EFFECT OF AQUATIC POLLUTANTS ON *CLARIAS BATRACHUS* (LINNAEUS, 1758) INHABITING DIFFERENT WATER BODIES OF VARANASI, INDIA

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

Zoology

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2026

CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**Effect of Aquatic pollutants on *Clarias batrachus* (Linnaeus, 1758) inhabiting different water bodies of Varanasi, India**" submitted in fulfillment of the requirement for the award of degree of Doctor of Philosophy (Ph.D.) in the Zoology Department, is a research work carried out by **Shivani Singh**, 11919648, is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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DECLARATION

I, hereby declare that the presented work in the thesis entitled "Effect of Aquatic pollutants on *Clarias batrachus* (Linnaeus, 1758) inhabiting different water bodies of Varanasi, India" in fulfillment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of Dr. Neeta Raj Sharma, working as Professor, in the School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India. In keeping with general practice of reporting scientific observations due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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List of Abbreviation

AAS	Atomic Absorption Spectrometer
APHA	American Public Health Association
As	Arsenic
Avg	Average
BIS	Bureau of Indian Standards
BOD	Biological Oxygen Demand,
CA	Cluster Analysis
Cl^-	Chloride
COD	Chemical oxygen Demand
CPCB	Central pollution control board
DO	Dissolved Oxygen
EC	Electrical Conductivity
FC	Faecal coliform
Max	Maximum
mg/l	Milligram per liter
Min	Minimum
ml	Milliliter
NO_3^-	Nitrate
NTU	National Turbidity Units
^{O}C	Degree Centigrade
OE	Degree East
ON	Degree North
Pb	Lead
PCA	Principal Component Analysis
PO_4^{3-}	Phosphate
POM	post-monsoon
PRM	Pre monsoon

SD	Standard Deviation
$SO4^{2-}$	Sulfate
T	Temperature
TDS	Total Dissolve Solid
TURB	Turbidity
WHO	World Health Organization
μg/l	Microgram per liter

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ABSTRACT

Background-

Clarias batrachus also recognized as Mangur or Catfish is a native of Asian continent and is one of the delicious foods consumed by human being due to its high nutritional value. It is omnivore and is active mainly at night and feeds on things such as insect larvae, fish eggs, fish and sometimes plant materials.

It is found all over the Asian countries including India, Myanmar, Sri lanka, Malaya, Archipelago, from Eocene to recent. It is of medicinal importance, and it recommended by most physicians in various diseases. *Clarias batrachus* is known as cat fish due to its cat-like whiskers or barbells present on either side of the mouth. It is generally found in natural ponds, pools, ditches, shallow water resources and behave like scavengers. It breeds during rainy season and can survive in very low oxygen content of water. This species is mostly found in fresh and brackish water. Morphologically, the body is divided into head trunk and tail regions.

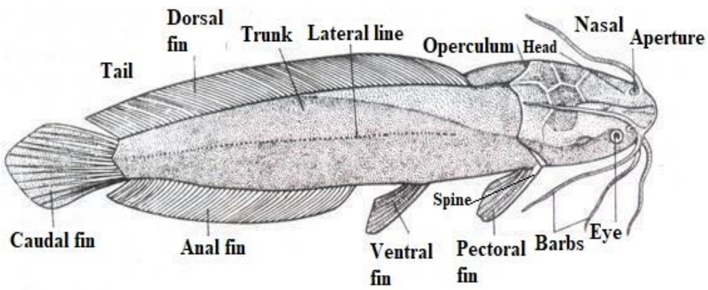


Figure. 1.1: Graphical representation of body parts of *Clarias batrachus*

Aim and Objectives-

The present study aims to analyze the physicochemical parameters of different water bodies containing *Calrias batrachus* fish. The parameters will help to determine the factors responsible for the depleting population of the fish. Morphometric and Histopathological variations in the fish during different seasons will provide detailed study of internal/external body parts and changes taking place due to environmental factors.

1. Impact of aquatic pollution in morphometry, histopathology and biochemical profiling in *Clarias batrachus*.

2.Comparative analysis of morphometry and histopathology studies of *Clarias batrachus* in different water bodies under investigation.

3.To correlate with the aquatic pollutants with the above mentioned parameters.

Materials And Methods-

All the materials and methods will follow the national and international standards/regulations of National Fisheries Development Board (NFDB), International organization for standardization (ISO), FAO (Food and agricultural organization), and American public health association (APHA).

Collection of water-

The water will be collected in January (resting), April (Late preparatory), July (early prespawning), and October (postspawning phases) months from the same sources and expose to physicochemical studies.

Physicochemical analysis-

This will be carried out prior to the experiment to quantify the concentrations of the metals and other parameters in the effluent. Double distilled water, borosil glass apparatus and analytical grade reagents will be used for preparation of all standards solutions. Physicochemical analysis is carried out according to international and national standard methods.

Collection of fish-

The healthy adult fish will be collected from several water bodies in different seasons. The fish will be cleaned using $KMnO_4$ solution to avoid any dermal infection. Fish will be collected, acclimatized, and grouped into control and experimental prior to exposure to heavy metals, pesticides, organic/inorganic pollutants and histopathological studies.

Morphometric analysis- The control and experimental fish will be exposed to the morphometric measurements i.e., total length, standard length, body weight, body width, body depth/height, body length, head length excluding snout, width of head, snout length, eye diameter, length of caudal fin, length of anal fin, length of pelvic fin, length of pectoral fin, height of caudal, pelvic and anal fin. The data will be used to calculate mean and standard deviation (SD) to obtain average value of all morphometric parameters. Moreover, condition factor, acute evaluation, chronic evaluation, and length weight relationship will be established using morphometric analysis.

Histopathological analysis-

Several statistical, taxonomical, Carcass, protein profiling, fatty acid profiling, and histopathological characterization methods will be employed. Stock solution of the chemicals will be prepared, used and all the tests will be repeated for reproducible results. The vital organs of interest including gills, liver, kidney and muscle tissues will be dissected out from both control and experimental fishes and will be immediately placed in Bouin's fixative (The Bouin's fixative consists of picric acid, formaldehyde and acetic acid. The process will be followed by dehydration, infiltration, impregnation, microtomy, embedding, stretching, and staining before being exposed to machines.

Result And Conclusion-The present study establishes a strong and consistent relationship between deteriorating aquatic environmental conditions and adverse biological responses in *Clarias batrachus*. Correlation analysis clearly demonstrated that variations in physicochemical parameters and pollutant concentrations were closely associated with changes in morphometric indices, tissue architecture and biochemical profiles of the fish. These findings provide compelling evidence that aquatic pollutants are the primary drivers of biological stress in the studied Pond ecosystems. Elevated levels of key physicochemical parameters, including electrical conductivity, total dissolved solids, biological oxygen demand and chemical oxygen demand, showed significant associations with morphometric impairment and structural damage in fish tissues. Reduced dissolved oxygen concentrations were strongly correlated with gill pathology, particularly epithelial lifting and lamellar fusion, indicating compromised respiratory efficiency under polluted conditions. Such correlations highlight the direct influence of organic pollution and oxygen depletion on fish health. The major findings of the study demonstrate that the ecological health of sacred Ponds in Varanasi is being adversely affected by a combination of organic pollution, trace metal contamination and seasonal variability. The integrated assessment of water quality parameters, biological responses of *Clarias batrachus* and multivariate statistical analysis provides robust scientific evidence of pollution-induced stress in these culturally significant freshwater ecosystems.

Chapter 1

INTRODUCTION

Humans have exploited water more than any other resource for the survival of life, despite it being an indestructible natural resource. Human activity, industrial pollution and careless sewage disposal are all contaminating this elixir of life. Dams serve as reservoirs for the majority of metropolitan areas water supplies. The detrimental effects of urbanization always fall on the dams. The careless discharge of sewage that has not been treated has poisoned the majority of the aquatic bodies. In India, big cities are located close to the dams. Runoff from industrial and agricultural areas makes rivers unsafe for consumption by humans. Water conditions are declining worldwide due to the growing population and human activities. Water quality data shows the amounts of different solutes in a specific location and time, helping to assess if the water is suitable for use and to upgrade current specifications (Zidni, *et al.*, 2019).

As the population and number of companies have grown, development has resulted in several unfavourable changes to the environment. People's ignorance and poor management have disrupted the ecosystem of water bodies. New concepts in the field of aquatic ecosystem monitoring and evaluation have emerged due to new environmental issues. The physical, chemical and biological properties of the aquatic system, which vary with the season and level of pollution are what determine its overall health. Unscientific trash dumping and poor waste management have significantly degraded the quality of the water. Increasing human activity in and near water bodies degrades aquatic systems and, eventually, the properties, both chemical and physical of water (Tiwari, P., & Paul, N., 2023).

The amount of different chemicals entering water resources has grown because of the release of garbage from cities, businesses, and farms, which has significantly changed the physicochemical properties of those resources. Eutrophication is accelerated by nutrients such as nitrogen and phosphorus from fertilizers and household garbage (Zidni, *et al.*, 2019). Natural mechanisms that cause eutrophication include dust, storms, runoff and mineral weathering (Tanushri Deb Nath, *et al.*, 2014). Because they serve as setup for pisciculture and as replenishing reservoirs for drinking water and household usage, lentic water bodies are extremely important (Yousafzai, *et al.*, 2017). Water quality is evaluated by measuring its temperature, turbidity, dissolved oxygen, pH, chloride, and total alkalinity, among other important parameters (Tiwari, *et al.*, 2003).

Water bodies that are contaminated may experience a change in their trophic position and become inappropriate for aquaculture. Numerous biological or physicochemical elements may function as stressors and have a negative influence on fish development and reproduction. To assess the health of lakes related fish culture, continuous monitoring of physicochemical and biological water quality indicators is necessary (Yadav, *et al.*, 2013). Anthropogenic activities, including burning fossil fuels, incorrect agricultural methods, industrialization and rapid urbanization, have grown significantly and are thought to be crucial in altering the natural state of freshwater ecosystems (Wokoma, *et al.*, 2022). Stream and riverine ecosystems have been significantly influenced by human activities, which have changed the water quality and the function of aquatic biota. Around the world, streams and rivers are dealing with a variety of environmental issues that are mostly related to human activity in their catchment regions. Concerns about freshwater resource contamination are widespread, but they are particularly acute in emerging nations. High levels of aquatic pollution are particularly problematic in developing nations (Weher, *et al.* 2018), where wastewater is carelessly dumped into rivers and streams either directly or indirectly without taking precautions to protect the environment (Velmurugan, *et al.*, 2019).

Rivers are vital to the nation's growth and the survival of life, yet they are becoming poisoned because of quick urbanization, industry and other developmental activities (Alam, *et al.*, 2005). Most of the water used in Indian agriculture originates from surface sources such as rivers, reservoirs, dams, etc. (Velmurugan, *et al.*, 2019). Aquatic species composition, distribution and abundance are greatly influenced by the interplay between the physical and chemical attributes of water (Ullah, *et al.*, 2017). Examining the physical and chemical features of the water bodies is frequently how the level of pollution is determined (Thyagarajan, *et al.*, 2015). Recent years have seen an increase in interest in studies concerning the water contamination of the Yamuna and Godavari rivers (Tasneem, *et al.*, 2018).

Any river's biological communities are lost because of diminishing water quality. The biotic markers of water pollution in a tributary or stream include aquatic creatures. The physical and chemical changes in stream water, which either directly or indirectly impact the biological populations, can be recognized by biological markers. According to their presence or absence, relative abundance, community structure and function, biological indicators may be used to assess the ecological health of an aquatic environment (Thomas, et al., 2013).

According to Tantarpale, aquatic animals, including fish, amphibians, algae and invertebrates, are crucial for comprehending how human activity affects stream ecosystems.

Fish have a broad tolerance range and are excellent markers of pollution. Fish react to shifts in the aquatic ecosystem's physical, chemical and biological circumstances brought on by human activity (Tanee, *et al.*, 2013). Both regional interactions and other local physical features affect stream fish distribution (Sunday, *et al.*, 2003).

According to Talapatra, *et al.*, (2014), fish are susceptible to any kind of human disturbance, including stream discharge, municipal garbage, industrial effluent input, habitat modification and fragmentation. Compared to tolerant species, sensitive species provide a more accurate indication of the health of the stream. However, tolerant fish species can quickly recover when the stress level drops since they are acclimated to adverse situations brought about by man-made or natural sources. Fish communities organization and function are influenced by natural variables, such as famine and flood conditions. Flood links ponds and offers excellent circumstances for the fish migration obstacles, increasing the intensity of fish bodies in irregular streams (Sunar, *et al.*, 2016). The distribution and pattern of fish congregation are influenced by the physical features of the stream concerning both the quantity and quality of water (Ghosh, *et al.*, 2023). The physical attributes of water vary significantly across time and space in most temperate streams, which also affects the fish grouping (Talapatra, *et al.*, 2024). The species composition of ecological communities is greatly impacted by human activity in catchment areas, leading to the emergence of foreign fish species and the extinction of local fish species et. An aquatic ecosystem's functional environmental veracity is disrupted when species composition changes in a stream, transforming a stable and intricate environment into one that is brittle (Sivamanik, *et al.*, 2015).

