 298-302.

Adam G, Duncan H. 2002. Influence of diesel fuel on seed germination. *Environ. Pollut.* 120, (2): 363-70.

Aguero M.V, Barg M.V, Yommi A, Camelo A, Roura S.I. 2008. Postharvest changes in water status and chlorophyll content of lettuce (*Lactuca Sativa* L.) and their relationship with overall visual quality. *J. Food Sci.* 73, (1): 47-55.

AL-Othman Z.A, Ali R, Naushad Mu. 2012. Hexavalent chromium removal from aqueous medium by activated carbon prepared from peanut shell: Adsorption kinetics, equilibrium and thermodynamic studies. *Chem. Eng. J.* 184, 238-247.

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55: 373-399.

Araujo A.S.F, Monteiro R.T.R. 2005. Plant bioassays to assess toxicity of textile sludge compost. *Sci. Agri. Piracicaba Brazil.* 62: 286-290.

Armstrong G.A, Hearst J.E. 1996. Carotenoids 2: Genetics and molecular biology of carotenoid pigment biosynthesis. *FASEB J.* 10, (2): 228–237.

Avci A, Kacmaz M, Durak I. 2005. Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. *Ecotoxicol. Environ. Saf.* 60, (1): 101-105.

Bagavathy S, Xavier G.S.A. 2007. Effects of aqueous extract of *Eucalyptus globules* on germination and seedling growth of sorghum. *Allelopathy J.* 20: 395-402.

Bailly C, Benamar A, Corbineau F, Come D. 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seed as related to deterioration during accelerated aging. *Physiol. Plant.* 97, (1): 104-110.

Banks M.K, Schultz K.E. 2005. Comparison of plants for germination toxicity tests in petroleum-contaminated soils. *Water Air Soil Poll.* 167: 211-219.

Bano A, Siddique S.A. 1991. Chlorinated hydrocarbons in the sediments from the coastal waters of Karachi (Pakistan). *Pak. J. Sci. Indust. Res.* 34: 70–74.

Barkosky R.R, Einhellig F.A. 2003. Allelopathic interference of plant water relationships by para-hydroxybenzoic acid. *Bot. Bull. Acad. Sin.* 44: 53-58.

Barondeau D.P, Kassmann C.J, Bruns C.K, Tainer J.A, Getzoff E.D. 2004. Nickel superoxide dismutase structure and mechanism. *Biochemistry*. 43, (25): 8038-8047.

Becerril J, Gonzalez Murua C, Munoz-Rueda A, De Felipe M.R. 1989. Changes induced by cadmium and lead in gas exchange and water relations of clover and lucerne. *Plant Physiol. Biochem (Paris)*. 27, (6): 913-918.

Besalatpour A, Khoshgoftarmanesh A.H, Hajabbasi M.A, Afyuni M. 2008. Germination and growth of selected plants in a petroleum contaminated calcareous Soil. *Soil & Sediment Contamin*. 17, (6): 665–676.

Bettinetti R, Giarei C, Provini A. 2003. A chemical analysis and sediment toxicity bioassays to assess the contamination of the River Lambro (Northen Italy). *Arch. Environ. Contam. Toxicol*. 45, (1): 72–78.

Bhatti M.T, Latif M. 2009. Assessment of water quality of a river using an indexing approach during the low-flow season. *Irrig. Drain*. 60, (1): 103-114.

Bindler R, Olofsson C, Renberg I, Frech W. 2001. Temporal trends in mercury accumulation in lake sediments in Sweden. *Water Air Soil Poll*. 1, (3-4): 343-355.

Blokhina O, Virolainen E, Fagerstedt K.V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot*. 91, (2): 179-194.

Bogatek R, Gniazdowska A. 2007. ROS and phytohormones in plant-plant allelopathic interaction. *Plant signal behav*. 2, (4): 317-318.

Bogatek R, Gniazdowska A, Zakrzewska W, Oracz K, Gawronski S.W. 2006. Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. *Biol. Plantarum*. 50, (1): 156-158.

Bona C, de Rezende I.M, Santos G.O, Souza L.A. 2011. Effect of soil contaminated by diesel oil on the germination of seeds and the growth of *Schinus terebinthifolius* Raddi (*Anacardiaceae*) Seedlings. *Braz. Arch. Biol. Technol*. 54, (6): 1379-1387.

Boonpragob K, Nash T.H. 1991. Physiological-responses of the lichen *Ramalina menziesii* tayl to the Los-Angeles urban-environment. *Environ. Exp. Bot*. 31, (2): 229-238.

Brandt K, Molgaard J.P. 2001. Organic agriculture: does it enhance or reduce the nutritional value of plant foods? *J. Sci. Food Agric*. 81, (9): 924-931.

Bressano M, Curetti M, Giachero L, Gil S.V, Cabello M, March G, Ducasse D.A, Luna C.M. 2010. Mycorrhizal Fungi Symbiosis as a Strategy against Oxidative stress in soybean plants. *J. Plant Physiol.* 167, (18): 1622-1626.

Brown J, McCaffrey J.P, Harmon B.L, Davis J.B, Brown A.P, Erickson D.A. 1999. Effect of late season insect infestation on yield, yield components and oil quality of *Brassica napus*, *B. rapa*, *B. juncea* and *Sinapis alba* in the Pacific Northwest region of the United States. *J. Agric. Sci.* 132, (3): 281-288.

