

Estimation of Pollution Load in Khanpur Dam and its Tributaries due to Anthropogenic Activities



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2022

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**A thesis submitted in partial fulfilment of the requirement for the degree of the
Degree of Master of Science in Environmental Science**

Institute of Environmental Sciences & Engineering

School of Civil & Environmental Engineering

National University of Sciences & Technology

Islamabad, Pakistan

2022

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Acknowledgements

First and foremost, all praises to Allah almighty for endowment of immense support, strengths, and courage to get through this research successfully. After that I am highly obliged to my supervisor Dr. Hira Amjad for all her support and guidance during this journey. Besides this, I would extend my gratitude to my co-supervisor Dr. Imran Hashmi for his continuous support, enthusiasm, and invaluable knowledge sharing, I would really like to thank him for providing me the platform and opportunity to learn at the highest forum. He has helped and motivated me immensely during the hard times of pandemic.

I owe my heartfelt gratitude to my guidance and examination committee Dr. Javed Iqbal and Dr. Shakil Ahmad for their utmost support and help wherever required during the research.

I am especially grateful to all the laboratory staff especially microbiology lab and wastewater lab, especially Engr. Amir Khan for his expertise and kindness.

Here, I would like to extend my gratitude and special thanks to my friends for being supportive, loving, caring, and always having my back. My life at NUST has been so enjoyable and fun by the presence of you guys, no matter what you guys have stood up for me and I will always be indebted for all the things you people have done for me.

At last, but not the least I would like to say this research is dedicated to my family for all the moral support, especially my father for his all struggle and hard work, if it wasn't for him, I would not be able to achieve whatever I have achieved in my life, my mother for all the prayers, love, and my siblings you all mean the whole world to me.

Thank you all from the bottom of my heart, Ali signing out.

Muhammad Ali Raja,

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List of Abbreviations

EC	Electrical Conductivity
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
TS	Total Solids
As	Arsenic
Cd	Cadmium
Cr	Chromium
Pb	Lead
AAS	Atomic Absorption Spectrophotometer
TKN	Total Kjeldahl's Nitrogen
NEQS	National Environmental Quality Standards
WHO	World Health Organization
D.O	Dissolved Oxygen
UN EP	United Nation's Environmental Programme
USDGs	United Nation's Sustainable Development Goals
USD	United States Dollar
BCM	Billion Cubic Meters
PWA	Pakistan Water Act
PCRWR	Pakistan Council of Research in Water Resources
IDS	Indus Drainage System
WWTPs	Wastewater Treatment Plants
BCF	Bio Concentration Factor
Mg/L	Milligram per Liter
PBS	Phosphate Buffer Saline
DNA	Deoxyribose Nucleic Acid

Abstract

Riverine water exposed to pollution is the major concern in the world because of its serious effects on ecosystem and human health. This study assessed the pollution status, sources, diffusion and potential risk of contaminants in Khanpur dam Pakistan. The present study is based on the measurement and characterization of various physicochemical parameters such as pH, Electrical Conductivity (EC), Turbidity, Total Dissolved Solid (TDS), Total Suspended Solids (TSS), Total Solids(TS), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), Alkalinity, Total Hardness, Nitrate-nitrite and Phosphate-phosphorus) and heavy metals that is Arsenic (As), Cadmium (Cd), Chromium (Cr), and Lead (Pb) in the subject area. Heavy metal concentrations investigated in sediment for the quantification of pollution and potential ecological risks of the Khanpur dam and its tributaries. Dam sediments were sampled across the five major sampling stations: the Nilan Kas, Haro River, Exit Stream, Nilan Nala, and Khanpur Dam. Special emphasis was on microbial profile related to the bacteria of gram-negative group, as many pathogenic bacterial species belong to the gram-negative group including those related to the spread of nonviral Gastroenteritis. The heavy metal concentrations of the sediment samples were determined by Atomic Absorption Spectrometry (Analyticjena AAS nova 800). Water quality assessment revealed that microbial pollution is widely spread all around the Khanpur Dam area, bacterial growth was observed in majority of the sites along with other harmful pollutants. Physicochemical parameters such as Total Hardness (70-130 mg/L), TKN (32-53 mg/L), Phosphate-Phosphorus (0.5-5.3 mg/L), and heavy metals like As (20-60 mg/kg) and Cd (80-160mg/kg) exceeded WHO permissible limits in majority of the sampling sites. Results revealed that most of the samples depicted higher values of physicochemical parameters with respect to the permissible limit set by the NEQS of WHO. The potential sources of contamination were identified to be the surrounding geogenic activities, industrial/municipal wastewater discharges, agricultural and surface runoffs by using multivariate statistics including correlation analysis. The major findings of the study showed that higher level of alteration in the physicochemical parameters and heavy metals in water will have great impact on freshwater resources and human health in the study area. Meanwhile other parameters such as pH, Dissolved Oxygen, Nitrite, Nitrate, TDS, TS, Cr, and Pb were found to be within the permissible limits. The bottom line suggests the water is deteriorating at a rapid pace with increasing urbanization in the hotspots of Khanpur Dam area.

1. Introduction

Water is being rendered amongst the most pivotal existing natural resource for the sustenance of life and it is present in abundance in the Earth's crust. It covers over 75% of the entire of the globe (Noreen *et al.*, 2019). Human body is made up of over 70% of the Water content, and loss of it even by a fraction can cause severe dehydration and if lost in large quantities may cause death. Human body can survive up to 30 days without food however without water functionality of the organs seizes and death may occur within 3 days, this tells the essentiality of water. Dependence of life highly on water depicts the origin of life on earth was about 3.7 billion years ago along with evolution of microorganisms in water. The Uniqueness of this natural resource has made the existence of life possible on the planet therefore, it is also referred as “the molecule that has made us”. Water is not only essential for human beings and but all the biotic ecosystems which exist in this world. Industrial, agricultural recreational and domestic activities all cease to exist without the constant supply of water.

The sustainable development goals won't be achieved without the availability of clean and fresh drinking water (Sharma *et al.*, 2021). Despite of presence of water in abundance still many of the developing nations are deprived of this vital natural resource. For adequate supply & security this resource needs paramount consideration (Mishra *et al.*, 2019). Open waste dumping and landfill leachates severely pollute a significant portion of freshwater bodies rendering it unfit for human consumption (Boadi & Kuitunen, 2002), consequently drinking water has become a scarce resource for the masses (Edokpayi *et al.*, 2017). Population bloom in cities has led to foundation of urban water pollution that ultimately ends up with increase in competition among people to get access to limited available fresh water (Saghir & Santoro, 2018). Clean and freshwater need has increased globally in various sectors such as, energy, domestic consumption agriculture and industries) due to elevated annual population growth rate. However, naturally derived frequency and intensity hydro-climatic extremes (e.g., droughts, floods) is exacerbating the severity of this issue. Increased water demand and reduced availability is also equally responsible for the decline in water quality, especially in the developing nations (Canas, 2012). Anthropogenically induced changes are challenging the sustainable development goals for 2030, which aim to ensure the availability of clean drinking water for all by 2030 (Olsson, 2018). In order to provide a comprehensive understanding of the quality-related parameters of water scarcity, scientists have been conducting water shortage tests on its various qualities for a number of years now. These

tests are essential in order to determine the severity of a water shortage and the best course of action to take. While most water shortages are caused by a lack of fresh water, other causes include contamination and drought (Haldar *et al.*, 2020). Fresh water is an increasingly scarce resource, with a growing population and increasing demand. With the world's population forecast to reach 9.7 billion by 2050, the challenge of finding more fresh water will become even more pressing. According to the World Health Organization, more than 1.5 billion people do not have access to safe drinking water and more than 2.5 billion people do not have access to adequate sanitation. The proportion of fresh water used relative to available water sources is an important consideration when planning for the future. This index has been used for several scientific studies and was also presented as a USDGs-indicator. The inadequacy of freshwater resources has been recognized for years and recently, the focus has shifted to groundwater. Groundwater is often thought of as a safe water source, but this is not always the case. Vanham in 2018 examined the shortcomings of existing water resources, including the lack of water quality, the negligence of using non-traditional water resources and the lack of management of polluted water. (Vanham *et al.*, 2018). Reasons for water scarcity must be understood by the people for instance how water quality and quantity affect human health and the environment (Vliet *et al.*, 2017).

The freshwater resources are under severe pressure from both natural and anthropogenic activities. These activities, such as mining, agrichemicals, industrial wastes, domestic sewage etc., have all released toxic metals, sediments, organic and inorganic compounds and pesticides into the water. This has led to a severe deterioration in the freshwater quality (Chen *et al.*, 2012). Water pollution occurs when pollutants are discharged directly or indirectly into water bodies without an adequate treatment. They can be generated by domestic, agricultural, and industrial activities. Water pollution has a number of consequences such as the impairment of water quality and the contamination of groundwater, surface water, and drinking water. Additionally, it causes the alteration of aquatic ecosystems, leading to the extinction of several species (Colic *et al.*, 2010; Noreen *et al.*, 2019). Water quality is a fundamental problem across the world owing to the prevalence of microbiological and harmful contaminants in water sources. Dams are often built as a solution to water pollution, but they can also be polluted by various factors such as input of excessive nutrients, heavy metal contamination, eutrophication, and obnoxious or abnormal fishing practices. The effects of water pollution have been well-documented, both for human and aquatic species. These inputs not only impact the socioeconomic activities of the country, but also

affect the native species residing in the water. Anthropogenic sources are important producers of pollutants in water. Water pollution in underdeveloped nations, like Pakistan, is mostly caused by a lack of resources and awareness coupled with rapid industrialization and population growth. (Akbar *et al.*, 2022).

1.1 National Scenario

Pakistan is an arid country and derives most of its water supplies from river flows. Other South Asian countries, like India, are comforted with a tropical monsoon climate, receiving average annual rainfall exceeding 1,000 mm. Pakistan, on the other hand, receives only 508 mm on average annually. This lack of rainfall has led to the country's vulnerability to water shortages. Therefore, Pakistan has to highly rely upon river water for countries supply of freshwater. This enhances the countries dependence on a single basin (river Indus basin), which is often over exploited. Furthermore, there is also a threat to its security due to pollution induced by anthropogenic activities. One of the major pollution sources is attributed to the wastewater intrusion from industries and households which pollute rivers, streams and nearby drains relentlessly. Unregulated industrial effluents, pesticides and other agricultural chemicals are continuously being discharge in aquifers and drains; it not only corrupts the freshwater bodies but groundwater reservoirs as well. Therefore, directly deteriorating the freshwater ecosystem (State Bank of Pakistan's Annual Report 2016-17).

The World Resource Institute reported in the year 2020, Pakistan is on course to experience severe water stress, the situation will even worsen by the year 2030 pushing the country amongst extreme water scarce countries in the world. The main driving force for this problem will be poor water management practices followed by limited storage capacity. Most of the water is lost to Arabian sea every year. Furthermore, trans boundary disputes are further aggravating the problem. Besides this, Water Distribution Networks obsolete, their inadequate supply and low productivity could not withstand the everyday increasing demand as a result of booming population. Ground water levels are declining rapidly due to excessive pumping out, incentives for water conservation are distorting due to extremely low tariffs, and underfunded water projects are leading to low recovery and high losses. Such issues can only be addressed by adequate water management practices.

1.2 Water Availability and Quality

Currently, Pakistan is not only subjected to limited water supply but also facing risks from extreme weather events aggravated due to global climate change. On the contrary, water demand is continuously increasing due to large scale urbanization and boom in population growth. Hence resulting into an imbalance pushing the country into a severe water shortage. In the space of few decades Pakistan has become a water scarce country from a water abundant country.

Pakistan is inhabited by 2.8% of the total population in the world and it only has 0.5% of global renewable water resources. As compared to other countries in the world Pakistan is ranked amongst the 36th in total renewable water resources (PIDE Report, 2022).

Water quality is altered by 3 main components which include where the water exists, magnitude of the anthropogenic activities in that area, and management of water resources. The quality of water can be determined by examining certain physicochemical and microbiological parameters such as (temperature, dissolved oxygen, presence of minerals etc.). Water quality in Pakistan is deteriorating at an alarming pace, majority of the water sources (rivers, canals, and underground aquifers) are highly contaminated due to industrial, municipal and domestic discharges while agricultural practices are also adding up to the issue as harmful chemicals in the form of pesticides, insecticides and fertilizers are being vigorously used (Shah *et al.*, 2022).

1.3 Socio-economic Status

A report published recently named as “*Water Crisis in Pakistan*” gave an insight regarding the socio-economic status related to water crisis. The report highlighted some eye-opening statistics. The report indicated the statistics regarding Pakistan’s excessive dependence on a single river basin is extremely dangerous rendering Pakistan highly vulnerable. As the entire country relies heavily on river Indus as it accounts for Pakistan’s 96% of total renewable water resources. Three fourth of water of its water is received from outside of the country. With respect to withdrawing rate Pakistan is ranked as the 8th lowest for generating per cubic water, putting this into perspective republic of Korea is ranked at 37th, Malaysia at 35th, Turkey 87th and China at 71st. In Pakistan only 36% of the population has the access to clean drinking water, cities are facing serious issues of erratic supply of drinking water. As per the global statistics out of 63% of produced water 52% is treated, 48% is released and 11% is reused. Whereas, in Pakistan merely

1% of wastewater is being treated making Pakistan one of the worst countries in terms of treatment rate as a result of poor water management practices and negligible recycling.