The form, environment and biology of fishes are incredibly diverse. All levels of biological structure may be evaluated ecologically using fish, and methods for doing so are available across the environment, population, individual and organ levels (Shrivastava, *et al.*, 2022). Their diverse physical, ecological, and behavioral adaptations to their natural behavior, fish may also be employed as indicators throughout time spans ranging from minutes to decades (Kumari, *et al.*, 2025). To effectively analyze and monitor rivers, it is necessary to comprehend the geographic differences that are linked to fish assemblage, structure and functions. Fish are so significant because they provide insight into ecological processes and producer-consumer relationships.

Deforestation, wood harvesting, urbanization, riparian vegetation loss, agricultural growth, land drainage and flood prevention have all been shown to have increasing geographical, hydrological and biological effects on rivers. Nutrient intake into rivers and soil erosion is often the outcome of catchment area deforestation. Numerous fish take refuge in the coarse stream bottom, which is harmed by mining-induced demolition of riparian flora. High amounts of suspended silt in rivers and sediment deposition on the riverbed may influence the ecosystem's health. Around the world, numerous plant and animal species are decreasing at a startling pace due to landscape changes such as habitat modification, division and loss (Shivakumar, *et al.*, 2014).

The construction of dams across rivers is the main factor in flow control. The fact that so many rivers have been reduced to man-made lake cascades demonstrates the extent of flow controls worldwide. Lotic habitats are eliminated by the flow management, while lentic habitats are created (Dahanukar *et al.*, 2004).

In recent years, the idea of river basin interconnection has gained significance. Numerous domestic and international initiatives have already been implemented. Fish distribution patterns, however, may be disturbed by the severe consequences regarding water balance and quality, the development of parasites and illnesses and changes to ecological biotic sections. Other indigenous fish may be displaced when other fish locate new, ideal homes and thrive there. As a result, many native fish species may become rare. Several natural species may become extinct throughout this process (Abalaka, *et al.*, 2013).

Fish is considered one of the greatest sources of protein. Subsistence fishing in streams, rivers, and tanks has been a source of revenue for the most vulnerable elements of society.

Global fisheries produce close to 100 million tons of commercial catch annually. The world's fish population has suffered because of the enormous pace of removal from the aquatic environment. Freshwater resource sustainability has been endangered by human activities. Fishing has become more intensive due to the demand for fish and a rise in the number of fishermen. To increase harvests, fishermen are resorting to damaging fishing tactics. Fishing uses chemical and herbal toxins like copper sulphate, bleaching powder, and tree extracts damage the aquatic body's entire fish population. Another disruptive technique that makes it easy for fisherman to catch large numbers of fish is dynamic meeting. Both target and non-target fish in the region may suffer greatly from the use of dragnets, which remove all the fish population in each area, including youngsters (Dahanukar, *et al.*, 2004).

Toxic substances in pollutants can be selective, eliminating a few vulnerable species or changing the ecosystem such that some species are preferred while others are not, or they can be complete, killing all fish species present (Dahanukar, *et al.*, 2004).

Clarias batrachus also recognized as Mangur or Catfish is a native of Asian continent and is one of the delicious foods consumed by human being due to its high nutritional value. It is omnivore and is active mainly at night and feeds on things such as insect larvae, fish eggs, fish and sometimes plant materials.

It is found all over the Asian countries, including India, Myanmar, Sri Lanka, Malaya, and the Archipelago, from the Eocene to the present. It is of medicinal importance, and it is recommended by most physicians in various diseases, e.g. Tuberculosis (TB), etc. *Clarias batrachus* is known as a catfish due to its cat-like whiskers or barbells present on either side of the mouth. It is generally found in natural Ponds, pools, ditches, shallow water resources and behaves like a scavenger. It breeds during the rainy season and can survive in very low oxygen content of water. This species is mostly found in fresh and brackish water. Morphologically, the body is divided into head, trunk and tail regions as shown in Figure 1.1.

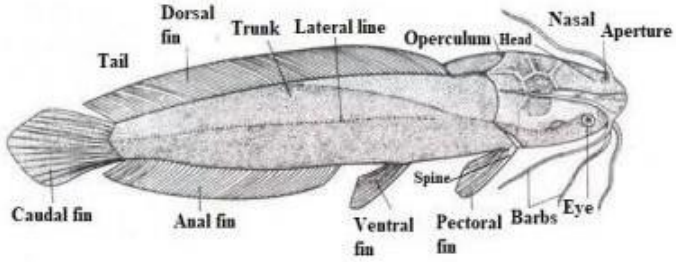


Figure 1.1: Graphical representation of body parts of *Clarias batrachus* (Courtesy: Google images).

Characteristically, this fish possesses a dorsal fin that extends from the operculum to the caudal fin. Anal fin is also found from the middle of the body to the caudal fin. Pectoral fin with spine and ventral fins are closely placed. The head is flat with four pairs of non-contractile sensory barbs. The head possesses reduced eyes and nostrils. The body of the fish is without scale, or naked skin. Dendritic accessory bronchial apparatus supplements gill respiration, and thus the fish can live for a significant period outside water. Spiracles are absent, the lateral line system is distinct, the tail is laterally compressed, diphycercal and has a rounded caudal fin. Weberian ossicles connecting the internal ear are present. An air bladder is also present, which is physostomous. This genus possesses an adjunct respiratory organ that supplements gill respiration. It can stay outside the water for a long time. It can travel more than half a kilometer on its paired fins and is therefore called a “walking fish” also. Due to the presence of four pairs of barbs, it is generally called a catfish. The pectoral fin of this fish possesses a pungent spine. With the help of this spine, it can attack the fingers of human beings if not conscious.

The body of *Clarias batrachus* is elongated, tapering toward the tail from a wider head. The typical coloured variation has a pale underside and a greyish to olive body. Other types include a pink variant with normal-coloured eyes and an albino with reddish eyes and a white body. The walking catfish reaches a length of around 0.5 meters (1.6 feet) and a weight of about 1.2 kilograms (2.6 pounds). The catfish belongs to the phylum Chordata, group Craniate, subphylum Vertebrata, division Gnathostomata, super class Pisces, class Osteichthyes, subclass Actinopterygii, super order Teleostei, order Cypriniformes, genus *Clarias*, species *batrachus*. Although, there are several catfish as shown in Figure 1.2 but they do not share the same importance as of *Clarias batrachus*.

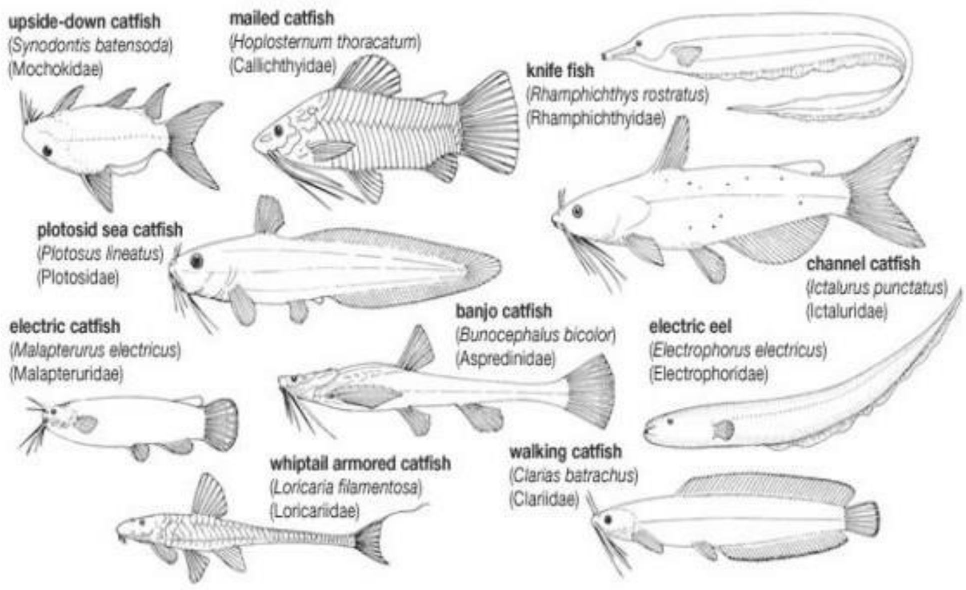


Fig. 1.2: Different types of catfish found in water bodies (Courtesy: Google images)

The natural occurring stock of a certain fish in a certain area of space behaving as a biological unit is limited. In other words, it can be defined as population size per unit of space. The abundance of population is the success of that species. The characteristics developed in fish depending on population and density are of utmost importance. The estimation of population is either done by direct count method or indirect methods. The population dynamics is assessed by the fluctuation in population abundance. These fluctuations are caused by natural factors, apart from those caused by human activities. This population dynamic is considered to reflect the biological features of a given fish species and its adaptation in the phase of altering ecology. Obviously, survival reflects adoptability and mortality, the adversity of ecology. The population dynamics include natality and mortality versus mortality, interspecific interactions in food chain, interspecific interaction on the spawning grounds, interspecific interactions that is competition, behavioral response to density of stock and physical parameters like temperature, space, salinity, dissolved oxygen, free CO_2, pH, calcium carbonate and specially pollution (Shivakumar, *et al.*, 2014).

The biochemical constituent of the fish shows variations from one species to another, in different seasons, depending on the age, size, growth etc. The significant constituents contain food, water, fat and minerals. Good fish flesh contains 60 to 84 percent water, 15 to 24 percent protein and 0.1 to 2 percent fat. *Clarias batrachus* is rich in 18 amino acids, saturated fat, monounsaturated fat and polyunsaturated (Omega 3) fat. It also contains Vitamins A, D, E, K and several minerals (Calcium, Phosphorus, Sodium, Potassium, Iron, Manganese, Selenium and Zinc). To promote optimum production of *Clarias batrachus* culture in the light of economic importance is one of the major factors to investigate the relevant causes hampering fish population (A.P.Mukharjee,1986).

The present study aims to analyze the physicochemical parameters of different water bodies containing *Clarias batrachus* fish. The parameters will help to determine the factors responsible for the depleting population of the fish. Morphometric and Histopathological variations in the fish during different seasons will provide detailed study of internal/external body parts and changes taking place due to environmental factors. *Clarias batrachus* (Linn) fish, commonly known as "mangur," is of medicinal importance and because of its delicious protein covers a large feedstock. *Clarias batrachus* is rich in polyunsaturated fatty acids (PUFA) or Omega-3 fatty acids that serve important roles in cardiovascular system to decrease the risk of heart attack. The fish's protein may be fragmented down into amino acids, that is responsible for the growth and repair of body tissue. Liver oil of the fish is an excellent source of vitamins A and D. Despite the exceptional nutritional value, the population of this fish is decreasing day by day due to (a) pollution, (b) human population, (c) mismanagement, (d) decreasing reservoirs and (e) low rainfall. Improvements to fish culture, causes of low fish populations, and the impact of industrial and agricultural waste will be examined through this work.

1.1 Research Objectives

The objectives of the research are:

- To analyze the impact of aquatic pollution in morphometry and histopathology profiling in *Clarias batrachus*.
- To perform a comparative analysis of morphometry and histopathology studies of *Clarias batrachus* in different water bodies under investigation.
- To correlate with the aquatic pollutants with the above-mentioned parameters.

1.2 Significance of Study

This research is significant to know whether different natural habitats have varying distribution of this species. In case of such variations the probable reason/s could be ascertained. The outcome of the research is beneficial to local farmers, contractors, sick people and drug companies due to its nutritional and medicinal values. It will not only help to improve the health of people but will also boost the economy.

Effect of Physico-chemical factors in a water body including temperature, pH, salinity, biological oxygen demand (BOD) and hardness, will provide information about the changes occurring in various water bodies. The population dynamics and analysis help in the increase of population density in different areas.

The use of *Clarias batrachus* in the interdisciplinary areas provides a cheap, easily available, non-toxic and environmentally benign alternative to other existing resources. Genetic studies provide the relevant data accounting for the changes in the morphology of the fish.

The protein found in the fish can be explored further with biological and chemical studies through extraction. For cow's milk, the protein is 20 per cent whey and 80 per cent casein. The nutrition is 60 per cent whey and 40 per cent casein in human milk. The protein fraction of whey makes up around 10 per cent of the total dried solids of whey. This protein is typically a mixture of beta-lactoglobulin (~65%), alpha-lactalbumin (~25%), bovine serum albumin (~8%) and immunoglobulins. These are soluble in their native forms, independent of pH. Now, the cost of fish protein is significantly less than that of whey protein supplied by national and international brands. So, this could be commercially exploited and used in bodybuilding and fitness industry. The fins are used to make the soup delicious in many countries, so more commercial usage can be exploited. The hormones found in the fish can be used to investigate the reason for variations in physiological parameters.

The pungent attack produced by the spine is another thrust area to examine. The origin of production is with the muscles involved for to and from movement that can cut human fingers. Due to this spine, it can walk up to certain kilometers, and the detailed study could provide some alternative medicine to improve muscle atrophy.

The mucus secretion from *Clarias batrachus* is another interesting phenomenon. Detailed report regarding this mucus is not available in present literature. Some studies have been done on the mucus for its antibacterial properties. So, this is good against bacteria and fungi. It can be used on the wounds to help them heal quickly.

The outcome of the research work provides details of the natural habitats with altering distribution of the species. Morphometric analysis would help to compare the variations among the fishes from different geographical areas. Also, it would help to analyze the normal distribution pattern with deviated patterns. The protein extracted from the fish would be a cheap solution to the existing proteins in the countries and areas where the fish is not available. The interdisciplinary work can establish a breakthrough with many products that can be used as eco-friendly substitutes of the existing products in different areas. All the products could be commercialized further as no work has been reported on them so far.