Cai F, Mei L.J, An X.L, Gao S, Tang L, Chen F. 2011. Lipid peroxidation and antioxidant responses during seed germination of *Jatropha curcas*. *Int. J. Agric. Biol.* 13, (1): 25-30.

Cao G, Prior R.L. 1998. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin. Chem.* 44, (6): 1309-1315.

Carter J.A, Tay K.L, Doe K.G, Mroz R.E. 1998. An evaluation of the use of soil and sediment bioassays in the assessment of three contaminated sites in atlantic Canada. *Water Qual. Res. J. Canada.* 33, (2): 295–317.

Chan L.S, Ng S.L, Davis A.M, Yim W.W, Yeung C.H. 2001. Magnetic properties and heavy-metal contents of contaminated seabed sediments of Penny's Bay, Hong Kong. *Marine. Pollut. Bull.* 42, (7): 569-583.

Chang C, Yang M, Wen H, Chern J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Analaysis.* 10, (3): 178-182.

Chartzoulakis K, Noitsakis B, Therios I. 1993. Photosynthesis, plant growth and dry matter distribution in kiwifruit as influenced by water deficits. *Irrigation Sci.* 14, (1): 1–5.

Chen L, Lin C.C, Kao C.H. 2000. Copper toxicity in rice seedlings: changes in antioxidative enzyme activities, H_2O_2 level, and cell wall peroxidase activity in roots. *Bot. Bull. Acad. Sin.* 41: 99-103.

Colton C.E, Einhellig F.A. 1980. Allelopathic mechanisms of Velvet leaf (*Abutilon Theophrasti* Medic. *Malvaceae*) on *Soybean. Amer. J. Bot.* 67, (10): 1407-1413.

Cornoy J.P, Virgon J.M, Smilie R.M, Barlow E.W. 1988. Influence of drought acclimation and CO_2 enrichment on osmotic adjustment and chlorophyll a florescence of *sunflower* during drought. *Plant Physiol.* 86, (4): 1108-1115.

Costa G, Spitz E. 1997. Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured *Lupinus albus*. *Plant Sci.* 128, (2): 131-140.

Croft K.D. 1998. The chemistry and biological effects of flavonoids and phenolic acids. In D. Harman, R. Holliday, & M. Meydani (Eds.). *Ann. N Y. Acad. Sci.* 854: 435-442.

Czabator F.J. 1962. Germination value: An index combining speed and completeness of pine seed germination. *J. For. Sci.* 8, 386-395.

Davies C.S, Nielsen S.S, Nielsen N.C. 1987. Flavor improvement of soybean preparations by genetic removal of lipoxygenase. *J. Am. Oil Chem. Soc.* 64, (10): 1428-1433.

Day K.E, Dutka B.J, Kwan K.K, Batista N, Reynoldson T.B, Metcalfe-Smith J.L. 1995. Correlations between solid-phase microbial screening assays, whole sediment toxicity tests with macro-invertebrates and in situ benthic community structure. *J. Great Lakes Res.* 21, (2): 192-206.

Dayan F.E, Romagni J.G, Tellez M, Duke S.O. 2000. Investigating the mode of action of natural phytotoxins. *J. Chem. Ecol.* 26, (9): 2079-2094.

Debus R, Hund K. 1997. Development of analytical methods for the assessment of ecotoxicological relevant soil contamination. Part B-Ecotoxicological analysis in soil and soil extracts. *Chemosphere.* 35, (1-2): 239-261.

Delfine S, Alvino A, Villiani M.C, loreto F. 1999. Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. *Plant Physiol.* 119, (3): 1101-1106.

Devasagayam T.P, Tilak J.C, Boloor K.K, Sane K.S, Ghaskadbi S.S, Lele R.D. 2004. Free radicals and antioxidants in human health: Current status and future prospects. *J. Assoc. Phys. India.* 52: 794-804.

Domash V.I, Sharpio T.P, Zabreiko S.A, Sosnovskaya T.F. 2008. Proteolytic Enzymes and Trypsin Inhibitors of Higher Plants under Stress Conditions. *Russ. J. Bioorg. Chem.* 34, (3): 318-322.

Dowdy R.H, Larson W.E. 1975. The availability of sludge borne metals to various vegetable crops. *J. Environ. Qual.* 4, (2): 278-282.

Dubey R.S. 1994. Protein synthesis by plants under stressful conditions. *In Handbook of Plant and Crop Stress* (M. Pessarakli, ed.) Marcel Dekker, Inc., New York. 277-299.

Dudonne S, Vitrac X, Coutiere P, Woillez M, Merillon J.M. 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agr. Food Chem.* 57, (5): 1768-1774.

Duh P. 1998. Antioxidant activity of burdock (*Arctium lappa* Linne). It's scavenging effect on free radical and active oxygen. *J. Am. Oil Chem. Soc.* 75, (4): 455-461.

Einhellig F.A. 1996. Interaction involving allelopathy in cropping systems. *Agron. J.*, 88: 886-893.

Ejieji C.J, Adeniran K.A. 2010. Effects of water and fertilizer stress on the yield, fresh and dry matter production of grain Amaranth (*Amaranthus cruentus*). *Aust. J. Agric. Eng.* 1, (1): 18-24.

Ellis R.H, Roberts E.H. 1980. Towards a rational basis for testing seed quality. In *Seed Production* (ed P.D. Hebblethwaite), *Butterworths*, London. 605-635.