1.4 Challenges and Major Sources of Water Contamination

Water crisis in Pakistan can be explained mainly due to massive population, occurrence of extreme climatic events such as (droughts and flash flooding), poor water management, in the agriculture sector, lack of infrastructure, and water pollution. All these reasons are increasing intra-state tensions among different provinces.

1.5.1 Rapid Population Growth and Unplanned Urbanization

Rapid population and unplanned urbanization are considered to be the biggest threat in deteriorating the water quality in Pakistan. Population boom has been observed with an increase of 2.6 times from 1970-2020, Pakistan jumped from the 9th rank to 5th. However, in comparison Bangladesh's population growth rate has been increased by 1.5 times in the same time span. Due to this issue Pakistan's water use rate has also increased by 0.7% meanwhile reserves remained stagnant at 246.8 billion cubic meters, resulting in decrease in per capita water resources availability from 3478 to 1117 cubic meter per year, subsequently leading to surging pressure on this precious resource. As the demand is increasing replenishment rate is decreasing. The population growth rate is projected to increase by 53% by the year 2050, reaching 338 million. The share of population in cities is also projected to increase from 37% in 2020 to 53% in year 2015. In this scenario water management inefficiency will also be an issue and water withdrawal to water resource ratio may exceed 100%.

1.5.2 Climate Change

In list of most vulnerable countries in the world to climate change, Pakistan is ranked amongst top 10. The extreme weather events had increased drastically in the past few decades. Events such as disturbed monsoon patterns, average temperature rise, glacier melting, flooding, and droughts have been observed with an increased intensity. Pakistan has also suffered from long spells of droughts and floods for instance in 2010, these calamities have caused severe loss of life and inflicted heavy damages to infrastructures worth exceeding 10 billion USD. Similarly, in Baluchistan droughts have increased over the years as Quetta had experienced an 8 year drought from 1997-2005. Overall climate change may change the aggregate water flows but projections show decline in water flow and increase in the occurrence of extreme events. Indus river basin is

considered to be the chief water resource of Pakistan, which depends on glacial melt and rainfall leaving it highly sensitive to climate change. It has already been observed the river has been reduced just to canal once it reaches Sindh province, whereas many farmers have migrated to urban areas due to lack of availability of water to sustain agricultural activities. Given the fact that Indus River accounts for 50-80% of its total load coming from ice and glacier melting.

1.5.3 Poor Water Management

Over 80% of the total water is used up by the agricultural sector which includes four major crops (wheat, cotton, rice and sugar cane) and in terms of revenue it only comprises of 5% of the total country's GDP. The productivity of the agriculture sector is extremely low in Pakistan as compared to other developed agrarian economies. Water canal pricing is very minimal which leads to misuse of the resources, it recovers only 1/5th of the total canal water charges including annual operating and maintenance cost, collection is total 60% of the receivables. Besides, farm sector accounts for half of the country's employment and comprises of only 1/5th of the GDP, less than 0.1% of the total tax revenues are generated thus limited finances are available for the maintenance of irrigation systems, substandard water management practices lead to enormous wastage of water as well. Unfortunately, Pakistan has one of the most inefficient irrigation systems in the world with an efficiency rate of 39%, with only 55 BCM of water is utilized by total of 143 BCM available, the remaining 87 BCM is lost during conveyance through canals, water courses, distributaries and minors. In addition, as compared to other countries Pakistan's efficiency is only 9% to the world's 40%.

1.5.4 Water Pollution

About half of the two million produced wet tons of human excreta go on to pollute water every year, according to a study. In Pakistan, 60 million people are at risk of exposure to high concentrations of arsenic in groundwater on the Indus Plain. The World Health Organization (WHO) has set a guideline of 10 micrograms per liter ($\mu\text{g/L}$) for arsenic in drinking water, but the new study found that, on the Indus Plain, concentrations of arsenic in groundwater can exceed this guideline by more than 10 times. The study, published in the journal *Science of The Total Environment*, was conducted by a team of Pakistani and Canadian researchers. Consumption of polluted water is causing a serious problem in Pakistan leaving 60% of people on risk to suffer from life threatening water borne diseases. Patients with water borne diseases fill in almost 1/3rd

of the hospital beds and account for 40% of all the premature deaths in Pakistan. According to the statistics in 2017, as a result of inefficient sanitation and inadequate water supply, approximately 60,000 people died prematurely and half of them were children ageing from 2-5 years old. Currently, Pakistan is also facing resurgence of diseases like diarrhea, polio, hepatitis A and E and dengue fever. 54,000 deaths are caused by diarrhea alone, meaning every hour a child dies in Pakistan due to diarrhea. The economic cost of floods, poor water sanitation and droughts is estimated to be around 12 billion USD which accounts for 4% of the total country's GDP.

1.5.5 Water Policies

Pakistan's first national water policy was formulated in year 2018, for the first time it was acknowledged that water is a finite resource, and it must be protected. It emphasized on the recovery of the water management and irrigation systems in the country. The policy also foresees the future impacts of climate change on this precious resource, therefore, demands cooperation at regional level. Furthermore, some provincial level initiatives were also devised i.e., Punjab Water Act 2018 (PWA), Sindh Agriculture policy 2018, KP Drinking Water Policy 2015, Baluchistan Irrigation Water Resource Management Policy 2006, and Climate Change Policy 2006. However, these initiatives still lack many aspects and completely ignore the scientific proportion, water quality, clear referencing, sustainable development goals, gender inclusion and targets etc. these gaps must be addressed to formulate a comprehensive water policy which can be realistically implemented. Also, the need is for political will, timeliness, financial capital and capacity.

1.5.6 Water Distribution Issue within Provinces

Due to increased water scarcity problem in Pakistan, conflict have arose among the different provinces regarding the distribution of water. Under Pakistan water accord 1991 canal water is being distributed among the provinces. A total volume of 145 BCF water is distributed in between 4 provinces; out of which 48% is allocated to Punjab, 42% to Sindh, 7% to Khyber Pakhtunkhwa and around 3% to Baluchistan. This accord was defined as per ample supply of water however it overlooks the apportionments for water shortage. Over the course of a year in months of lesser rainfall and excessive heat supply is reduced thus causing the conflict in between upstream province Punjab and downstream provinces Sindh and Baluchistan. Punjab is alleged for water theft by Sindh and Baluchistan blames Sindh for not providing its share from Sukkur and Guddu barrages.

1.5 Estimation of Pollution in some Rivers and Dams of Pakistan

Rivers and dams transport the fresh water and minerals in entire country, and they play a significant role in influencing the hydrological cycle by serving outlet channels for surface runoffs. Pakistan is blessed with one of the world's largest glaciers but still holds the 36th position in the list of water stressed countries. Majority of the freshwater lakes present in land are being vigorously polluted, those which are not yet polluted their ecological status is under extreme threat. Water quality is deteriorated in majority of the reservoirs and have fallen below the standards devised by World Health Organization (WHO), and as a result of increasing temperatures glaciers are losing their flora and fauna at an alarming pace (PCRWR 2020).

1.6 Present Study

In the current study, an attempt has been made for qualitative and quantitative estimation water quality assessment, purely based on the suburbs of Khanpur Dam Lake reservoir. Construction work at Khanpur Dam Lake was completed the year 1988 and it is built on the Haro River which extends 31.7 Kilometers, and it acts as a major water source for Khanpur Dam. For almost 40-year Khanpur Dam is providing consumable water supply to the neighboring areas as well as considered as a renowned spot for tourism related activities. Along with drinking water it also meets the demand from agricultural sector in the areas of district Haripur. Unfortunately, watershed meeting challenges of rapid deforestation and urbanization which has led to acceleration in soil erosion, land sliding, surface runoffs vigorously. Such factors are deteriorating the quality of water in the dam (Zaheer *et al.*, 2016). Besides human settlements and other anthropogenic activities are damaging its flora and fauna (Akbar *et al.*, 2022). In this regard, increased sediment load in the river discharge can negatively affect the water clarity, creating favorable conditions for lake eutrophication (Hayashi *et al.* 2015), which renders the reservoir water quality unfavorable for safe drinking and consumption (Faizi *et al.*, 2022).

1.7 Study Objectives

- Water quality assessment by monitoring of physicochemical and microbiological parameters in the upstream, mid-stream and downstream of Khanpur dam area.
- Assessment and monitoring of variation in pathogenic microbial profile related to gastroenteritis diseases and assessment of prevalence of heavy metals in Khanpur dam area.

2. Literature Review

River Indus along with its eastern tributaries (River Soan, Chenab, Ravi, Jhelum and Sutlej) and western tributaries (River Kabul and Swat) comprising of Pakistan's largest inland River System called Indus Drainage System (IDS). Along with IDS River Indus is the major south flowing river with total drainage area of 1,165,000 km² that running for about 3610 km with annual flow of 6600.01 m³/s approximately. Drainage system not only playing key role in driving economy of Pakistan by fulfilling their industrial, domestic and agricultural water demands but also supporting plains, arid farmland, and temperate forests ecosystems across the country. The total available annual water in Indus River Basin is approximately 229 billion cubic meter (BCM) (Young *et al.*, 2019). From the total available water, industrial sector is consuming about 80%, domestic consumption is approximately 23% and the agriculture sector being the largest water consumer is consuming 69% (Khosro *et al.*, 2015; Young *et al.*, 2019). Recent developments such as Industrialization, modern agricultural activities and Urbanization along the IDS basin subjected the pressure on sewage system. Furthermore, industrial and domestic effluents are discharged directly into the river system (Nawab *et al.*, 2018; Khan *et al.*, 2022). Different anthropogenic activities along with basin subjecting river water and its surrounding atmosphere to serious pollution because of direct discharge of untreated domestic and industrial waste and effluents (Bajwa *et al.*, 2016).

From main eastern tributaries of River Indus, River Soan in northern Punjab is the main watershed that starting from springs of Bun village near Murree and running about 250 km along the pothohar region before emptying into the Indus River at Pirpiyahi near Kalabagh (Aziz *et al.*, 2014). Increasing chemical drainage from Sihala Industrial and Urban state along with wastewater treatment plants (WWTPs) in Islamabad and Rawalpindi cities that are posing directly threat to freshwater ecosystem in river water by deteriorating the water quality with contamination of untreated effluents. River Chenab from main eastern tributaries of River Indus is originating from Lahaul and Spiti districts of Himachal Pradesh, India and flowing through the Jammu and Kashmir, draining about 960 km² with annual flow of 800.60 m³/s in total drainage area of 67,500 km² (Alamdar *et al.*, 2017). Major urbanized areas of Gujrat, Sialkot, Gujranwala, Jhang, Multan and Faisalabad are courtesously polluting the River Chenab (Hanif *et al.*, 2016).

River Ravi is a transboundary river between India and Pakistan that runs for about 725 km with a mean annual flow of 267.50 m³/s and a total basin of 14,442 km². The river provides water for irrigation and domestic uses in Panjab, Pakistan. Its overall pollution level is reportedly very high, due to careless disposal of large amounts of industrial effluents, urban and agricultural runoffs and drainage sewages from both countries (Syed *et al.*, 2014). In general, the overall annual water availability in the drainage basin of associated IDS tributaries is approximately 229 BCM, that plays a key role in Pakistan's economy to meet the agricultural, domestic as well as industrial water demand, and support the ecosystems of temperate forests, plains and arid farmlands. However, the increasing anthropogenic activities along the Indus basin are seriously polluting the riverine water and atmosphere around it (Younas *et al.*, 2022).

A transboundary river between Pakistan and India that runs for about 725 km with total basin of 14,442 km² that counts for mean annual flow of 267.50 m³/s, is known as River Ravi that is the third main eastern tributary of River Indus. These rivers providing water for the purpose of irrigation and to fulfill domestic water needs in Punjab, Pakistan. Overall pollution level in Indus River is alarmingly very high due to careless disposal of large amount of urban and agricultural runoffs, drainage sewages and industrial effluents from both countries (Syed *et al.*, 2014). On average the available water in drainage basin of associated IDS tributaries is about 229 BCM, that plays vital role in driving the Pakistan's economy to sustain the ecosystems of the country and enhance the overall economic growth rate. Anthropogenic activities are the main hurdles in slowing down the country growth rate.(Younas *et al.*, 2022).

The minor tributary of River Indus is Soan River that originates from southern range of the Murree hills, which located at longitude 71° 45' to 73° 35'E and latitude 32° 45' to 33° 55'N and passes through the Attock, Rawalpindi and Jhelum districts in Punjab (Pakistan). Soan River total length is 272 km with total drainage catchment area of 11, 085 km². A drainage channel called Nallah Lai, originates from Margalla hills and ultimately falls into this river. Along with this channel wastewater is also draining into the river near the Soan bus bay. Soan River is providing its water to the Khanpur dam that is the largest source of drinking water for the Islamabad and its surrounding areas. As agricultural lands are located along this watershed therefore, herbicides and insecticides application is common in these farm lands that leads to the contamination of water.

Streamflow, precipitation and snow melting from Murree hills are the main sources of water to recharge this water body (Ejaz *et al.*, 2020).