Chapter 2

REVIEW OF LITERATURE

J. Selvanathan (2012) established that the discharge of heavy metals into aquatic environments is a major cause of water pollution concerns. Heavy metals are known to be poisonous, to last for long periods of time and to have the tendency to bioaccumulation. The freshwater fish *Clarias batrachus* was the subject of an experiment that was carried out within the research facility to investigate the effect that exposure to heavy metals like mercury and cadmium had on the fish. Over the course of 30 days, fish that weighed 10 0.5 g and were 10 1 cm in length were given sublethal doses of mercury at 0.19, 0.09, 0.05 and 0.03 ppm and sublethal doses of cadmium chloride at 0.66, 0.30, 0.17 and 0.12 ppm. The fish were exposed to these levels of mercury and cadmium chloride. Experiments on acute toxicity were used as a basis for the calculation of these values. During this historical period, fish were fed food that had been created in a laboratory. This continued till the present day. Fish were taken from the tank, slain and tissues from their gills and livers were harvested on the tenth, twenty and thirty-first day of the experiment, respectively. The autopsy took a little over three and a half minutes to complete in its entirety. The results indicated that the extent of deformation in the gills and liver correlated with the duration of metal exposure. The concentration of the metals was found to vary with the dosage and the period. In addition, the results proved that the concentration of the metals was time-dependent.

Shephard, *et al.*, (2020) reported that our natural surroundings are degrading at an alarming rate and we are losing more of our ecosystems daily as a direct consequence of an increase in environmental pollution brought on by a range of human activities. This is a direct effect of the rise in environmental pollution brought on by human activities. It is common practice to discharge industrial effluents into bodies of water, even though these effluents might be dangerous since they include heavy metals, herbicides, and other substances. Any pollutant that is discharged into water will change the surface tension, thermal properties, conductivity, density and pH value of the water. Examples of biodegradable pollutants include proteins, fats, insecticides and fungicides. Non-biodegradable pollutants include things like other pollutants. Because of the existence of acidic and alkaline pollutants, most of the invertebrates and microorganisms are eradicated.

According to Ackerman (2020), the flora and fauna of aquatic settings are adversely affected as a direct result of this, which in turn produces bioaccumulation in aquatic species and bioconcentration in higher vertebrates. A world that is both ecologically mindful and capable of self-sufficiency is a prerequisite for the well-being and contentment of humans. It is usual practice to utilize this approach for assessing the level of contamination existing in aquatic habitats. This method involves making a comparison between the containment concentrations in related biota. Since the bioconcentration of compounds in the environment has been studied, everyone believes that there are a lot of quantitative links between the structure of chemicals and the biological activity that they display in aquatic systems. These correlations have been recognized because of the measurement of the bioconcentration of compounds in the environment. These connections have been forged in contexts including water. The gills and the digestive tracts of the organism are, however, the principal sites of absorption and accumulation of these heavy metals in the organism. The abundance of lead in the environment is mostly attributable to three different types of pollution: urban wastewater, industrial discharges and agricultural runoff. Lead is a typical heavy metal that may be discovered in the natural world. Because it is an anti-knock agent that is added to fuel, there is a greater chance that it will be discovered in the air. From there, it is taken to streams and rivers by runoff, where fish and other aquatic species devour it and absorb it into their bodies. This process continues until the substance is completely gone.

Gnana Veera Subhashini (2018) studied the successful growth of fish and produced fish that are healthy, and reported that it is very necessary to do research on environmental risk factors and elements that cause stress. In the present study, the significant effects of ozone against the exposure of various environmental factors on walking catfish, *Clarias batrachus* was divulge in terms of hematological and biochemical parameters such as total cholesterol, triglycerides, High-density lipoprotein (HDL), Low-density lipoprotein (LDL), Very low-density lipoprotein (VLDL), total cholesterol/HDL, Total RBC's, Hemoglobin (Hb), Packed cell volume (PCV) and Blood Glucose. These characteristics were subjected to statistical analysis, and the findings revealed significant shifts in the lipid and hematological profiles of the *Clarias batrachus* population. When taken together, our results imply that *Clarias batrachus* might be a bioindicator to monitor the quality of lake water and the health status of fish in the marine communities of Varanasi, India. This is because *Clarias batrachus* has been found in both freshwater and saltwater environments.

Kamal Sarma (2012) reported that the purpose of this study was to evaluate the effect that salinity has on the growth and biochemical composition of freshwater catfish, *namely Clarias batrachus*. The LC50 of salt was found to be 12.52% after 96 hours of exposure to fingerling as part of a static non-renewable acute toxicity bioassay test. The test was performed on fingerlings that were 14.5 centimeters in length. Considering these results, two sublethal salinity levels, namely 4% and 8%, were selected for the aim of exploring the long-term repercussions of salinity on *Clarias batrachus* over the course of ninety days. These values were 4% and 8%, respectively. The results of the study indicated that growth and survival rates were both lower in saline water (four per cent and eight per cent, respectively). The freshwater environment (0 per cent salinity) had the greatest growth and survival rates, followed by the 4 and 8% salinity levels in the measurements. To have an indication of the metabolic shift, a few key biomarkers were assessed. At the end of the rearing period of ninety days, it was revealed that the amount of glucose that was present in both the brain and the blood of *C. batrachus* decreased with an increase in the salinity of the environment. The fish that were grown in an environment with 4% oxygen had lower amounts of glycogen in their livers and muscles compared to the fish that served as the control. As the quantity of salt in the diet grew, it was discovered that the amount of ascorbic acid that was present in any of the organs that were under investigation reduced. This was connected to the finding that vitamin C reduces the effects of stress. The quantity of acetylcholine esterase activity that was measured steadily reduced as the salinity rose. In liver and muscle tissues, the activity of metabolic enzymes, such as alkaline phosphatase (ALP) activity and adenosine triphosphatase (ATPase) activity, decreased as the concentration of salt in the environment increased. The findings of this experiment allow for the conclusion to be drawn that *Clarias batrachus* is sensitive to having its development and physiological response significantly (P 0.01) affected when it is exposed to higher levels of salt than it is typically found in its natural environment.

Sivamanik and Ahmed (2015) focused on the influence of physicochemical factors on the bacterial population in the Mullaiperiyar river, which is in the Theni district and discovered the existence of bacterial colonies such as Streptococcus,*E. coli*, *Klebsiella,* *Proteus, Salmonella* and *Shigella dysentery*. This means that the water in the river is not safe for people to drink, and proper sanitation and drainage systems must be put in place.

Sharma, *et al.* (2014) carried out preliminary physicochemical and microbiological tests on the water taken from the Bahini River in Guwahati, which is in the state of Assam in India. According to the data, each area had a count of viable organisms that was rather high overall. MPN index of water samples was also found to be extraordinarily high, with values more than 2400/100ml for each of the sites. The isolates were characterized and identified as E. coli, Bacillus sp., *Enterobacter sp., Klebsiella sp.* and *Pseudomonas sp.*, in that order. The outcomes of the study indicate that rapid action is necessary to eliminate the pollution that is present in river water and that the water itself must first be treated acceptably.

Rawway, *et al.* (2016) kept an eye on the River Nile's water quality and analyzed microbial pollution by determining total bacterial counts as well as bacterial indicators (TC, FC and FS). All samples were found to be contaminated by all these pathogenic bacteria, and it was determined that the Nile River's water quality varied from mediocre to excellent according to Egyptian laws of the Nile River protection agency. This information was gleaned from monitoring the Nile River's water quality.

Bhagra, *et al.* (2017) reported that there was an investigation into the bacteriological profile of drinking water samples found in and around the Shimla hills. A total of 1098 water samples were isolated, and the results showed the presence of *E. coli* (35.6%), *Klebsiella pneumoniae* (31.6%), *Klebsiella oxytoca* (19.3%), *Enterobacter sp.* (8%) and *Citrobacter sp.* (2%).

Sephar, *et al.* (2020) performed physicochemical and bacteriological tests on water samples collected from 20 different villages located within the districts of Pune and

Satara in the Indian state of Maharashtra. A total of 206 samples were analyzed, each coming from a unique source and being analyzed by one of two distinct WQIs, one of which included *Fecal coliform* and the other of which did not include FC. The findings showed that the water bodies included the highest amount of *fecal coliform* and intestinal enterococci (IE), it is seen as a major reason for concern since it might have long-term effects on human health.

Wokoma, (2022) explored the impact that pollution of freshwater bodies has on the overall health of the aquatic species that are endemic to those freshwater bodies. Even though fresh water only accounts for a very small percentage of the total volume of water found on the surface of the globe (less than one per cent), this category of water is very necessary for the continued existence of all kinds of life on the planet. It has been shown that both natural and human activities, such as residential, industrial and commercial, have the potential to damage the quality of surface water. This, in turn, has a detrimental effect on the aquatic (freshwater) species that may be found in that water. The reactions that organisms have when they are exposed to pollutants may be different. These responses might include things like migratory, morphological, behavioral, histopathological, respiratory, reproductive, or biochemical alterations and most significantly, mortality. The method in which an organism responds to a pollutant is contingent upon several elements, including but not limited to the kind of pollutant, the concentration of the pollutant, the species of the organism and the size of the organism. It is possible to enhance the quality of contaminated water by using procedures such as nanofiltration, electrodeposition, sequestration, activated carbon treatment, ion exchange and other similar methods. On the other hand, it is also possible to help stem the tide of aquatic pollution through practices such as reducing the excessive application of pesticides, educating and enlightening the public about environmental issues, regulating products and processes, limiting the discharge of pollutants and other methods that are very similar. On the other hand, the answer to decreasing the amount of pollution in fresh water to the barest possible minimum is to adhere strictly to all environmental laws and regulations on the local, national and international levels. This is the solution to reducing the amount of pollution in fresh water to the barest possible minimum.

Senger, *et al.* (2016) give a very simple definition: pollution is the discharge of thermal energy or potentially hazardous materials into the environment because of human activity.

Jay R. *et al.* (2004) defined that the pollution is an unwelcome change in the physical, chemical, and biological properties of our air, land, and water that either wastes or harms our material resources or might hurt our businesses, living circumstances, and cultural values. Additionally, these modifications may have a detrimental effect on our material resources.

According to Wokoma *et al.* (2010) pollution is defined as the act of releasing waste materials into the air, land, or water from either residential or industrial sources. This waste material may be gaseous, liquid, or solid; it could be harmful or not; it could be biodegradable or not; it could be untreated or just partially treated; and so on. Most people think that the most well-known definition of aquatic pollution was created by the Joint Group of Experts on the Scientific Aspects of Marine pollution (GESAMP, 1976). The first publication of this word was in 1976. Marine and aquatic pollution, according to their definition, is the introduction of substances or energy into the marine and aquatic environment, including estuaries, by humans, either directly or indirectly. This may harm living and non-living resources, make people sick, stop people from using the sea for lawful purposes (like fishing), and lower the quality of the water used there. They took into consideration pollution in marine and aquatic habitats, which is defined as the entry of chemicals or energy into the respective environments (M. N. Islam, 2007).

Jayakumar, *et al.* (2017) stated that any substance that can generate or has a high possibility of causing any of the undesirable effects in water that were stated before, then that substance is regarded as a pollutant that enters the water supply and should be avoided. Lotic water and lentic water are the two classifications that are used to define bodies of freshwater. The existence of rivers, streams, or other forms of flowing water is the defining characteristic of what are known as "lotic" aquatic habitats. The bodies of water may constantly oscillate or flow in only one direction. They consist of rivers, streams and creeks, in addition to seas and the ocean, since all these different bodies of water are subject to the influence of the tides. Lakes and Ponds, which are examples of lentic bodies of water, often have rather tranquil water.

Sephar, (2020) discovered that the buildup of metals like chromium, lead, mercury, zinc, and copper in fish tissues not only affects their physiology but also messes with their metabolism and slows down their growth and development.

Shephard, *et al.* (2020) studied the levels of heavy metals (Cd and Zn) in several different places around Palestine lake in Indiana, as well as the levels in the stream that brings metal-bearing wastes to the lake. The average content of Cd in the lake was 17.3 g/l, while the average concentration of Zn was 293 g/l. Both numbers are high.

Jemse, (2003) analyzed the presence of aqueous metals in fish and found that the visceral (intestinal) organs had better potential to retain metals than the gills did. This is because the gut is directly associated with nutrition from its surroundings.

Metal pollution of Umtata River was studied by Fatoki *et al.* (2022), who showed that there were significant levels of Al, Cd, Pb, Zn and Cu, all of these things might affect the health of the aquatic environment and the health of the people who live in the country and use the river water directly for home purposes without treating it beforehand.

Shrivastava *et al.* (2022) explored a lake in Bhopal that gets its water from sewage and tested it for heavy metal pollution. It was found that the levels of manganese and iron were below the acceptable limit, but the levels of chromium, nickel, zinc, and lead were not safe for people to drink. This lake in Bhopal is not considered to have water quality that is suitable for human consumption.

Asonye, *et al.* (2022) found heavy metal profiles in the waterways of Nigeria (rivers, streams and waterways). The fact that the findings indicated concentrations of lead, cadmium, chromium, zinc and manganese that were higher than what was suggested by the WHO indicates that these surface waters represent substantial dangers to the health of the people who are located nearby and make use of these waters for commercial, household and societal uses.