Eqani S.A, Malik R.N, Katsoyiannis A, Zhang G, Chakraborty P, Mohammad A, Jones K.C. 2012. Distribution and risk assessment of organochlorine contaminants in surface water from river Chenab, Pakistan. *J. Environ. Monit.* 14, (6): 1645-54.

Eqani S.A, Malik R.N, Mohammad A. 2011. The level and distribution of selected organochlorine pesticides in the sediments from River Chenab, Pakistan. *Environ. Geochem. Health.* 33, (1): 33-47.

Ericson M.C, Alfinito S.H. 1984. Proteins produced during salt stress in tobacco cell cultures. *Plant Physiol.* 74, (3): 506-509.

Erkan N, Ayranci G, Ayranci E. 2009. A kinetic study of oxidation development in sunflower oil under microwave heating: Effect of natural antioxidants, *Food Res. Int.* 42, (8): 1171-1177.

Escudero A, Albert M.J, Pita J.M, Perez-Garcia F. 2000. Inhibitory effects of *Artemesia herba-alba* on the germination of the *gypsophyte Helianthemum squamatum*. *Plant Ecol.* 148: 71-80.

Evans C.A.R, Miller N.J, Paganga G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* 20, (7): 933–956.

Floegel A, Kim D.O, Chung S.J, Song W.O, Fernandez M.L, Bruno R.S, Koo S.I, Chun O.K. 2010. Development and validation of an algorithm to establish a

total antioxidant capacity database of the US diet. *Int. J. Food Sci. Nutr.* 61, (6): 600-623.

Foyer C.H. 2005. Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. *Plant Cell.* 17, (7): 1866-1875.

Frische T. 2003. Ecotoxicological evaluation of in situ bioremediation of soils contaminated by the explosive 2,4,6-trinitrotoluene (TNT), *Environ. Pollut.* 121, (1): 103-113.

Fuentes D, Disante K.B, Valdecantos A, Cortina J, Vallejo V.R. 2007. Sensitivity of Mediterranean woody seedlings to copper, nickel and zinc. *Chemosphere.* 66, (3): 412-420.

Gaspar T, Penel C, Castillo F.J, Greppin H. 2006. A two-step control of basic and acidic peroxidases and its significance for growth and development. *Physiol. Plant.* 64, (3): 418–423.

Ghozlene I, Reda D.M, Nedjoud G, Houria B, Ali C. 2013. Oxidative stress, chlorophyll content and ROS production and localization in *Triticum durum* seed. *Ann. Biol. Res.* 4, (5): 11-15.

Gniazdowska A, Bogatek R. 2005. Allelopathic interaction between plants: Multi site action of allelochemicals. *Acta Physiol. Plant*, 27 (3): 395-407.

Gong P, Wilke B.M, Strozzi E, Fleischmann S. 2001. Evaluation and refinement of a continuous seed germination and early seedling growth test for the use in the ecotoxicological assessment of soils. *Chemosphere.* 44, (3): 491-500.

Gordon M.H. 1990. The mechanism of the antioxidant action *in-vitro*. In: Hudson B.J.F. (ed.), *Food Antioxidants.* 1-18.

Gorinstein S, Haruenkit R, Poovarodom S, Vearasilp S, Ruamsuke P, Namiesnik J, Leontowicz M, Leontowicz H, Suhaj M, Sheng G.P. 2010. Some analytical assays for the determination of bioactivity of exotic fruits. *Phytochem. Anal.* 21, (4): 355-362.

Grodszinsky A.M. 1989. General and specific mechanisms of biochemical interactions between plants. *Biol. Plant.* 31, (6): 448-457.

Gruis D, Schulze J, Jung R. 2004. Storage protein accumulation in the absence of the vacuolar processing enzyme family of cysteine proteases. *Plant Cell.* 16, (1): 270-290.

Gupta S, Heinen J.L, Holaday A.S, Burke J.J, Allen R.D. 1993. Increased resistance to oxidative stress in transgenic plants that over express chloroplastic Cu/Zn superoxide dismutase, *Proc. Natl. Acad. Sci. USA.* 90, (4): 1629-1633.

Haimi J. 2000. Decomposer animals and bioremediation of Soils. *Environ. Pollut.* 107, (2): 233-238.

Halvorsen B.L, Holte K, Myhrstad M.C.W, Barikmo I, Hvattum E, Remberg S.F, Wold A.B, Haffner K, Baugerod H, Andersen L.F, Moskaug J.O, Jacobs D.R, Blomhoff R. 2002. A systematic screening of total antioxidants in dietary plants. *J. Nutr.* 132, (3): 461-471.

Hammann M, Gupta S.K, Zihler J. 1998. Protection of soils from contamination in Swiss legislation. *Adv. Geo Ecol.* 31, 629-636.

Hanley M.E, Whiting M.D. 2005. Insecticides and arable weeds: effects on germination and seedling growth. *Ecotoxicology.* 14, (4): 483-490.

Haq I.U, Ullah N, Bibi G, Kanwal S, Ahmad M.S, Mirza B. 2012. Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. *Iran J. Pharm. Res.* 11, (1): 241-249.

Harinasut P, Poonsopa D, Roengmongkol K, Charoensataporn R. 2003. Salinity effects on antioxidant enzymes in mulberry cultivar. *Sci. Asia.* 29, 109-113.