2.1 Water Quality Parameters

2.1.1 pH

The pH measurement reflects the acidity or alkalinity of the water sources that can produce sour or alkaline tastes. The pH is a measurement of hydrogen and hydroxyl ions in a solution, using a logarithmic scale. The measure indicates if the solution under consideration is alkaline ($\text{pH} > 7$) or acidic ($\text{pH} < 7$). Fresh water pH normally ranges between pH 6 and pH 8. The pH affects the availability and solubility of nutrients that are needed by aquatic organisms for utilization. Increased pH in a stream or other body of water is caused by carbonates rich soils such as limestone as well as presence of nitrate-based fertilizers while low pH is caused by the presence of phosphoric acid in phosphorous fertilizers (Bell, 2019). Decomposing matter from domestic wastes produces carbonic acid which can lower pH in a river.

2.1.2 Temperature

The temperature of surface waters is influenced by latitude, altitude, season, time of day, air circulation, cloud cover and the flow and depth of the water body. Many of the physical, chemical and biological processes in water are affected by temperature and this makes the temperature to be a general variable that concentration of other variables depends on. As water temperature increases, the rate of chemical reactions generally increases together with the evaporation and volatilization of substances from the water. Increased temperature also decreases the solubility of gases in water, such as oxygen, carbon dioxide, nitrogen, methane and others. Aquatic organisms also depend on temperature for the functioning of their body processes such as metabolic rate and respirations rates, for example in warm water respiration rate is high which increase oxygen consumption and then causes an increase in decomposition of organic matter. Growth rates of organisms also increase and this is most noticeable bacteria and phytoplankton whose population double in very short time periods hence leading to an increase in water turbidity and algal blooms, when nutrients conditions are suitable

2.1.3 Turbidity

Turbidity is a measure of water clarity. Turbidity in water is caused by fine suspended matter such as clay and silt along with small grains of organic and inorganic matter. Also plankton

and other microscopic organisms can increase turbidity (Ullberg, 2015). Apart from these causes turbidity is also caused by agriculture activities mostly siltation as a result of cultivating along riverbanks. Sand mining also causes an increase in turbidity (Ullberg, 2015). When the level of turbidity is high the availability of macroinvertebrates will be low. This is because increased turbidity may alter prey-predator relationship since some of the aquatic organisms depend on other organisms as their food so when the water is more turbid it will make it difficult for other organisms to see their preys

2.1.4 Electrical Conductivity

Electrical conductivity is an indicator of water quality. The EC value is an index that represents the concentration of soluble salts that affect the taste of the drinking water source. Conductivity data can determine concentration of solutions, detect contaminants and determine the purity of water. The electrical conductivity (EC) of water is a measure of the ability of a solution to carry or conduct an electrical current. Since the electrical current is carried by ions in solution, the conductivity increases as the concentration of ions increases. Therefore, it is one of the main parameters used to determine the suitability of water for irrigation and firefighting.

2.1.5 Nitrate

Natural nitrate levels are relatively below such as 1 mg/L or lesser in some surface waters. At this level, nitrate is not harmful, but adverse health effects can be seen in aquatic species when nitrate concentration gets too high. When excessive nutrients such as nitrate and phosphorus – wash into a body of water, harmful algal blooms (HABs) are often the result. These blooms can produce dangerous toxins and cause a drop in dissolved oxygen, resulting in fish kills. Algal blooms in water bodies that serve as drinking water sources are especially problematic. Nitrate can cause other health issues when consumed, especially in young children. Exposure to drinking water with high nitrate levels can result in methemoglobinemia, also known as "blue baby syndrome." This condition often occurs when water with excessive nitrate is used to prepare infant formula. The level of NO^3 and NO^2 in drinking water causes diseases such as blue baby syndrome, cancer and bleeding of the spleen (Aydin, 2007).

2.1.6 Dissolve Oxygen

Most aquatic organisms require dissolved oxygen, often abbreviated as DO. DO is one of the most commonly measured water quality parameters, but the reason for measuring it varies

based on the environment. Dissolved oxygen is a direct indicator of a water body's ability to support aquatic life as aquatic organisms need DO to survive. An imbalance of DO occurs when there is a harmful algal bloom (HAB). Microbes consume waste and transform it into harmless end products in the treatment process at wastewater treatment plants. DO plays a critical role in this process, as these microbes rely on it to break down wastewater contaminants, such as organics or ammonia.

3. Materials and Methods

This research was carried out in four phases as depicted in the flowchart diagram below. The present study was aimed at monitoring the water quality as well as soil/sediment to verify whether the water present in selected study area was suitable for the drinking and other intended purposes. The present study also assisted in determining the magnitude of anthropogenic activities going on in the surroundings of Khanpur Dam area. Focus was to determine the microbial, physicochemical, and heavy metal concentrations in order to make necessary recommendations.

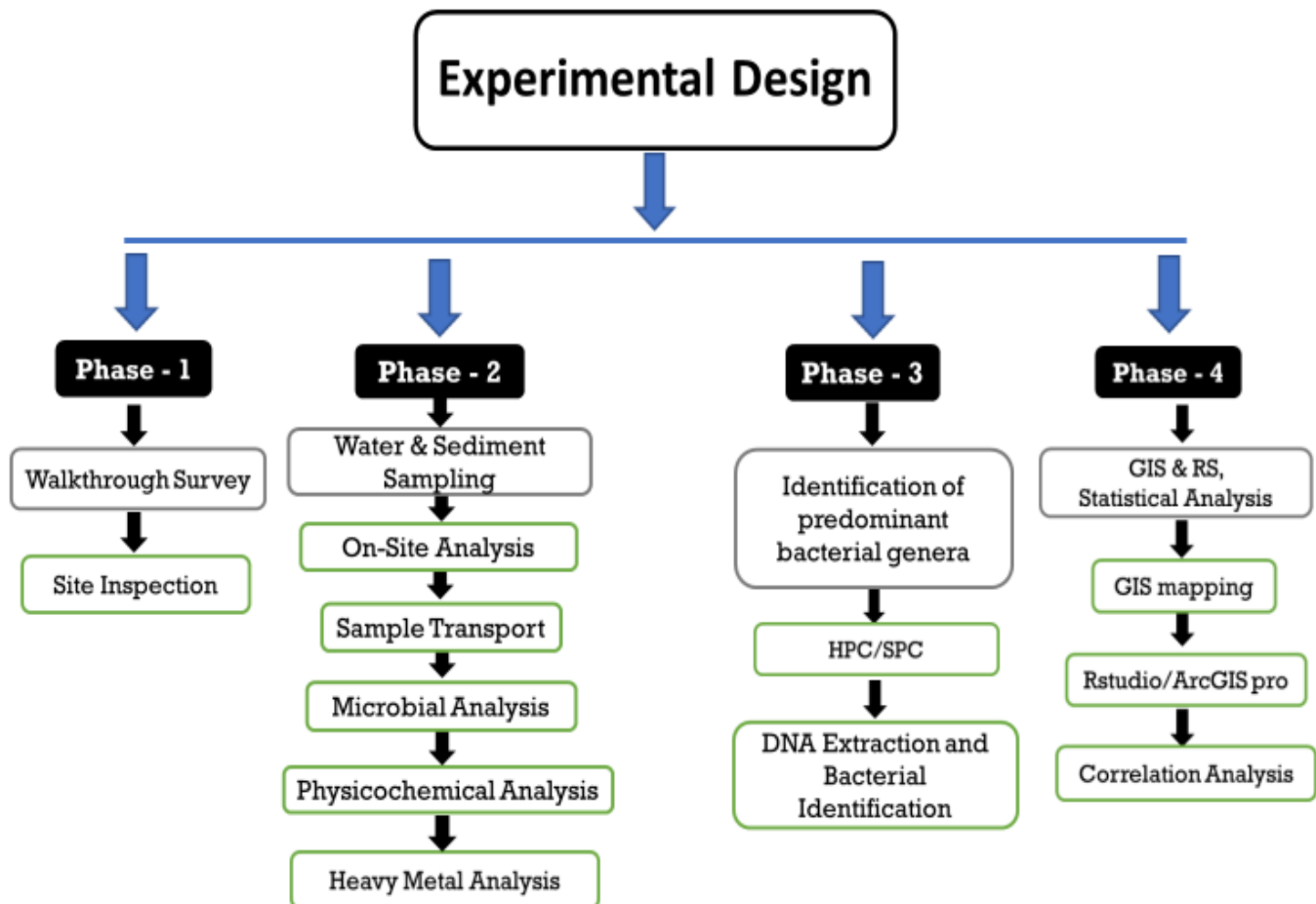


Figure 3.1: Experimental Design

3.1 Data Acquisition and General Overview

Data acquisition involved walkthrough survey to establish a comprehensive picture of the study area besides it aided in determining the sampling locations. A complete site inspection was followed by the sample collection. Water, soil and sediment samples were collected in triplicates from the designated sampling sites as mentioned in table 3.1. On-site parameters were tested on each of sampling sites which included pH, Temperature, Dissolved Oxygen and Electrical Conductivity. The acquired samples were transported to laboratory for further analysis which included microbiological parameters, physicochemical and heavy metal analysis according to APHA standards.

3.2 Study Area Map and Sampling Points

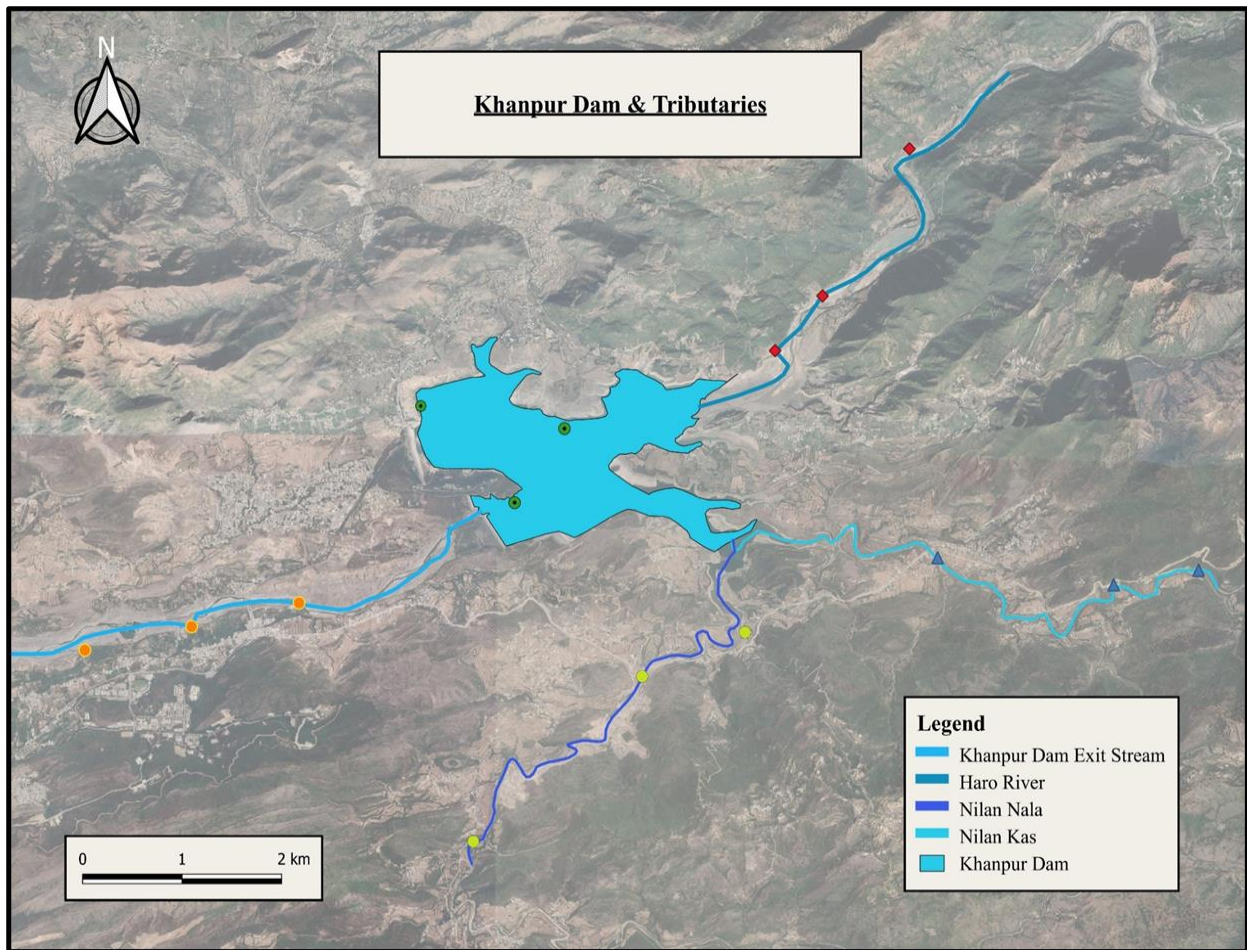


Figure 3.2: Study Area Map and Sampling Points

3.3 Microbiological Analysis

For microbial analysis (500mL) sterile Pyrex glass bottles were prepared, the sterilization process included rinsing followed by autoclaving at 121°C for 15 minutes and oven drying for two hours at the temperature of 115°C. Same procedure was carried out for sampling bottles used for physicochemical, and soil/sediment sample collection. Once completely dried sampling bottles were tightly sealed and transported to sampling stations in the icebox.