Ayas, *et al.* (2022) investigated the quantities of heavy metals such as lead, cadmium, copper and nickel that were found in the water, sediments and fish in the Nallihan bird paradise in Turkey. Because of the observation, it was established that the concentrations of the metals had dropped beyond the boundaries of detectability (BDL). In addition, it was found that the deposit of metal was greater in wells than in carp or bleak due to the differences in the feeding patterns of these three different species of fish (I.V. Brahim,1999).

Weher, (2018) studied the levels of three heavy metals (Cd, Cu and Zn) in a variety of tissues (muscle, bone, skin, scales and gills) of three species of fish that were collected from the Northern Jordan Valley in Jordan. For Oreochromis aureus, the pattern of accumulation of Cd level was gills>skin>bone>scale>muscle; for Cyprinus carpio and

Clarias lazera, the order was gills>scales>bone>skin muscle; and for *Clarias lazera*, the order was gills> scales >skin muscle. The copper concentration showed the order of accumulation in *Oreochromis aureus* was gills> skin≥ muscle≥ bone≥ scales and in *Cyprinus carpio* and *Clarias lazera* was scales>gills>skin>bone>muscle, and Zn concentration in *Oreochromis aureus* was skin>bone>scale>muscle>gills and in *Clarias lazera* was skin>gills≥bone>muscle and in Cyprinus carpio was skin>scales≥muscle> gills>bone. It was determined that the levels of heavy metals (Cd, Cu and Zn) in the muscles of the three different species of fish fell within the acceptable ranges established by the FAO criteria.

Ahmed, *et al.* (2009) observed the concentration of heavy metals such as As, Pb, Cr, Ni, Hg and Cd in different organs such as the liver, intestine, gills, scales and muscles of three fish species found in the river Meghna. They concluded that all of the measured heavy metals in different organs of fish were within permissible limits, except Pb, which was highly concentrated. The sequence in which the cumulative mean concentrations of heavy metals were found in the various organs of the fish that were investigated was as follows: liver > gut > gills > scale > muscle.

Moghaddam, *et al.* (2007) determined the distribution of trace metals in fish tissue, sediments and water from the Okumeshi river, Nigeria, and it was concluded that the order of heavy metal concentration in water was Ni>Mn>Cr>Cd>Pb, in sediments it was Mn>Cd>Ni>Cr>Pb. The trend of metal accumulation in Tilapia fish was observed to be Cd>Mn>Ni>Cr>Pb in gills, Mn>Cd>Cr>Ni>Pb in liver, Mn>Cd>Ni>Cr>Pb in muscle and Mn>Ni>Cr and Cd>Pb in bones and trend in different tissues of catfish showed in the sequence Cd>Mn>Ni>Cr>Pb in gills, Mn>Cd>Cr>Ni>Pb in liver, Mn>Cd>Ni>Cr>Pb in muscle and Mn>Cd>Ni>Cr>Pb in bones and it was concluded that in most of the fish samples, cadmium concentration was found above the maximum tolerable values provided by international institutions.

Kpee, *et al.* (2019) undertook a study on the seasonal variation of four heavy metals, viz. Cd, Ni, Cu and Pb in muscles of catfish, sediment and water sample of IPO Stream, and the results revealed the metal concentration in the fish sample in the order Pb, Cu , Ni and Cd which were 24.14µg/g,17.85µg/g, 2.79µg/g and1.15µg/g respectively and tin sediments in the order of Pb > Cu >Ni > Cd which were 25.73µg/g, 18.48µg/g, 2.91µg/g and 1.35µg/g respectively. A similar pattern was seen in the water sample's concentrations of heavy metals, which came out to be 23.32 g/l for lead, 17.32 g/l for copper, 2.42 g/l for nickel and 1.15 g/l for cadmium, respectively. These readings fell short of the benchmark that the W.H.O. and FAO have set for the quality of aquatic environments.

Raja, *et al.* (2009) After conducting research on the concentrations of 10 different heavy metals in four commercial marine edible fish species off the coast of Parangpetti, the researchers concluded that the order of accumulation in the muscles of all of the fishes was as follows: Fe > Zn > Al > Ni > Mg > Mn > Cr > Cd > Cu > Co. The quantities of heavy metals that were discovered to be present in the edible sections of the fish that were tested were determined to be well within the acceptable range for consumption by humans.

Malik, *et al.* (2010) conducted research on the bioaccumulation of seven heavy metals in water samples and the tissues of two fish species found in the lake of Bhopal. The species studied were the *Labeo rohita* and the *Ctenopharygodon idella*. Zn > Ni > Cr > PB > Cu > Cd > Hg was the sequence of accumulation in the water samples, and the quantity was acceptable according to BIS standards. The accumulation of heavy metals followed the pattern liver > kidney > gills > muscles in *L. rohita*, but in *C. idella* it followed the pattern gills > liver > kidney > muscles. It was found that the concentrations of the heavy metals under study did not exceed the FAO-mandated safe standards for their presence.

Nwani, *et al.* (2010) conducted a study to determine the concentration of Fe, Zn, Cu, Mn, Pb and Cr in the gills and muscles of six different fish species from a lotic freshwater ecosystem in Nigeria. These fish species included *Chrysicthys nigrodigitatus*, *Clarias angullaris, Tilapiazilli, Mormyrus rume, Mormyrus macropthhalmus and Mormyrus tapirus.* The order of heavy metals concentration in both the gills and the muscles was Fe>Zn>Mn>Cu>Cr>Pb, and the concentration of Zn, Cu and Pb were within the permissible limits as prescribed by WHO and FEPA. On the other hand, the concentration of Fe (except in the muscles), Mn and Cr were above the permissible limits, which indicates that the fish species have been contaminated by these metals.

Reza (2010) investigated the seasonal shifts in the levels of heavy metal pollution in the River Brahmani. The Brahmani River is in the Angul district of the state of Odisha, India. According to the data that was collected and analyzed, the levels of the trace metals Cadmium, Chromium, Copper, Cobalt, Iron, Manganese, Nickel, Lead, Mercury, and Zinc were all much lower than the thresholds established by the standard IS: 10500. The average values for the Heavy Metal Pollution Index (HPI) during the Pre-monsoon are 36.19, while the mean values during the winter are 32.37. Because none of these readings is higher than the critical index limit of 100, it may be concluded that the water does not contain any trace amounts of metal contamination.

Eneji, *et al.* (2011) determined the concentration of seven heavy metals viz. Cr, Zn, Cu, Fe, Mn, Cd and Pb in three different tissues of two fish species, *i.e. Tilapia zilli* and *Clarias gariepinus,* from the River India. "The accumulation of metals in *Tilapia zilli* was Gills (52.2%) > Intestine (26.3%)> Muscle (21.5%). Similarly, the order of accumulation in *Clarias gariepinus* was Gills (40.3%)> Intestine (31.6%)> Muscle (28.1%). The trend of heavy metals concentration in Tilapia zilli, was Cr > Zn > Cu > Fe > Mn > Cd >Pb, and that of *Clarias gariepinus* was Cr > Zn > Fe > Cu > Mn > Cd > Pb.

Jabeen, *et al.* (2011) studied the toxicity of heavy metals such as Al, As, Ba, Cr, Ni and Zn in different fish tissues of three fish species, including *Labeo rohita*, *Catla catla* and *Cirrhina mrigala* at three sites of the river Ravi, including Shahdara bridge, Baloki headworks and Sidhnai barrage. The results showed that the toxicity of metals fluctuated significantly in fish at all sampling stations with the season. The order in which metals were found to accumulate in fish was as follows: fish liver > kidney > gills > gut > reproductive organs > skin > scales > fins > bones > muscle. It was determined that the amount of Al, As, Ni, Ba, Cr and Zn was excessive in comparison to the allowable values that were advised.

Javed, (2011) examined the accumulation of heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) in three commercially important fishes, namely *Channa punctatus*, *Clarias gariepinus* and *Labeo rohita*. The results revealed that the Fe and Zn concentrations were the highest in all tissues analyzed followed by Ni, Cu, Co, Mn and Cr in almost all three species. In the muscles of *Channa punctatus* the order of accumulation was observed to be Fe >Zn > Ni > Cu > Co> Mn, whereas in *Clarias gariepinus* it was Fe > Zn > Ni > Cu=Mn> Co > Cr. In *Labeo rohita* the sequence of accumulation was Zn > Fe > Ni > Cu > Co > Mn.

Kaur, *et al.* (2013) measured the content of heavy metals in eight species of edible fish from two contaminated tributaries of the Chenab River in Pakistan: *Cirrhinus reba, Channa punctata, Heteropneustes fossilis, Labeo rohita, Mystus cavasius, Oreochromis niloticus, Wallago attu* and *Puntius sophore*. The sequence of metal accumulation in the organs that were examined was as follows: Cr > Pb > Cu > Cd. The concentration of metals was greatest in the liver, followed by the gills, kidneys and muscles. It was determined that the concentration of metals was greater in the pre-monsoon period compared to the post-monsoon period. The levels of lead, cadmium and chrome that were found in the muscle tissue of some species, including *C. punctata, W. attu, L. rohita, O. noloticus* and *P. sophore*, were found to be too high.

Khan, *et al.* (2012) studied that the presence of four separate heavy metals was studied in the muscles, livers and gills of three distinct species of freshwater fish. *Cyprinus carpio, Cirrihinus mrigala* and *Mystus bleekri* were the species of fish in question. As a result of this, the following is the sequence in which heavy metal concentration was discovered to occur in tissues: The buildup of heavy metals in fish species was shown to occur in the following order: liver > gills > muscles copper > zinc > manganese > lead. It was also determined that the levels of heavy metals were below the limits set by the Food and Agriculture Organization (FAO) for each metal that was detected in fish bodies.

Opaluwa *et al.* (2012) evaluated the levels of six different heavy metals in the water, sediments and various body sections of two different species of catfish that were collected from the UKE stream in Nigeria. These species were *Clarias gariepinus* and *Synodontis schall*. The conclusion that can be drawn from this is that the sediments have a higher concentration of metals than the water does. Fe>Zn>Cu>Mn>Pb>Cd, and the order of bioconcentration of metals in various body parts of *Clarias gariepinus* was Zn>Fe>Cu>Cd>Pb>Mn, while that of *Synodontis schall* was Zn>Cu>Fe>Cd>Mn>Pb. The metals in the water and sediments were found to be above the safe levels, but the metals in the fish were below the safe levels. This means that the stream was polluted with heavy metals.

Patil, *et al.* (2012) analyzed the bioaccumulation of seven heavy metals, i.e. Zn, Cu, Cd, Pb, NI, Co and Cr in different organs of six freshwater fish species from the river Pauna, Maharashtra. The order of bioaccumulation of various heavy metals in different organs of all the test fishes was Zn (liver) >Cr (liver)> Cu (gill) >Pb (gill) >Co (gill) > Ni (liver) >Cd (liver). The observed concentration of Zn, Cu and Cr was above the permissible limit, and that of Cd, Pb, Ni and Co were below the permissible limits as prescribed by FAO/ WHO.

Baskaran and Prabhahar (2013) conducted seasonal research on the bioaccumulation of five heavy metals (zinc, chromium, copper, cadmium and lead) in several organs (gill, gut, liver, kidney and muscle) of the fish Cyprinus carpio from Vettar Estuary, Nagapattinam Coastal region, Tamil Nadu over the course of one year. Zinc, chromium, copper, cadmium and lead are some of the metals that fall into this category. These data led one to the conclusion that the rate of bioaccumulation was at its maximum during the monsoon season and at its lowest during the Pre-monsoon season. This was the conclusion that could be derived from the facts (Jabeen *et al*.2011).

Khound *et al.* (2012) undertook a study of six heavy metals in water samples and tissues (gills, muscle, liver) of three edible fishes namely *Oreochromis niloticus*, *Labeobar busintermedius* and *Clarias gariepinus* from two lakes- Awassa and Koka rift valley of Ethopia and concluded that the mean metal concentration of metals in water was in the order Cr>As>Pb>Cd>Se>Hg. Also, the mean concentration of metals in the gills were in the order Cr>Pb>Hg>As>Cd>Se; in muscles the order was Cr>Hg>As>Pb>Cd>Se and in liver Cr>Hg>Cd>As>Pb>Se. Also, it was observed that both the lakes were affected by industrial effluence.

Elnabris, *et al.* (2013) researched the levels of six different heavy metals in the muscles of six different commercial fish species, including *Merluccius hubbsi, Micropogonias furnieri, Pangasius hypothalamus*, *Oreochromis niloticus*, *Mugil cephalus* and *Sparus aurata*. The mean concentration of heavy metals found in the muscles of all fish species was Zn>Ni>Cu>Mn>Pb>Cd, and the levels of all metals were lower than the limits authorized by FAO/WHO/EU/MAFF. Zn had the highest concentration, followed by Ni, then Cu, then Mn, then Pb and finally Cd (Rafiquel, *et al.* 2013).

Natali Janet Delorme (2005) investigated the accumulation of heavy metals in the muscle tissue of African catfish (*Clarias gariepinus*), as well as in the water and sediments of the Nile River. According to the findings, the largest concentrations of the elements Zn, Cu and Fe were found in the fluids and muscles, followed by Mn, Cr, Pb, Cd, Ni and Hg. The cycle of deposition of elements in sediments was as follows: Fe > Zn > Mn > Cu > Pb > Cd > Cr > Hg. Because of the rise in temperature, it was determined that the growth and bioaccumulation factors of heavy metals, particularly zinc, iron and copper, were at their highest during the Pre-monsoon months.