Hodgson J.G, Mackey J.M.L. 1986. The ecological specialization of dicotyledonous families within a local flora: some factors constraining optimization of seed size and other possible evolutionary significance. *New Phytol.* 104, (3): 497-515.

Hoke R.A, Giesy J.P, Kreis R.G.Jr. 1992. Sediment pore water toxicity identification in the Lower Fox River and Green Bay, Wisconsin, using the Microtox assay. *Ecotoxicol. Environ. Saf.* 23, (3): 343-354.

Horsfall M, Spiff A.I. 2002. Distribution and partitioning of trace metals in sediments of the lower reaches of the new Carlabar river, port Harcourt, Nigeria. *Environ. Monit. Assess.* 78, (3): 309-326.

Inderjit, Nilsen E.T. 2003. Bioassays and field studies for allelopathy in terrestrial plants: Progress and problems. *Crit. Rev. Plant. Sci.* 22, (3-4): 221-238.

Jabbar A, Masud S.Z, Parveen Z, Ali M. 1993. Pesticide residues in cropland soils and shallow groundwater in Punjab Pakistan. *Bull. Environ. Contam. Toxicol.* 51, (2): 268–273.

Jacobs M.W, Coates J.A, Delfino J.J, Bitton G, Davis W.M, Garcia K.L. 1993. Comparison of sediment extract microtox® toxicity with semi-volatile organic priority pollutant concentrations. *Arch. Environ. Contam. Toxicol.* 24, (4): 461-468.

Javanmardia J, Stushnoffb C, Lockeb E, Vivancob J.M. 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chem.* 83, (4): 547–550.

Jefferson L.V, Pennacchio M. 2003. Allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination. *J. Arid Environ.* 55, (2): 275-285.

Jidere C.M, Akamigbo F.O, Ugwuanyi J.O. 2012. Phytoremediation potentials of cowpea (*Vignia unguiculata*) and maize (*Zea mays*) for hydrocarbon degradiation in organic and inorganic manure-amended tropical paleustults. *Int. J. Phy.* 14, (4): 362-373.

Joshi B, Sarika, Pandey N, Rao P.B. 2009. Allelopathic effect of weed species extraction germination, growth and biochemical aspects in different varieties of wheat (*Triticum aestivum* L). *Indian J. Agric. Res.* 43, (2): 79-87.

Justesen U, Knuthsen P. 2001. Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes. *Food Chem.* 73, (2): 245-250.

Kamkar A, Javan A.J, Asadi F, Kamalinejad M. 2010. The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil. *Food Chem. Toxicol.* 48, (7): 1796-1800.

Kandil A.A, Sharief A.E, Nassar E.S.E. 2012. Response of some rice (*Oryza sativa* L.) cultivars to germination under salinity stress. *Int. J. Agr. Sc.* 4, (6): 272-277.

Karaguzel O, Cakmakci S, Ortacesme V, Aydinoglu B. 2004. Influence of seed coat treatments on germination and early seedling growth of *Lupinus varius* L. *Pak. J. Bot.* 36, (1): 65-74.

Karim M.A, Nawata E, Shigenaga S. 1993. Influence of flooding with diluted sea water on morphological and physiological characteristics in hexaploid triticale. *Plant Cell Environ.* 16, (6): 663–671.

Keddy C.J, Greene J.C, Bonnell M.A. 1995. Review of whole-organism bioassays: soil, freshwater sediment, and freshwater assessment in Canada. *Ecotoxicol. Environ. Saf.* 30, (3): 221-251.

Khan R.A, Khan M.R, Sahreen S, Ahmed M. 2012. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill. *Chem. Cent. J.* 6, (1): 12.

Khoddami A. 2013. Techniques for analysis of plant phenolic compounds. *Molecules*. 18, (2): 2328-2375.

Kirnak H, Kaya C, Ismail T.A.S, Higgs D. 2001. The influence of water deficit on vegetative growth, physiology, fruit yield and quality in Eggplants. *Bulg. J. Plant Physiol.* 27, (3-4): 34-46.

Koger C.H, Reddy K.N, Poston D.H. 2004. Factors affecting seed germination, seedling emergence, and survival of Texas weed (*Caperonia palustris*). *Weed Sci.* 52: 989-995.

Kucera B, Cohn M.A, Metzger G.L. 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Sci. Res.* 15, 281-307.

Kumi S.A, Kawano M, Shiki Y, Yeboah P.O, Carboo D, Pwamang J, Morita M, Suzuki N. 2010. Organochlorine pesticides (OCPs), dioxin-like polychlorinated biphenyls (dl PCBs), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo furans (PCDD/Fs) in edible fish from Lake Volta, Lake Bosumtwi and Weija Lake in Ghana. *Chemosphere*. 81, (6): 675-684.

Lagrimini L.M. 1991. Wound-induced deposition of polyphenols in transgenic plants over expressing peroxidase. *Plant Physiol.* 96, (2): 577-583.

Lamb C, Dixon R.A. 1997. The oxidative burst in plant disease resistance. *Annu.* Rev. *Plant Physiol. Plant Mol. Biol.* 48: 251-275.

Lapaquellerie Y, Maillet N, Jouanneau J.M, Latouche C. (1995). Cadmium pollution in sediments of the Lot River (France): Estimate of the mass of Cadmium. *Environ. Technol.* 16: 1145-1154.