3.4 Methodology Adopted for Sample Collection

Simple grab sampling approach was adopted for water samples and sample collection was done in biological triplicates from the respective sampling sites. However, composites samples were collected for soil/sediment samples which were further subjected to heavy metal analysis after microbial analysis. Grab sample is obtained in a single time as time limit should not exceed the window of 15 minutes, it is usually collected manually but, in some cases, automated samplers may also be used where frequent sampling is required. On the contrary composite sampling can be obtained in an extended period of time, individual (grab) samples are being collected and mixed together over a certain period of time such as 12 hours.

At time of sample collection GPS coordinates were noted down by using GARMIN GPSMAP 64x, besides certain on-site parameters i.e., Temperature, Dissolved Oxygen (D.O), Electrical Conductivity, and pH were also analyzed using Lutron multimeter WA-2015. Once completed with sampling, the obtained samples were preserved and quickly transported to laboratory. Samples were stored in an ice box to avoid deterioration and maintain the temperature. Sample collection was performed with accordance to SOP's devised by Environmental Protection Agency (EPA), once the sample was filled head space of approximately 2 cm was left in the bottle and caps were tightened immediately. Prior to that bottles were rinsed with sampling water to entirely homogenize it with sampling water.

Table 3.1: List of Sampling sites along with GPS Coordinates

Sampling Sites	Latitude	Longitude
Nilan Kas Upstream	33.797041	73.007359
Nilan Kas Midstream	33.795528	72.998135
Nilan Kas Downstream	33.798333	72.979027
Nilan Nala Upstream	33.769001	72.928627
Nilan Nala Midstream	33.78607	72.946949
Nilan Nala Downstream	33.790632	72.958125
Exit Stream Upstream	33.79366	72.909707
Exit Stream Midstream	33.791209	72.898051
Exit Stream Downstream	33.788782	72.886455
Haro River Upstream	33.840606	72.975962
Haro River Midstream	33.825395	72.966538
Haro River Downstream	33.819741	72.961384
Khanpur Dam Upstream	33.814028	72.922924
Khanpur Dam Midstream	33.81169	72.938524
Khanpur Dam Downstream	33.814028	72.932172

Sampling was carried out in time span of 5 months from (August 2021- December 2021). Upstream, midstream, and downstream were determined from each sampling station based on the accessibility. As soon as reaching the laboratory samples were subjected to microbiological analysis HPC (Heterotrophic plate count) technique, followed by physicochemical analysis which included Nitrite-Nitrogen, Nitrate-Nitrogen, Total Kjeldahl's Nitrogen, Phosphate-phosphorus, Total Alkalinity, Total Hardness, Chemical Oxygen Demand, Total Suspended Solids, Total Dissolved Solids and Total Solids.

3.5 Heterotrophic plate count technique

HPC is an experimental procedure which is used to enumerate the living bacterial species of the specimen under the examination. It uses different selective and non-selective agar medias

to inhibit microbial colonies. In this research MacConkey agar was used, it is a selective media and inhibits the growth of bacteria belonging to the gram-negative enteric genus such as *Escherichia coli*, *Salmonella typhimorium*, *Enterobacter aerogenes* etc.

MacConkey agar was weighed and thoroughly mixed with distilled water with the help of magnetic stirrer. Once completely dissolved then mixture of distilled water and agar was autoclaved at 121°C and 15 psi for 15 minutes. Once the media was cooled down to 50°C it was poured on to petri dishes and kept in level two safety cabinet until media got solidified. After the solidification petri plates were inverted and placed into the incubator at 37°C for 24 hours for sterility test. After 24 hours when no growth was observed the sterility test was passed hence the sample spreading was initiated (APHA, 2017).

Water, soil/sediment samples those were obtained from designated sampling locations mentioned above were subjected to serial dilutions of raw samples. Three-fold 10^{-3} dilutions were prepared using Phosphate Buffer Saline (PBS) to take the bacterial colony growth down to countable range (30-300) for each dilution 1 mL of aliquot was pipetted from the sample and poured into a sterile test tube filled up with 9 mL of autoclaved PBS and the mixture was homogenized using vortex mixer. 100µl from each dilution was used to spread on the media plates and placed in incubator at 37°C for 24-48 hours to assess the bacteriological growth. After incubation growth was observed and colonies were being counted using 560 Suntax Colony Counter.

3.5.1 Bacteriological Colony Morphology

Bacterial colony morphology is an immensely useful tool for the initial determination of appeared bacteriological colonies. It involves a close visual examination of the grown colonies. A colony morphological table was made based on the appearance and attributes according to the sites.

4. Results and Discussions

This research was proposed to investigate the matter of growing concern of water pollution in the suburbs of Khanpur dam due to rapid increase in urbanization. A special emphasis was given to microbiological aspect of the water bodies under inspection along with physicochemical, and heavy metal analysis of soil/sediment samples. All the parameters were tested according to APHA 2017 standards and compared to WHO standards for drinking water quality to assess the present situation.

Table 4.1 List of Physicochemical Parameters and Standards (WHO and PSQCA)

Parameters	Units	WHO	PSQCA
pH	-	6.5-8.5	6.5-8.5
Temperature	°C	-	-
Turbidity	NTU	<5	<5
Dissolved Oxygen	mg/L	-	-
Electrical Conductivity	µS/cm	750	1000
Total Alkalinity	mg/L of CaCO ₃	500	-
COD	mg/L	-	-
Total Hardness		100	150
TDS		500	1000
Total Kjeldahl Nitrogen		0.5	-
NO ₃ -N		10	10
NO ₂ -N		3	-
PO ₄ -P		0.3	-

Table 4.2 Standards Heavy Metals in Soil/Sediment

Heavy Metal	WHO	USEPA	WSRA
Cadmium (Cd)	3	<6	0.3
Chromium (Cr)	100	<25	71
Lead (Pb)	100	<40	16
Arsenic (As)	20	ND	10

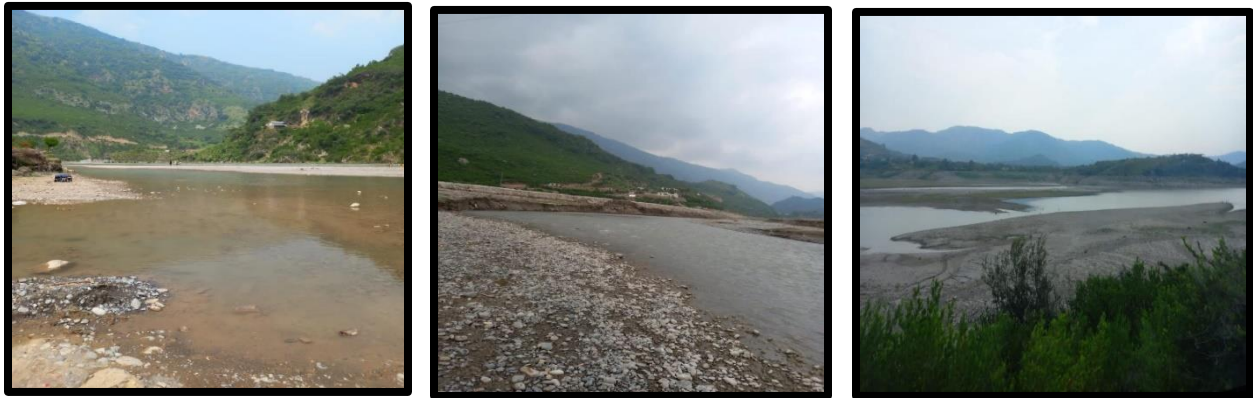
4.1 Site inspection and walkthrough survey

Site inspection and walkthrough survey was important to get familiar with the study area and for the identification of sampling points. Key observations were noted down to correlate with the finding of the parameters tested mentioned above.

Site 1: Haro River

At Haro river upstream slight algal growth, excessive vegetation and recreational activities were observed. Besides this plastic waste such as shopping bags and wrappers were also noticed. midstream and downstream were relatively cleaner however, construction waste and plastic bags were observed here as well.

Figure: 4.1 Haro River observations



Site 2: Nilan Kas

Nilan Kas upstream was relatively cleaner, terrain was rocky and high vegetative growth was present, but as we moved to midstream to downstream water started to become more turbid. Furthermore, animal waste and wastewater intrusion were also witnessed.

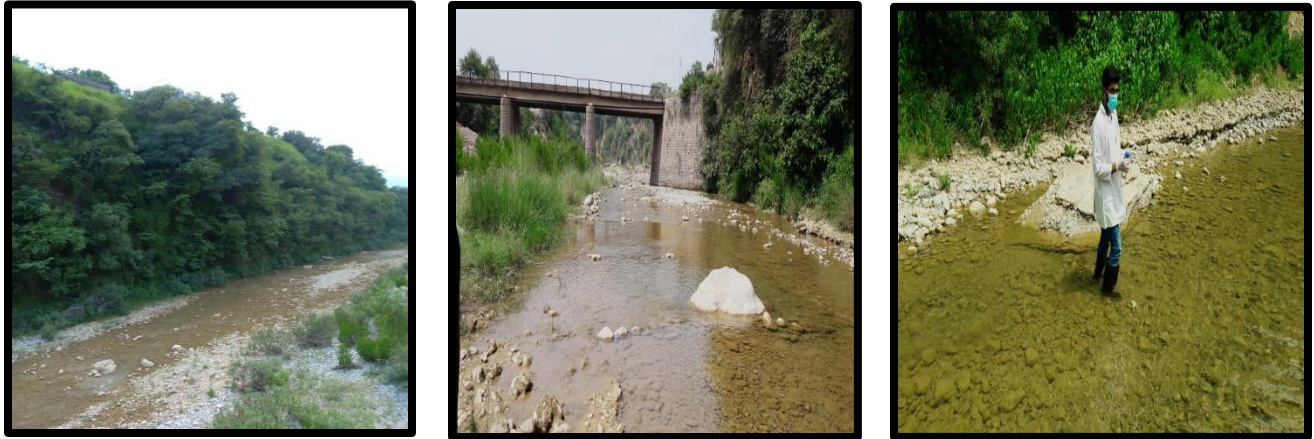


Figure 4.2 Nilan Kas observations

Site 3: Nilan Nala

Water at Nilan Nala appeared turbid besides algal growth was evident in the downstream due to presence of animal waste since animal manure was also observed.



Figure 4.3 Nilan Nala observations

Site 4: Khanpur Dam

In the Upstream of Khanpur dam, excessive boating activity was going on, due to which water had become muddy and highly turbid. Tourists were present in large numbers and littering was also observed however, at midstream and downstream water was relatively clear.



Figure 4.4 Khanpur Dam observations

Site 5: Exit Stream

Water quality in Exit Stream was observed in highly deteriorated form, high algal growth was observed in the upstream, littering, car washing, human intervention and recreational activities were observed in mid-stream and down-Stream.



Figure 4.5 Exit Stream observations

4.2 On-site Analysis

During the sampling initially several onsite parameters were tested such as pH, DO, EC and temperature using the Luton multimeter W.A. 2015.

4.2.1 pH

pH is very basic yet one of the most important water quality testing parameters, it indicates if water is either acidic or alkaline in nature (Sailaja *et al.*, 2015). pH in the range of 6.5-8.5 is considered to be in neutral range therefore rendering water acceptable for usage without any harm. pH was tested from all 15 sampling sites and results revealed that it was found out to be within the WHO permissible guidelines. However, some variation was detected among sites of Khanpur dam ranging from 6.7 to 7.4 which can be seen in figure 4.6.

4.2.2 Dissolved Oxygen

It is the amount of oxygen dissolved in water, it is a significantly important parameter in determining the physical and biological activities occurring in the water (Sialja *et al.*, 2015). D.O, in water can be transferred from the contact of water to the atmosphere, photosynthesis or aeration of water. More the movement of water more will be the D.O. present in it. Cool and fresh water tends to have more D.O. than warm and saline water, the reason is the oxygen in abruptly moving warm water escapes easily. D.O. values measure in Khanpur dam vicinity are depicted in figure 4.7. The results of present study indicate that D.O. levels in all the sampling stations were found within permissible limits for drinking water. (6.5-8.5).

4.2.3 Electrical Conductivity

E.C. determines the capacity of water to carry electric current. It is a measure of dissolved ionic components present in the water. It is directly influenced by the presence of total dissolved solids in water (Vunain *et al.*, 2019). In this research E.C. in majority of the sites was found out to be within the permissible limits however Nilan Nala showed E.C. in high numbers ranging from (550-650 μ S). Besides, it was on the borderline in Haro River and Khanpur Dam. The reason could be the presence of higher dissolved ionic content due to ongoing recreational activities and construction waste as shown in figure 4.8.