In a study by Mohanambal and Puvaneswari (2013), the concentrations of heavy metals in different tissues of adults *Catla catla* were assessed after exposure to a sublethal quantity of lead. The fish were exposed to 6.52 mg/l of lead, which is 1/25th of the 96-hour LC50 value, over a period of 120 days. This study aimed to assess how prolonged exposure to low levels of lead affects heavy metal accumulation in fish tissues. The purpose of this study was to find out how many heavy metals were present. The liver collected the most lead after the kidneys. The gills, ovary, testis, and brain followed. Prolonged lead exposure has been shown to cause chronic lead nephropathy, hypertension, pathological alterations, and reproductive problems.

Sunday, *et al.* (2013) had examined water samples and residues from Bindare stream, Chikaji Industrial Area Sabon Gari, to determine the levels of nine different heavy metals. These metals included Al, Ti, Fe, Cr, Zn, Cu, Mn and Cd. The order of the metals in the sediments was as follows: Al > Ti > Zn > Fe > Mn > Cr > Ni > Cd. In water, however, the metal concentration order was slightly different: Al > Ti > Zn > Fe > Mn > Cr > CD. The amounts of metals, including aluminum, titanium, zinc, and copper, were found to be greater than what the Nigerian Industrial Standards say they should be.

Tanee, *et al.* (2013) wanted to find out how four distinct heavy metals (zinc, lead, copper, and iron) build up in seven different kinds of fish from the Chi River in Thailand's Maha Sarakham Province. The order of accumulation of metals in water was Fe > Cu > Zn > Pb. The order of accumulation of metals in sediments was Fe > Pb > Cu > Zn, while the order of accumulation of metals in fish was Fe > Pb > Zn > Cu. The accumulation of heavy metals was shown to be much larger in the liver compared to the accumulation in other tissues (liver > stomach- intestine > gill > muscle).

Thomas and Mohaideen (2013) researched the seasonal changes of five heavy metals along the Marina Sea beach in Chennai. The findings indicated that all the heavy metals were below the limit that the WHO says is safe for their presence. The heavy metals studied included As, Cd, Cr, Pb and Hg. It was noted that seasonal variations did not significantly influence the metal concentrations in fish samples. The highest levels of heavy metals in water samples were identified during the monsoon season, whereas the highest levels of heavy metals in sediment samples were found during the pre-monsoon season.

Afshan, *et al.* (2014) explored the impacts of sublethal concentrations of cadmium (Cd), zinc (Zn), chromium (Cr) and lead (Pb) on common carp (*Cyprinus carpio*). The accumulation of heavy metals in different tissues showed specific patterns: in the liver and gills, the metals accumulated in the order of Pb > Cd > Ni >Cr and Cd > Pb > Ni > Cr, respectively. For kidneys and flesh tissues, the accumulation followed the order Pb > Cd > Cr >Ni and Pb > Cr > Cd > Ni, respectively.

Kordylewska, (2022) in Nigeria investigated the quantities of heavy metals in the tissues of *Syndontis membranaceous* along the lowest section of Taylor Creek. They discovered that zinc, manganese, lead and cadmium were accumulated in tissues in the following order: zinc > manganese > lead > cadmium. The amounts of manganese, lead, and cadmium in the tissues were substantially higher than what the WHO says is safe. Based on the facts given here, one might conclude that fishing in the stream is not safe for people to eat.

Asmah and Biney (2014) investigated the accumulation sequence of certain chosen heavy metals in the tissues and organs of tuna from West Africa (Cu, Zn, Cd, Pb and Mn) in Frigate Tuna was Zn > Mn > Cu > Pb > Cd; Atlantic Little Tuna was Zn > Cu > Mn > Pb >Cd and Skipjack tuna was Zn > Mn > Cu > Pb > Cd. It was determined that the concentration of necessary elements (Cu, Mn, and Zn) was predominantly greater than that of non-essential metals (Cd and Pb).

El. Moselhy, *et al.* (2014) determined the concentration of heavy metals (copper, zinc, lead, cadmium, and manganese) that had accumulated in the liver, gills, and muscles of fourteen separate species of benthic and pelagic fish inhabiting the Egyptian Red Sea. The overall amounts of heavy metals discovered in various organs and types of fish were quite different from each other. Because of the way the tissue is made, it was not surprising that muscles always contained the lowest quantities of metal of any form of tissue. Most of the fish that were looked at had a lot of copper, zinc, and iron in their livers, which was the organ that was looked at. The gills, on the other hand, have the most lead and manganese of any portion of the fish. It was also found that the amounts of metals were either lower than what was found in worldwide studies of similar species or about the same as what was found in those studies. The metals found in the muscles of the fish that are presently available are within the limitations set by international law. Because of this, people may consume these fish without worrying.

Kumar, *et al.* (2014) examined the seasonal variations of three heavy metals, lead, copper, and arsenic, in water and fish sourced from the River Yamuna in Allahabad. It was found that the levels of Pb and Cu were greater than the permitted limits of WHO, which indicates that there is a dangerous risk to human health; on the other h and, Arsenic was identified at a level that was lower than the permissible limit.

Kupeli *et al.* (2014) studied the Al, B, Ba, Cr, Cu, Fe, Mn, Ni, Sr and Zn, some of the trace and essential elements that were analyzed in 17 distinct fish species that were collected from the seas around the Sakarya area. It was found that the concentrations were usually far lower than the limits set by the Turkish Food Codex (TFC), the Food and Agriculture Organization (FAO), and the World Health Organization (WHO). It is conceivable to infer that consuming fish from the Sakarya region poses no risk, given the minimal concentrations of heavy metals present in these species (Verma, *et al*. 2025).

Masood, *et al.* (2014) analyzed the heavy metal profiles of Barilius bendelisis, spirogyra plants, and water samples from the Shnebaye stream, revealing a decreasing trend in metal accumulation in fish, that is Cd>Ni>Cu (head); Cd>Ni>Zn>Cu(abdomen); Ni>Cu>Cd (tail). The recorded value of heavy metal in plant (spirogyra) and soil were Cd>Ni>Cu>Zn and Cd>Ni>Cu>Zn respectively, while the values of selected heavy metals in surface, middle and startup water of stream were as follows: Ni>Cu>Cd>Zn (surface water), Ni>Cu>Cd>Zn (mid water) and Ni>Cu>Cd>Zn (startup water), respectively.

Mohamed and Osman (2014) assessed the concentrations of heavy metals (Cd, Cr, Ni, Pb, Cu, Zn, Fe, and Sr) in the muscle tissues and gills of Nile Tilapia (*O. niloticus*) and in water samples collected from the effluent of a sewage treatment facility. The absorption of the heavy metal that was observed in the order's tissues and gills was in the order of Sr > Fe > Zn > Cu > Pb > Ni > Cr >Cd, and the order for the water sample was Sr > Pb > Cr > Cd > Ni > Cu > Zn>Fe. In conclusion, the amounts of metals would have a bad influence on the health of people living in the research region.

Sharma and Chaudhary (2014) examined the existence of five distinct heavy metals in the surface water around three kinds of facilities (a sugar mill, a paper mill, and a thermal power plant) and the Western Yamuna Canal (WYC) throughout the pre-monsoon and winter seasons. It was noted that the levels of Cr and Cd were higher before the monsoon, whereas the levels of Mn, Ni, and Cu were higher in the winter. The results revealed that the water samples obtained from the region surrounding the sugar mill had more heavy metals than the ones taken from the paper mill and the thermal power plant. Along with the WYC, researchers discovered that the concentration of metals in water samples dropped as the distance from the nearest industrial region increased. It was found that the overall average level of heavy metals went down in the order of Ni > Cu > Cr > Mn >Cd and Mn > Ni > Cu > Cr > Cd in WYC and surrounding industrial water samples, respectively.

Shivakumar, *et al.* (2014) attempted to assess the significant ecotoxic heavy metals that had accumulated in the organs of commonly consumed fish species, including *Etroplus maculatus*, *Cirrhinus reba*, and *Ompok bimaculatus*, collected from the region of the Bhadra River in India. The concentration of metals in the organs of *Etroplus maculates* was in order of Fe>Zn>Cu > Pb>Ni>Cd, followed by Zn>Cu>Fe>Pb>Ni>Cd in

Cirrhinus reba and Fe>Zn> Cu > Pb>Ni>Cd in *Ompok bimaculatus*. Muscle, intestine, and gills exhibited a higher buildup of metals. The levels of Cd and Pb found in fish species were close to the World Health Organization's safe limit.

Kupeli, *et al.* (2014) carried out an initial investigation of the presence of four heavy metals in the water and sediments of the Karnaphulli River in Bangladesh. They found a declining tendency of metal in the water as Cr > As > Pb > Cd, and they found a similar pattern in the sediments as Cr > Pb > As > Cd. The range of heavy metals found in the water sample was found to be higher than the permissible limit, which indicates that the water cannot be used by humans without risk (O. Fagbuaro *et al.*, 2014).

Giripunje, *et al.* (2016) looked at how much heavy metals (Zn, Pb, Ni, Cu, and Cd) were in the gills and muscles of Tilapia fish. The result indicated that the gills of Tilapia fish had a greater concentration of metals compared to the muscles. The following patterns were found for the number of metals in the muscles and gills: Zn > Pb > Ni > Cu > Cd. The amount of lead and cadmium in the muscles of fish was far greater than what the FAO and WHO say is safe. A health risk assessment of lead and cadmium in Tilapia fish from Naik Lake indicated that consumption of these fish might pose a threat to human health.

Malakootian, *et al.* (2016) measured the levels of Ni, Cr, Hg, and Pb in the muscular tissue of three types of fish *Thunnus tonggol*, *Pleuronectiformes*, and *Liza klunzingeri*. The result indicated the trend of bioconcentration of Cr and Ni ranked as *Thunnus tonggol* > *Pleuronectiformes* > *Liza klunzingeri* and in terms of the amount of Hg, they were ranked as *Pleuronectiformes* > *Thunnus tonggol* > *Liza klunzingeri*, and contamination levels of Pb were observed as *Liza klunzingeri*> *Pleuronectiformes* > *Thunnus tonggol*.

Ullah *et al.* (2016) examined the levels of seven different heavy metals, including manganese, iron, chromium, zinc, lead and nickel, which were measured in the liver, kidneys, gills and muscle of three different fish species that were collected from the river Panjkora in Pakistan. The findings indicated that the concentrations of heavy metals exhibited considerable variation based on the tissue type and fish species. The species that had the heaviest metals were the *Cyprinus carpio*, followed by the *Garra gotyla* and then the *Cyprinion watsoni*. The order in which tissues accumulated and then decreased was as follows: liver > kidney > muscles > gills.

Yousafzai, *et al.* (2017) investigated the bioaccumulation of several heavy metals in *Cyprinus carpio and Labeo rohita* from *Sardaryab, Khyber Pakhtunkhwa*. These metals included Fe, Zn, Pb and Cu. The number of metals in the liver was found to be substantially higher than in the other tissues. The liver had the heaviest metals, followed by the gills and finally the muscles of both types of fish.

Saxena and Garg (2022) reported that the effects of insecticide pollution on the ovaries of the teleost fish Channa punctatus were investigated, and the findings showed a degradation of the oocytes and a decrease in the size of the ovary. The influence of mercury chloride on the reproductive process of teleost fish, during the research conducted on Channa punctatus, atretic follicles and the degeneration of stage I and stage II oocytes were found in the treated fish ovary samples.

Baruah and Das (2022) observed histological alterations in the ovaries of fish called *Heteropneustus fossilis* once they were exposed to waste from paper mills. There was a lot of atresia in the ovary, and the younger oocytes were quite damaged. There were also changes in the position of the nucleus, as well as partial lysis and swelling atresia (S. Debnath *et al*. 2010).

James, *et al.* (2003) observed that after 140 days, copper poisoning affects growth, gonadal development, and reproductive function. The researchers observed a significant decline in reproductive indices, including gonad weight, gonadosomatic index, and fertility.

Gupta and Guha, (2016) said that when the toxicity of mycrocystin was tested on the ovaries of *Heteropneustus fossilis*, the researchers observed a reduced amount of yolk in developing oocytes, along with deformed follicular cells and necrosis resulting from toxin exposure.

Olfat and El-Greisy, (2017) assessed the effects of several zinc-containing water sources in *Siganus rivulatus*, revealing significant necrosis, enlargement, and hyperplasia of oocyte follicular cells by histological analysis of the ovaries.

Mazrouh and Mahmoud, (2019) watched the histological alterations that happened in gonads when they were exposed to different contaminants. They determined that fish exposed to higher levels of contaminants had a greater prevalence of gonadal abnormalities. These anomalies consisted of malformed oocytes and spermatocytes, along with a decrease in their quantity and a lack of active oogenesis and spermatogenesis.

Akande, *et al.* (2010) stated that the impact of sewage effluents on the reproductive parameters of Danio rerio was assessed, yielding several anomalies. Some of these problems included a delayed or stopped start to spawning, fewer females spawning, a reduced rate of egg fertilization and hatching, and a lower rate of egg fertilization and hatching.

Ebrahimi and Taherianfard, (2020) observed heavy metal pollution in fish health, and that may alter the normal steroidogenesis pattern in fish, resulting in reduced hormone synthesis in both male and female fish, hence lowering the quality and quantity of sperm and ova formation. They also reported the impact of heavy metal exposure on the reproductive system of cyprinid fish from the Kor River.

