Assessment of Plastic Fractions and Selected Heavy Metals in Groundwater and Wastewater Used for Irrigation Practices in Peri-Urban Areas of Okara City, Pakistan

Master of Philosophy

In

Environmental Sciences

By

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Department of Environmental Sciences

Faculty of Biological Sciences

Quaid-i-Azam University, Islamabad

2020-2022

Assessment of Plastic Fractions and Selected Heavy Metals in Groundwater and Wastewater Used for Irrigation Practices in Peri-Urban Areas of Okara City, Pakistan

QUAID-I-AZAM UNIVERSITY
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A Dissertation Submitted in Partial Fulfillment of Requirement for the Degree of Master of Philosophy in Environmental Science

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APPROVAL CERTIFICATE

It is to certify that the research work presented in this thesis, entitled **"Assessment of Plastic Fractions and Selected Heavy Metals in Groundwater and Wastewater Used for Irrigation Practices in Peri-Urban Areas of Okara City, Pakistan"** was conducted by **Ms. Amen Sana (Reg. No. 02312011012)** under the supervision of **Prof. Dr. Riffat Naseem Malik *(T.I)*.** No part of this thesis has been submitted else for any other degree. This thesis is submitted to the **Department of Environmental Sciences,** in the partial fulfillment of the requirements for the degree of **Master of Philosophy in the field of Environmental Science,** Department of Environmental Sciences, Quaid-i-Azam University Islamabad, Pakistan.

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To, mama & baba

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LIST OF ABBREVIATIONS

PCs	Pharmaceutical compounds
PhACs	Pharmaceutical active compounds
NEQS	National environmental quality standards
RQ	Risk quotient
ESAC	European surveillance of antimicrobial consumption
WWTPs	Wastewater treatment plants
mg/g	Milligram per gram
PAC	Powdered activated carbon
AC	Activated carbon
l/kg	Litre per kilogram
EC	Electrical conductivity
SEM	Scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy
FAO	Food and Agriculture Organization
WWF	World Wildlife Fund
GPS	Geo Positioning System
AAS	Atomic Absorption Spectrophotometer
SWW	Samples from South Sewage Line
GDP	Gross Urban Product
N/A	Not Any
WPO	Wet peroxidation
EC	Electrical Conductivity
pH	Potential of Hydrogen
UNW-DPC	United Nations-Water Decade Program on Capacity Development
E	East
N	North
W	West

NWW1	North Wastewater site 1
NWW2	North Wastewater site 2
NDS	North Disposal Station
HCA	Hierarchal Cluster Analysis
HDPE	High Density Polyethylene
PP	Polypropylene
BOPP	Biaxially oriented Polypropylene
LDPE	Low Density Polyethylene
SSL	South Sewage Line
GW	Fresh water
Min.	minute
g	gram
ml	milliliter
H_2O_2	Hydrogen Peroxide
$Zn\ Cl_2$	Zinc Chloride
µm	Micro meter
mm	millimeter
mg/L	Milligram per liter
H^+	Hydrogen Ion
ft	feet
HNO_3	Hydric Acid
v/v	Volume by Volume
kV	Kilo volt
Cu	Copper
Zn	Zinc
Cd	Cadmium
Mn	Manganese
Fe	Iron
Pb	Lead

Ni	Nickle
Cr	Chromium
Hg	Mercury
As	Arsenic
m^3	Meter cube
km^3	Cubic kilometer
mg/l	Milligram per liter
mg	Milligram
ml	Milliliter
µg/ml	Microgram per milliliter
BC	Bio char
g/l	Gram per liter
kJ^2/mol^2	Kilo joule square per mole square
J/K/mol	Joule per Kelvin per mole
kJ/mol	Kilo joule per mole
l/g	Litre per gram
ng/l	Nanogram per litre
µg/l	Microgram per litre
µg/kg	Microgram per kilogram
mg/l	Milligram per litre
mg/g	Milligram per gram
OM	Organic Matter
cm	Centimeter
mS/cm	Micro Siemens per centimeter
^{o}C	Degree Celsius

ABSTRACT:

Background: Fresh water and groundwater scarcity is one of the major concern around the globe. Pakistan is also facing major water crisis which has highlighted the country as a water stressed nation. Pakistan earns a major portion, (21%), of its GDP from agriculture. Therefore irrigation water is one of the most water demanding sector. Due to unavailability of groundwateron such a large scale, farmers have shifted toward wastewater irrigation. Other than the fact that wastewater is rich in organic matter and nutrients, many harmful elements such as heavy metals, pathogens, plastic fraction etc. are also a part of its chemistry. They can cause significant environmental concerns such as polluting soil and groundwater table and health concerns for people consuming the agricultural products grown is wastewater.

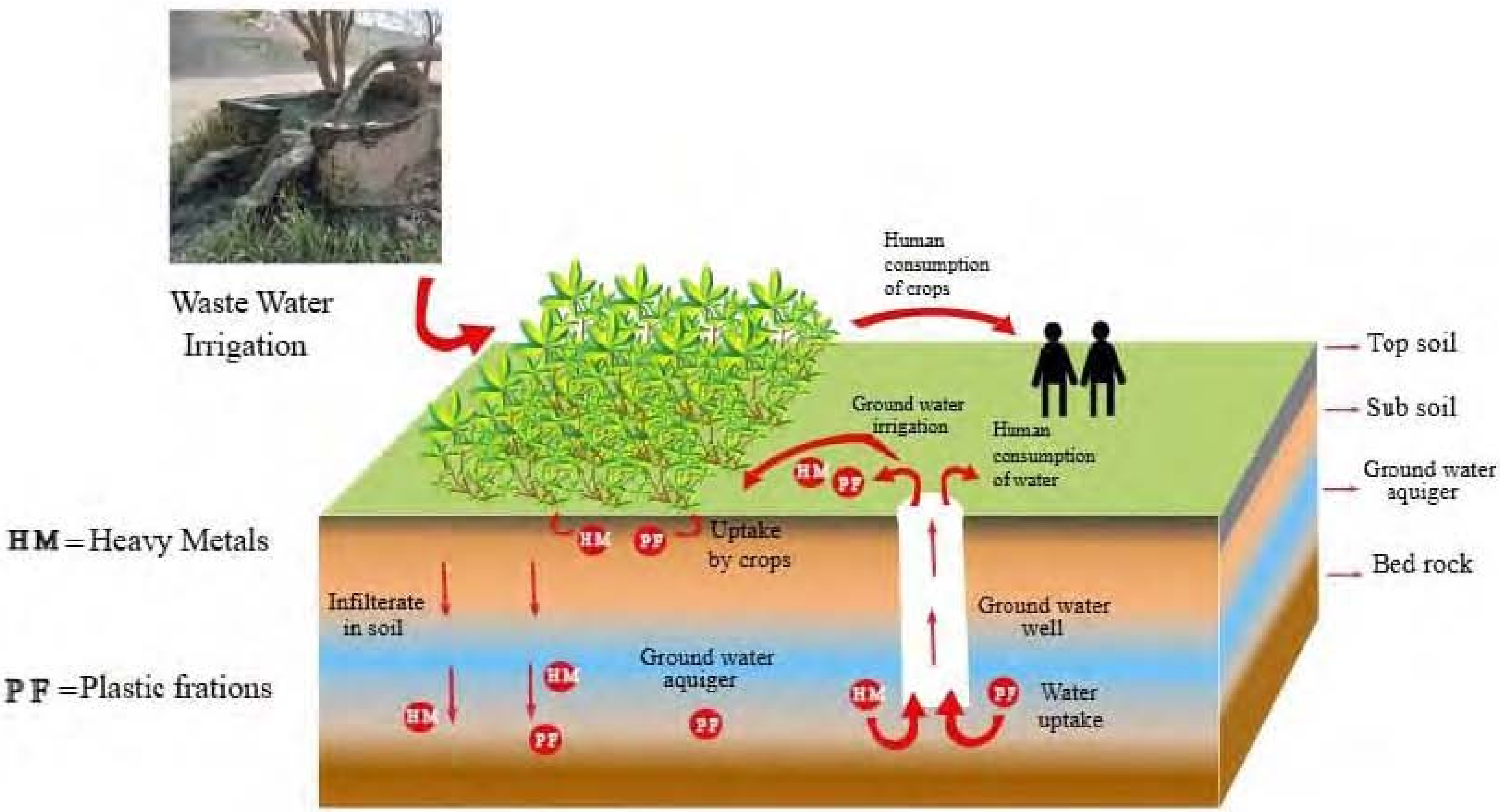
Objective: District Okara is one of the major agricultural districts of Punjab, Pakistan. This area is famous for fertile lands and good agricultural yield. Along with groundwater and canal water irrigation, wastewater irrigation is one of the major irrigation source in the district. The objective of this study was to observe the quality of wastewater and groundwater used for irrigation by evaluating their physiochemical properties and quantifying the concentrations of heavy metal and plastic fractions (macro- and micro-plastics), as their concentrations can impact the quality of food produced in the soil irrigated using these water systems. Basic quantification and characterization of plastic fraction is also an objective of this study since plastic pollution is of serious concern as an emerging pollutant around the globe. Health risk assessment of waste was also conducted in order to determine whether or not the selected groundwater wells are safe for human consumption.

Methodology: The study design was based on two north wastewater disposal stations and a south wastewater sewage line, which provide wastewater to agricultural fields of northern and southern parts of the city and 11 groundwater wells distributed at random locations in the peri-urban and rural areas of Okara were selected. Samples were collected, the on-field physiochemical parameter were recorded and the samples were preserved accordingly. All the key points of quality control and quality assurance were kept in check throughout the study. Heavy metals analysis were performed on the wastewater and groundwater samples using atomic absorption spectrometry and then the data was evaluated based on different statistical analysis. Plastic fractions were segregated and then visually identified and recorded. MPs were identified and enumerated using a compound

microscope equipped with digital camera. Health risk assessment of groundwater was performed using the standardized procedure.

Results and conclusions: Heavy metal analysis of urban wastewater samples revealed that the concentrations of Cd, Zn and Cu were higher than the acceptable FAO limits for irrigation at most wastewater site. 8 out of 11 groundwater samples showed high concentration of Cd ranges between 0.01-0.02 mg/L. Health risk assessment of the selected groundwater sites revealed the risk of Cd exposure in both adults and children upon consumption, however children were relatively more prone to long term health effects by Cd exposure than adults. The quantification of plastic fractions in urban wastewater and groundwater (25±6 and 413±2828 particles/L), with fiber as the most abundant particle shape and red was the most abundant color pigment in both wastewater and groundwater samples. The size of plastic particle had an inverse relation with particle abundance, which means more particles were present in the smaller size class and vice versa. Most plastic fractions were observed in the size range of 100µm-1mm for both groundwater and wastewater samples i.e. 212 and 239 particles/L.

GRAPHICAL ABSTRACT



CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Agriculture in Pakistan:

Agriculture plays a crucial role in the economic growth of developing countries such as Pakistan, India, and Bangladesh etc. Many factors such as fertile soils, suitable warm and cold weather patterns and good average rainfall contributes toward high agricultural yields in these areas. Pakistan is a known agriculture based economy. Agriculture contributes a huge value of 21% to Pakistan's Gross Urban Product (GDP) along with 2.7% annual growth(Wada et al., 2012). In Pakistan the agricultural sector is responsible for as much as 44% of employment opportunities to the labor force and since a large number of people belongs to the rural areas of the country, the livelihood of 62% of rural population is connected to this sector.

Water scarcity in Pakistan:

In countries such as Pakistan where the economic growth heavily depends upon agricultural sector, it contributes its role in regulating more than one economic parameter such as urban food production, suppling goods and services in the national and international market, and source for earning foreign exchange ultimately leading to food security, employment stability, poverty reduction etc. (Timer, CP, 2002). In A report by (Ashraf et al., 2016) the current Water Resources Vulnerability Index (WRVI) for Pakistan is 77%, reflecting a severe water scarcity condition. Pakistan is among the top ten countries with largest water withdrawal for agriculture. India is by far the leading country (90%), followed by China, the USA and Pakistan (Scheierling and Treguer, 2018).

1.2. Irrigation sources for agriculture:

In Pakistan there are three major types of sources used for irrigation practices i.e. canal irrigation, groundwater irrigation and wastewater irrigation. Only 15% of the water requirement for total crop growth is met with rainfall, the remaining water requirement is achieved via irrigation(Progress & Fy, 2005). For continuous production and better agricultural yield, farmers requires a constant and effective irrigation source.

There are three types of irrigation practices mostly utilized by farmers in Pakistan i.e. groundwater irrigation, canal water irrigation and wastewater irrigation. Indus basin irrigation system is the major surface water resource for agricultural irrigation in Pakistan which is divided into further tributaries to irrigate the main agricultural regions of Punjab region. Out of the 75% of water diverted to the canal irrigation network only 34% reaches the agricultural lands(Qureshi & Perry, 2021). The loss is due to improperly designed distribution facilities so this system solely, is not sufficient to fulfil the irrigation needs of the farmers. Also the Indus river system is under major water stress so it is impossible to meet the irrigation water demand of the region(Yang et al., 2016). Thus, almost 40 to 60% of agricultural needs are fulfilled via groundwater irrigation systems (Qureshi, McCornick, et al., 2010) that includes, tube wells, ponds and bore holes etc. But these practices are beyond the reach of lower or lower-middle class farmers due to their cost of installation and maintenance as well as the monthly electricity bills for running them. Other than that framers have a third option of wastewater irrigation, it is cost effective and readily available source of irrigation. Since wastewater have a large concentration of organic matter and essential nutrients, using wastewater reduces the extra cost of using fertilizers and it naturally enrich the soil with nutrients.

1.3. Groundwater irrigation:

1.3.1. Global groundwater usage in agriculture:

Earth's surface is covered with 71% of water out of which only 3% is groundwater which meets all our daily demands from drinking to industrial usage. Agriculture sector is one of the major consumer of groundwater which accounts for almost 70% of global groundwater usage. With the rapidly growing population, the food demand is also increasing, therefore agricultural sector is expanding, so the water demand for irrigation is also increasing. Countries with largest agricultural output are consuming the largest number of their groundwater reserves, among these countries India, Pakistan, the United States, Iran, China, Mexico, and Saudi Arabia has the highest nonrenewable groundwater uptake(Sarwar & Eggers, 2006). The fast pace agricultural growth is making it difficult for the groundwater table to recharge at a natural speed. In areas where the groundwater uptake exceeds the rate of groundwater recharge for a long period of time, the groundwater tables lower to unreachable lengths causing water scarcity.