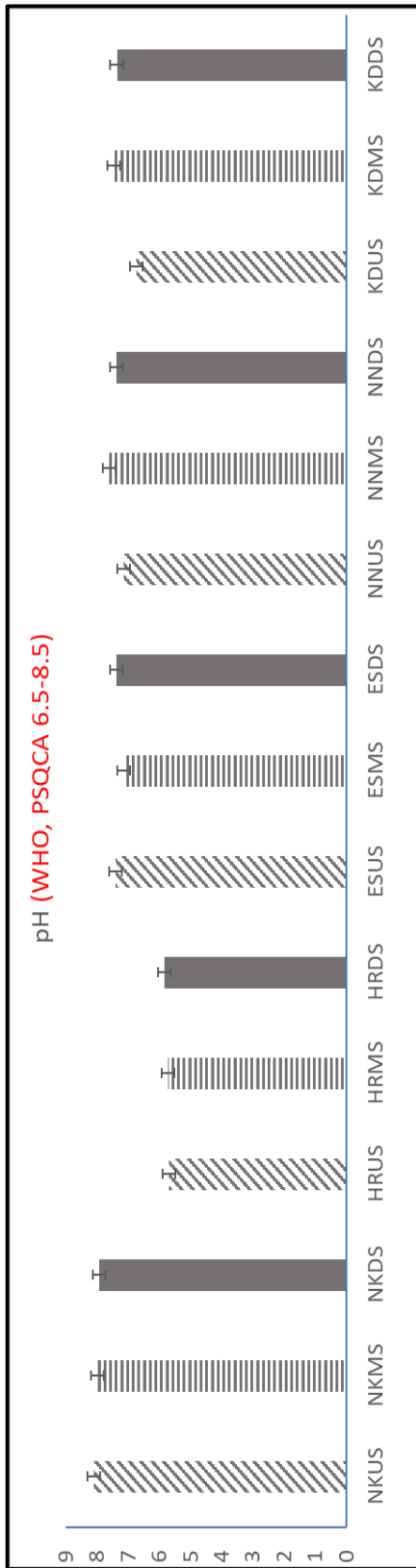


Figure 4.6 Graphical Illustration of pH

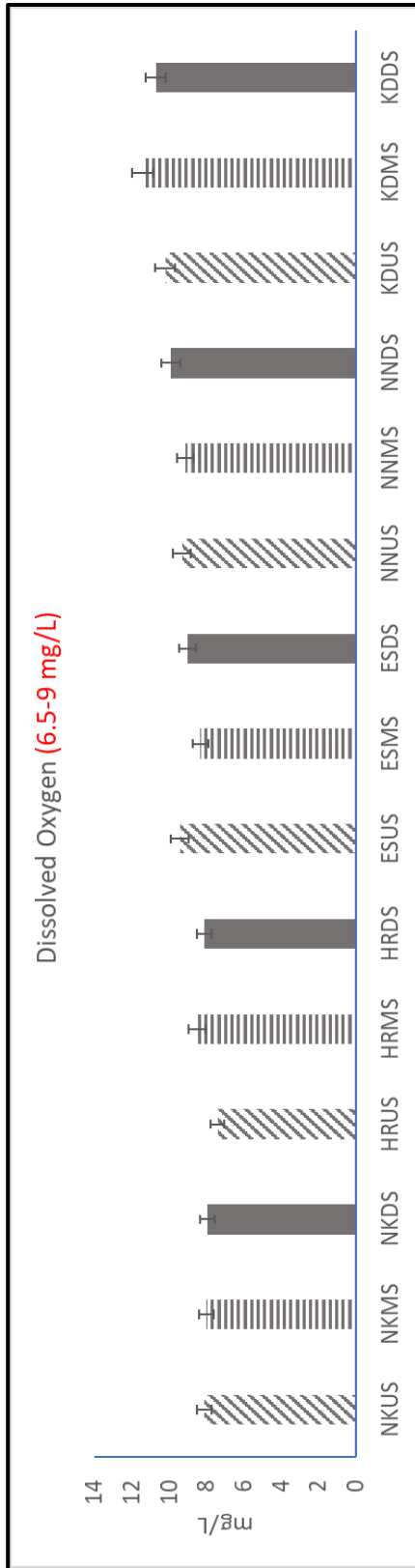


Figure 4.7 Graphical Illustration of Dissolved Oxygen

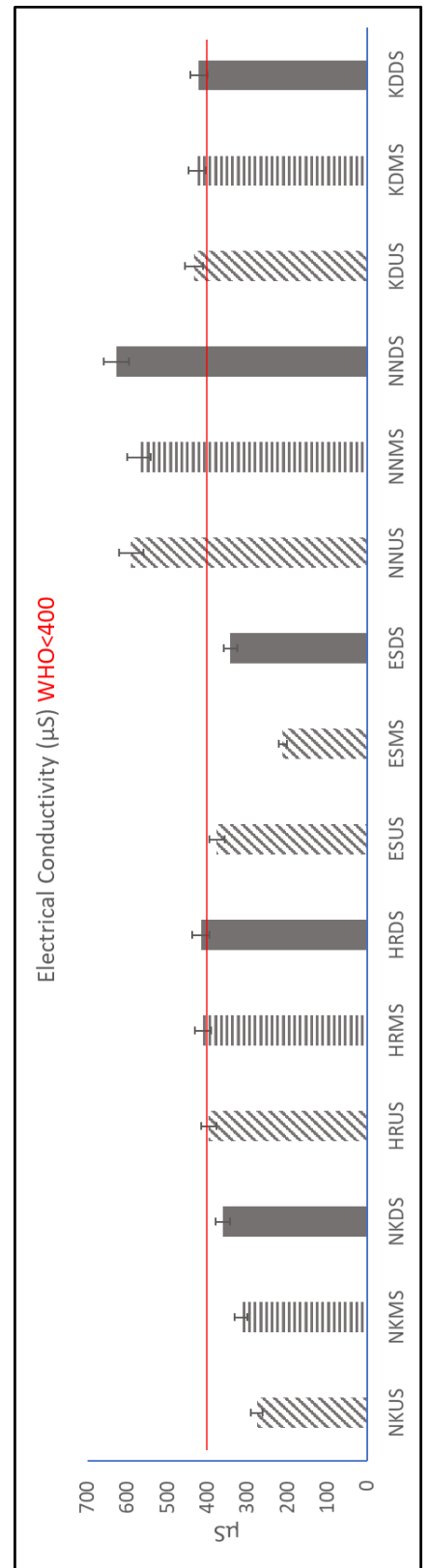


Figure 4.8 Graphical Illustration of Electrical Conductivity

4.3 Microbiological Analysis

Soon after the transportation of water and soil/sediment samples, to assess the microbial profile microbial analysis was carried out. Growth was observed and colonies were counted from all the sites, mean values of the colony forming units/mL of sample were calculated and graphically depicted.

Assessing the graphs of microbial profile, it is evident that prevalence of microbial contamination is very high among all the sites except for Khanpur dam main catchment area, the main reason for growth to be not seen in Khanpur dam is high dilution factor however, all the remaining sites were severely polluted rendering water unfit for drinking. This is the evidence of fecal and sewage intrusion in the water bodies entering the Khanpur dam as shown in figure no. 4.9.

Similarly, for soil/sediment samples dilutions were prepared and it was justifiably noticed that high number of bacterial growth was observed in soil/sediment as compared to water samples as shown in figure 4.10 Colony Morphology table along with cfu/mL colony count has been attached in the Annexure A for reference.

4.3.1 DNA Extraction of Purified bacterial Cultures

After the careful examination of observed microbial colonies, predominant species colonies were selected and were subjected to four rounds of streaking to attain pure cultures. Once the isolation of pure cultures was completed DNA extraction was carried out using ZOKEO DNA extraction Kit. Extracted DNA has been sealed with paraffin and preserved at -50°C for further analysis such as genome sequencing. Gel electrophoresis results of DNA extractions are following.

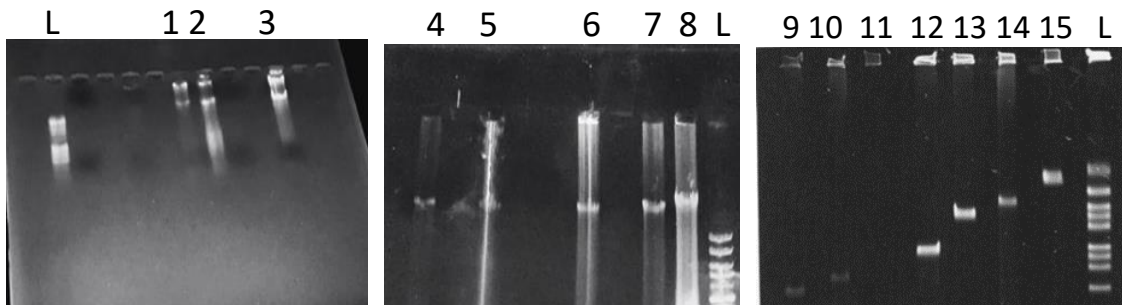


Figure 4.11 Gel electrophoresis results

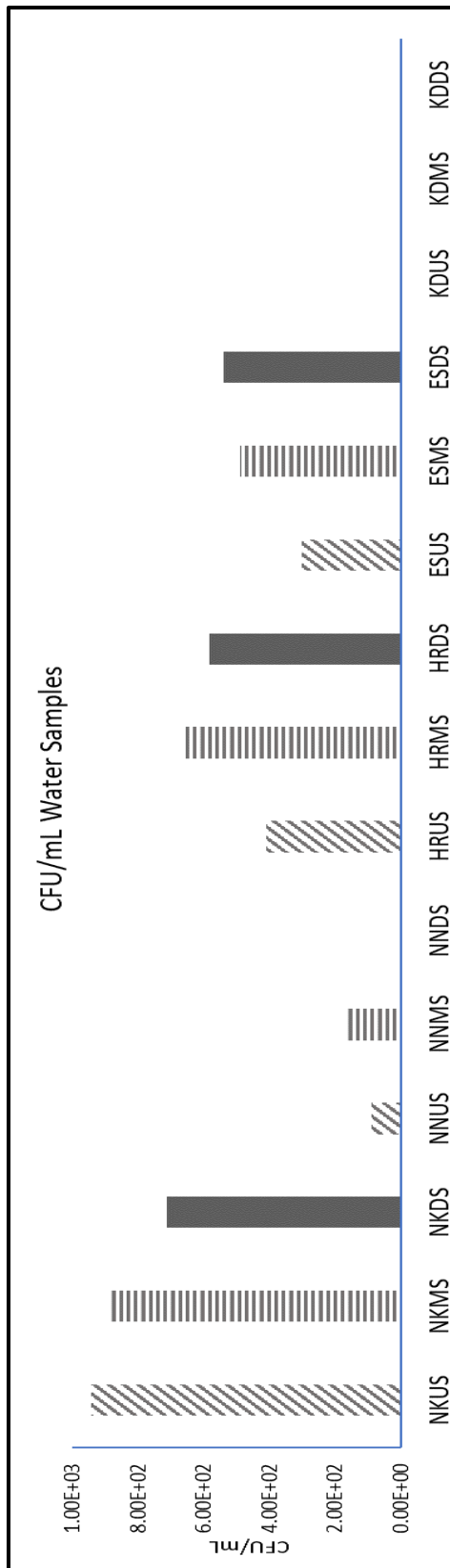


Figure 4.9 Graphical Illustration of CFU/mL count water samples

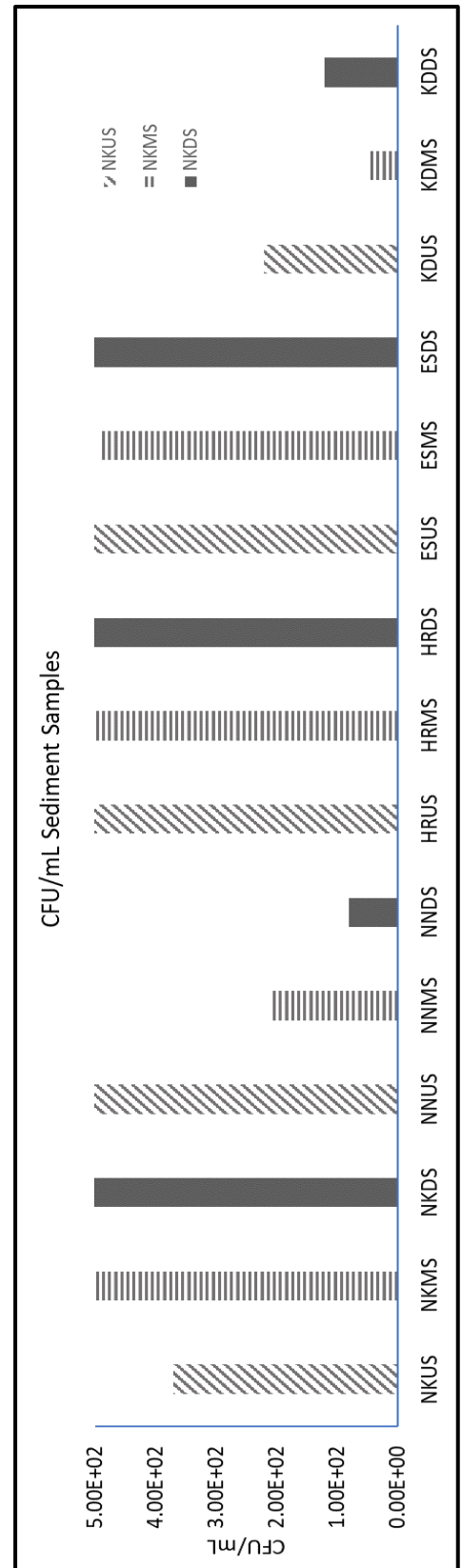


Figure 4.10 Graphical Illustration of CFU/mL count sediment samples

4.4 Physicochemical Analysis

Similarly, 12 physicochemical parameters were analyzed for all the 5 sites, samples were obtained in triplicates and their mean values were calculated. Complete table of results is attached in Annexure B.

4.5 Heavy Metal Analysis

Heavy metal analysis was carried out, based on the literature pollutants of concern 4 heavy metals were shortlisted for testing (Cadmium, Arsenic, Lead and Chromium).