Deka and Mahanta, (2021) examined the histological characteristics of the ovaries in *Heteropneustus fossilis* subjected to malathion treatment, documenting the adhesion of primary follicles, cytoplasmic retraction, cytoplasmic degradation, an increase in atretic oocytes, and the partial destruction of the ovigerous lamellae and vitrellogenic membrane due to malathion exposure.

Magar and Bias, (2013) reported the histopathological effects of Malathion on the ovary of *Channa punctatus*. Acute exposure results in degenerative changes such as a reduction in the size of mature oocytes and disruption and vacuolation in the cytoplasm. In contrast, chronic exposure leads to alterations, including a complete loss of normal ovarian configuration, necrosis, elongated ovarian follicles, and fragmented ova with abnormal shapes.

Marutirao, (2013) conducted research to investigate the impact that dimethoate poisoning has on the ovaries of *Puntius ticto*. The histology of the ovaries exhibited notable changes, including the existence of atretic oocytes and an increase in interfollicular gaps.

El-Morshedi, *et al.* (2014) revealed that water pollution has an impact on the gonads of lake sea bass fish. The examination of Manzalah indicated a delay in spermatogenesis, disruption of spermatogenic cells, and a decrease in spermatids. Water contamination caused atresia, a distorted zona radiate, necrotic oocytes, and a damaged tunica albuginea in the ovary. Because of the severe effects of heavy metal toxicants, the total amounts of polysaccharides, proteins, DNA, and RNA in the testicular and ovarian tissues of fish went down.

Masarat, *et al.* (2014) studied about the effects of a sublethal dosage of mercuric chloride were tested on the fish *Clarias gariepinus* and the results obtained demonstrated degenerative alterations in the ovary. The researchers also found vacuolization in the ooplasm and the breakdown of the connective tissue and follicular walls.

Rokade *et al.* (2014) reported that the effects of industrial effluents on the ovaries of Poecilia reticulate were investigated, and the findings included the liquefaction of the cytoplasm, the development of atresia and the degeneration of the ovarian wall.

Tantarpale and Rathod, (2014) studied the effects of cypermethrin on the ovary of *Channa striatus*, and the researchers found that the ovaries underwent degenerative changes. These changes included interfollicular oedema, the destruction of yoked oocytes and the rupture of oocyte membranes.

Ambani, (2015) analyzed the effects of heavy metal contamination on *Labeo rohita* from the Harike wetland and the findings of histological investigations, therefore, include gonadal abnormalities. These gonadal abnormalities consist of misshapen oocytes, a reduction in their quantity, and a lack of active oogenesis. It was shown that putting untreated wastewater into wetlands is a big threat to the biodiversity that is already there.

Sengar, (2016) published a review article examining the hazardous effects of water pollution and cadmium chloride on fish and water resources. The research identified cadmium chloride as an endocrine disruptor that impedes the biological functions of fish and may induce significant histopathological, biochemical, and physiological anomalies across many fish species.

Khillare, *et al.* (2017) analyzed the histological alterations that occurred in the ovaries of Channa punctatus after exposure to chromium poisoning, which led to gonadal abnormalities in the form of a malformed nucleus and a decrease in the number of oocytes, which ultimately affected the fish population.

Sharma *et al.* (2017) evaluated the sub-lethal amount of manganese (0.64 mg/l) that was administered to *Garra gotyla gotyla* over a period of nine weeks, and the impact it had on the ovaries was studied. Throughout the course of the experiment, the quantity of oocytes and the width of stage I, stage II and stage III oocytes shrank in the metal-treated groups. This was seen in all three stages. In the treated ovaries of *Garra gotyla gotyla*, further histological abnormalities such as necrosis, vacuolation, an increase in follicular space and the presence of atretic oocytes were discovered.

Paulose, (2022) studied the gills of *Labeo rohita* that had been exposed to methylmercury exhibited symptoms of vacuolation, fusion of adjacent lamellae due to hyperplasia, desquamation in lamellae, and necrosis.

K. Pandey and Shukla, (2005) performed a histopathological analysis of the gills and liver of *Labeo rohita* due to copper exposure. They observed total fusion of adjacent secondary gill lamellae, detachment of the epithelium, an elevated count of mucus cells, vacuolated hepatocytes, and degeneration of hepatic cells. These alterations resulted from copper exposure.

Athikesavan, *et al.* (2016) investigated the impact of sublethal nickel dosages (5.7 mg/l) on the histopathology of several organs in *Hypophthalmichthys molitrix*, including the gut, liver, kidney, and gills. Histopathological abnormalities included mucus proliferation, fusion of gill lamellae, hypertrophy of gill tissue, necrosis of hepatocytes, degeneration of blood vessels, hypertrophy of the liver, vacuolation and pyknotic nuclei, and degeneration of kidney tubular cells and hyperplasia. Deterioration of blood vessels and hyperplasia of renal tissue were further changes.

Camarago and Martinez, (2017) studied the Neotropical fish *Prochilodus lineatus* to investigate the histological changes that took place in the fish's gills, liver and kidneys during the study. Changes included epithelial lifting, hyperplasia and hypertrophy of the respiratory epithelium, lamellar fusion, and aneurysms in the gills, all of which were directly attributable to this. It was also shown that lamellar fusion had happened. Researchers found that the hepatocytes in the liver had grown too big, the cytoplasm and nucleus had broken down, there were areas of necrosis, and there were groups of Melano macrophages. These changes were all seen. The kidneys showed an extension of the glomerulus, a shrinkage of the region known as Bowmann's, a hazy swelling, and a breakdown of the hyaline droplets.

Figueiredo Fernandes, *et al.* (2007) stated that *Oreochromis niloticus* had its gill and liver tissue exposed to copper at concentrations of 0.5, 1.0, and 2.5 mg/l during a duration of 21 days. They looked at the findings. The predominant histological abnormalities seen in the gills were the elevation of lamellar epithelia and the presence of oedema. Some lamellar aneurysms and significant vasodilation of the lamellar vascular axis were also seen. The changes that were found showed necrosis and vacuolation in the liver. It was shown that both tissues changed more when they were exposed to 1.0 and 2.5 mg/l than when they were exposed to 0.5 mg/l.

Atamanalp, *et al.* (2018) studied the histological changes that happened in the liver of rainbow trout (*Oncorhynchus mykiss*) when they were given a sublethal dose of copper sulfate ($CuSO_4$) and left to be exposed to it for 28 days. After handling, they saw changes like non-homogeneous areas, congestion of the central vein, darkly stained hepatocytes, more Kupffer cells, vascular deterioration, and sinusoidal collapse (Hogstrand, 2021).

Mohamed, (2018) investigated the impact of heavy metal pollution on histological changes in the liver, gills, intestine, testes, heart, and muscle of *O.* and *L. niloticus* specimens taken from four khors of Lake Nasser in Egypt. We also found out how much metal there was by using metal detection. The findings revealed that Fe > Zn > Pb > Cu > Cd > Co. The intestinal mucosa and seminiferous tubules of both fishes exhibited substantial degenerative and necrotic alterations due to the accumulation of metals, histological modifications, proliferation in the gill filament epithelium, and fusion of secondary lamellae. These alterations included localized necrosis accompanied by vacuolar degeneration in the liver, degeneration and atrophy of cardiac muscle fibers, and degeneration of muscular bundles.

Velmurugan, *et al.* (2019) established the histological effects of sublethal concentrations of the organophosphate pesticide dichlorvos (0.91 ppm and 1.82 ppm) over a duration of 10 days on the gill and liver tissues of the freshwater fish *Cirrhinus mrigala*, alongside a parallel untreated control. The observed alterations in gill tissue included hyperplasia, desquamation and necrosis of epithelial cells, oedema, epithelial lifting, lamellar fusion, curling of secondary lamellae, and aneurysms in secondary lamellae. The liver exhibited characteristics of cloudy swelling of hepatocytes, vacuolar degeneration, dilation of sinusoids, and nuclear hypertrophy (Qadir, 2011).

Jegede, (2013) studied the histological analysis of the gill tissue, which showed swelling, erosion of the gill lamellae, and a change in the structure of the tissue. When a static bioassay was performed to find the LC50 of $CuSO_4$ and look at histological alterations in the gill, liver, kidney, intestine, and heart of *Heterobranchus bidorsalis* (African giant catfish) fingerlings that had been exposed to $CuSO_4$, this is what was found. At this point, the liver's pathology comprised misshapen liver cells, a lot of hepatocytes vacuolation, fibrosis, coagulative necrosis, and round cell infiltration in the hepatic parenchyma. Hepatitis was the main reason for all these alterations. Researchers found that the mucosa cells and the submucosa in the gut had undergone serious necrotic and degenerative alterations. Conversely, renal modifications, including an expanded globular lumen and proximal tubule damage, may result after extended exposure to elevated levels of copper sulfate. The heart also goes through changes, such as atrophy and degeneration of the cardiac muscle fibers.

Prabhahar, *et al.* (2012) investigated the effect of cadmium sulfate on the histology of the gills, liver, and kidneys of the edible freshwater fish *Cirrhinus mrigala*. The main and secondary lamellae, pillar cells, epithelial cells, and cartilaginous cells were all shown to be hurt. There was also fusion of the gill lamellae, as well as necrosis and bulging of the tip gill lamellae. In the liver, the abnormalities seen post-treatment included ruptured hepatocytes devoid of their polygonal architecture, blood cells that exhibited dilation and coagulation, and substantial vacuoles inside the hepatocytes with displaced nuclei. There were broken tubular cells, vacuoles between cells, brown pigment, and Melano macrophages in the kidney tissue of fish that had been treated with cadmium.

Das, *et al.* (2013) examined histological changes in the liver of *Esomus danricus,* an Indian flying barb, after exposure to three sublethal doses of 0.005, 0.0025, and 0.001 g/l during a 28-day duration. The liver exhibited necrosis, lipid-laden cytoplasm or foamy hepatocyte cytoplasm, lymphocytic infiltration, and extensive cytoplasmic degeneration. The liver was more likely to be hurt when it was exposed to higher amounts.

Mokhtar and Abd-Elhafeez, (2013) administered sublethal dosages of lead acetate (0.4 and 0.7 mg/l) to *Oreochromis niloticus* for three weeks, during which the researchers examined the histological abnormalities occurring in the fish's gills, ovaries, liver, and hepatopancreas. A control group received lead acetate in the same amounts. The findings collected indicate that lead acetate may adversely affect the organs examined. The damage to the gills includes hyperplasia of the epithelial cells that line the gill filaments, as well as a lot of bleeding in the gill lamellae and a lot of atresia in the ovaries. The gill lamellae and gill filaments are also very damaged. Due to the changes, the histological analysis of fish liver showed that hepatocytes were vacuolar degenerating. Hepatic sinusoids were also discovered to be dilated.

Muthukumar and Rajaraman, (2013) conducted research on the effects of a sublethal dose of chromium, namely 10% (3.5ppm) of 96h LC50, on the histopathology of gill and liver tissue from *Labeo rohita* fingerlings during a period of 10, 20 and 30 days. According to the results, there was only a slight histological alteration over the first ten days of exposure. However, the gill lamellae fused, the epithelium degenerated and there was hypertrophy after 30 days of exposure. The liver lesions included vacuolation, hepatocyte degeneration and hepatocyte cell boundary disintegration.

Chavan and Muley, (2016) exhibited the impact of sublethal concentrations of mercuric chloride (0.0206 ppm and 0.0402 ppm) on the liver and gills of *Cirrihinus mrigala*, revealing significant pathological changes in the gills, characterized by lamellar degeneration, epithelial lifting, and necrotic alterations in intercellular epithelial cells. In the liver, there was a loss of cellular architecture, necrosis in hepatocytes, and fat accumulation in parenchymal cells. It was advised that effluents be treated before discharge into the resources to prevent adverse effects on aquatic biota.

Paul, *et al.* (2014) stated that the liver histology of air-breathing catfish (*Heteropneustus fossilis*) with different pH values (40.5, 70.5 and 80.5) was investigated following exposure to 36 times the LC50 level of copper sulphate ($CuSO_4.5H_2O$) and cadmium chloride ($CdCl_2.H_2O$). Histological changes, including necrosis, hepatocyte degradation, blood vessel degeneration, dilated sinusoids with pyknotic nuclei, and cell vacuolation, were seen in the liver tissue due to the exposure. The extent of hepatic tissue harm was closely associated with the nominal concentration of the used metals.

Atabati, *et al.* (2015) investigated how copper sulfate affected the gill histopathology of *Ctenopharyngodon idella* (grass carp) that had been given 2.5 and 5 mg/l $CuSO_4$.

The researchers examined the gill sample after 96 hours of treatment and found hyperplasia in the treated specimens. They also observed significant gill mucus in all treatment groups, indicating a clear correlation between high concentration and increased mucus production. An increase in the concentration of the chemical directly affects the amplification of lesions at higher densities and the degeneration of primary and secondary lamellar epithelial cells.

Thyagarajan, *et al.* (2015) saw that copper sulfate affected the fish's liver, gills, and muscles *Ariopsis seemani*. The fish were given a sublethal dose of copper sulfate at two, four, and six parts per million, five, ten, and fifteen days later. Histopathological abnormalities in the liver included hazy enlargement of cells with large vacuoles, degeneration of nuclei, vacuolation in stroma, disordered hepatic cords, loss of hepatocyte shape, pycnotic nuclei, and severe necrosis. The histological alterations include the rupture of blood sinusoids inside the gill and muscular tissues.