1.3.2. Status of groundwater irrigation in Pakistan:

Groundwater irrigation is the major source of irrigation in areas that lack access to the Indus basin irrigation systems or don't get enough rainfall, in order to maintain the agricultural yield. Since 1970's Pakistan has seen a major boom in groundwater irrigation through privately or publicly owned tube wells. Groundwater irrigation fulfills more than 50% of Pakistan's irrigation requirements (Qureshi, Gill, et al., 2010). It provides a continuous and cleaner source for irrigating crops without damaging the crops and the soil it irrigates. Due the unrestricted access to groundwater, it provides food security and livelihood to the people of that region and contributes to the economic growth of the country.

1.3.3. Effect of groundwater scarcity on irrigation:

Groundwater is an important source of freshwater for at least two billion people worldwide and is used for drinking, agricultural, urban, and industrial purposes (Re et al,, 2019)**.** In the last few years groundwater abstraction has raised to 60 km^3 which is considerably higher than the 55 km^3, the annual recharge rate(Qureshi, Gill, et al., 2010). Since the government has not set in place any particular regulations regarding the use of groundwater for irrigation, the unsustainable consumption has led to a drop in the water table. It is reported that the groundwater table has declined to about 5-15% due to unchecked irrigation practices especially in the region of Punjab (Qureshi et al., 2008).

1.3.4. Heavy metals in groundwater:

Heavy metals can reach environment through various sources. Anthropogenic activities such as industrialization, agricultural activites etc are the major sources. Long term heavy metal contamination results in their mobilization to the surface and groundwater bodies, which in conclusion reach human and animals through ingestion and dermal contacts. Studies in various region in the world has identified different heavy metals in their groundwater reservoirs, some of them are reported in the Table 1.

Table 1.1 Various studies reporting heavymetal contamination in groundwater and their possible sources

Sr. No	Location	Detected heavy metals	Sources	Referencs
1.	Kassena Nankana, Ghanna	Cr and Pb	Corrosion of lead-containing pump fixtures on the boreholes contributed to Pb in water. Fertilizers, Improper disposal of waste and wastewater cased high levels of Cr.	Zakaria et al., 2022
2.	Tapiza, Albania	As, Cd, Co, Cu and Pb	anthropogenic activities i.e. agriculture, transport, construction and housing	Deda et al., 2019
3.	Ergene Basin, Türkiye	Highest Cr; Cr, and Pb detected	Fertilizers, impurities from galvanized pipes and plumbing systems	Arkoc, 2014
4.	Northeast China	Cr and Cd	landfill and long-term waste water irrigation	Dong et al., 2009
5.	Pakistan	Cd	fertilizers and pesticides in agriculture	Niazi et al., 2019
6.	Chandigarh, India	Cd, Ni, Pb, Zn	Agricultural activities i.e. heavy usage of fertilizers in the agricultural fields and landfill sites	Ravindra & Mor, 2019
7.	Iran	Cu	Agricultural activites	Nouri et al., 2008
8.	Tamil Nadu, India	Ag, Pb, Ni, Al, B, Cd, Cu, Fe and Mn	Heavy use of fertilizers in agricultural fields, wastewater emission from industries. seawater intrusion caused by intensive pumping for agriculture.	Vetrimurugan et al., 2017
9.	Reasi District, Jammu Kashmir	Pb, Cr and Cd	different anthropogenic activities, agricultural discharge, sewage discharge, combusting gases and dust from brick kiln	Kaur et al., 2020
10.	Neyshabur Plain, Iran	Fe, As, Pb and Cd	Agricultural activities	Saleh et al., 2019

1.3.5. Plastic fractions in groundwater:

Different environmental matrixes plays a vital role in the regional or global transport of plastic fractions. Intensive use of plastic wastage has impaced our environment greatly, which has left our groundwater sources vary prone to any kind of environmental pollution i.e. heavy metals and plastic pollution etc. the presences of plastic fractions have been reported in various groundwater samples all around the world, some of them are mentioned in the table Presence of MPs has been reported in atmospheric compartment in different studies showed in Table 1.2

Table 1.2: Concentrations of MPs in groundwater and abundant shapes reported in different studies around the globe

Sr no.	**MPs abundance (untreated wastewater)**	**Abundant Shape**	**Location**	**References**
1	$1.5x10^4$	Fibers	Sweden	Magnusson and Noren, 2014
2	$3.2x10^5$	Fibers	France	Dris et al., 2015
3	$1.5x10^4$	Films	Scotland	Murphy et al., 2016
4	$1.6x10^5$	Black particles	Finland	HELCOM, 2014
5	$1.0 x10^3$	N/A	US	Carr et al., 2016

1.4 Wastewater irrigation:

1.4.1. Global usage of wastewater in irrigation:

Approximately 60% of the world population expected to suffer from water scarce conditions by the year 2025(Qadir et al., 2007). Since irrigation is one of the largest consumer of water, global water scarcity has taken a toll on the agricultural yield. With the rapidly increasing population food demand is also increasing and the current pace of agriculture has failed to keep up with the food demand. Therefore the world is now shifting to the century's old practice of using wastewater to fulfil the irrigation demand of the crops. Wastewater irrigation is a very sustainable way of practice as it provide natural essential nutrients to the soil, preserves water resources, prevent contamination of groundwater resources, reduce artificial fertilizer usage and saves the wastewater purification and disposal cost, thus wastewater irrigation is a cost-effective, unlimited and environmental friendly irrigation resource.

Countries such as Vietnam, Mexico and China has been involved in wastewater irrigation practices for decades(Irrigation, 1990). Pakistan, India, Jordan and Saudi Arabia are also commonly practicing wastewater irrigation as a potential source of irrigation. In European countries, wastewater is treated 70 to 100% but the use of treated water for irrigation is very low(Singh et al., 2016). Regarding wastewater usage in agriculture most number of studies have been conducted in Mediterranean countries i.e 44 %, this excess of wastewater usage in agriculture is due to sever water stressed conditions in the countries, Asia is places on the second position with 24% studies on wastewater usage due to poor economic conditions in most of the areas, then the American continents lies in the third position with 19 % studies conducted in the region and lastly only 10% of studies on wastewater usage in agriculture has been conducted on Africa, Europe and Oceania (Singh et al., 2016) since these countries are not very big agricultural produces as the other regions and they are able to fulfil their irrigation needs via groundwater irrigation.

1.4.2. Wastewater irrigation in Pakistan:

Since Pakistan is a semi-arid country, water scarcity is one of the major concern in the region and as an agricultural economy, irrigation practices takes up a large sum of water resources of the country. Indus Basin Irrigation System in Pakistan has the largest contiguous planned irrigation system around the globe. But with an increasing demand of food due to rapidly increasing population it is impossible to solely rely on this canal irrigation system. Therefore shifting to an alternative resource is a reasonable option and wastewater is one of the most accessible choice in this regard. In most of the peri-urban areas of Pakistan, wastewater is now a major irrigation source for crops especially for vegetable farming. Since most of the farmers in this region have leased lands or own small land areas, switching from groundwater to wastewater irrigation is a more cost-effective option.

Using wastewater for irrigation is also a solution for proper wastewater disposal in the country. Out of 388 cities in Pakistan it is estimate that only 2% of the cities have working wastewater treatment plants(Ensink et al., 2004). However most of these wastewater treatment plants have a capacity of receiving only 30% of the total wastewater produced in the city, the remaining is disposed of untreated in the canals and rivers, so it ends up contaminating groundwater bodies.

Studies have revealed that a total of 2,400,000 m^3/day of wastewater is directly used in agriculture and the amount of 400,000 m^3 untreated wastewater is disposed of in the irrigation canal on daily basis.

Total amount of direct wastewater used daily in agriculture was 2,400,000 m^3 while an additional 400,000 m^3/day of untreated wastewater was directly disposed of in irrigation canals(Keraita & Drechsel, 2004), which represents 31% and 5%, respectively of the total amount of wastewater produced per day. The remaining 64% was either disposed of in rivers or in the Arabian Sea.

1.5. Characteristics of wastewater:

Wastewater is rich with a large number of chemical, physical and biological properties that makes it an interesting study matrix. Wastewater consists of a large number of microorganisms, inorganic minerals, dissolved and suspended organic matter and a large number of different heavy metals such as zinc, copper, lead, cadmium etc. (Pescod, 1992), pharmaceuticals, pesticides, detergents, cloth fibers, fats and nitrogenous organic matter etc.(Cizmas et al., 2015). Other than these pollutants a major portion of wastewater consists of polymer fibers, polypropylene, polyethylene terephthalate, polystyrene, polyethylene, and micro-plastics(Carr et al., 2016) they are expected to cause hazardous effects on living organisms. Keeping in view the different properties of wastewater, different studies have focused on various parameters of wastewater.

1.5.1. Heavy metals in wastewater:

Heavy metals are high density metallic chemical elements, occur naturally in the environment in varying concentrations. Heavy metals have caused a major health and environmental concern globally as they are known to cause toxicity at even very low concentrations (Metal & Development, n.d.) As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn are those heavy metals that cause major concern in the environmental matrices i.e. soil and water(Ali et al., 2019).

Wastewater is known to have inorganic toxic substances, such as heavy metals that are present in large quantities. The sources of heavy metals in the sewage system varies in different areas accordingly but most common sources reported are corrosion in urban plumbing, household activates such as use of tools made up of alloys and metals for cooking, storing etc., and groundwater infiltration(Houhou et al., 2009). Since wastewater irrigation is a common practice in many areas, continuous application of wastewater causes accumulation of heavy metals in the soil in a substantial amount. They remains in the soil for an indefinite period, thus altering soil properties (Pether, 1949). Studies have revealed that there is a linear relationship between accumulation of heavy metals and time span of wastewater irrigation in a certain area (Lucho-Constantino et al., 2005).

1.5.2. Plastic fractions in wastewater:

Plastics are chemically stable compounds made up of miscellaneous chemical elements of synthetic polymers, these polymers are assorted to create plastics that are used for various purposes in everyday life(Wright et al., 2013). Since plastics are non-corrosive, malleable, resilient materials, they do not decompose instead only breakdown from larger particles into smaller ones due to various physical, chemical and biological processes and they persist in the environment for a long time. Among these plastic particles, Micro-plastics (MPs) are referred to fibers, fragments, films and foams of length of <5 mm(Galloway et al., 2017) and macro-plastics are those particles of length >5 mm. Studies in the recent years have revealed that MPs can effect several environmental compartments such as aquatic, terrestrial and atmospheric environment. Although a large number of research has been carried out in the past few years in context to MPs and their impacts on the environment and living organisms but this issue is still up for discussion since MPs sources, concentrations, retention and characteristics vary according to the surrounding and its conditions (Andrady et al., 2017).

Presence of MPs in wastewater has been a major concern due to the fact that in most region of the world wastewater is disposed of in the waterbodies where it can alter the water chemistry and in many cases not only affect the life of aquatic organisms but proved to be deadly for them. Wastewater, in the form of wastewater irrigation, contributes as one of the major source of MPs in the soil. Wastewater irrigation is a known source for the accumulation of hazardous materials such as pharmaceuticals and heavy metals etc. (Dalkmann et al., 2012), it apparently contributes towards the accumulation of micro-plastics in agricultural soils. In many parts of the world treated and untreated wastewater has become an important or only source of irrigating agricultural fields. Approximately 7% of the total irrigated land in the world which is estimated to be 20 million hectares are irrigated using treated or untreated wastewater. Researchers have reported that the food demands of 10% of the world population is met using agricultural products grown via wastewater irrigation. (UNW-DPC, 2013). Plastic waste is a main constituent of untreated or partially treated wastewater. Household activates such as washing textile goods, daily personal care goods such as shampoos, scrubs, facewash etc. contains large amounts of small plastic particles (Ziajahromi et al., 2016). Many studies have reported different concentrations and shapes of MPs in wastewater around the globe.

1.7. Problem statement:

Wastewater irrigation is one of the main cause of terrestrial pollution. Wastewater contains heavy metals, pharmaceuticals, petrochemicals, plastic fractions etc. which upon irrigation will be transported to the soil. It can alter soil properties such as pH, EC, OM and HM content [1], etc. resulting in deterioration of soil quality. Bioaccumulation of heavy metals in the crops grown in such soils can enter the food chain thus they can cause toxic effects on human health upon consumption. Agricultural drainage from irrigation also contributes toward the addition of pollutants in water bodies. Wastewater irrigation is a common practice since the beginning of agricultural activities in Okara. However, its continuous practice may leads to potentially negative implications on the agricultural soil quality due to the presence of various pollutants, also it can damage crop quality and crop yield.

Many studies have reported the exceeded levels of heavy metals from the existing standards in irrigation wastewater in Pakistan, a significant correlation has been found in the heavy metals present in the wastewater (Haroon et al., 2018). However in Pakistan, most studies do not take into account the plastic pollution that can be introduced in agricultural soils along with heavy metals due to prolonged wastewater exposure. This study is designed to anazyle and report both heavymetals, macro- and micro-plastic fractions as a pollutant in irrigation wastewater.

1.8. Objectives:

The present study is designed:

- To quantify the selected heavy metals contents in the wastewater and groundwater samples collected from 3 wastewater sites and randomly selected 11 groundwater wells from the peri-urban areas of Okara, in order to determine irrigation as a source of heavy metals contamination in of soil and the crops grown in it
- To identify the role of wastewater irrigation as a source of plastic pollution in agricultural soils by thir quantitative and qualitative assessment
- To identify wastewater irrigation as a source of heavymetal and plastic pollution in groundwater samples
- To assess risk of groundwater consumption from the selected groundwater wells on human health (adults and children)

1.9. Significance of study:

District Okara, Punjab, Pakistan is famous for its fertile lands. It is one of the major agricultural district of Pakistan for the production of Potato, wheat, maize etc. Since agriculture is the biggest source of livelihood here, it is important to study the factors that can directly or indirectly affect the agricultural productivity in the area. Irrigation is one of the key factor that contributes toward the quality and continuity of agricultural yeild. Apart from groundwater and canal water, wastewater irrigation is a also reliable source of irrigation, since the urban wastewater is collected at disposal stations and provided to the farmers via sullage carriers to fulfil their irrigation needs.