Ortega R.C, Lara Nunez A, Anaya A.L. 2007. Allelochemical stress can trigger oxidative damage in receptor plants mode of action of phytotoxicity. *Plant Signal Behav.* 2, (4): 269-270.

Lee S.K, Mbwambo Z.H, Chung H, Luyengi L, Gamez E.J.C, Mehta R.G, Kinghorn A.D, Pezzuto J.M. 1998. Evalution of the antioxidant potential of natural products. *Comb. Chem. High Throughput Screen.* 1, (1): 35- 46.

Liu M, Xu Z, Yang Y, Feng Y.J. 2011. Effects of different spectral lights on Oncidium PLBs induction, proliferation, and plant regeneration. *Plant Cell Tiss. Organ Cult.* 106: 1-10.

Loibner A.P, Szolar O.H.J, Braun R, Hirmann D. 2003. Ecological assessment and toxicity screening in contaminated land analysis. In: Thompson, K.C., Nathanail, C.P. (Eds.), *Chem. Anal. Contam. Land.* 229-267.

Long E.R, McDonald D.D, Smith S.L, Calder F.D. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *J. Environ. Manage.* 19, (1): 81-97.

Lopez N.G, Otero R.R, Grande B.C, Gandara J.S, Gonzalez B.S. 2005. Occurrence of organochlorine pesticides in stream sediments from an industrial Area. *Arch. Environ. Contam. Toxicol.* 48, (3): 296-302.

Larson L.J. 1989. Method for the preliminary assessment of aquatic contamination sites using sediment extract toxicity tests. *Bull. Environ. Contam. Toxicol.* 42: 218-225.

Lowry O.H, Rosebrough N.J, Farr A.L, Randall R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Ma J.K, Drake P.M, Christou P. 2003. The production of recombinant pharmaceutical proteins in plants. *Nat. Rev. Genet.* 4, (10): 794-805.

Macias F.A, Castellano D, Molinillo J.M. 2000. Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. *J. Agric. Food Chem.* 48, (6): 2512-2521.

Mai B.X, Fu J.M, Sheng G.Y, Kang Y.H, Lin Z, Zhang G, Min YS, Zeng E.Y. 2002. Chlorinated and polycyclic aromatic hydrocarbons in riverine and estuarine sediments from Pearl River Delta, China. *Environ. Pollut.* 117, (3): 457-474.

Maila M.P, Cloete T.E. 2002. Germination of *Lepidium sativum* as a method to evaluate polycyclic aromatic hydrocarbons (PAHs) removal from contaminated soil, *Internat. Biodet. Biodegrad.* 50, (2): 107-113.

Malik R.N, Nadeem M. 2011. Spatial and temporal characterization of trace elements and nutrients in the Rawal Lake Reservoir, Pakistan using multivariate analysis techniques. *Environ. Geochem. Health.* 33, (6): 525-541.

Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. 2004. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 79, (5): 727-747.

Marin M.V, Nguez S.D, Dirzo R. 2006. Simulated seed predation reveals a variety of germination responses of neotropical rain forest species. *Am. J. Bot.* 93, (3): 369-376.

Matekaire T, Maroyi A. 2009. Thermotolerance and osmotic potential studies in germinating seeds and growing seedlings of *Acacia sieberiana* and *Acacia tortilis*. *J. Sustain. Dev. Afr.* 10, (4): 95-105.

Maxam G, Rila J.P, Dott W, Eisentraeger A. 2000. Use of bioassays for assessment ofwater-extractable ecotoxic potential of soils. *Ecotoxicol. Environ. Saf.* 45, 240-246.

Mazorra L.M, Nunez M, Hechavarria M, Coll F, Sanchez-Blanco M.J. 2002. Influence of brassino-steroids on antioxidant enzymes activity in tomato under different temperatures, *Biol. Plant.* 45, (4): 593-596.

Medini H, Elaissi A, Khouja M.L, Chemli R. 2013. Phytochemical screening and antioxidant activity of *Juniperus phoenicea ssp. phoenicea* L. Extracts from two Tunisian locations. *J. Exp. Biol. Agr. Sci.* 1, (1): 2.

Menzie C.A, Potocki B.B, Santodonato J. 1992. Exposure to carcinogenic PAHs in the environment. *Environ. Sci. and technol.* 26, (7): 1278-1284.

Mersie W, Singh M. 1993. Phenolic acids affect photosynthesis and protein synthesis by isolated leaf cells of velvet-leaf. *J. Chem. Ecol.* 19, (7): 1293-1301.

Michalak A. 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish J. of Environ. Stud.* 15, (4): 523-530.

Milosevic M, Vujakovic M, Karagic D. 2010. Vigour tests as indicators of seed viability. *Genetika.* 42, (1): 103-118.

Mishra S, Jha A.B, Dubey R.S. 2011. Arsenite treatment induces oxidative stress, upregulates antioxidant system, and causes phytochelatin synthesis in rice seedlings. *Protoplasma.* 248, (3): 565–577.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7, (9): 405-410.

Mittler R, Vanderauwera S, Gollery M, Breusegem V.F. 2004. Reactive oxygen gene network of plants. *Trends Plant Sci.* 9: 490-498.

Moreno C.S, Larrauri J.A, Saura-Calixto F. 1998. Procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agr.* 76, (2): 270-276.