Jayakumar and Subburaj, (2017) examined the species of freshwater stinging catfish, *Heteropneustus fossilis*, exposed to sublethal levels of cadmium to evaluate the heavy metal's toxicity. The most prevalent changes in the gills of fish were the following: erosion of secondary lamellae, lamellar fusion, shrinking of blood vessels, secondary lamellar damage, hyperplasia, vacuolation, epithelial lifting, and shortening of secondary lamellae. Other changes included hyperplasia, vacuolation, epithelial lifting, and the raising of secondary lamellae. Vacuolation, epithelial raising, and hyperplasia were other common changes. Hyperplasia was another prevalent alteration. They found broken hepatocytes, vacuolation, bleeding in the liver sinuses, cellular necrosis, an expanded pycnotic nucleus, and Melano macrophages in the liver histology. Also, they saw that the glomerulus in the kidney had become worse, the peritubular gap had gotten bigger, the glomerulus had gotten smaller, vacuolation had happened, and cytoplasm had been lost. They also showed that cadmium is a possible heavy metal poison that might affect fish at the tissue level even when it is present in very small amounts. Even though the levels were relatively low, this was the case.

Kalaiyarasi, *et al.* (2017) examined the histological changes in the gills and liver of marine spotted catfish, Arius maculatus, obtained from a sewage disposal site at Therespuram, situated off the coast of Thoothkudi on India's southeastern coast. Because of this finding, many abnormal changes were found in the fish obtained from the study site, namely in their gills and livers. Histological analysis of the gills exhibited vacuolation, deformation of the cartilage core, lamellar fusion, epithelial elevation, epithelial rupture, and haemorrhage. In contrast, the liver histology demonstrated hepatocyte rupture, vacuolation, an increased number of pycnotic nuclei, augmented Kupffer cells, and elevated Melano macrophages. The results showed that the correct steps need to be taken to prohibit untreated sewage from getting into the ocean.

Padrilah, *et al.* (2018) reviewed how fish histopathology may make fish build up copper in their bodies. Different organs have different levels of copper accumulation, and this depends on several things, such as how long an organ is exposed to copper, how the metal is absorbed, the conditions of the environment, and factors that are specific to the organ itself. Copper buildup has a bad effect on fish, and therefore, there are various histopathological abnormalities in fish organs.

Pavlica, *et al.* (2020) revealed a dose-dependent rise in micronuclei frequency in hemocytes from both species, namely the zebra mussel and snail, indicating that hemolymph serves as a suitable test tissue for genotoxicity evaluation.

Nagarani, *et al.* (2009) looked at the micronucleus test in *Therapon jaruba* after 96 hours of exposure to mercuric chloride and found that erythrocytes subjected to 0.25 ppm $HgCl_2$ had a much higher MN frequency. They thought the micronucleus test was a good way to screen fish for genotoxic chemicals in a controlled setting.

Malla, *et al.* (2011) sought to find out whether synthetic sindoor was genotoxic in freshwater catfish *Heteropneustus fossilis* (Bloch) by utilizing a micronucleus test. This was accomplished by giving six separate groups of fish six different substances: 0 ppm, 50 ppm, 100 ppm, 500 ppm, 1000 ppm, and 2000 ppm. It was demonstrated that the incidence of micronuclei in renal and peripheral blood erythrocytes significantly increased with elevated concentrations of sindoor. It was hypothesized that synthetic sindoor acts as a potent clastogen at higher concentrations and induces stress in fish when present in greater amounts (K.C. Osterhoudt, 2011).

Okonowo *et al.* (2011) investigated the micronuclei profiles of two freshwater fish, *Synodontis Clarias* and *Tilapia nilotica*, and indicated that the average micronucleus frequency in *Synodontis Clarias* exhibited significant variation, being 2.2 times more than the values seen in the corresponding *T. nilotica*. However, there was no statistically significant difference ($P>0.01$) between the two fish species.

Rocha *et al.* (2006) assessed the frequencies of micronuclei and other nuclear anomalies in red blood cells from three distinct groups of *Colossoma macropomum* subjected to 2 mg/l of methyl mercury (MeHg). A third group served as a control and was not subjected to MeHg exposure. The first two groups were exposed to MeHg for 24 and 120 hours, respectively. It was shown that there were no significant changes in the MN frequency among the treatment groups; however, there were extremely significant differences ($p < 0.02$) between the frequencies of the group treated for 120 hours and the control group. Consequently, the researchers concluded that MeHg adversely affects the erythrocytes of *Collossoma macropomum* during extended exposure.

Kaur, *et al.* (2013) determined whether the effluent from the dyeing company was genotoxic to a type of freshwater fish known as *Cirrhinus mrigala*. A micronucleus test was conducted after processing the blood from the fish's anterior kidneys for a period of 24, 48, 72 and 96 hours, and the micronuclei that were found in the erythrocytes of the fish were examined. The fish that had been treated and those that had not been treated were different in ways that may be regarded statistically significant (p 0.05). The findings indicate that the highest quantity of micronuclei was identified in erythrocytes at a concentration of 24.48% and an exposure duration of 96 hours. In contrast to the other concentrations that were looked at throughout different time periods and the control group, this was the case.

Mahboob, *et al.* (2014) looked at chromosomal alterations and sister chromatid swaps to see how mercuric chloride affected *Clarias gariepinus*. Over the course of seven days, the fish got varying levels of mercuric chloride. The results of the experiment suggest that $HgCl_2$ had genotoxic effects on fish. This is proven by an increase in the average frequencies of chromosomal abnormalities, micronuclei, and sister chromatid exchanges compared to the control group.

Natali Janet Delorme, *et al.* (2005) examined how cadmium and zinc, both on their own and in pairs, affected the genotoxicity of the tilapia fish *Oreochromis niloticus* using the micronucleus test. The results showed that the rates of micronuclei and nuclear abnormalities in the erythrocytes were much higher in all the treatment groups than in the control group, which did not receive any treatments. Furthermore, the study demonstrated that the genotoxic potential of these metal complexes, together with the concurrent administration of Cd and Zn, showed the presence of antagonistic interactions.

Dubey and Tripathi, (2014) examined the freshwater fish *Channa punctatus*, subjecting its red blood cells (RBCs), kidney cells, and gill cells to the micronucleus test to study the genotoxic effects of cadmium chloride. We examined the effects of dosage and duration on the frequency of produced micronuclei in different tissues. The gills had the most micronuclei, followed by the RBCs, while the kidney cells had the least. This was found by looking at how often micronuclei appeared in each of the tissues. The study's results suggest that the micronucleus test should be done on fish tissues to check for water contamination. The results also showed that the test might be used to check for and study water pollution and mutagens that are present in water (Choudhury, 2016). Talapatra and Nandy, (2014) concluded on how to find genotoxicity, especially how micronucleation in fish species erythrocytes happens when water is polluted. They stated that a genotoxicity test using erythrocytes is a simple way to find out how polluted water is in bodies of water that get industrial, agricultural, and residential wastewater.

Chaudhari and Sexana, (2015) assessed the micronucleus test in RBC to the genotoxicity induced by the pyrethroid pesticide fenvalerate in *Channa punctatus*. Three groups of fish were given three different sublethal amounts (0.0157, 0.0314, and 0.0625 ppm) during a period of up to thirty days. Researchers found that the induction of micronuclei was highest on the fifth day at a dosage of 0.0625 ppm of pesticide and then slowly went down (Sabina, et al. 2019).

Kouser and Javed, (2025) indicated that *Cirrihinus mrigala* had a mean value of 16.98+ 11.70% of micronuclei, which was far greater than the other three fish species: *Labeo rohita, Catla catla,* and *Ctenopharyngodon Idella*. The research lasted thirty days and looked at how three sublethal amounts of copper (17, 25, and 33, 50% of 96-hr LC50) affected each of the three fish species: *Ctenopharyngodon Idella, Catla catla, and Labeo rohita*. But the number of additional nuclear anomalies went in this order: *Labeo rohita> Cirrihinus mrigala> Ctenopharyngodon Idella> Catla catla.*

Jesus, *et al.* (2016) examined the *Oreochromis niloticus, Hoplias malabarius,* *Geophagus brasiliensis* and *Serrasalmus branditi* to resolve the frequency of nuclear abnormalities (NA). As a result of these findings, it was determined that *S. branditi* and *H. malabarius* had a much greater prevalence of nuclear abnormalities, such as bulbs, binuclei, lobes, micronuclei, notches and vacuoles. Also, fish taken in metropolitan areas exhibited a high frequency of NA, although O. niloticus appeared to be more adaptable to environmental conditions than other species.

Tasneem and Yasmeen, (2018) evaluated the effect of a sublethal dose of the pesticide karanjin on *Cyprinus carpio* over a 21-day period, using a dose equal to one- tenth of the 96-hour LC_{50} value (0.28 ppm). The micronuclei were totally missing in both the treatment and the control group; nonetheless, various nuclear abnormalities were seen, including karyolysed nuclei, blebbed nuclei, notched nuclei, pear-shaped nuclei, circular nuclei, lobed nuclei and blebbed nuclei. It was also determined that the different kinds of aberration and the total quantity of them grew in proportion to the length of the exposure time.

McLeese, *et al.*, (2021) said that oil and refined products of oil are formed of 75 percent short and long hydrocarbon chains. This makes oil one of the most difficult and unexpected mixtures to analyze from a toxicological aspect, since it is one of the mixes that is composed of both short and long hydrocarbon chains. It was seen and recorded that the occurrence of coal liquid dispersion, in which phenol is one of the key components, caused fathead minnows and rainbow trout to lose their capacity to reproduce completely. Phenol is one of the primary components of coal liquid dispersion. Because the short chains are ephemeral, they spend a much shorter amount of time in environments containing water. It has been shown that fish possess a significant capacity to provide a threat to other forms of aquatic life. Even though there is a significant degree of dilution in the waters around offshore locations, it has been shown that the total quantity of C1-C4 APs may reach concentrations of up to 140 ng/l in these waters. This is the case even though there is a large amount of dilution. Although the most dangerous compounds are volatile, there is still a chance that fish may quickly absorb some of the WSF. This may have a detrimental impact on the fish' overall biological organization. The phenol leak that took place in June 1993 and involved a road tanker accident was the root cause of the pollution that took place in the Peechi reservoir, which supplies drinking water to the central region of Kerala. The water storage facility may be found in the southern Indian state of Kerala. The water-soluble fraction (WSF) of crude oil may include a mixture of polyaromatic hydrocarbons (PAH), phenols and heterocyclic compounds that contain nitrogen or Sulphur. This mixture may also be found in crude oil. It has been hypothesized that the components of crude oil that dissolve in water are an important element in determining the toxicity of petroleum if it is released into the environment unintentionally. It is possible for the total amount of phenol and alkylphenols (APs) in water to range anywhere from 0.6 to 10 mg/l and this range is determined by the industrial field from where the water was collected. Produced water may include APs that have a higher degree of alkylation (butyl through heptyl, C4-C7), although these APs are typically present in smaller levels, ranging from 2-237 g/l. The reason for this is because phenol and other APs in the C1-C3 range have a water solubility that is rather high. These two categories are responsible for more than 95% of the total phenols found in water that have been manufactured. In general, the water solubility and degradation rate of APs decrease with increasing degrees of alkylation, however the bioaccumulation factor increases. This is because the bioaccumulation factor is proportional to the amount of AP that has been accumulated. On the other hand, the bioaccumulation factor can potentially become more prominent. Both the acute and the chronic impacts that these chemicals have on marine species are significantly influenced by the chemical structure of alkylphenols as well as the degree to which they have been alkylated (Mason-Johnes *et al.*2022).

P. Olmedo, *et al.* (2013) used perch, yearling trout and trout fry as experimental animals to assess the toxicity of a variety of chemicals present in tar. There are many different types of compounds in tar. Reports of the symptoms caused by the phenol and the cresols were produced. Higher concentrations caused a very typical rapid loss of balance; the fish turned on its side and showed a wild, dashing movement; the gill covers were first widely open and then tightly closed; the respiratory movements became weak and irregular; and the fish changed into a turtle just before it died. Phenol has been demonstrated to induce fibrillary twitching in frog muscles, which is followed by tonic convulsions and, ultimately, complete central nervous system paralysis. But according to other theories, phenol causes fish muscles to twitch in a fibrillatory manner. The researchers tested the toxicity of phenol on goldfish and discovered that it might paralyze the neuromuscular systems.