This is an initial study which has focused on the presence of heavy metal contamination as well as plastic pollution, in the groundwater and wastewater irrigation sources used in Okara city and its peri-urban areas. No such research has been conducted in Okara city so far. The comparative analysis of different parameters among bothwastewater and groundwater irrigation sources will help identify their long term impacts on agricultural lands in future studies. Health risk assessment of groundwater sources will determine whether it is fit for human consumption or not.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study area:

2.1.1: Geographical data:

Okara district is one of the major agricultural district of Pakistan, with a total cover area of 4623, located at **30° 48' 30.6000'' N**, **73° 27' 33.8256'' E** longitude and latitude. Okara district has three tehsils i.e. Okara city, Depalpur, Renala Khurd and a total of 86 chaks are also included in the district. Okara city is headquarter of the District Okara with the road length of 199 km and a total population of 4, 61,600 according to the District Council. The recorded annual rainfall in the

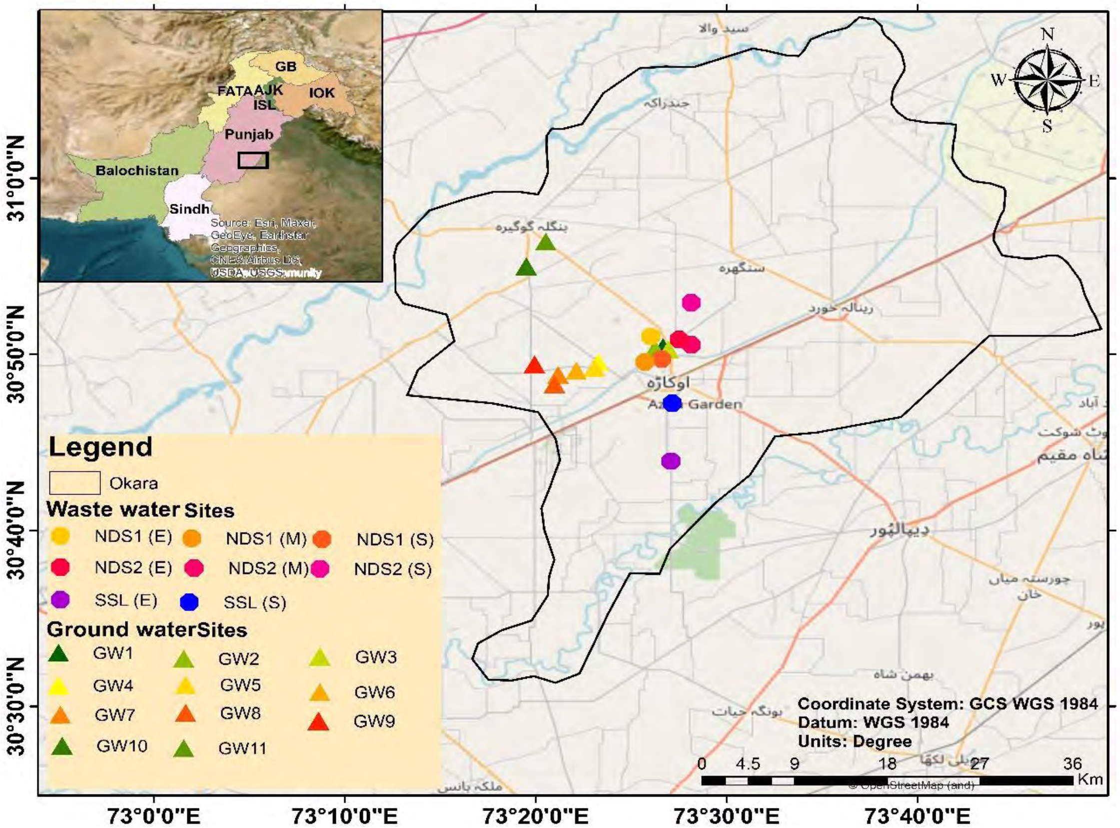


Fig 2.1: Sampling points on the study area map

city is 250-265 mm. The climate of the region is mostly warm and dry with moderate rainfall. Seasonal distribution in the area remains persistent as the summer season ranges from April to October with May, June and July recorded with the highest temperatures from 24° to 45°C and the winter season ranges from November to February with temperature dropping to 3°C.

The railway track divides the city into two parts, Northern side and Southern side of the railway track. The north side of the city is well-developed and commercialized area which is divided into block system i.e. A block, B block, C block, D block, E block, F block. Its population was estimated to be 154121 in 2016. Main vegetable and fruit markets, shopping centers, trading centers, restaurants, food vendors, schools, collages, private academies and offices are all present on this side of the city.

South side of the city is not developed with relatively poor living standards and limited access to clean water and food sources, most of the population in this area travels to the north side of the city to buy their living necessities or rely on what little resources are available there. South side of the city covers a large number of chaks therefore the population density of this area is higher i.e. 226850 estimated in 2016.

2.1.2: Irrigation practices in Okara city:

Major crops that are cultivated in the region are maize, wheat, potato, sugar cane, rice and large variety of vegetables. Water requirement for irrigation vary with each type of crop, such as wastewater is considered to be a more suitable irrigation medium for vegetables in the area etc.

The water table of the city has brackish water, therefore the drinking water demand of the city is fulfilled via varied depth of tube wells installed on the bank of Lower Bari Doab canal that passes through the territory of Okara city. Lower Bari Doab is one of the major source of irrigation in Okara city apart from tube wells and wastewater. Due to the high cost of tube wells the government has provided the farmers with wastewater via the sewerage system of the city for irrigation practices. The sewerage system consists of two parts based on the side of the city from where the sewerage was collected.

2.1.3. Urban wastewater collection in Okara city:

2.1.3.1. Northern side sewage system:

The sewerage system on the northern side comprises of 9″ to 48″ i/d sewerage pipelines that are linked to the disposal station of the city. Two disposal stations are present on the north side of the city. These disposal stations are specifically designed structure consists of a screening chamber, two collecting chambers and a pumping chamber. The urban wastewater collected in the stations are discharged into the discharge sump, which will throw it into the katcha sullage carrier having a length of 10,000 Rft each. The sullage carrier passes through the agricultural fields where it is used for irrigation purpose by the framers into the main disposal drain.

2.1.3.2. Southern side sewage system:

The southern side of the city is a relatively underprivileged and underdeveloped area. It is a low elevation area then the rest of the city therefore a pumping and distribution station was not required. After collection of urban wastewater in the main sewage line, the wastewater flows through the sullage carrier under the influence of gravity due to the difference in elevation. The sullage carrier runs along the agricultural lands, due to lack of resources most of the farmers irrigate their lands using the sewage water.

2.1.4. Groundwater irrigation system in Okara:

Northern side of Okara city has a large number of public and private tube wells installed in order to fulfill the irrigation requirements of the crops. Due to good economic conditions in northern side of the city, farmers can afford to install tube wells therefore, groundwater irrigation is a more popular practice. However on the southern side of the city very few public tube wells are installed, of which mostly are out of order and only a few private tube wells that are used to pump groundwater for the crops rarely. Therefore hardly any running groundwater tube wells were located in this side.

Wastewater and groundwater samples were collected from of Okara city during the month of March

2.2. Sampling strategy:

Figure 2.2: Sampling stratergy of groundwater and wastewater samples from Okara (A) Sample collection of plastic fractions from wastewater sulliage carriers using menta trawl (B) pouring the collected sample on 220 µm sieve (C) drying the sample (D) storing and labeling the samples in plastic zipper bags (E) wastewater well (F) wastewater sample collection for heavy metal analysis (G) measuring pH and EC of wastewater samples using multimeter (H) acidifying samples with 2-3 drops of conc. HNO_3 for heavy metal analysis (I) sealing and labeling the samples in plastic bottles (J) taking 4 samples from groundwater wells (K) measuring pH and EC of groundwater samples using multimeter (L)) acidifying 2 samples with 2-3 drops of conc. HNO_3 for heavy metal analysis

2.2.1. Wastewater sample collection:

. Wastewater samples from northern sides were collected from the sullage carrier running from each disposal station 30°49'50.6"N 73°26'32.5"E and 30°52'56.2"N 73°28'08.7"E denoted as NWW1 and NWW2 in this study, whereas the samples from the southern side were collected from

30°47'13.7"N 73°27'09.0"E from the running sullage carrier. 7 cucic of wastewater was pushed in the sullage carrier with the help of motors installed in the disposal station running at the speed of 1400 rotations/min. 70 ml water samples were directly collected in each of the two plastic bottles from the start, middle and discharge end of the sullage carrier, then they were acidified, labeled and sealed.

2.2.2. Wastewater sludge samples collection:

The wastewater sludge samples were collected using a menta trawl for microplastics collection. Menta trawl has the mesh size of 220 µm. The menta trawl was placed in the sullage carrier till the net is completely filled with the suspended waste particles, which takes almost 15 to 20 minutes. The sample was collected and washed on a 220 µm sieve and then collected and labeled on a zipper bag. From each site two types of duplicate samples were collected from starting point, mid-point and ending point of the sullage carrier.

Samples collected from the south sewage line are denoted as SWW. Samples for wastewater and sludge were collected from the start and discharge end of the sewage line in the same way as the samples for north side sampling sites.

2.2.3. Groundwater Sample collection:

Groundwater samples were randomly collected from 11 tube wells. Due to the unavailability of groundwater tube wells in the southern side of the city, we could locate only one running tube well which was irrigating 15 acres of land. Other 10 groundwater samples were collected from northern side of the city. Two types of wastewater samples were collected: Acidified samples for heavymetal analysis and unacidified samples for detection of plastic fractions in groundwater. The 70 ml samples were collected directly into each of the 4 plastic bottles, and then sealed and labeled.

2.3. Sampling prerequisites:

The samples were taken in 100ml polyethylene bottles that were treated with acid water and then rinsed with deionized water before taken to the field. Duplicates of treated and untreated samples were collected on site. One set of the samples were treated with 2-3 drops of conc. HNO_3 in order to lower the pH to ≤ 2 to avoid any changes in the chemical properties of water. Acidification of the samples make the metals available in dissolved form. Then the samples were taken to the laboratory and stored at 4°C.

2.4. Field analysis:

The longitude and latitude of the sample pH and EC were measured using portable OAKTON PCTSTestr 50 pocket tester. The instrument is calibrated on regularly before analysis with the buffer solutions of pH 4, 7 and EC 84, 1413 µS/cm respectively. The pH and EC of each sample was recorded three times in order to obtain accuracy.

Latitude, the longitude of the study area was noted at the spot with the use of GPS services. Some parameters were needed to analyze at the sampling spot quickly after samples were taken with the help of field kits like electrical conductivity (EC), and pH. Field data is quickly recorded for further evaluation of results. Interviews of farmers were conducted in order to collect the information about the field area.

2.5. Instrumentation and chemicals:

2.5.1. Instruments:

All instruments were used for the specific chemical analysis after proper calibration. The list of various instruments used are:

1. Electronic balance
2. Digital Probe/ multi-meter
3. Atomic absorption spectrophotometry
4. Digital compound microscope

2.5.2. Chemical analysis:

All the chemicals used for analysis are of 99% analytical grade to get better results. All apparatus washing is done by use of deionized water for avoiding contamination. All standard solutions were prepared by diluting the required chemical of the desired quantity. For precision the amount of chemicals required is measured by weight balance for better investigation of results.

2.6. Laboratory analysis:

The following parameters were analyzed in the lab by using the standard method of examination.

2.6.1. Heavy metals in wastewater:

Several steps are involved for the analysis of heavy metals in the wastewater samples. These steps are as follows:

2.6.1.1 Digestion of wastewater samples:

Wastewater samples contains a large number of organic matter and impurities, therefore these pollutants can interfere with the readings of atomic absorption spectrophotometer (AAS) analysis. So the wastewater samples were first digested and then ran on the atomic absorption spectrophotometer. Selected heavy metals Cu, Zn, Cd, Mn, Fe and Pb were analyzed on AAS.

2.6.1.2 Procedure:

5 mL of concentrated HNO3 were added to each 50 mL wastewater sample in a 100 mL conical flask after this the mixture was slowly boiled on a hotplate until the volume reduced to 10 mL. This reaction can be violent so the complete procedure was carried out in a fume hood. Then the solution was cooled to room temperature and then 2.5 mL of concentrated HNO was added to the solution. Until the digestion is done, heating will continue with the successive addition of 2.5 mL portions of concentrated HNO3 at regular interval. The aqueous mixture will be evaporated till it is dryed and then cooled at room temperature and reconstituted in a 2.5 mL of HCl solution (1:1 v/v). Then the solution was warmed at low flame before the next addition of 2.5 mL NaOH (5 M). The mixture was then filtered using a Whatmann No. 1 filter paper. Lastly, the filtrate was transferred to a 100 mL volumetric flask and then raised up to the mark using distilled water **[1].**

2.6.2. Heavy metals in groundwater:

2 acidified groundwater samples of all sites were filtered using Whatman's filter paper No.40 in a 50ml conical flask, the filterate was stored in plastic bottles and then labled carefully.

2.6.3. Analysis on atomic absorption spectrophotometer (AAS):

Both wastewater and groundwater samples after treatment, were analyzed in atomic absorption spectrophotometer (AAS) for heavy metals analysis at reported wavelengths for each (Fe^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, $Cd^{2+,}$ Cu^{2+}). For each trace metal standards of required concentrations were first made and then the calibration curve was checked for accuracy in a reading of every water sample. Standards solutions of various concentrations 0.5, 0.1, 0.05, 0.01 ppm were analyzed on the AAS for calibration. Procedural blank samples were also analyzed before any trace metal analysis in were performed in the AAS, the value of blanks were below zero, which is an acceptable range.

2.6.4. Health risk assessment of groundwater:

Health risk assessment (HRA) is a procedure designed to estimate the exposure risk of a substance of concern to a designated population. In this case health risk assessment will provide the magnitude of adverse effect of human health when exposed to heavy metal contamination in groundwater for a certain period of time.

Literature has suggested that there are two main exposure pathways of heavymetal through groundwater: first oral intake or ingestion and second dermal contact. The HRA was conducted for two different age groups in this study i.e. children and adults to increase the diversity of data.