Moysich K.B, Mendola P, Schisterman E.F, Freudenheim J.L, Ambrosone C.B, Vena J.E, Shields P.G, Kostyniak P, Greizerstein H, Graham S, Marshall J.R. 1999. An evaluation of proposed frameworks for grouping polychlorinated biphenyl (PCB) congener data into meaningful analytic units. *Am. J. Ind. Med.* 35, (3): 223-231.

Muntz K, Belozersky M.A, Dunaevsky Y.E, Schlereth A, Tiedemann J. 2001. Stored proteases and the initiation of storage protein mobilization on seeds during germination and seedling growth. *J. Exp. Bot.* 52, (362): 1741-1752.

Murphy D.J, Vance J. 1999. Mechanisms of lipid-body formation. *Trends Biochem. Sci.* 24, (3): 109-115.

Musil A.F. 1950. Identification of *Brassicas* by seedling growth or later vegetative stages. A handbook of *US Dept. Agric.* 857, 26.

Nayyar H, Gupta D. 2006. Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. *Environ. Exp. Bot.* 58, (1-3): 106-113.

Oracz K, Bailly C, Gniazdowska A, Come D, Corbineau F, Bogatek R. 2007. Induction of oxidative stress by sunflower phytotoxins in germinating mustard seeds. *Chem. Ecol.* 33, (2): 251-64.

Ou B, Huang D, Woodill H.M, Flanagan J.A, deemer E.K. 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity and ferric reducing antioxidant power (FRAP) assays: a comparative study. *J. Agric. Food Chem.* 50, (11): 3122-3128.

Ou B, Huang D, Hampsch-Woodili M, Flanagan J.A. 2003. When the east meets west: the relationship between yin-yang and antioxidation–oxidation. *FASEB J.* 17, (2): 127–129.

Palma J.M, Sandalio L.M, Javier F.C, Romero-Puertas M.C, McCarthy I, Del L.A.R. 2002. Plant proteases protein degradation and oxidative stress: role of peroxisomes. *Plant Physiol. Biochem.* 40, (6-8): 521-530.

Parvanova D, Ivanov S, Konstantinova T, Karanov E, Atanassov A, Tsvetkov T, Alexieva V, Djilianov D. 2004. Transgenic tobacco plants accumulating osmolytes show reduced oxidative damage under freezing stress. *Plant Physiol. and Biochem.* 42, (1): 57-63.

Prasad K.V.S.K, Saradhi P.P, Sharmila P. 1999. Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. *Environ Exp. Botany*. 42, (1): 1–10.

Prati D, Bossdorf O. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (*Brassicaceae*). *Am. J. Bot*. 91, (2): 285-288.

Prieto P, Pineda M, Aguilar M, 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Ann. Biochem*. 269, (2): 337-341.

Ramel F, Birtic S, Cuine S, Triantaphylides C, Ravanat J.L, and Havaux M. 2012. Chemical quenching of singlet oxygen by carotenoids in plants. *Plant Physiol*. 158, (3): 1267–1278.

Ranal M.A, Santana D.G. 2006. How and why to measure the germination process? *Revista Brasil. Bot*. 29, (1): 1-11.

Renault S, Zwiazek J.J, Fung M, Tuttle S. 2000. Germination, growth and gas exchange of selected boreal forest seedlings in soil containing oil sands tailing. *Environ. Pollut*. 107, (3): 357-365.

Roach T, Beckett R.P, Minibayeva F.V, Whitaker C, Chen H, Bailly C, Kranner I. 2010. Extracellular superoxide production, viability and redox poise in response to desiccation in recalcitrant *Castanea sativa* seeds. *Plant, Cell Environ*. 33, (1): 59-75.

Romero C, Brenes M, Yousfi K, Garcia P, Garcia A, Garrido A. 2004. Effect of cultivar and processing method on the contents of polyphenols in table olives. *J. Agric. Food Chem*. 52, (3): 479-484.

Rosa M. Martínez-Alvarez, Amalia E. Ana Sanz. 2005. Antioxidant defenses in fish: Biotic and abiotic factors. Rev. *Fish Biol. Fish*. 15: 75-88.

Ruiz J.M, Rivero R.M, Lopez-Cantarero I, Romero L. 2003. Role of Ca 2+ in metabolism of phenolic compounds in tobacco leaves (*Nicotiana tabacum* l). *Plant Growth Reg*. 41: 173-177.

Santos C.L.V, Campos A, Azevedo H, Caldeira G. 2001. *In-situ* and *in-vitro* senescence induced by KCl stress: nutritional imbalance, lipid peroxidation and antioxidant metabolism. *J. Exp. Bot*. 52, (355): 351-360.

Saraee R.E.K, Abdi M.R, Naghavi K, Saion E, Shafaei M.A, Soltani N. 2011. Distribution of heavy metals in surface sediments from the South China Sea ecosystem, Malaysia. *Environ. Monit. Assess.* 183, (1-4): 545-554.

Sarikurkcu C. 2011. Antioxidant activities of solvent extracts from endemic *Cyclamen mirabile* Hildebr. tubers and leaves. *Afr. J. Biotechnol.*10, (5): 831-839.

Schaller A. 2004. Cut above the rest: the regulatory function of plant proteases. *Planta.* 220, (2): 183-197.