4.6 Statistical Analysis

For statistical analysis Pearson Correlation was found on RStudio software and pollution-based risk assessment was carried out to further authenticate the results.

4.7 Physicochemical Profile of Sampling Stations

4.7.1 Nitrite-Nitrogen

Nitrite is an intermediate in oxidation of ammonia to nitrate. Increased nitrite concentration is often attributed to sewage because of its high ammonia concentration. Nitrite must be assessed within the first 24 hours of sampling due to its unstable nature. All samples were well within the WHO permissible limit as shown in Figure 4.12.

4.7.2 Nitrate Nitrogen

All sampling sites showed positive nitrate values but well below WHO permissible limits. Exit Stream showed highest value which is attributed to open defecation and animal waste at Mid and Downstream. Nitrate values of all sampling sites are shown in Figure 4.13.

4.7.3 Total Kjeldahl's Nitrogen

TKN is a sum of measure of ammonia and total organic nitrogen. Water samples at Haro River, Exit stream and Nilan Nala downstream exceeded the WHO permissible limits. Highest value measured was 53.2 mg/L at Exit Stream site as shown in Figure 4.14.

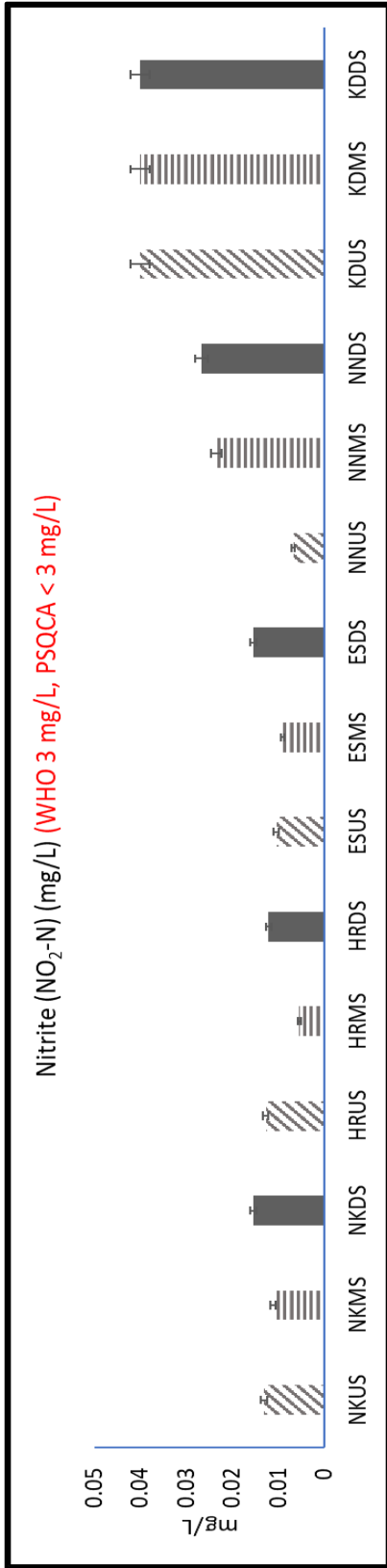


Figure 4.12 Graphical Illustration of Nitrite-Nitrogen

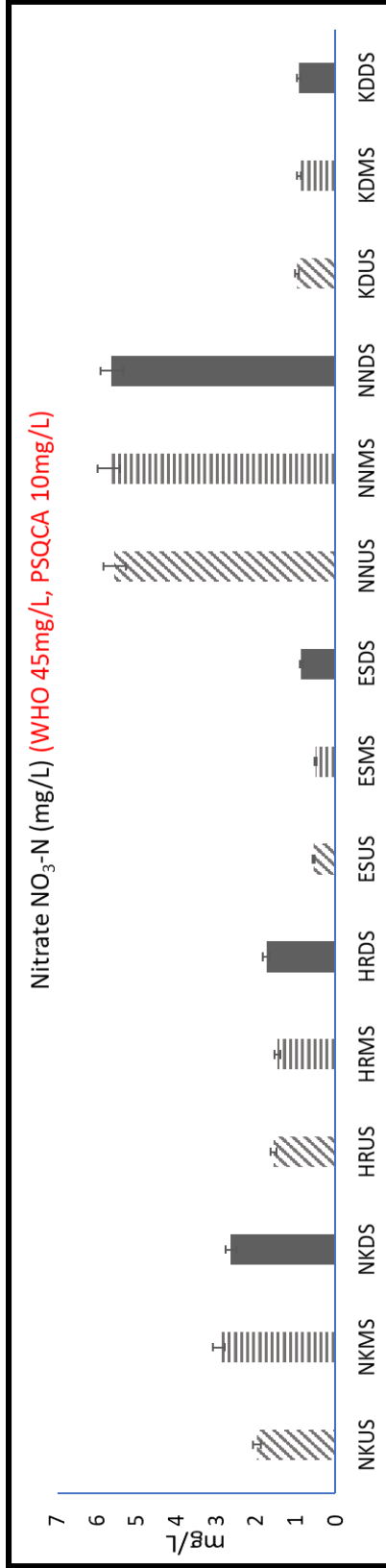


Figure 4.13 Graphical Illustration of Nitrate-Nitrogen

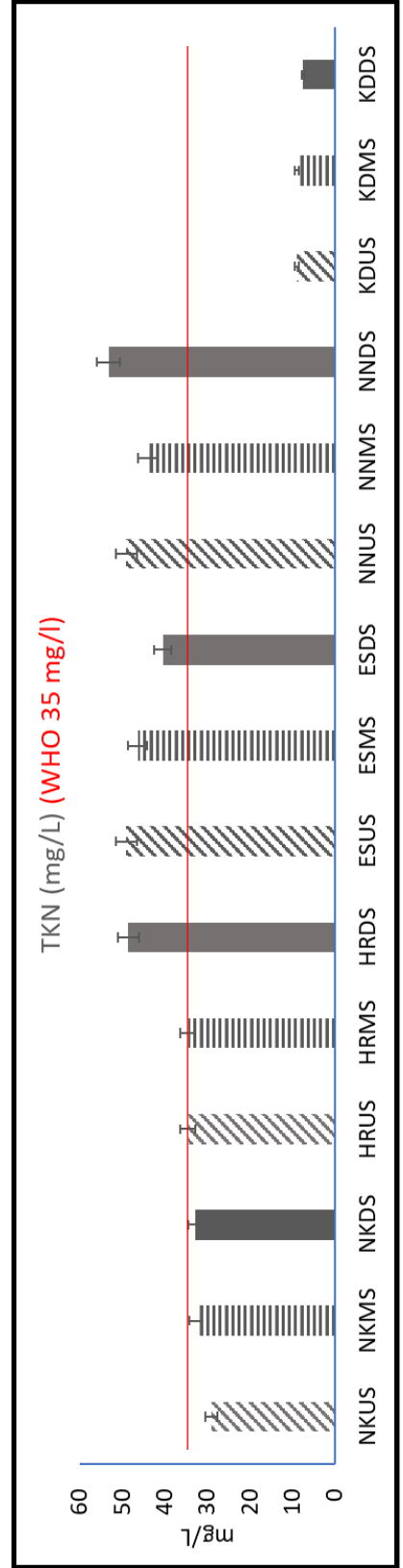


Figure 4.14 Graphical Illustration of Total Kjeldahl's Nitrogen

4.7.4 Total Dissolved Solids (TDS)

TDS is directly proportional to EC. All samples were found out to be within the permissible limits as indicated in Figure 4.15.

4.7.5 Total Alkalinity

Total Alkalinity was found in very high concentrations at Nilan Kas due to anthropogenic activities such as detergent washing and car wash activities in the water body other than that it was observed within the WHO permissible limits for all sites as indicated in figure 4.16

4.7.6 Total Hardness

Total Hardness was exceeding WHO permissible limits in Nilan Nala and Khanpur Dam and it was on the borderline on the remaining sites, the reason could be domestic wastewater that may contain calcium, magnesium, and other cations from the cleaning agent, food residue and human waste as shown in figure 4.17. (Memon et al., 2016) reported similar results for total hardness in water samples from Indus River (High sediment load due to rocky surface & less vegetation).

4.7.7 Chemical Oxygen Demand

COD was well within the limits at all the sites Except for Exit stream and Haro River downstream as depicted in figure 4.18. COD has an inverse relation with DO indicating the organic loading in the water bodies.

4.7.8 Phosphate Phosphorus

Phosphate Phosphorus was observed alarmingly in high concentrations due to presence of organic matter in water bodies, chicken and dairy farm farms have been noticed along the Haro River and Nilan Kas which contribute to higher concentrations of Phosphate phosphorus as shown in the figure 4.19.