The average daily intake (ADI) of heavy metals through oral intake or ingestion was calculated by using Eq. (i).

$$ADI_{ing} = \frac{C \times IR \times EF \times ED}{BW \times AT} \text{................(i)}$$

The average daily intake (ADI) of heavy metals through dermal contact was calculated by using Eq. (ii)

$$ADI_{derm} = \frac{C \times SA \times k_p \times ET \times EF \times ED \times CF}{BW \times AT} \text{.................(ii)}$$

in the above equations 1 and 2, C is the concentration of heavy metal in groundwater in mg/L, IR is ingestion rate (2 L/day for adults and 0. 95 L/day for children)**,** EF is exposure frequency that is 360 days/year**,** ED is exposure duration which is designated to be 70 years for adults and 6 years for children, BW is the body weight as 70 kg average weight for adults and 15 kg for children, SA represents exposed skin area whish is estimated to be 18,000 cm^2 for adults and 6600 cm^2 for children, ET represents exposure time that is 0.6 h/day for adult and 1h/day for children, ATis the average time (ED×365 days) that is 25,550 days for adults and 2190 for children, CF is unit conversion factor which is 0.001. kp is the value of dermal permeability coefficient. Its values are given in litrature as 0.001 for Fe, Cu, Cd and Mn, 0.0006 for Zn , 0.004 for Pb **(USEPA 2004).**

Hazard Quotient determines the potential risk of an individual heavy metal in groundwater along with its overall associated risk in humans due to the consumption of the water The hazard quotient of the heavy metals in groundwater was calculated using Eq. (iii).

$$HQ = \frac{ADI}{RfD}\ldots\ldots\ldots\ldots\ldots(iii)$$

In the above equation, HQ is the hazard quotient of , RfD is the reference dose. The reference dose of oral toxicity for the heavy metals are given as 0.04 for Cu, 0.0035 for Pb, 0.14 for Mn, 0.3 for Zn, 0.7 for Fe and 0.0005 for Cd (USEPA 2006) and the values of reference dose of dermal toxicity are given as 0.012 for Cu, 0.00042 for Pb, 0.045 for Fe, 0.0008 for Mn, 0.06 for Zn, 0.0005 for Cd (Wu et al. 2009). The hazard index (HI) was calculated using Eq. (iv)

$$HI = HQ_{ing} + HQ_{derm}\ldots\ldots\ldots\ldots\ldots (iv)$$

In the above equation, HQ_{ing} is hazard quotient from ingestion and HQ_{derm} is hazard quotient from dermal exposure.

2.6.5. Plastic fraction in groundwaters:

2 unacidified groundwater samples were directly passed through a 1.2µm pore size glass fiber filter paper with a diameter of 47mm in a vaccum pump assembly. After complete filteration, the filter papers was removed and dried for 24 hours and placed in a preheated petri dish which was properly labeled and sealed.

2.6.6. Plastic fractions in wastewater:

Several steps are involved in the analysis of plastic fractions in the wastewater sludge samples from sample preparation to extraction and lastly detection. These steps are as follows:

2.6.6.1. Digestion of Wastewater samples:

Digestion of organic matter is one of the most important step in the laboratory analysis of wastewater samples because the presence of organic matter can hinder the identification and counting process of micro-plastics in a sample **[2].** The organic material in the wastewater samples was removed through wet peroxidation (WPO) method, this method has been proved as the most effective method for the removal of organic matter because Fenton reagent used in this process does not damage the integrity of micro-plastic particles in the sample **[3]**

The volume of 20ml of 0.05M Fe (II) was added in the 1000ml of beaker containing 1g of sewage sludge sample. Then a solution of 20ml of 30 % H_2O_2 was added in the breaker with the help of a pipette and leave the sample for 5 min in the lab. Place the beaker on a hot plate at 75°C and add a magnetic stirrer as well for thorough mixing then cover the beaker with a watch glass. A magnetic stirrer was added to the beaker and beaker was covered with watch glass. The beaker will be removed from the hot plate when bubbles emerge from the solution. Addition of deionized water will reduce the intensity of the reaction. Reheat the mixture at 75°C for 30 min. if organic matter is still visible remove add 20ml of 30% H_2O_2 again with moderate heating till the organic matter disappears.

2.6.6.2. Separation of Micro-plastics:

In order to effectively remove the plastic particles from the water sample a saturated salt i.e. $ZnCl_2$ will be used as it effectively separate dense micro-plastics from the water samples. $ZnCl_2$ stock solution was prepared in the laboratory and used for the purpose of density separation.

2.6.6.3. Density Based Separation of Micro-plastics:

The prepared $ZnCl_2$ solution was added in the digestate and then it was shaken well for 10 mins to separate the particles that were adhered together. Then the solution was emptied in a separatory funnel, the beaker was rinsed well with distilled water to remove the complete sample. This solution was left overnight in order to let it settle. The micro-plastic particles due to low density will accumulate on the top and the solution at the bottom will be discharged from the funnel. The supernatant was then removed from the funnel into a separate beaker and then vacuum filtered by placing a 1.2µm pore size glass fiber filter paper with a diameter of 47mm. lastly the filter papers were dried for 24 hours and placed in a preheated petri dish **[4]** and sealed.

2.6.6.4. Microscopic Observations:

First of all visual identification of MPs was carried out. For the plastic particles with the size range > 500µm visual identification is an effective method **[5]**. A binocular microscope (CX41; Olympus Co. Ltd., Japan) with a magnification power up to 100X with digital camera (ISH500; U-TV0.5XC-3, Co. Ltd, Japan) was used for identification and quantification of the micro-plastic particles suspected to be present on the filter papers under magnification of 4X and 10X. With the help of microscopy, physical characteristics such as size, color and shape can be easily identified.

2.7. Role of Contamination Control and Quality Assurance:

All the experimental work was carried out in controlled environmental conditions. Precautionary measures were taken to avoid the contamination during analysis. In order to evade chances of the contamination during analytical procedure, all laboratory surfaces were wiped out with deionized water and bright colored sponge, cotton lab coat was worn, hands were covered with latex gloves, all lab windows were closed during experiment. A glass petri dish filled with deionized water was placed on a surface in the laboratory where all analysis was carried out in order to have an estimate of amount of airborne contamination during analysis. All the sieves used during laboratory procedures were washed with distilled water and covered with aluminum foil before and after sieving process.

Moreover, reagents used during the process of density separation and digestion were sieved through a sieve of 75µm.Furthermore, during atmospheric sampling procedural blanks were carried out in order to have an estimate of rate of contamination during sampling. Before use all the glassware and equipment were washed and rinsed properly with deionized water, and remained covered with aluminum foil before and after use. Metal forceps and petri dished were heated in oven for one hours at the temperature of 200 °C in order to remove the plastic contamination. During whole experiment use of plastic apparatus was avoided, as use of plastic material can affect the experimental results.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Descriptive statistics of physiochemical parameters and heavy metals in groundwater and wastewater:

Groundwater samples from 11 sites and wastewater samples from 3 different sites including 3 subsamples from each wastewater site were taken and their physiological parameters including pH and EC were measured on site. After in-lab analysis, the heavy metal concentrations for both types of samples were also determined. The statistical summary including minimum, maximum, range, mean and standard deviation represents the concentrations of heavy metals including Manganese (Mn), Zinc (Zn), iron (Fe), Cadmium (Cd), lead (Pb) and Copper (Cu) etc. and physiochemical parameters including pH and EC of both groundwater and wastewater sites in the Table 3.1. Analytical results of groundwater and wastewater parameters were compared with the standards of Food and Agriculture Organization (FAO).

The pH of groundwater samples ranges between 5.7-6.10 with an average value of 5.91±0.15 which falls in the slightly acidic range on the pH scale. The pH on three wastewater sites NDS1, NDS2 and SSL ranges between 5.6-6.10, 5.9-7.00 and 5.8-6.20 with an average value of 5.91±0.15, 5.86±0.23 and 6±0.24 which indicates that the pH of wastewater falls in slightly acidic to neutral range on a pH scale. The overall results of pH values lies within the permissible range of FAO standards i.e. 6.5-8

The EC is an important physiochemical parameter of water which indicates t that is an indication of the conducting capacity of water affected by the existence of ions as it is directly allied to the concentration of ions in water. The EC of groundwater samples ranges from 0.61-5.19 with an average value of 1.57±1.29 whereas the EC at wastewater sites NDS1, NDS2 and SSL is 2.40±0.10, 1.07±0.90 and 3.76±0.06. The values of EC falls at groundwater falls in the permissible range of EC of 0.7-3.0 dS/m mentioned in the FAO standards, but the EC of wastewater is slightly high at SSL.

The concentrations of all heavy metals in the groundwater samples lies within the permissible limits suggested by FAO standards except for the values of Cd which ranges between 0.01-0.02 mg/L, a slightly higher value than the given permissible limit in water used for agricultural purposes and the average value of Cd is 0.01±0.01. However, in the case of wastewater samples, the concentrations of various heavy metals were found to be above their permissible limits such as the value of Cd were higher in all three wastewater sites NDS1, NDS2 and SSL with the average values of 0.33±0.34, 0.92±1.28 and 0.19±0.02. Similar results showed in various studies indicates that the major causes of cadmium contamination in groundwater are anthropogenic activities including industrial activities, vehicular traffic and agriculture etc. among which agriculture is one of the biggest source (Hoareau et al., 2022). Various agricultural activities such as sewage irrigation, fertilizers and pesticides use, atmospheric deposition etc. have largely contributed to cadmium contamination in groundwater and soil (Zhao et al. 2015). The pH of soil and water is one of the most significant factor which affects the mobility of cadmium. The lower the pH of water (acidic), the more mobile cadmium is, resulting in the formation of organic and inorganic soluble complexes including $CdCL^+$, $CdSO_4$, $CdCl_2$ and $CdOH^+$ (kubier and pichler 2019), these soluble complexes can readily infiltrate into the groundwater, thus contaminating this natural reservoir.

In wastewater samples the values of Cu were beyond the permissible limits in NDS1 and NDS2 with average values of 0.30±0.09 and 0.26±0.07 and the values of Zn in NDS1 and NDS2 are also beyond their permissible limits with average values of 2.29±0.44 and 2.02±0.17.The cause of higher concentrations of Cu and Zn in urban wastewater is associated to their abundance in households materials and commercial activities. Studies have revealed that sources of these heavy metals are widely understood and the largest sources of Cu are water supply system at homes and building materials. Cu is widely known for its anti-corrosion quality therefore other than Fe, Cu is the most commonly used metal in domestic and commercial settings and for the sources of Zn in urban wastewater, corrosion of galvanized materials, wear and tear of breaks and tires and car washes are commonly referred (Sorme et al., 2022),(Legret et al., 1999).

Table 3.1: Descriptive statistics of heavy metal concentrations and physiochemical properties of groundwater and wastewater samples. All the concentrations of heavymetals are in mg/L

Location	**Variables**	**Min-Max**	**Mean ± Std.**
GW	Mn	0.02-0.13	0.08±0.04
	Zn	0.10-0.91	0.29±0.24
	Fe	0.01-0.16	0.08±0.05
	Cd	0.01-0.02	0.01±0.01
	Pb	0.01-0.03	0.01±0.01
	Cu	0.01-0.03	0.02±0.01
	pH	5.7-6.10	5.91±0.15
	EC	0.61-5.19	1.57±1.29
NDS1	Mn	0.09-0.13	0.11±0.02
	Zn	1.99-2.77	2.29±0.44
	Fe	2.79-3.09	2.91±0.16
	Cd	0.11-0.72	0.33±0.34
	Pb	0.22-0.47	0.30±0.14
	Cu	0.21-0.39	0.30±0.09
	pH	5.6-6.10	5.86±0.23
	EC	2.29-2.48	2.40±0.10
NDS2	Mn	0.09-0.12	0.11±0.02
	Zn	1.85-2.20	2.02±0.17
	Fe	2.81-3.65	3.18±0.43
	Cd	0.17-2.39	0.92±1.28
	Pb	0.24-0.31	0.27±0.04
	Cu	0.21-0.34	0.26±0.07
	pH	5.9-7.00	6.32±0.57
	EC	0.38-2.09	1.07±0.90
SSL	Mn	0.09-0.10	0.09±0.01
	Zn	1.07-1.45	1.26±0.27
	Fe	2.13-2.53	2.33±0.29
	Cd	0.18-0.21	0.19±0.02
	Pb	0.19-0.22	0.21±0.02
	Cu	0.11-0.14	0.12±0.02
	pH	5.8-6.20	6±0.24
	EC	3.71-3.80	3.76±0.06

FAO*	**Mn**	**Pb**	**Cu**	**pH**	**EC dS/m**
	0.2	5	**3.2**	6.5-8	0.7-3.0

3.2. Correlation analysis of physiochemical parameters and heavy metals in groundwater and wastewater:

Correlation between different parameters indicates the strength and relationship between various parameters.Therefore the correlation between the relevant parameters was quantified using Pearson correlation analysis between pH, EC, heavy metals i.e. Zn, Cu, Cd, Mn, Pd, Fe are showed in the table 3.3. A positive correlation is shown by a linear correlation coefficient greater than zero, whilst a negative relationship is indicated by a number less than zero. All the data were analyzed for correlational analysis on IBM SPSS Statistics 19, and the statistical significance level for this analysis was $P < 0.05$.

Heavy metals have showed strong to moderately positive correlation with each other. Cu-Zn (p<o.o1), Fe-Zn (p<o.o1) and Fe-Cu (p<o.o1) have showed strong positive correlation with each other which indicate that the increase in one metal will result in the increase of the other metal as well. Various studies have suggested that the positive correlation between these heavy metals is influenced by many factors that affect their levels in water, for this, their source identification (natural geological abundance and anthropogenic discharge) is one of the most important factor. Fe, Cu and Zn showed highest concentrations in wastewater due to the heavy metal discharge from industries. These metals showed a positive correlation in the study conducted in Palta, India (Shah et al., 2005). This may be due to the extensive use of copper, zinc and iron-containing pesticides and fertilizers in regions with high level of agricultural activities. Fe, Zn, Cu and Pb concentrations in soil and water could be related to the deposition of chemical emissions from vehicular traffic, fossil fuel combustion and suspension of street dust (Friedlander 1973). The positive correlation between copper and iron levels in the wastewater have also suggested that this may be due to the presence of iron oxide minerals in the soil and bedrock, which can conveniently adsorb copper ions.

Results showed that pH and EC have moderate negative correlation with each other. The pH of water can affect its EC level but the strength and direction of the correlation can vary depending on the specific heavy metal and the other physiochemical factors e.g. BOD, pharmaceuticals etc. present in the water. Therefore, pin pointing the reason behind negative relation of pH and EC is a rigorous task and require much elaborated data. A study on Mekong River in Thailand showed a significant negative correlation between pH and EC, indicating that with the decrease in pH, the EC of water has increased. Physiochemical parameters and heavy metals mobility are closely associated with each other. Results showed a major influence of EC on Cd solubility in water, this can be attributed to the fact that Cd has relatively high affinity to make complexes with anions in water e.g. chloride, leading to an increased Cd solubility in water (Machado et al., 2016). The presence of various other pollutants such as Cu and Zn etc. will increase the cation concentrations in the water, which increases its acidity, resulting in low pH and high EC (Prathumratama et al., 2008) this may be the why the mobility of Cd is high in water samples with acidic pH.