Sera B. 2012. Effects of soil substrate contaminated by Knotweed leaves on seed development. *Pol. J. Environ. Stud.* 21, (3): 713-717.

Shahidi F, Wanasundara P.K. 1992. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* 32, (1): 67-103.

Shah K, Dubey R.S. 1998. 18 kDa cadmium inducible protein complex: its isolation and characterization from rice (*Oryza sativa* L.) seedlings. *J. Plant Physiol.* 152, (4-5): 448–454.

Sharififar F, Moshafi M.H, Mansouri S.H, Khodashenas M, Khoshnoodi M. 2007. *In-vitro* evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multi-flora Boiss. *Food Control.* 18, (7): 800-805.

Sharma O.P, Bhat T.K. 2009. DPPH antioxidant assay revisited. *Food Chem.* 113, (4): 1202-1205.

Sibley P.K, Ankley G.T, Benoit D.A. 2001. Factors affecting reproduction and the importance of adult size on reproductive output of the midge *Chironomus tentans*. *Environ. Toxicol. Chem.* 20, (6): 1296-1303.

Singh N, Rajini P.S. 2004. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem.* 85, (4): 611-616.

Singh N.B, Singh D, Singh A. 2009. Modification of physiological responses of water stressed *Zea mays* seedlings by leachate of *Nicotiana plumbaginifolia*. *Gen. Appl. Plant. Physiol.* 35, (1-2): 51-63.

Singleton V.L, Orthofer R, Lamuela-Raventos R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol.* 299: 152-178.

Smirnoff N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist.* 125, (1): 27-58.

Sodaeizadeh H, Rafieiolhossaini M, Havlik J, Van P.D. 2009. Allelopathic activity of different plant parts of *Peganum harmala* L. and identification of their growth inhibitors substances. *Plant Growth Regul.* 59, (3): 227-236.

Song X.Y, Song Y.F, Sun T.H, Li X.X, Zhang W, Zhou Q.X. 2006. Soil-based eco-toxicity of diesel oil contamination to seed germination and seedling growth of wheat. *J. Agro-Environ. Sci.* 25, (3): 554-559.

Starner D.E, Hamama A.A, Bhardwaj L.1999. *Canola* oil yield and quality as affected by production practices in Virginia, Perspectives on new crops and new uses. *ASHS Press Alexandria*, 254-256.

Stoeva N, Bineva T. 2003. Oxidative changes and photosynthesis in oat plants grown in As-contaminated soil. *Bulg. J. Plant. Physiol.* 29, (1-2): 87-95.

Sun O.J, Sweet G.B, Whitehead D, Buchan G.D. 1995. Physiological responses to water stress and water logging in *Nothofagus* species. *Tree Physiology*. 15, 629-638.

Sung J.M. 1996. Lipid peroxidation and peroxide-scavenging in soybean seeds during aging. *Physiol. Plant.* 97, (1): 85-89.

Sverdrup L.E, Krogh P.H, Nielsen T, Kjaer C, Stenersen J. 2003. Toxicity of eight poly-cyclic aromatic compounds to red clover (*Trifolium pretense*), ryegrass (*Lolium perenne*) and mustard (*Sinapsis alba*). *Chemosphere*. 53, (8): 993-1003.

Tanaka T, Hasegawa A, Yamamoto S, Lee U.S, Sugiura Y, Ueno Y. 1988. Worldwide contamination of cereals by the *Fusarium* mycotoxins, nivalenol, deoxynivalenol, and zearalenone. *J. Agric. Food Chem.* 36, (5): 979-983.

Tarazona J.V, Vega M.M. 2002. Hazard and risk assessment of chemicals for terrestrial ecosystems. *Toxicology*. 181-182, 187-191.

Tewari R.K, Kumar P, Sharma P.N, Bisht S.S. 2002. Modulation of oxidative stress responsive enzymes by excess cobalt. *Plant Science*. 162, (3): 381-388.

Turker A.U, Camper N.D. 2002. Biological activity of common mullein, a medicinal plant. *J. Ethnopharmacol.* 82, (2-3): 117-125.

Turner N.C. 1986. Crop water deficit: a decade of progress. *Adv. Agron.* 39, 1-51.

Ullah N, Haq I.U, Mirza B. 2013. Phytotoxicity evaluation and phytochemical analysis of three medicinally important plants from Pakistan. *Toxicol. Ind. Health.*, 1-7.

Valladares F, Niinemets U. 2008. Shade tolerance, a key plant feature of complex nature and consequences. *Annu. Rev. Ecol. Evol. Syst.* 39, 237-257.

Velikova V, Yordanov I, Ederev A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Sci.* 151, (1): 59-66.

Velioglu Y.S, Mazza G, Gao L, Oomah B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agr. Food Chem.* 46, (10): 4113-4117.

Wang J.R, Li S.X, Li K.I. 2001. Effect of water limited deficit stress during different growth stages on leaf enzymes of winter wheat. *Acta Bot. Borel Occident. Sin.* 21, (1): 47-52.

Wang L.I, Giovannucci E.L, Hunter D, Neuberg D, Su L, Christiani D.C. 2004. Dietary intake of cruciferous vegetables, Glutathione S-transftrase (GST) polymorphisms and lung cancer risk in a Caucasian population. *Cancer Causes Control.* 15, (10): 977-985.

Weatherley P.E. 1950. Studies in the water relations of the cotton plant. The field measurement of water deficits in leaves. *New Phlytol.* 49, (1): 81-97.