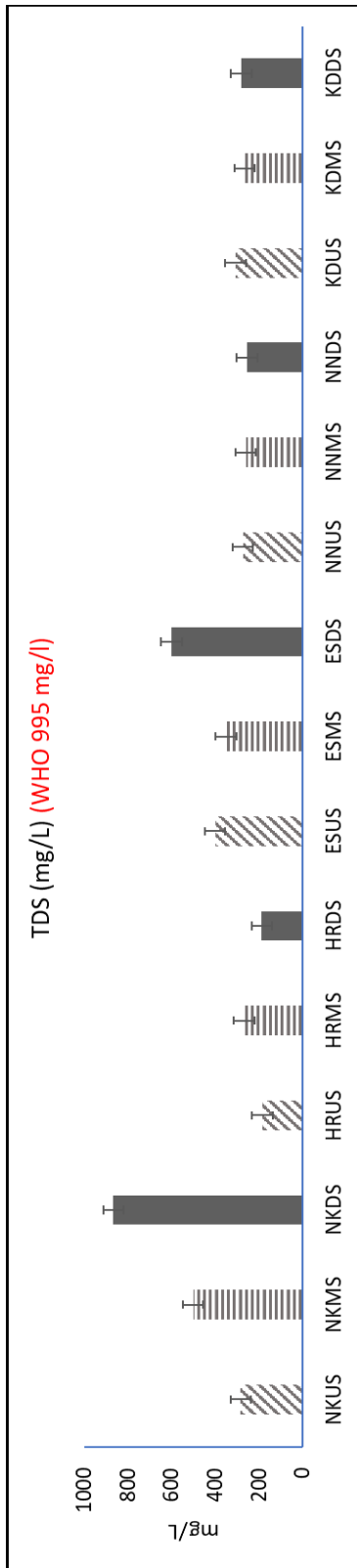


Figure 4.15 Graphical Illustration of total dissolved solids

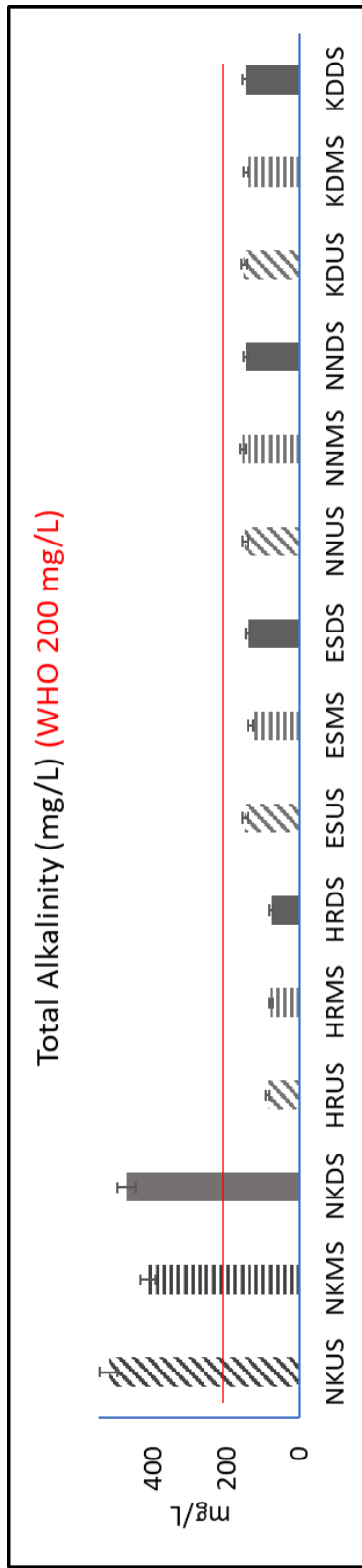


Figure 4.16 Graphical Illustration of total alkalinity

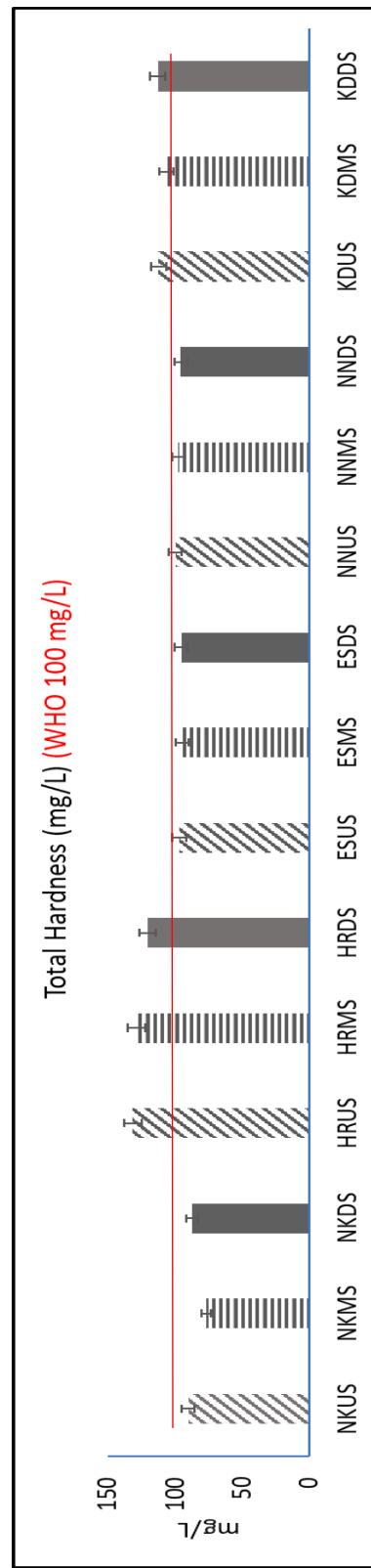


Figure 4.17 Graphical Illustration of total hardness

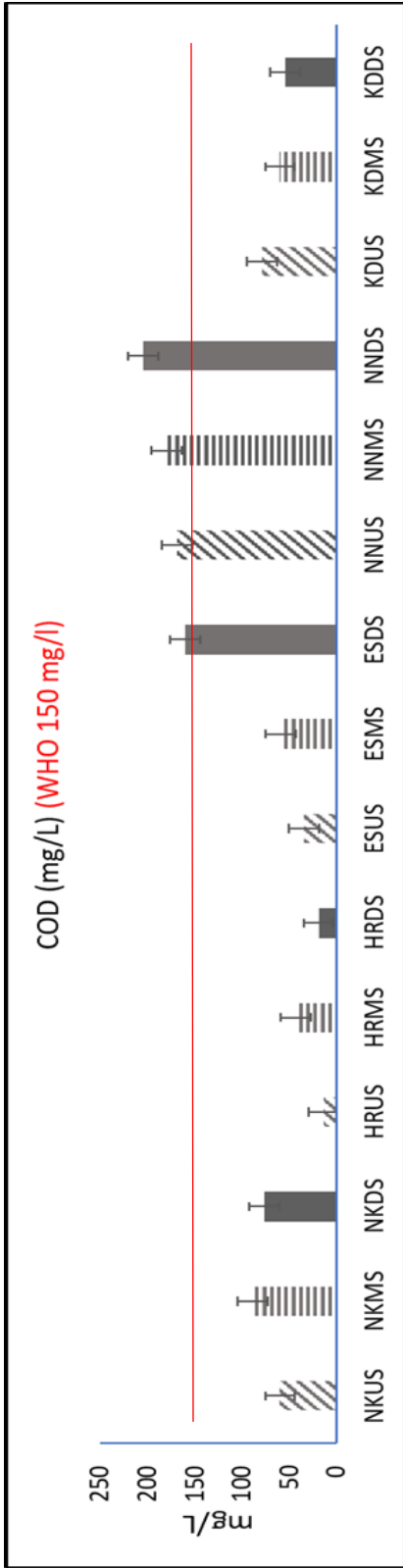


Figure 4.18 Graphical Illustration of chemical oxygen demand

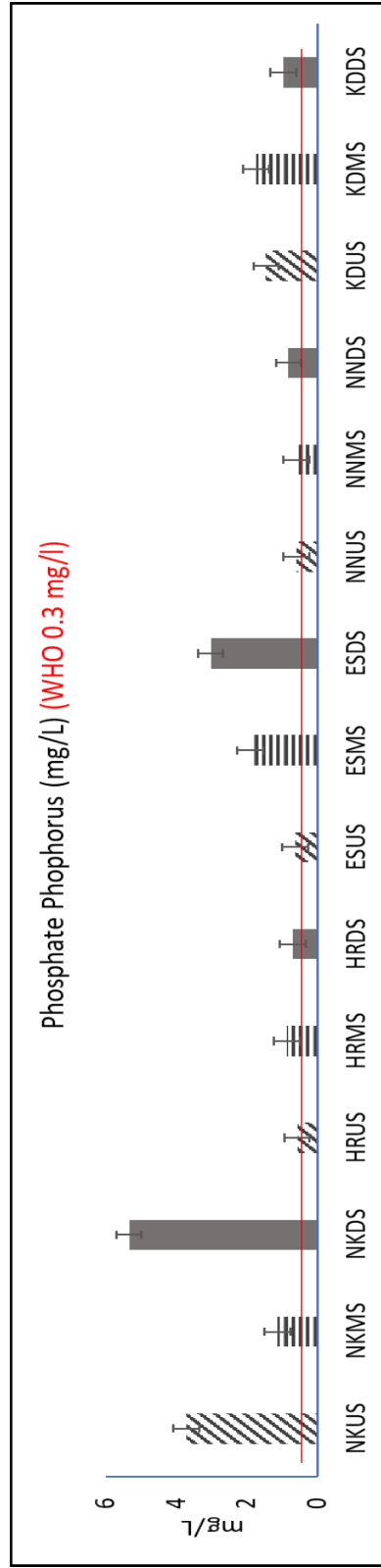


Figure 4.19 Graphical Illustration of phosphate-phosphorus

4.8 Heavy Metal Analysis

4.8.1 Cadmium

The results showed that Cadmium was below the permissible limits in Nilan Nala and Haro River however it was found in alarmingly higher concentrations in Khanpur Dam Nilan Kas and Exit Stream. major industrial releases of cadmium are due to waste streams and leaching of landfills, and from a variety of operations i.e., it can also add up from the car wash facilities present nearby the waterbodies. The graphical illustration can be seen in figure 4.20.

In a similar study (Astatkie et al., 2022) found out ‘toxic’ contamination and ‘severe’ ecological risk due to Cadmium in Dolo Stream, Ethiopia.

4.8.2 Arsenic

Similarly Arsenic was also observed in large quantities in most of the sediment samples Leaching from Topsoil and rocks is the most important natural source. Arsenic values observed in Khanpur dam region can be seen in the figure 4.21. (Jabeen *et al.*, 2011) observed Arsenic in alarming quantities in River Ravi in a similar study.

4.8.3 Lead

Lead was within permissible limits, but it was present in higher quantities in some parts of Nilan Nala, most probably due to vehicular emissions as depicted in figure 4.22.

4.8.4 Chromium

Chromium was found out in permissible limits however higher concentration levels for were observed at all sites with highest value at Nilan Nala and Haro river which can be observed in figure 4.23. Electroplating, leather tanning, and textile industries release relatively large amounts of chromium. (Iqbal and Shah., 2014) found out chromium within permissible limits in Khanpur Dam, however other trace elements were found in higher concentrations.

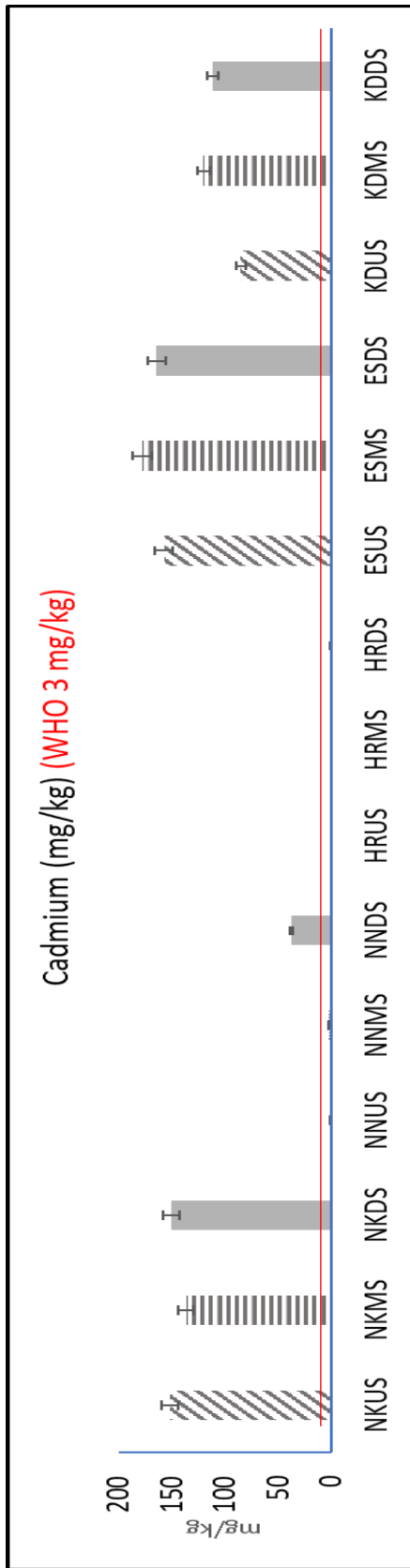


Figure 4.20 Graphical Illustration of cadmium

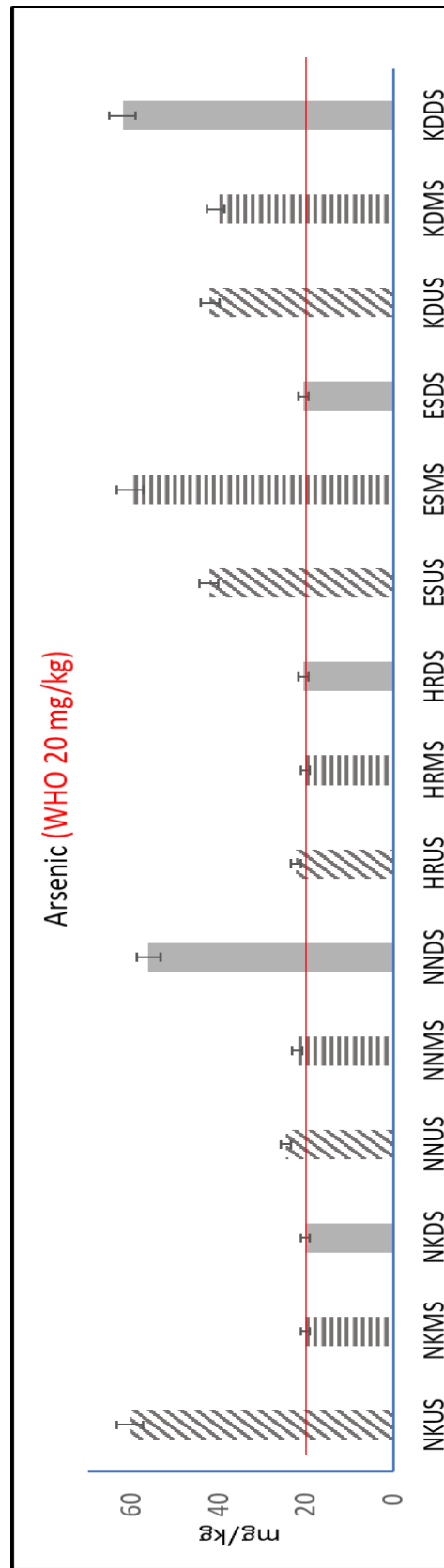


Figure 4.21 Graphical Illustration of arsenic

4.9 Statistical Analysis

4.9.1 Pearson Correlation of physicochemical and heavy metal profile

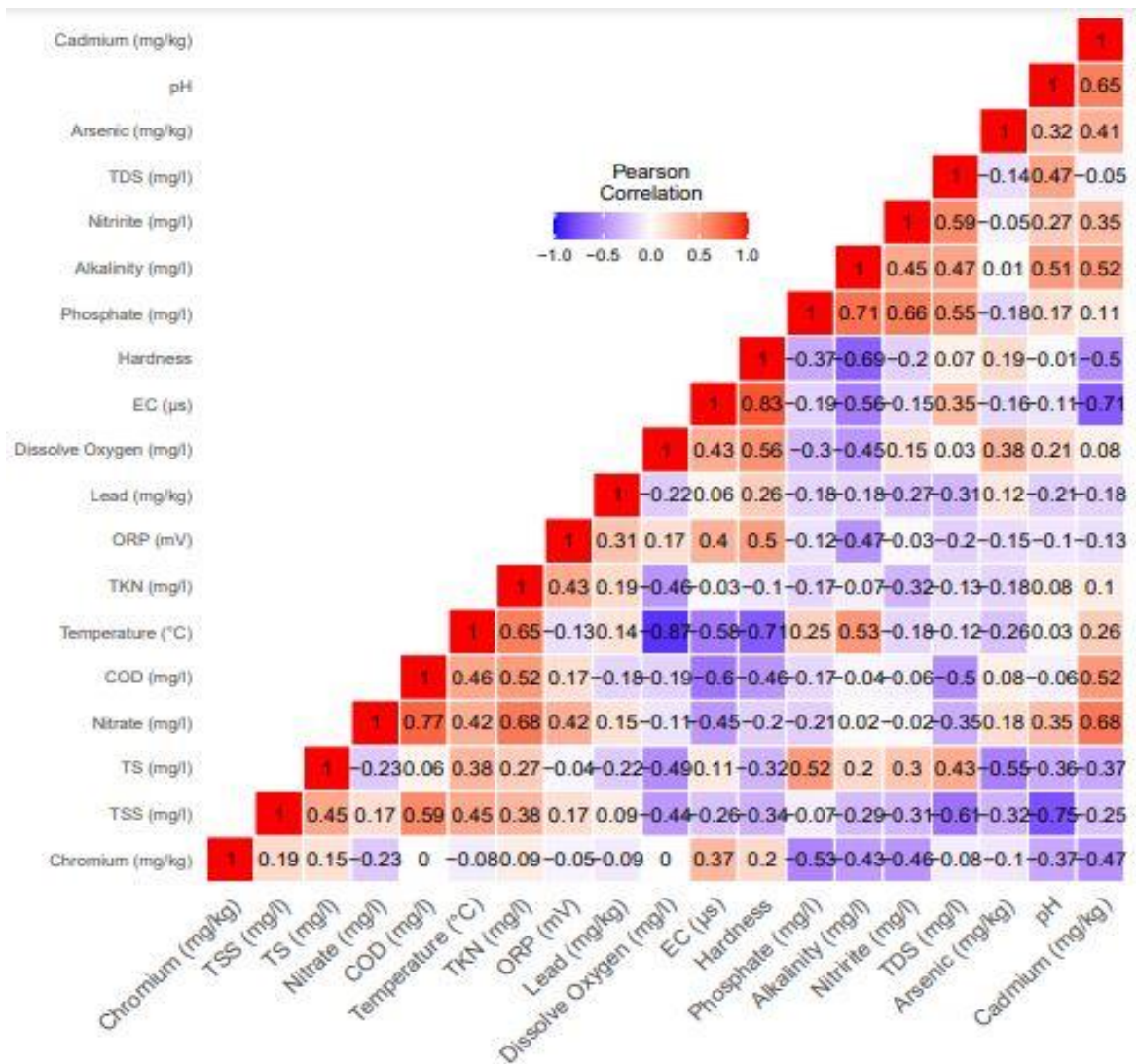


Figure 4.24 Pearson Correlation

P-value- 2.2e-16 or $p < 0.001$ the p value would indicate a significant result.