Table 3.2. Correlation between physiochemical and heavy metals in groundwater and wastewater samples.

	Mn	Zn	Fe	Cd	Pb	Cu	pH	EC
Mn	1							
Zn	0.415	1						
Fe	0.442	.947**	1					
Cd	0.289	.496*	.576**	1				
Pb	0.381	.874**	.928**	.578**	1			
Cu	0.447	.965**	.936**	.602**	.886**	1		
pH	.578**	0.174	0.24	0.226	0.148	0.223	1	
EC	-0.228	0.265	0.162	-0.13	0.182	0.105	-0.354	1
****. Correlation is significant at the 0.01 level (2-tailed).**								
***. Correlation is significant at the 0.05 level (2-tailed).**								

3.3. Concentrations of heavy metals in groundwater and wastewater:

For groundwater and wastewater samples different sites were selected in order to observe the concentrations of heavy metals including Manganese (Mn), Zinc (Zn), iron (Fe), Cadmium (Cd), lead (Pb) and Copper (Cu), and box and whisker plots were made in order to observe their concentrations at groundwater and wastewater sites separately. Figure 3.1 (a), (c), (e), (g), (j) and (k) shows that the concentrations of heavy metals in groundwater sites and (b), (d), (f), (h), (i) and (l) shows that the concentrations of heavy metals in wastewater sites.

At groundwater sites, the concentrations of Cd were highest at GW11 and GW4 i.e. 0.02 mg/L at both sites and the Cd concentrations were lowest at GW2, GW5, GW6, and GW8 i.e. 0.01 mg/L at all sites. The highest values of Cd at both sites were above the permissible limit of 0.01mg/L for Cd in groundwater used for irrigation purposes given by FAO. Fe showed the highest concentrations at groundwater sites, GW5 and GW6 with a value of 0.02 mg/L at both sites which was well below the permissible limit of 5mg/L for Fe and lowest concentration at site GW10 which as observed to be 0.01 mg/L. Mn was dominant at sites GW2 and GW5 at values of 0.13 mg/L at both sites, with in the permissible limit for Mn that is 0.2mg/L and the lowest values at the site GW11 having a value of 0.02 mg/L. The value of Pb was highest at GW10 with a value of 0.03 mg/L and lowest at GW11, GW5 and GW9 with a value of 0.01 at all sites, all of which remains within the permissible limit for Pb that is 5mg/L. The value of Pb at site GW2 showed a negligible concentration due to no variation between the mean value and the median value. Cu showed the highest value of 0.03mg/L at GW1 and GW8 and lowest values of 0.01mg/L at GW3, GW6 and GW10, none of the values of Cu exceeded the permissible limit. Lastly the concentrations of Zn were significantly high at GW1 and GW2 with the values of 0.91 and 0.54 mg/L respectively and lowest value at GW10 at the value of 0.10 mg/L, however all the values lie within the safe permissible limits. The concentration of Zn at GW8 showed a negligible difference in concentration due to no variation between the mean value and the median value. Since most of the groundwater well are present in the vicinity of agricultural farms and roadsides they showed high concentrations of some heavy metals however all of them lies within the permissible limit, other than Cd.

At wastewater sites, the box and whiskers plot showed that site NDS1(E) has the highest concentrations of Mn, Pb, Cu and Zn (0.13, 0.22, 0.39 and 2.77mg/L) in addition to that site NDS2(E) also has the highest concentrations of all the heavy metals including Cd, Fe, Mn, Pb, Cu and Zn (2.39, 3.65, 0.12, 0.13, 0.34 and 0.12mg/L). The values of The wastewater site SSL(S) has the lowest concentrations of all the heavy metals including Cd, Fe, Mn, Pb,Cu and Zn (0.18, 2.13 0.09, 0.19, 0.11 and 1.07mg/L). Other than these the lowest concentrations of Cd and Mn (0.11mg/L and 0.09mg/L) were also found at the site NDS1(S) and NDS2(S) also reports the lowest concentration of Mn (0.09mg/L). The data of wastewater showed that the concentrations of Cd at all sites were well above its permissible limits i.e. 0.01mg/L. The concentrations of Zn also exceeded their limit at the sites NDS1(E) and SSL(S) and the Cu values were also above their limits at various wastewater sites such as NDS(E).

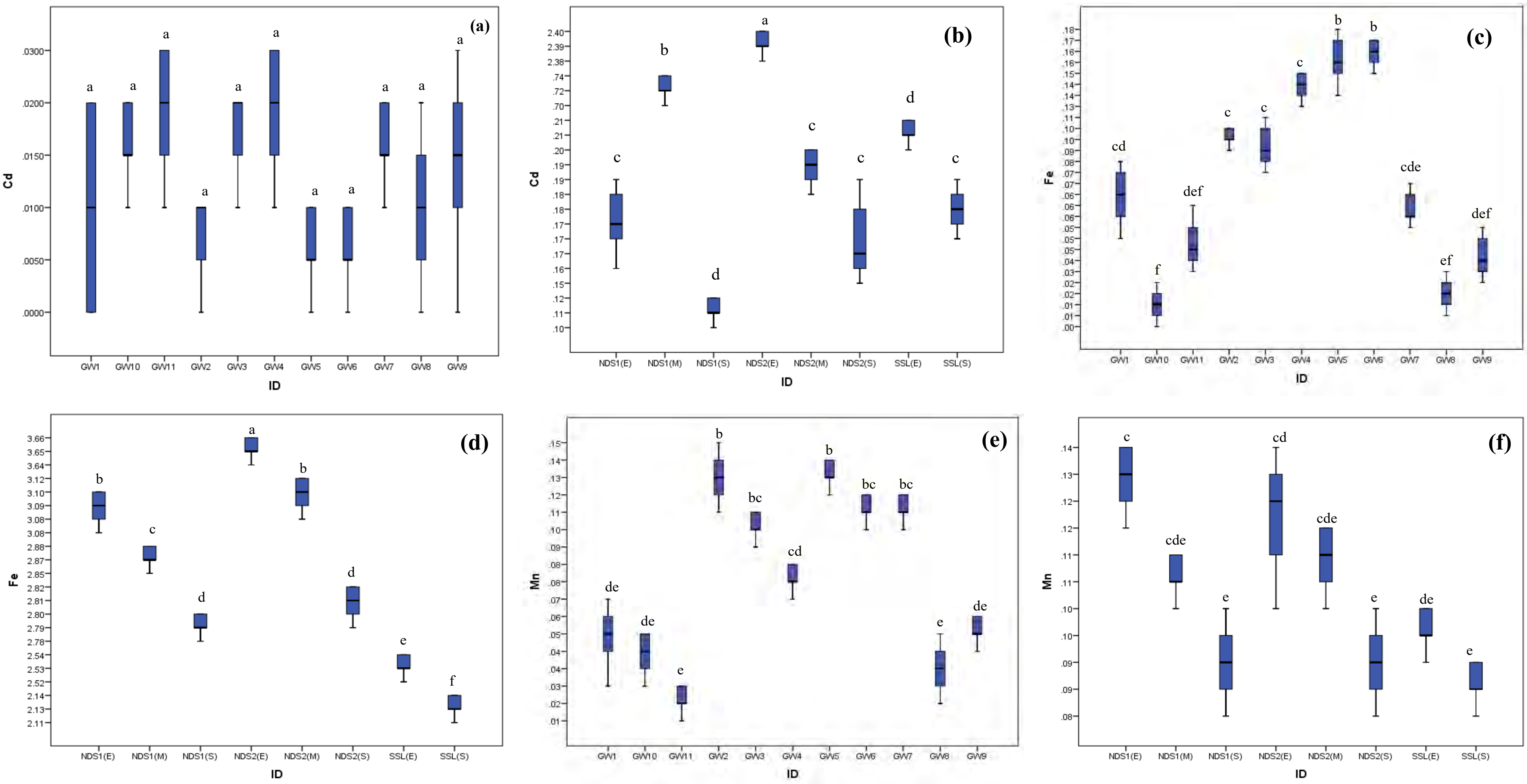
In order to find significance among heavy metal concentrations at different sites one way ANNOVA was applied on the data. There was no significant variation in Cd values at different groundwater sites ($p > 0.05$). The values of iron were significantly different at various groundwater sites. The sites GW2, GW3, and GW4 showed no significant difference however, they showed significant difference from GW5 and GW6. The values of Mn at GW2, GW3, GW5, GW6 and GW7 showed no significant difference among each other however, these sites showed significant difference from the groundwater sites GW1, GW10 and GW9. Pb values at the sites GW1, GW2, GW3, GW4, GW6, GW7 and GW8 showed no significant difference with each other while showing significance to some extent with the sites GW10, GW11, GW5 and GW9 as well. The values of Cu at the sites GW2, GW4, GW5, GW7 and GW9 showed no significant difference with each other. The values of Zn showed significant difference with each other at most of the sites i.e. GW1, GW2, GW3, GW4, GW5, GW6, GW9 and GW10 all are significantly different from each other.

In case of wastewater sites, the values of Cd were not significantly different at site NDS1(E), NDS2(M), NDS2(S) and SSL(S), however they showed significant difference from the site

NDS2(E). Fe concentrations showed no significant difference at sites NDS1(E) and NDS2(M) as well as at sites NDS1(S) and NDS2(S), the remaining sites showed significant difference with these sites and with each other. For the values of Mn, the sites NDS1(S), NDS2(S) and SSL(S) showed no significant difference with each other. The values of NDS1(M) and NDS2(M) were also not significantly different, all other sites showed significant difference to some extent. The site NDS1(S), NDS1(E), NDS2(S) and SSL(E) showed no significant difference with each other for Pb values, the remaining sites were significantly different from each other. The values of Cu showed no significant differences at sites NDS1(S), NDS2(M) and NDS2(S) as well as at sites SSL(S) and SSL(E), and at sites NDS1(M) and NDS2(E). The value of Cu at site NDS1(E) was significantly different from all other sites. At the wastewater sites NDS1(S), NDS1(M) and NDS2(S) the value of Zn was not significantly different from each other however, all other sites were significantly different from each other.

Most heavy metals in wastewater samples followed a trend, with highest concentrations at NDS2 (E) and NDS1 (E) at the wastewater sites, this may be due to the fact that the ending sites of NDS1 and NDS2 were the major dumping site of both sullage carriers into their respective wastewater channels which eventually leads to the underground sewage system, therefore the wastewater was more concentrated at this end. At the starting point of the sullage carrier the water flow rate was much higher due to high pumping force of electric motors from the disposal stations. However the flow rate decreased gradually as the water moved toward the end of the sullage carrier, due to very low flow rate suspended particles emerges at the surface of wastewater containing organic matter, fine soil or sediment particles, biological solids, microbes and other discharged particles in wastewater. The fine suspended particles in wastewater can provides surface area for the adsorption to pollutants such as heavy metals (Jain et al., 2008). Heavy metals from anthropogenic sources are mostly released in the environment in the form of inorganic complexes, which can form weak bonds (both physical and chemical) with the surface of fine soil or sediment particles (Pempkowiase et al. 1999), thus contributing towards an increased heavy metal concentration in wastewater samples.

The groundwater wells selected in this study were present in the peri-urban and rural areas of District Okara, at quite some distance from the central Okara city. The high values of Cd in groundwater wells indicate intense anthropogenic activities in the prei-urabn and rural areas including intensive agricultural, fertilization (wastewater and fertilizers) and livestock activities (Çiçek et al. 2013). The pH values of groundwater system in Okara lies between acidic to moderate, according to literature acidic pH supports the mobility of Cd in water.


(a)
Cd
ID
GW1 GW10 GW11 GW2 GW3 GW4 GW5 GW6 GW7 GW8 GW9
(b)
Cd
ID
NDS1(E) NDS1(M) NDS1(S) NDS2(E) NDS2(M) NDS2(S) SSL(E) SSL(S)
(c)
Fe
ID
GW1 GW10 GW11 GW2 GW3 GW4 GW5 GW6 GW7 GW8 GW9
(d)
Fe
ID
NDS1(E) NDS1(M) NDS1(S) NDS2(E) NDS2(M) NDS2(S) SSL(E) SSL(S)
(e)
Mn
ID
GW1 GW10 GW11 GW2 GW3 GW4 GW5 GW6 GW7 GW8 GW9
(f)
Mn
ID
NDS1(E) NDS1(M) NDS1(S) NDS2(E) NDS2(M) NDS2(S) SSL(E) SSL(S)