Weatherley P.E. 1951. Studies of the water relations of the cotton plant. II. Diuinal and seasonal variations in relative turgidity and environmental factors. *New Phytol.* 50, 36-51.

Weir T.L, Park S.W, Vivianco J.M. 2004. Biochemical and physiological mechanisms mediated by allelochemicals. *Curr. Opin. Plant Biol.* 7, (4): 472-479.

Xia J, Tian Q. 2009. Early stage toxicity of excess copper to photosystem II of *Chlorella pyrenoidosa*-OJIP chlorophyll a fluorescence analysis. *J. Environ. Sci.* 21, (11): 1569-1574.

Xiong Z.T, Wang H. 2005. Copper toxicity and bioaccumulation in Chinese cabbage (*Brassica pekinensis Rupr*) *Environ. Toxicol.* 20, (2): 188-194.

Yamasaki S, Dillenburg L.R. 1999. Measurements of leaf relative water content in *Araucaria angustifolia*. *Rev. Bras. Fisiol.* 11, (2): 69-75.

Yongqing M.A. 2005. Allelopathic studies of common wheat (*Triticum aestivum* L.). *Weed Biol. Manag.* 5, (3): 93-104.

Zaefyzadeh M, Quliyev R.A, Babayeva S.M, Abbasov M.A. 2009. The effect of the interaction between genotypes and drought stress on the superoxide

dismutase and chlorophyll content in durum wheat landraces. *Turk. J. Biol.* 33, (1): 1–7.

Zhang G, Chakrabotry P, Li J, Sampathkumar P, Balasubramanian T, Kathiresan K, Takahashi S, Subramanian A, Tanabe S, Jones K.C. 2008. Passive atmospheric sampling of organochlorine pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers in Urban, Rural and Wetland Sites along the coastal length of India. *Environ. Sci. Technol.* 42, (22): 8218-8223.

Electronic reference

1. http://upload.wikimedia.org/wikipedia/commons/thumb/c/ce/*Brassica_napus*_2.jpg (visited on 27-05-2013)

Appendix

Preparation of Buffers for use in Enzyme studies

By G. Gomori

The buffers described in this section are suitable for use either in enzymatic or histochemical studies. The accuracy of the tables is within ± 0.05 pH at 23 °C. In most cases the pH values will not be off by more than ± 0.02 pH even at 37 °C and at molarities slightly different from those given (usually 0.05 M).

The methods of preparation described are not necessarily identical with those of the original authors. The titration curves of the majority of the buffers recommended have been redetermined by the writer. The buffers are arranged in the order of ascending pH range.

1. Citrate Buffer

Stocks solutions

A: 0.1 M solution of citric acid (21.01 g in 1 L)

B: 0.1 M solution of sodium citrate (29.41 g $C_6H_5O_7Na_3.2H_2O$ in 1 L. the use of the salt with 5½ H_2O is not recommended).

x mL of A + y mL of B, diluted to a total of 100 mL.

x	y	pH
46.5	3.5	3.0
43.7	6.3	3.2
40.0	10.0	3.4
37.0	13.0	3.6
35.0	15.0	3.8
33.0	17.0	4.0
31.5	18.5	4.2
28.0	22.0	4.4
25.5	24.5	4.6
23.0	27.0	4.8
20.5	29.5	5.0
18.0	32.0	5.2
16.0	34.0	5.4
13.7	36.3	5.6
11.8	38.2	5.8
9.5	41.5	6.0
7.2	42.8	6.2

2. Citrate phosphate Buffer

Stocks solutions

A: 0.1 M solution of citric acid (19.21 g in 1 L)

B: 0.2 M solution of dibasic sodium phosphate (53.65 g of $Na_2HPO_4.7H_2O$ or 71.7 g of $Na_2HPO_4.12H_2O$ in 1 L).

x mL of A + y mL of B, diluted to a total of 100 mL

x	y	pH
44.6	5.4	2.6
42.2	7.8	2.8
39.8	10.2	3.0
37.7	12.3	3.2
35.9	14.1	3.4
33.9	16.1	3.6
32.3	17.7	3.8
30.7	19.3	4.0
29.4	20.6	4.2
27.8	22.2	4.4
26.7	23.3	4.6
25.2	24.8	4.8
24.3	25.7	5.0
23.3	26.7	5.2
22.2	27.8	5.4
21.0	29.0	5.6
19.7	30.3	5.8
17.9	32.1	6.0
16.9	33.1	6.2
15.4	34.6	6.4
13.6	36.4	6.6
9.1	40.9	6.8
6.5	43.6	7.0

3. Acetate Buffer

Stocks solutions

A: 0.2 M solution of acetic acid (11.55 g in 1 L)

B: 0.2 M solution of sodium acetate (16.4 g $C_2H_3O_2Na$ or 27.2 g of $C_2H_3O_2Na.3H_2O$ in 1 L).

x mL of A + y mL of B, diluted to a total of 100 mL

x	y	pH
46.3	3.7	3.6
44.0	6.0	3.8
41.0	9.0	4.0
36.8	13.2	4.2
30.5	19.5	4.4
25.5	24.5	4.6
20.0	30.0	4.8
14.8	35.2	5.0
10.5	39.5	5.2
8.8	41.2	5.4
4.8	45.2	5.6

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