Correlation among physicochemical parameters and heavy metals

- TKN showed significant linear relation with COD because both of these parameters indicate intrusion of organic matter.
- pH depicted an inverse significant relationship with total suspended solids, and EC. DO show significant inverse relation with temperature, COD and direct relation with turbidity as increased turbidity reduces sunlight penetration thus reducing temperature and increasing DO, DO correlated positively with nitrite & inversely with COD, Alkalinity and Hardness
- Temperature also shows an inverse significant correlation with DO & total hardness, COD showed a positive correlation with TKN.
- Due to discharge of agricultural and domestic waste, a significant correlation was observed between heavy metals. Cadmium showed positive linear correlation with Arsenic. However, it showed negative correlation with Lead and Chromium.
- Chromium showed strong inverse relationship with phosphate-phosphorus. Cadmium depicted a positive correlation with Nitrate whereas lead showed inverse relationship with total dissolved solids as depicted in Table 4.24.

4.10 Pollution based risk assessment by ARCGIS pro

The maps below include the Parameters which are Exceeding WHO Limits in majority of the sampling stations.

4.10.1 Total Kjeldahl's Nitrogen

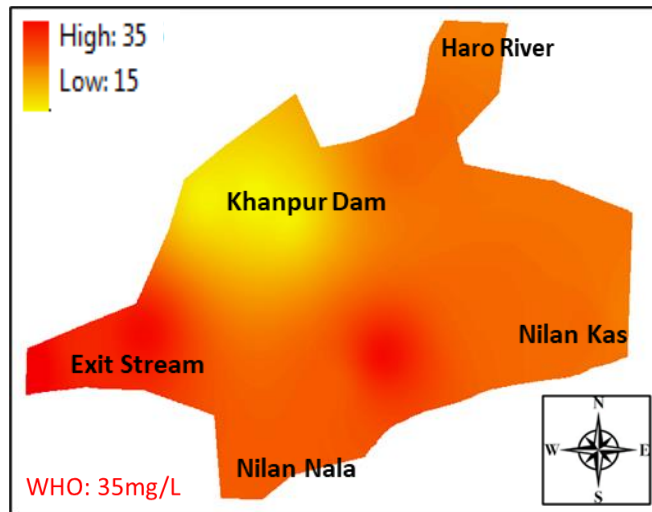


Figure 4.25 Pollution based Risk Assessment total Kjeldahl's nitrogen

The image above indicates severe pollution of Total Kjeldahl's Nitrogen in all the sites ranging from (32-53 mg/L). It is a measure of both ammonia and organics form of nitrogen. Common sources of ammonia/ammonium include human and animal wastes, as well as certain fertilizers and industrial wastes. This image explains how wide this pollutant has spread along the area of Khanpur dam and its tributaries.

4.10.2 Phosphate Phosphorus

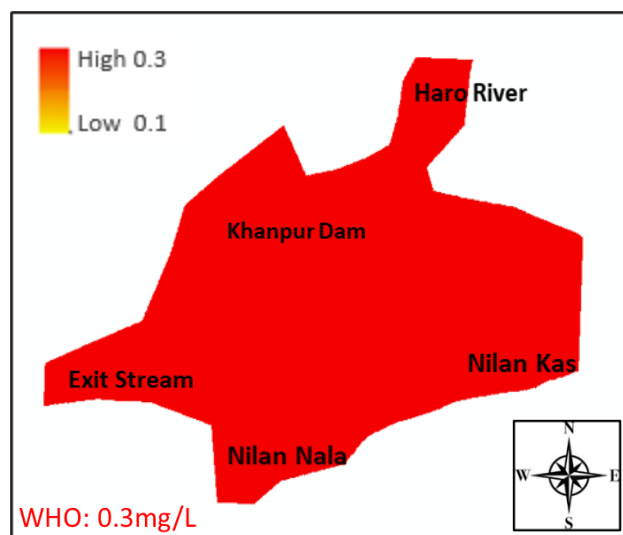


Figure 4.26: Pollution based Risk Assessment Phosphate-Phosphorus

Phosphate Phosphorus was observed ranging (0.5-5.3 mg/L) which is an extremely high concentration, WHO permissible limits were exceeded in all the sites.

4.10.3 Cadmium

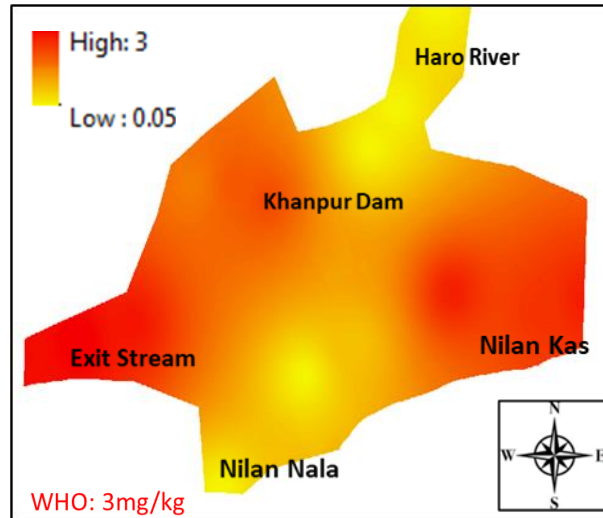


Figure 4.27 Pollution based Risk Assessment Cadmium

Cadmium had been observed in high concentrations in majority of the sites especially in Nilan Kas, Exit Stream and some parts of the Khanpur dam ranging from (80-160mg/kg).

4.10.4 Arsenic

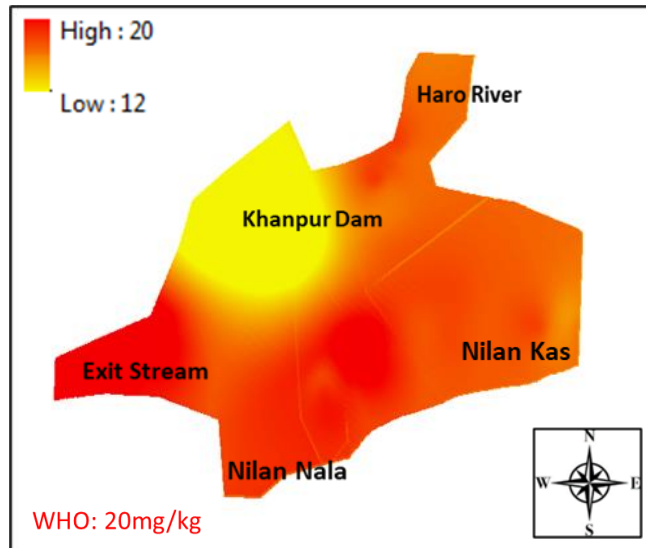


Figure 4.28 Pollution based Risk Assessment Arsenic

Similarly, Arsenic has also been found in alarmingly large quantities in all the sites except for Khanpur Dam ranging from **(20-60 mg/kg)**.

Chapter # 5

5. Conclusions and Recommendations**5.1 Conclusions**

High Total Hardness (70-130 mg/L), Phosphate Phosphorus (0.5-5.3 mg/L), TKN values (32-53 mg/L) were observed at Exit stream, Nilan nala and Nilan kas indicate wastewater intrusion and presence of organic matter. However, Microbial contamination (Gram Negative bacteria) has been observed in all the samples except for Khanpur Dam indicates the intrusion of sewage sludge including fecal contamination rendering it unfit for human consumption and plays a significant role in spreading gastroenteric diseases. Similarly, Heavy metals such as Arsenic (20-60 mg/kg) and Cadmium (80-160mg/kg) were detected in higher concentrations in the soil/sediment samples of many sites possessing a serious concern in health issues for the people consuming water from these sources.

5.2 Recommendations

- i. Localities must be informed and educated about heavy metal contamination in the water bodies to avoid further deterioration of health.
- ii. Car Wash stations and Poultry Farms must be regulated vigorously and must comply with proper sewerage systems.
- iii. Tourism department must impose strict actions against illegal waste dumping and effluent discharges in the water bodies.
- iv. Environmental survey of predominant Antibiotic Resistant Bacteria genes, and their genomic context in Khanpur dam along with its tributaries should be carried out in future research.
- v. Furthermore, Estimation of transmission of ABR organisms and genes in Khanpur dam must also be investigated

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Annexures

Annexure-A, Colony Morphology Table based on appearance of microbial species observed.

Specie Code	Color	Opacity	Size	Texture
Specie A	Light Pink	Opaque	Medium	Dry
Specie B	Yellow	Transluscent	Medium	Sticky
Specie C	Red	Opaque	Small	Dry
Specie D	Dense Pink	Opaque	Medium	Matte
Specie E	Off-White	Opaque	Large	Matte
Specie F	Colorless	Transparent	Small	Sticky
Specie G	Light Pink (Dense Centre)	Transluscent	Medium	Sticky
Specie H	Light Pink (Convex)	Opaque	Small	Dry

Annexure B, Mean values of all the physicochemical parameters

Sampling Sites	pH	Temp (°C)	D.O. (mg/l)	ORP (mV)	EC (µs)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphate (mg/l)	TKN (mg/l)	Hardness (mg/l)	COD (mg/l)	TDS (mg/l)	TSS (mg/l)	TS (mg/l)	Alkalinity (mg/l)
Nilan Kas Upstream	8.1	33.4	7.8	-31	276	1.85	0.013	3.71	28.9	90	59.7	283.3	50	333	523
Nilan Kas Midstream	8	33	8.1	-41	316	1.65	0.011	1.12	32.6	76.7	88.4	533.3	51.7	585	416.7
Nilan Kas Downstream	7.93	29.3	8.2	-24	361	2.6	0.105	5.33	32.9	86.7	76.7	866.6	50	916.6	506
Nilan Nala Upstream	7.15	21.2	9.3	-15	590	1.55	0.01	1.72	34.5	131.3	9.3	416.7	33.3	450	86.7
Nilan Nala Mid-Stream	7.63	21	9.1	-21	571	1.45	0.011	0.83	34.7	128.6	49.4	386.7	50	436.7	78
Nilan Nala Downstream	7.38	21.1	9.9	-22	628	1.73	0.013	0.7	48.5	123.3	67.2	576.7	80	656.7	77.3
Exit Stream Upstream	7.42	30.3	9.4	-13	376	5.56	0.01	0.59	49	99.7	154.5	26.6	380	406.7	150
Exit Stream Midstream	7.16	29.8	8.3	-29	211	5.7	0.02	0.59	43.9	97.3	181.1	33.3	346.6	380	156.7
Exit Stream Downstream	7.39	29.1	9	-13	342	5.63	0.02	0.81	53.2	95.7	158.4	33.3	366.6	400	146.7
Haro River Upstream	5.7	28.3	7.4	-27	396	0.54	0.01	0.63	28.9	96.76	44.7	88.3	400	566.7	150
Haro River Midstream	5.73	28.4	8.5	-30	410	0.5	0.01	1.91	32.7	94.3	88.3	150	566.7	733.7	133.3
Haro River Downstream	5.84	28.2	8.1	-22	416	0.85	0.02	3.02	32.9	95.3	132.4	200	716.7	916.7	141.7
Khanpur Dam Upstream	6.74	14.8	10.2	-21	433	0.96	0.04	1.45	8.87	112	78.6	306.7	40	346.7	152.7
Khanpur Dam Midstream	7.47	14.1	11.4	-31	425	0.91	0.04	1.74	8.87	106	59.7	266.7	40	306.7	147.3
Khanpur Dam Downstream	7.37	14.3	10.7	-29	421	0.91	0.04	0.97	7.47	112.7	54.3	280	33.3	313.3	148