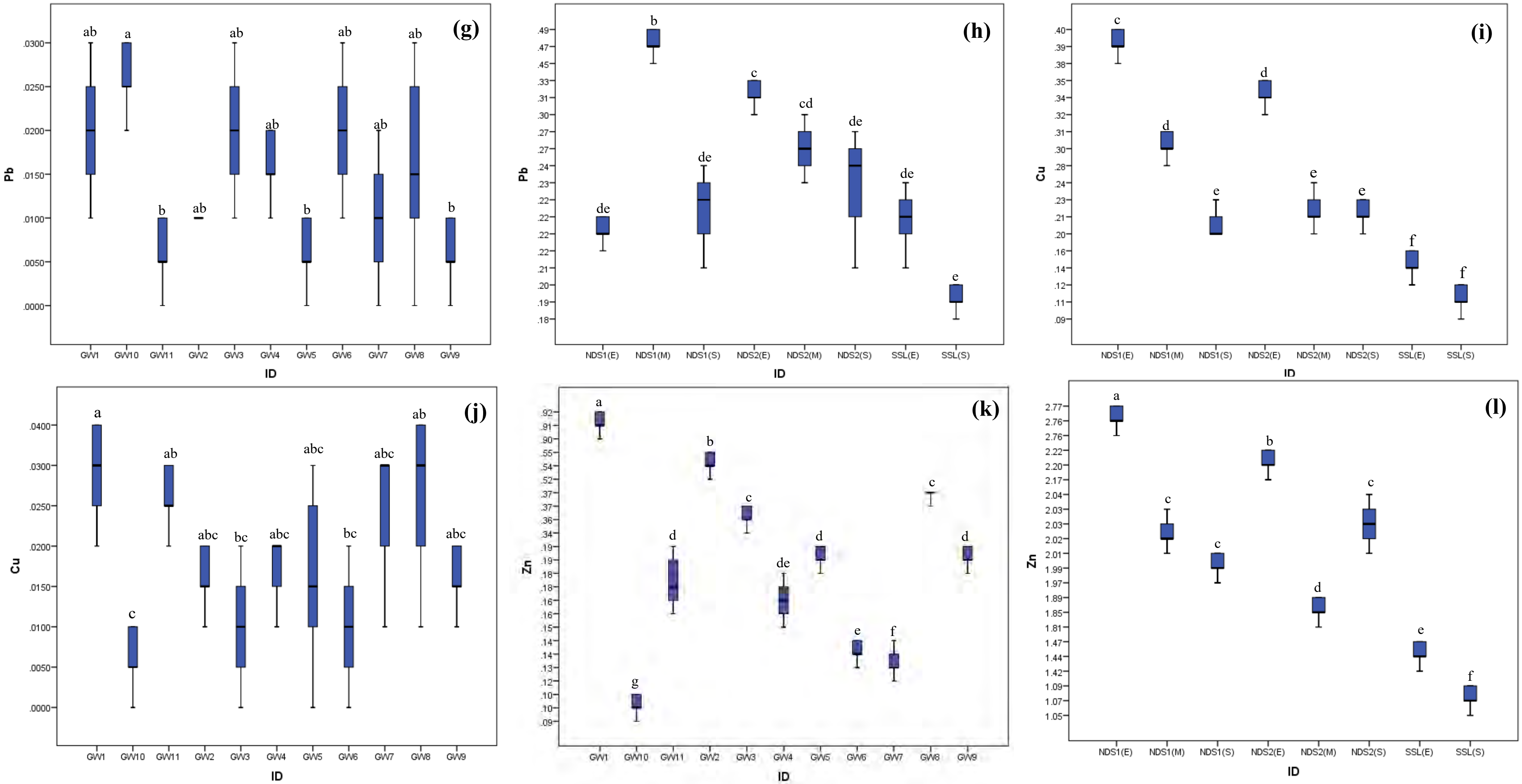


Fig 3.1: Box and whisker plots shows the concentrations of heavy metals in groundwater and wastewater sites (a) Cd in groundwater (b) Cd in wastewater (c) Fe in groundwater (d) Fe wastewater (e) Mn in groundwater (f) Mn in wastewater (g) Pb in groundwater (h) Pb in wastewater (i) Cu in wastewater (j) Cu in groundwater (k) Zn in groundwater (l) Zn in wastewater

3.4. Human health risk assessment of groundwater:

The hazard index (HI) of selected heavy metals for adults and children are shown in the table 3.3, that represents the potential risk to human health due to exposure to heavy metal contamination in the groundwater samples. The HI total was calculated using the values of health index via ingestion pathway (HI_{ing}) and health index via dermal exposure (HI_{derm}).

In order to determine the heavy metal contamination in groundwater the threshold value was established at 1.0 both children and adults (USEPA 2001), if the HI value of a metal lies below 1 in water it will be deemed fit for human consumption otherwise the probability of adverse effect caused by the exposure to the heavy metal will increase over period.

The HI values for Mn was highest at GW2 and GW5 (0.0509), however since the value is less than 1, Mn lies within the acceptable limit at all groundwater sites. For Zn the highest HI value was observed at GW1 (0.0869) which lines within the acceptable limit for adults, therefore Zn do not pose any risk at all groundwater sites. The HI values of Fe also lies within the acceptable limit for adults all sites and do not pose any health risk, however the highest HI value of Fe was observed at the site GW5and GW6 with a value of 0.0070 at both sites. The HI value of Cd was observed to greater than 1 at 7grounwater sites. Cd showed the highest concentration of 1.7359 at sites GW4 and GW11, exceeding far above the acceptable value for adults. Sites GW1, GW3, GW7, GW9 and GW10 also showed higher HI values of Cd 1.3019 respectively. This determines the fact that groundwater consumption at these sites can cause health risk in adults when exposure to the Cd in water. The values of Pb and Cu also lies within the acceptable limit in adults, thus posing no health risk upon exposure in water.

With respect to HI values (adults) of heavy metals at all groundwater site, this is the order of risk from highest to lowest is: Cd > Pb > Fe > Mn > Zn > Cu and the HI values (children) the order of risk from highest to lowest is: Cd > Pb > Mn > Zn > Cu > Fe. In case of children, the HI value of Mn lied below the acceptable limit at all groundwater sites, with GW6 and GW7 having the highest value of 0.1088 respectively. The HI values of Zn and Fe were observed to be below the acceptable limit i.e. 1.0, for children. The value of Cd were above the acceptable limit of 1 in all the groundwater sites. The highest HI value of Cd at GW4 and GW11 (4.2345) was approximately 4 times higher than the acceptable limit for children, therefore it can pose serious health risk in children upon exposure. The HI values of Pb and Cu were observed to be within the acceptable limit for children, leading to no health risk upon exposure in water.

In this study the health risk assessment of heavymetals at grounwater sites revealed high risk index for Cd in case of both adults and children, however it showed potentially higher health risk for children then adults upon the consumption of water from these selected groundwater sites. A study conducted in Charsadda, Pakistan revealed that Cd contamination in groundwater drinking sources was found in the of rural areas of the district, this may be due to continuous agricultural activites such as usage of phosphate fertilizers, wastewater irrigation and corrosion of some galvanized water distribution and pumping stystem (Sardar et al. 2013).

There are three main primary exposure pathways of Cd i.e. inhalation, ingestion and dermal contact through various matrices such as air, water and soil sediments. Due to low excreation level of Cd from human body, it can accumulate in in tissues and organs for over a time span of 10 to 30 years, which is the half-life of Cd (Suwazono et al. 2009). Cadmium is a known carcinogenic metal which can cause oxidative stress on tissues and organs, causing various anatomical abnormalities and higer risk of breast, prostate, lung, genitourinary and colon cancer in humans (Matović et al. 2015) (Satarug et al. 2012). Various non-cancerious disease are also associated with the short term and long term exposure of Cd, comprises of hypertension, itai-itai disease, renal dysfunction, type 2 diabetes mellitus and bone disease in adults (Nogawa et al. 2004) and memory loss, attention deficit and hyperactive disorder, learning difficulties and various genetic mutations leading to autism in children (Heyer and Meredith 2017).

Table 3.3. Summary of hazard index (HI) of selected heavy metals in adults and children

	Risk assessment (Hazard index total) for adults						Risk assessment (Hazard index total) for children					
Sample ID	**Mn**	**Zn**	**Fe**	**Cd**	**Pb**	**Cu**	**Mn**	**Zn**	**Fe**	**Cd**	**Pb**	**Cu**
GW1	0.0157	0.0869	0.0028	1.3019	0.1900	0.0215	0.0395	0.1934	0.0064	3.1759	0.4396	0.0479
GW2	0.0509	0.0511	0.0041	0.4340	0.0950	0.0108	0.1285	0.1137	0.0094	1.0586	0.2198	0.0240
GW3	0.0392	0.0339	0.0039	1.3019	0.1900	0.0072	0.0989	0.0755	0.0089	3.1759	0.4396	0.0160
GW4	0.0294	0.0158	0.0061	1.7359	0.1425	0.0143	0.0741	0.0351	0.0138	4.2345	0.3297	0.0320
GW5	0.0509	0.0177	0.0070	0.4340	0.0475	0.0108	0.1285	0.0393	0.0158	1.0586	0.1099	0.0240
GW6	0.0431	0.0129	0.0070	0.4340	0.1900	0.0072	0.1088	0.0287	0.0158	1.0586	0.4396	0.0160
GW7	0.0431	0.0124	0.0026	1.3019	0.0950	0.0215	0.1088	0.0276	0.0059	3.1759	0.2198	0.0479
GW8	0.0137	0.0353	0.0008	0.8679	0.1425	0.0287	0.0346	0.0786	0.0019	2.1173	0.3297	0.0639
GW9	0.0196	0.0177	0.0015	1.3019	0.0475	0.0108	0.0494	0.0393	0.0035	3.1759	0.1099	0.0240
GW10	0.0157	0.0091	0.0004	1.3019	0.2375	0.0036	0.0395	0.0202	0.0009	3.1759	0.5495	0.0080
GW11	0.0078	0.0167	0.0020	1.7359	0.0475	0.0179	0.0198	0.0372	0.0044	4.2345	0.1099	0.0399

3.4. Physical characterization of plastic fractions:

Micro and macro-plastic particles were extracted from nineteen water samples (i.e. 11 groundwater and 8 wastewater samples), after the microscopic analysis, the data was used to classify the plastic fractions based on their physical characteristics including abundance, shape, color and size. These physical parameters of plastics fractions are of great concern not only for their identification but because particle size, shape and abundance is most significant in identifying their role in the transportation of pollutants such as heavy metals, antibiotics and pesticides etc due to their adsorption (Dennis et al. 2016). According to laboratory studies, several factors (e.g., particle size, type, shape, density) seem to influence the transport of MPs in subsoil (Ren et al.. 2021).

3.4.1. Abundance:

Abundance of plastic fractions varies greatly in both groundwater and wastewater samples. Results showed the presence of different concentrations of plastic fractions in both groundwater and wastewater samples.

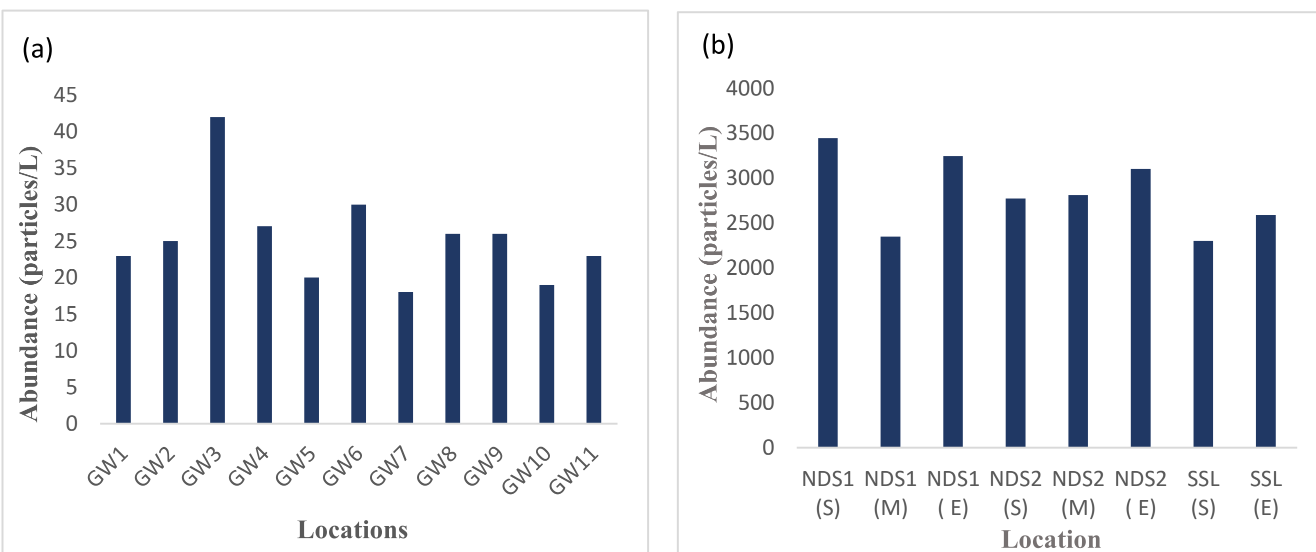


Fig 3.2: Numeric abundance of plastic fractions in **(a)** groundwater samples **(b)** wastewater samples (particle/L)

The average number of plastic fractions observed in the groundwater samples were 25±6 particles/L, ranges from 18 particle/L in GW7 and 42 particles in GW3 whereas the average number of plastic fractions observed in wastewater samples were 413±2828 particles/L, ranged from 2305 particle/L in SSL (E) and 3444 particles/L in NDS1 (S) as shown in the figure 3.2.

Regardless of the fact that global research community is showing keen interest in plastic pollution, only a handful of research data is available regarding plastic pollution in groundwater due to various obstacles that awaits in the sampling, extracting and detection phases. The concentration of plastic fractions shows a heterogeneous variation in all the groundwater sites. Present studies have indicated that the concentration of plastic fractions largely depend on different factors such as of aquifer type, sampling and detection methods. Natural and artificial methods of aquifers recovery can also contribute in the buildup of plastic pollution in the groundwater aquifers (Re et al., 2019). Authentication of results is compromised due to no commonly devised procedure for the identification of plastic contaminations in the groundwater and lack of availability of hydrogeological information (i.e. subsoil information etc.) of the designated area (Stefano et al., 2022). At present only a very few studies are available for the comparison, however they have recorded only a few micro-plastic particles per liter. (Subharthe et al., 2022)(Panno et al., 2019) have recorded 38 ± 8 particles/L and 7± 4.3 MP/L, other studies do not show any significant difference either.

In comparison to groundwater, wastewater samples showed a large quantity of micro-plastic particles per liter. Simon et al., 2018 obtained samples from 10 wastewater treatment plants, the count for micro-plastics in the influent samples showed a median value of 7216 particles/L. plastic fraction concentration in wastewater largely depend upon factors such as the population density distribution, economic development and transportation network. China is the most populated country in the world with a population of 1.39 billion people recorded in 2017, in a study conducted in 2010, a total of 4.8-12.7 million Mt plastic waste was recorded from a data collected from 192 coastal countries and China has the highest input quantity. (Jambeck et al., 2015) high population density, large scale goods manufacturing and assembling units most of which uses plastic as a raw material contributes the most input of plastic pollution in wastewater systems in a region.

3.4.2. Shape:

Fibers, films and fragments are the three main shape classes that were observed in this study. The variation in the shapes of plastic particles in the samples showed similar trends in both wastewater and groundwater samples **fiber > fragment > film**. Micro-plastic fibers are the most dominant shape found, ranging from 50-80% in both groundwater and wastewater samples. Fragments constitutes 20-40% of the samples and films from 20-10% were the least observable shape. Various studies have revealed that fiber and fragments are the most existing micro-plastic in the groundwater environment **(P1)**(Wagner et al., 2014).

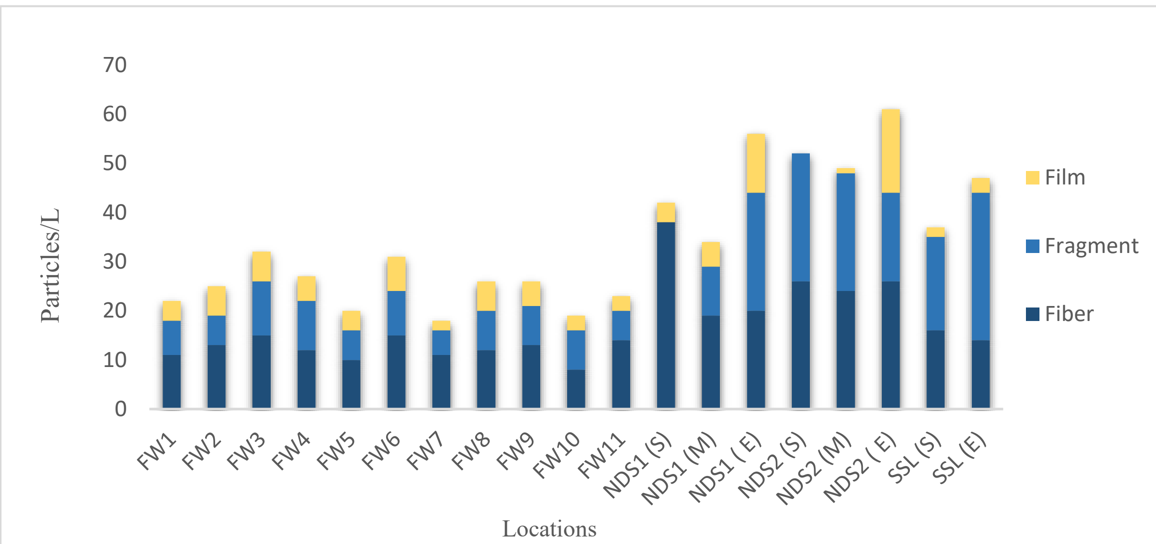


Fig 3.3: Concentrations of different plastic particle shapes identified in the wastewater and groundwater samples.

Micro-plastic fibers are more prevalent than other micro-plastic shapes because a large variety of sources contributes toward their dispersal in the environment. More than 45 million tons per year of polyester is produced for the production of synthetic textile alone (Aizenshtein et al., 2014) along with other materials such as nylon and acrylic etc. Textile products are the primary source of micro-plastic fibers, they can easily shed off from their surfaces. Due to their extremely small size i.e. 100–800 mm (Hernandez et al. 2017) almost up to 40% of micro-plastic fibers remain unfiltered in the wastewater treatment plants (Messinger et al., 2016), and are consequently released in the urban drains or groundwater bodies e.g. rivers, lakes and oceans. Other sources of micro-plastic fibers are packaging materials and containers etc., weathering over time eventually breaks them into particles small enough to be classified as fibers.

After fibers, fragments are the second most abundant particle shape. Tear and wear of larger plastic particles results in the formation of plastic fragments. Plastic products become fragile due to external stress such as sunlight, weathering etc. (Liu et al., 2019). Over time and eventually breaks down into plastic fragments, these micro-plastic fragments are solid and angular particles that tends to accumulate in larger concentrations. Plastic films are the least abundant type of plastic fractions in the natural environment forms due to the break dowm of plastic bags. The durability of plastic fibers and fragments is the main factor for their greater persistence in the environment whereas plastic films are thin and flexible (Ebere et al., 2019). They further breaks down into smaller particles in a short interval of time therefore their retention time in the environment is very short.

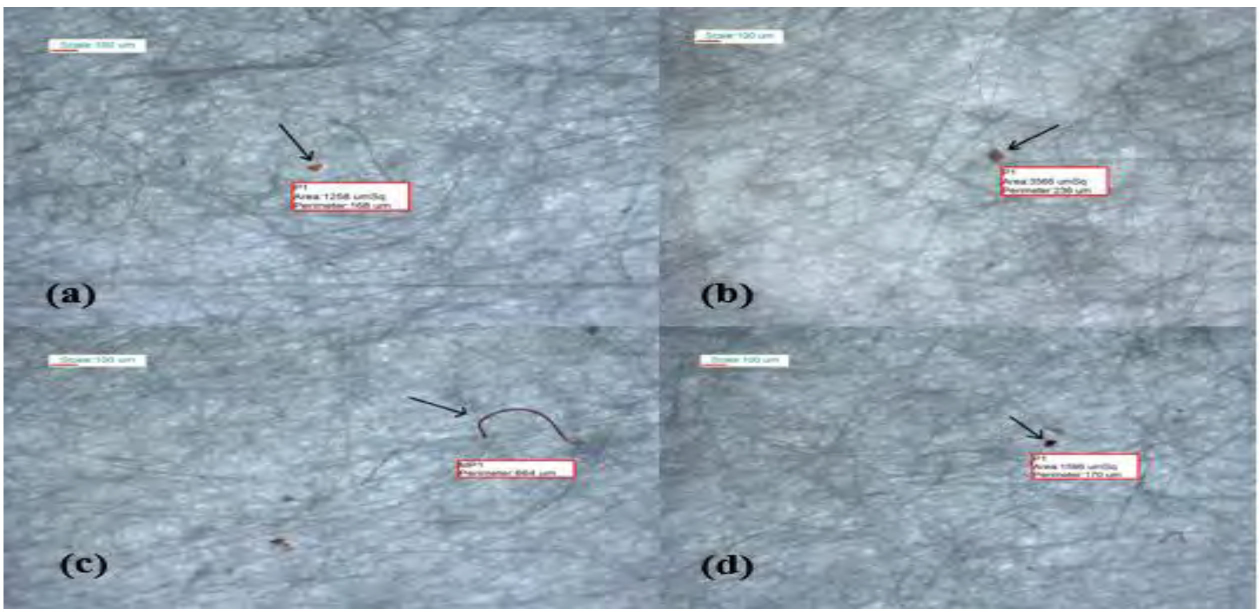


Fig 3.4: Micro-plastic particles identified in the groundwater samples under the Digital Stereo-microscope (a) red fragment (b) opaque film (c) blue fiber (c) blue fragment

Various studies have reported the same order of plastic particles shape in the wastewater systems. (Gündoğdu et al., 2018)**(** Julia et al., 2017) have identified highest concentrations of fiber followed by fragments and then films in the wastewater influent of wastewater treatment plants. And even after all removal techniques fibers remain the most predominant plastic particle shape. Textile fibers were the most common shape of plastic fractions found both treated and untreated wastewaters.

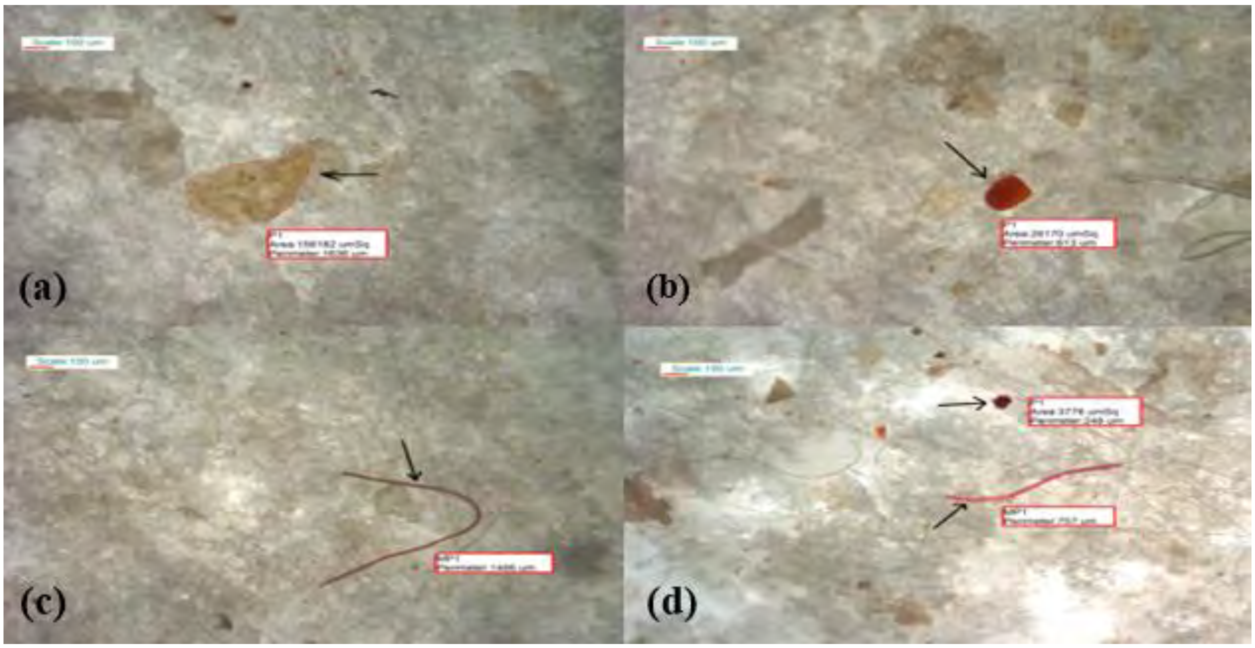


Fig 3.5: Micro-plastic particles identified in the wastewater samples under the Digital Stereo-microscope (a) transparent film (b) red fragment (c) blue fiber (d) black fragment and blue fiber

3.4.3. Color:

Color pigment is one of the characteristics which is included in the study of physical attributes of plastic fractions i.e. micro- and macro-plastics. A large variety of colors have been linked to plastic fractions in different studies, they help recognize the possible origin of a plastic particles (Hartmann et al., 2019)**.**

In this study we have recorded colors based on their abundance in the groundwater and wastewater samples. "Red, Blue, Green, Black" are the major color pigments found in both sample types.

Colors that are less abundant and unidentified are recognized as "others". The trend of color variations in groundwater is slightly different than that in wastewater samples. In groundwater samples red is the most abundant particle with a proportion of 93 particles/L, 78 particles/L were black, 66 particles/L were blue, whereas green particles were recorded as 21 particles/L. The least prevalent particles in the samples fall in the category of others with included transparent, multicolor, purple and brown etc. This trend of groundwater samples is shown in a descending order as: **Red>Black>Blue>Green>Others**

Whereas wastewater samples presented a slightly different trend as red showed the highest proportion with 109 particles/L, then black, blue and others shows a proportion of 90, 78 and 64 particles/L. The wastewater samples showed slightly variation in trend than the groundwater samples as green particles were present in least proportion with an amount of 25 particles/L. This trend of wastewater samples is shown in a descending order as:

Red>Black>Blue>Others>Green

Abundance of colored plastic fractions red, blue and black in the samples indicate extensive use of colored pigments in manufacturing urban products and packaging materials(Zhang et al., 2018).

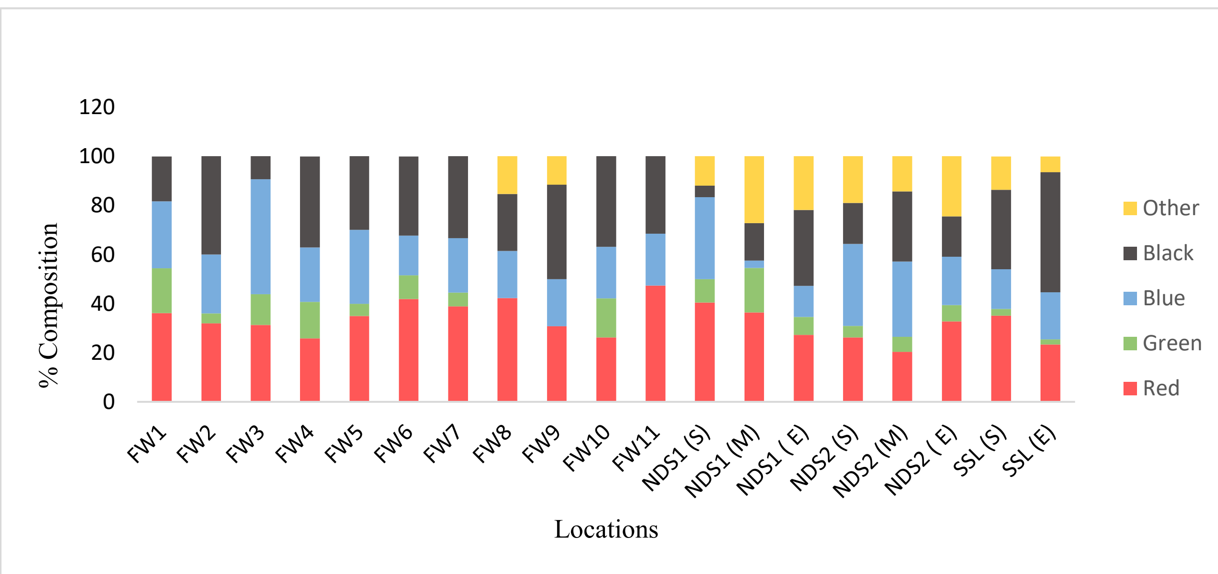


Fig 3.6: Percentage composition of various colors of plastic fractions in groundwater and wastewater samples.

The abundance of bright colored plastic fractions red and blue id linked to the fact that they are more visible thus easy to identify under microscope. Particles that are transparent and white seems to be in negligible maybe because they are less visible and therefore miscalculated (Hartmann et al., 2019).

3.4.4. Size:

For size measurements we calculated the particle length using a microscopic scale. Size classes of 50µm-100µm, 100µm-1mm, 1mm-2mm, 2mm-3mm, 3mm-4mm, 4mm-5mm were categorized for wastewater and groundwater samples based on the range of the plastic fractions obtained from the microscopic results, an additional size class of 50-100µm was also added in the category of groundwater samples. For wastewater samples, a size class of particles >5mm was also added, due to the presence of particles larger than 5mm at several wastewater sites, according to past studies particles of range >5mm are called macro-plastics (Lambert et al., 2014).

After the counting of plastic particles and designating them into their associated class size, it was observed that largest concentration of plastic fractions were observed in the category of 100µm-1mm fo groundwater i.e. 212 and for wastewater the catagory 50µm- 100µm has the largest number of particles i.e. 239 particles/L.

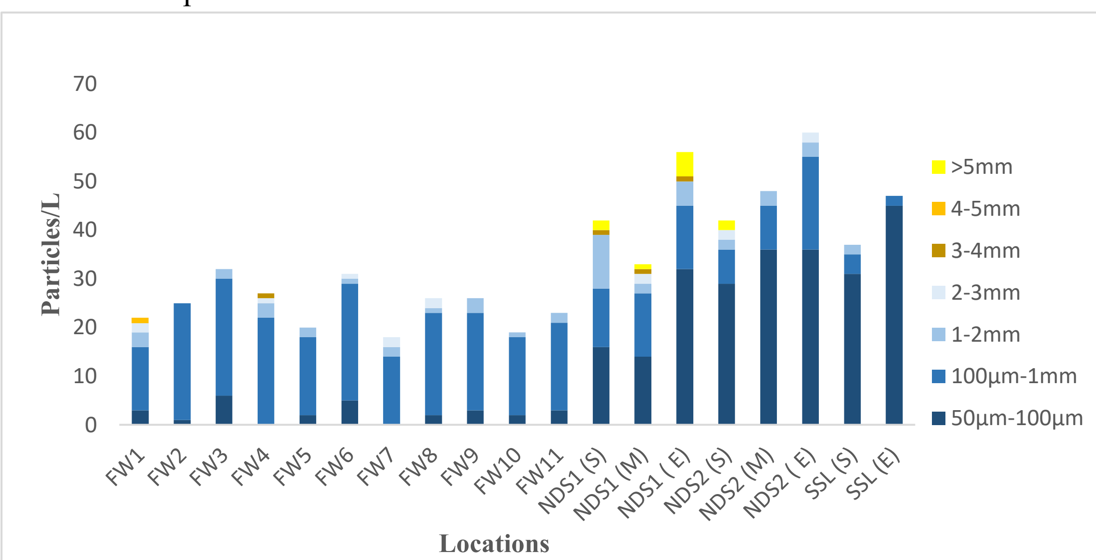


Fig 3.7: Abundance of plastic fractions between the saize range of 50µm- 5mm in groundwater samples and wastewater samples

A steep decrease in particle concentration can be seen with the increase in particle size. The largest class size 4-5mm has the least amount of particles in both groundwater and wastewater samples i.e. 1 and 3 particles/L as shown in figure 3.7.

Samandra et al., 2022 has discussed that it is possible that the only extremely small particles can travel through the soil profile into the groundwater table and all other larger particles are trapped within the topsoil and subsoil. The study also reports that the contamination may have been a result of accumulation of plastic contamination over a period of decades, therefore identifying its origin requires long term data and strenuous efforts. The presence of both macro- and micro-plastics in wastewater points toward higher abundance of plastic fractions in wastewater samples.

CONCLUSION

The reason of conducting this study was to thoroughly examine the urban wastewater used for the purpose of agricultural irrigation and its assessment along with groundwater irrigation system. For conducting this study, Okara city and its peri-urban areas were selected, since it serves as a major agricultural economy in Punjab. The wastewater irrigation system in Okara consists of disposal stations and sullage carriers that are wastewater distribution channels between disposal stations and agricultural lands, which have been developed here since 1970's. Groundwater well were mostly present away from the central Okara city, in the peri-urban and rural settings of the district, in the vicinity of agricultural fields.

In order to examine the quality of wastewater and groundwater used for irrigation, physiochemical properties (pH and EC), selected heavy metals (Fe, Mn, Cu, Zn, Pb and Cd) and plastic fractions (micro- and macro-plastics) were analyzed, in an attempt to investigate these three parameter together. The health risk assessment of samples from groundwater wells was performed in order to determine whether the people of that area can consume water from these sites or not. The conclusions drawn from this study are as follows:

- The physical parameters of wastewater and groundwater samples revealed that the values of pH were higher than the permissible limits whereas the value of EC at all sites, except for one wastewater site (SSL), fall within the permissible limit given by FAO for irrigation water. The pH of both wastewater and groundwater at all sites in Okara was slightly acidic.
- The concentrations of heavy metals including Zn, Cu and Cd were beyond the safe limits given by FAO in all three urban wastewater sites, however Cd was the only heavy metal beyond its permissible limit at 8 out of 11 groundwater sites. The acidic nature of water and intense anthropogenic activities on the agricultural lands such as wastewater irrigation, fertilizer application, insecticides and pesticides usage etc. many have been contributed toward the contamination of groundwater table in the peri-urban and rural areas of Okara.
- Human health risk assessment of groundwater revealed the HI value of heavy metals in adults is in the order of **Cd > Pb > Fe > Mn > Zn > Cu** and HI values in children showed an order of **Cd > Pb > Mn > Zn > Cu > Fe**. The hazard index (HI) of Cd in both adults

and children was above 1 which indicates that upon exposure Cd can cause significant health risks in humans, whereas the exposure risk is much greater in children then in adults.

- The average number of plastic fractions observed in the groundwater samples were 25±6 particles/L and in wastewater samples the average amount was 413±2828. The amounts showed significant abundance of plastic fractions in the both water irrigation sources which represents that they are contaminated with plastic pollution.
- The most abundant shape of plastic fractions in in both wastewater and groundwater samples was in an order of **fiber > fragment > film**. 50-80% of fibers, 20-40% fragments and 20-10% films were observed in all water samples.
- The trend of plastic color pigment in groundwater samples was as follows **Red > Black > Blue > Green > others** whereas in wastewater samples abundance of plastic color was as follows **Red > Black > Blue > Others > Green.** Red was the most abundant color pigment in both water sources.
- The size class of 100µm-1mm has the highest number of plastic particles i.e. 212 and 239 particles/L in both groundwater and wastewater samples. Particle size and particle abundance has an inverse relationship in both water sources. Both micro-plastic (< 5mm) and macro-plastic (> 5mm) particles were observed in the wastewater samples.

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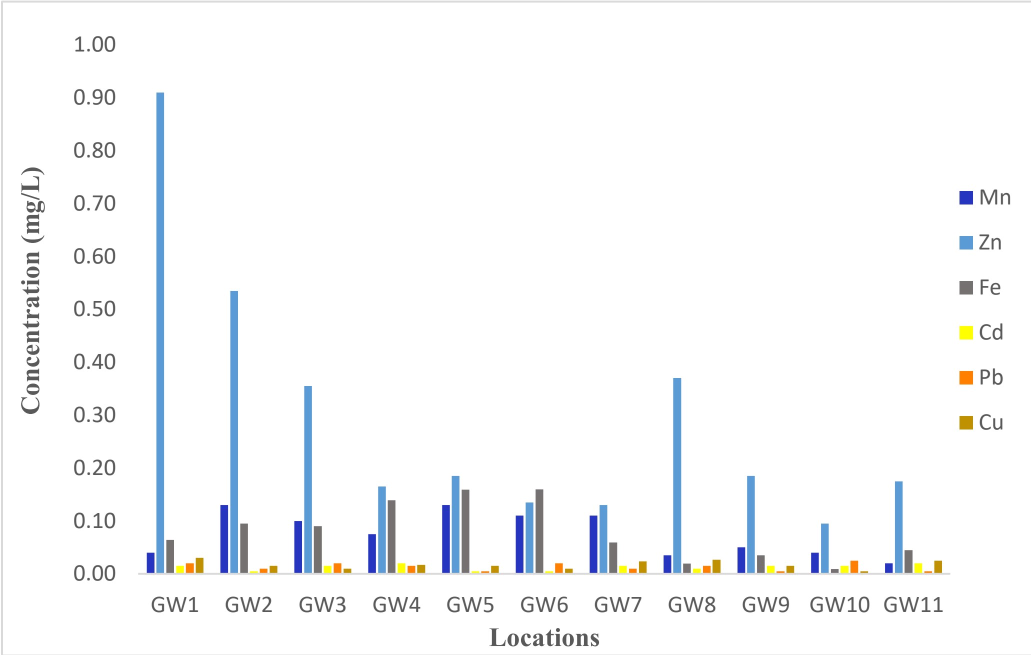
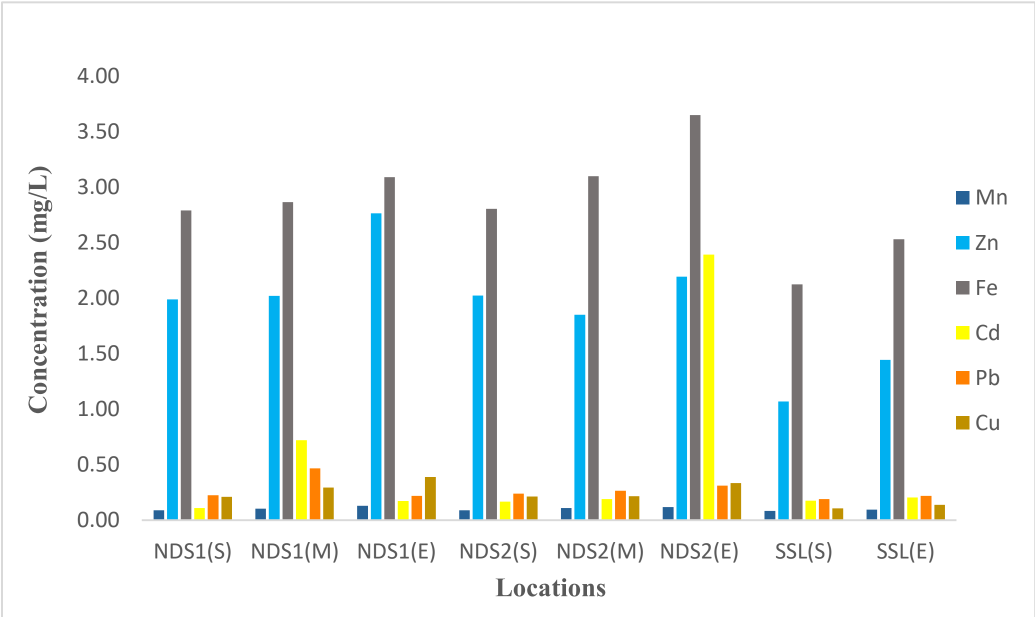
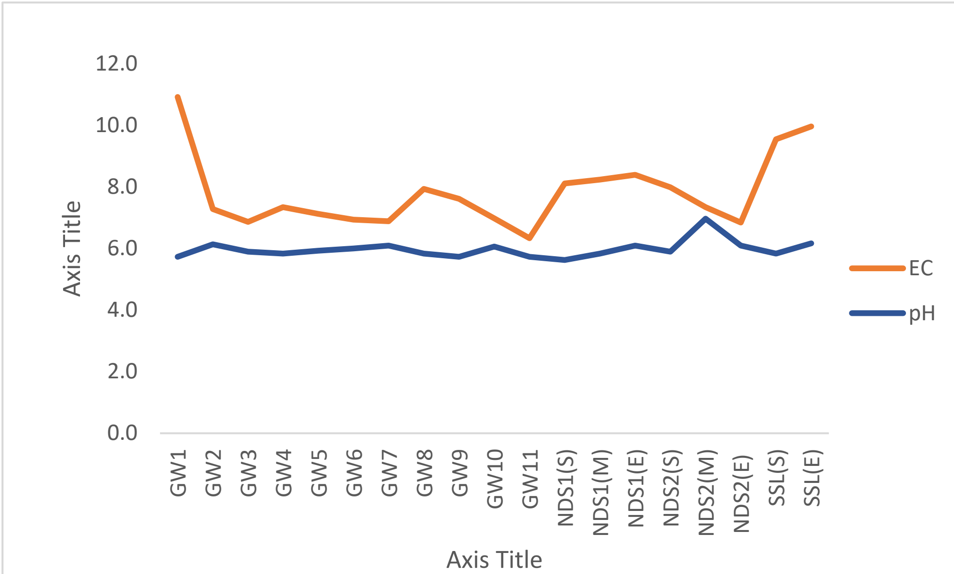
ANNEXURE



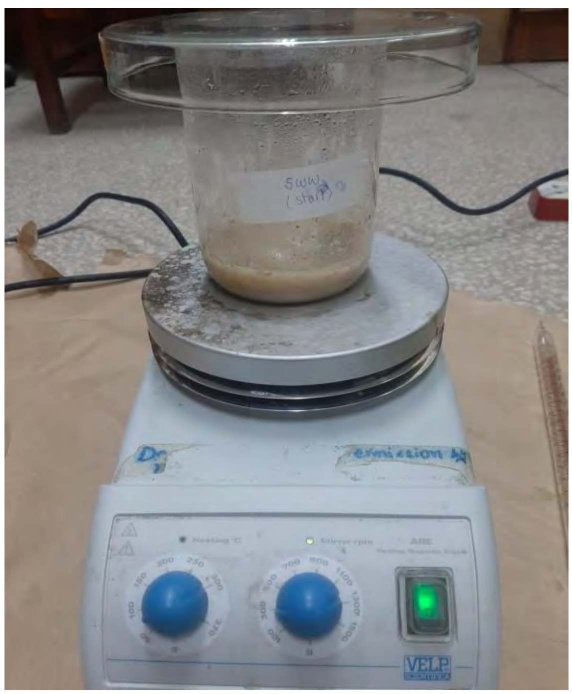
Annexure 1.1. Heavy metal concentrations at the selected groundwater sites



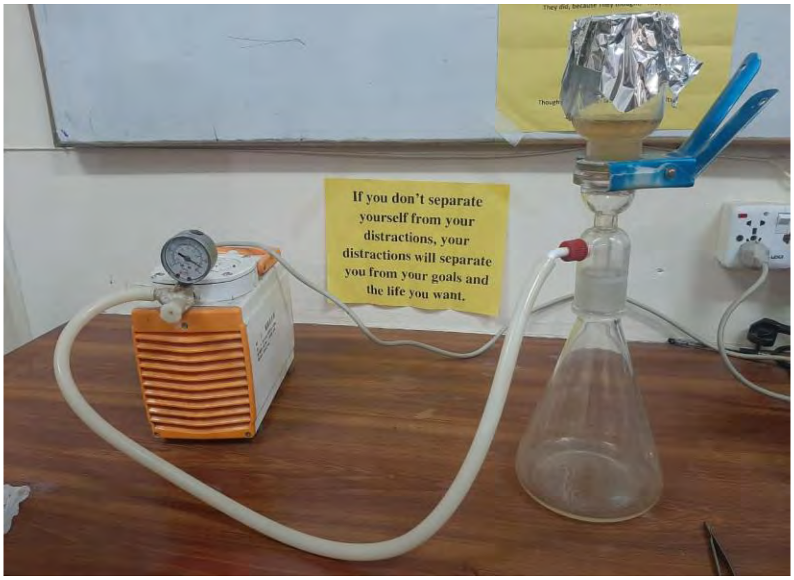
Annexure 1.2. Heavy metal concentrations at the wastewater sites



Annexure 1.3. Physiochemical parameters at selected groundwater and wastewater sites



Annexure 1.4. Chemical digestion of wastewater samples on hot plate



Annexure 1.4. Vaccum filteration of wastewater samples for microplastic extraction

Annexure 1.5. Heavy metal analysis of water samples in Atomic Absorption Spectrophotometer.

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