Kordylewska, (2022) observed that after being exposed to 100 mg of phenol per cubic meter of air for 15 days, there was a discernible shift in the normal functioning of the central nervous systems of the rats. There is some evidence to suggest that phenol has a deleterious influence on the reproductive processes of aquatic invertebrates, such as gastropods, fish, sea urchins and prawns. It was shown that the egg production of a species of copepod known as *Acartia clausi* was inhibited when the organism was exposed to phenol at a concentration of 0.5 mg l-1 over a period of eight days. Because phenol has a high level of lipophilicity, the nonionic forms of its chloro derivatives may be taken in by the process of passive diffusion. This is because phenol is a lipophilic compound. Phenol is known to be lipophilic, which means that there is a chance that it will accumulate at different levels of the food chain. Because of this, not only does phenol constitute a threat to the natural environment, but it also endangers the well-being of people. Poisoning from phenol is a concern that must be considered in fish- rearing methods. Phenol is produced spontaneously in the digestive tracts of animals and humans from tyrosine and its derivatives as part of the process of tyrosine metabolism. An organism known as *Hoplosternum littorale*, which is a facultative air breather, had its gas exchange and ion regulation altered when it was subjected to 12.5, 25 and 37.5% of the water-soluble fraction (WSF) of Urucu crude oil. They are typically found in the ocean and in fish tissues, where they cause acute or chronic toxicity when they are present. They are capable of a wide range of behaviour, the majority of which are hostile in nature. They are toxic to both the immune system and DNA and there is a possibility that they may induce cancer. revealed that persistent exposure to phenol at 0.1 mg l-1 can diminish the quality of sperm and reproductive success in sea urchins, which may provide a threat to the continuing survival of these ecologically important species.

Routledge and Sumpter, (2020) reported the estrogenic effects of these compounds and the huge amounts that are formed from the breakdown of APEOs, 4-t-OP and the various isomers of 4-NP have been the APs that have been the focus of the study that has been conducted on the topic of chronic effects. found that the placement of the alkyl group (para > meta > ortho), the branching of the alkyl group (tertiary > secondary = normal) and the size of the alkyl group all have a role in the estrogenic effect of alkylphenols, with 4-t-OP being characterized as the most potent estrogen. *Dicentrarchus labrax* was used as a test subject to evaluate the effects of phenolic compounds (phenol and OH-phenols) on an organism that was living in its natural habitat. According to the results, fish that had been treated with OH-phenols displayed metabolic difficulties such as hypoglycemia, a low blood urea nitrogen level (BUN) and a reduction in the activity of alkaline phosphatase (Saikia *et al.*, 2018).

These problems were caused by a decrease in the activity of alkaline phosphatase. It has been shown that environmental toxicants including o- and m-cresol may inhibit cyclooxygenase activity, platelet aggregation and thromboxane B2 production. This was one of the discoveries that was discovered. After being injected into the rats' stomachs by gastric intubation, it was discovered that cresol was absorbed immediately into the animals' bloodstreams. This took place in both the stomach and the small intestine at the same time. The researchers investigated the effect that the effluent from a paper mill had on the ATPases found in the gills of the freshwater fish *Channa punctatus*. They found that the effluent decreased the activity of total ATPase, ouabain-insensitive ATPase and Na+, K+-ATPase, with the Na+, K+-ATPase activity exhibiting the largest degree of impairment. ATPases are enzymes that produce energy from ATP and ADP. As an in vitro model, we investigated the effects of pentachlorophenol (PCP) on primary cultures of hepatocytes derived from freshwater crucian carp (*Carassius carassius*). PCP was shown to increase the quantity of Ca^{2+} that was present inside the cell, even though it produced a reduction in Ca^{2+}, Mg^{2+} ATPase activity and ATP content. These changes were brought about by PCP (Rocha, *et al.*, 2011).

Pradhan, *et al.,* (2020) studied that phenolic compounds are agents that may create reactive oxygen species. This can result in considerable damage to cells, such as the oxidation of polyunsaturated lipids found in membranes. Phenolics are a kind of compound. Some of them are assumed to be agents that create reactive oxygen species, while others are thought to be scavengers for free radical species. Both hypotheses are supported by the available evidence. When compared to the control group, the long-term exposure of the freshwater fish Carassius auratus to 2, 4-dichlorophenol demonstrated that the activities of catalase (CAT) and selenium-dependent glutathione peroxidase (Se-GPx) as well as the amount of oxidized glutathione (GSSG) were significantly higher overall. After being exposed to petroleum (oil in water dispersions), it was shown that all the tissues of the catfish, *Clarias gariepinus*, had increased levels of lipid peroxidation, superoxide dismutase and catalase activity. *Carassius auratus* fish were given varying doses of 2, 4 and 6-trichlorophenol through intraperitoneal injections to investigate the effects on hydroxyl radical production and oxidative stress in the liver of these fish. Under the action of 2, 4, 6-trichlorophenol, the formation of free radicals was discovered to have greatly increased, whereas the activities of antioxidant enzymes such as CAT, SOD and GST were shown to have reduced. This was the conclusion drawn from the research. This was a direct result of the acts that were carried out by the chemical. It was also discovered that the ratio of GSH to GSSG had decreased and there was a significant spike in the quantity of MDA that was present; these results provided evidence that C. auratus had been injured because of oxidative stress. evaluated the possible effects of the wastewater pollution induced by a petroleum industry on the oxidant/antioxidant status of muscle and liver tissues obtained from fish that were gathered from the Kizilirmak River in Kirikkale, Turkey. These fish were taken from the Kizilirmak River. According to the results, some contaminants from the petrochemical industry are to blame for the oxidation that occurs in the muscular tissues of fish. This is because these pollutants cause the antioxidant system to become dysfunctional. shown that 3-(dimethylamino) phenol increased the quantity of free radicals and altered the properties of the cell membrane. In addition to this, it caused a significant oxidation of the hemoglobin in human erythrocytes and changed the activities of glutathione peroxidase, catalase, superoxide dismutase and acetylcholinesterase.

Mulcahy, (2021) observed that fish blood is increasingly used in environmental monitoring and toxicological research as a reliable indicator of physiological and pathological changes, particularly in disease assessment and fisheries management. In environmental toxicology, alterations in the physiological and biochemical characteristics of fish following exposure to toxicants provide an effective approach for evaluating water quality, as blood circulating through the gills is in direct contact with the surrounding aquatic environment and can rapidly reflect adverse changes in water conditions. Such hematological studies are therefore useful for assessing both water quality and the overall health status of fish populations. Blood chemistry has long been recognized as an important diagnostic tool in clinical, toxicological, and pathological investigations, and fish hematological parameters have been proposed as valid biomarkers for evaluating pollutant-induced risks (Roche & Boge, 1996). Because fish live in close contact with their environment, they are highly sensitive to physical and chemical changes, which are often reflected in their blood components. Blood reflects the physiological condition of an organism at any given time, as it facilitates intercellular and intracellular transport and maintains direct contact with various organs and tissues. Although hematological responses to chemical stressors may not be highly specific, they provide valuable information for impact assessment studies by indicating general physiological condition, metabolic status, and health. Key hematological markers, such as hemoglobin concentration (Hb), packed cell volume (PCV), and red blood cell (RBC) count, are widely used to assess physiological responses to sublethal exposure to toxic substances, as changes in erythrocyte count or hemoglobin levels are critical indicators of blood volume alterations, including hemodilution or hemoconcentration, particularly under chronic stress conditions.

McLeese *et al..*, (2022) From an ecotoxicological perspective, the interactions between toxic products and biological membranes are based on two fundamental elements: first, the disruptions caused at the level of structure and membrane functions that pose a threat to the cell's life and second, the crossing of these barriers, which is a prerequisite for the contaminants' accessibility into the cell or organism. Understanding the nature of the interactions between hazardous chemicals and biological membranes requires knowledge of both factors. Before any of these two aspects can be regarded as completely understood, it is necessary to understand the nature of the interactions that take place between biological membrane and dangerous substances. Erythrocytes are a good model for the study of membrane stability because, when they die, they produce the protein hemoglobin, which is easily detectable using spectrophotometry. This makes erythrocytes an ideal candidate for this kind of research. Because of this, erythrocytes are an excellent model to employ for researching the stability of membranes. In addition, since both its structure and function are so simple, it is an ideal candidate for use as a model organism in toxicity research because it is so easily implementable. This makes it an excellent option. There is a relationship between the osmotic stability of erythrocytes and their resistance to lysis because of changes in the tonicity of the solvent. This relationship exists because there is a link between the two. After a predetermined amount of time has elapsed, during which the blood is allowed to incubate in solutions containing gradually decreasing concentrations of sodium chloride, the osmotic stability of erythrocytes may be evaluated by measuring the quantity of hemoglobin that has been released from the cells. This can be done after blood has been allowed to incubate for an allocated amount of time in solutions containing progressively decreasing concentrations of sodium chloride. The effects of phenol on the phospholipid and fatty acid makeup of the plasma membrane of carp erythrocytes at doses lower than those necessary for mortality. Fluidity and stability are two characteristics that at first glance could seem to be at odds with one another. However, for cell membranes to efficiently carry out their functions, they need to include both characteristics. For signaling and transit to function well, there must be a certain degree of fluidity in the environment. This is required. On the other hand, an excessive quantity of movement may make the structure less stable and vice versa; this is true whether there is a little bit of mobility or a large amount of mobility. The capability of this biological complex to retain its structure in the presence of chaotropic circumstances, such as oxidative stress, hypotonicity, pH extremes, heat and the presence of solutes (such as ethanol, urea and guanidine), is referred to as membrane stability. These chaotropic situations include oxidative stress, hypotonicity, pH extremes, heat and the presence of solutes. Oxidative stress, hypotonicity, pH extremes, heat and the presence of solutes are all examples of chaotropic conditions. Changes that occur biochemically, immunologically, physically, or chemically in the blood might be responsible for hemolysis. To be more precise, it may be caused by extrinsic factors such as immune system diseases, infections, drugs, exposure to toxins, or damage to the red cells that are induced by mechanical stress. The erythrocyte membrane is made up of a lipid bilayer that has membrane-spanning proteins, hydrophilic sugar residues (sialic acid and galactose) and a protein meshwork (spectrin) on the cytoplasmic side of the lipid bilayer. The membrane also has hydrophilic sugar residues (sialic acid and galactose) on the exterior side. The erythrocyte membrane is made up of membrane-spanning proteins, which are responsible for its development. This protein meshwork is related to transmembrane proteins by a noncovalent binding process that is mediated by ankyrin and b and 4.1 proteins, respectively. Some examples of these proteins are glycophorin and b and 3 proteins. Erythrocyte membranes not only provide the necessary amount of mechanical stability to Withs and the shear stress that is endured when the blood is flowing through tiny blood arteries, but they also perform essential activities such as acting as cell-surface receptors, signal transducers and immunological functions. This is because erythrocyte membranes are composed of two layers: the inner and outer layers. Maintaining this level of mechanistic steadiness is necessary for one's continuing existence. It has been shown that the theory that the toxicity of phenol, which is related to the generation of oxygen between cells, directly affects the membrane lipids of an aquatic animal (fish), most likely because of its direct effect on the enzymes that directly influence fatty acid metabolism, is correct. This is because it has been shown that phenol directly affects the enzymes that directly influence fatty acid metabolism. This is most likely because phenol has a direct influence on the enzymes that are involved in the metabolic process of fatty acids. It is common for the acute toxicity of alkylphenols (APs) to grow in tandem with the hydrophobicity of the compounds, and the concentrations of the most poisonous alkylphenols that affect aquatic creatures, such as fish, are in the range of mg/l. Even though most compounds produce either polar or non-polar narcosis, several chemicals exhibit a greater degree of toxicity than can be expected only based on the narcosis mode of action. This is the case even though most compounds generate either polar or non-polar narcosis. Even though most drugs elicit either polar or non-polar narcosis, this is nonetheless the case. It is thought that the presence of these substances induces a particular uncoupling of oxidative phosphorylation. This uncoupling, in turn, leads to a decrease in the activity of metabolic processes inside organisms, which in turn leads to a reduction in the amount of energy used by the organisms. It is hypothesized that nonylphenol and some alkyl phenol ethoxylates are to blame for variations in the permeability of the mitochondrial membrane, which ultimately leads to a decrease in the quantity of metabolic activity. Considering these facts, an effort has been made to investigate, under in vitro settings, how the stability of erythrocyte membrane is influenced by the impacts of some phenolic compounds (phenol, m-cresol and 4-nonylphenol). This investigation has been carried out with the intention of finding out how the erythrocyte membrane is affected. This experiment was carried out to determine how the effects of these compounds affect the erythrocyte membrane. The fact that erythrocytes are the targets of a substantial number of xenobiotic chemicals is a fact that has been well recognized. As a direct result of this fact, toxicity endpoints that are related to the functions of erythrocytes have been used largely over the course of the past few years to evaluate the harmful effects of a wide variety of toxicants and to investigate the mechanisms of their action utilizing appropriate in vitro systems. This has been done to determine whether the toxic effects of these toxicants are harmful to living organisms and to determine how they work. This has been done to safeguard human beings from the possibly hazardous consequences that these toxicants may have.

In 1992, Murugesan and his team did a lot of research and made it public. The authors assessed the physico-chemical and biological quality of the Chittar River in Courtallam, located in Tamil Nadu, India. The analysis of many water samples revealed that, while the river exhibited a relatively low degree of pollution, its biological condition was very poor. The remaining physico-chemical parameters that were tested, except for sulfate, were found to be within the permitted limits. But the levels of both total and fecal coliform were greater than what is permissible, which shows that the river is in bad shape.

Kumar, *et al.* (2010) studied few of the rivers' physical and chemical parameters (temperature, pH, total hardness, total alkalinity, BOD, COD, and turbidity) and found that Haridwar was the most polluted of the Uttarakhand rivers (Devprayag, Gangotri, Haridwar, Rudraprayag, Dakpathar, and Yamunotri).

Helen, *et al.* (2011) examined water samples for physico-chemical parameters, including pH, electrical conductivity, turbidity, total dissolved solids, alkalinity, total hardness, dissolved oxygen, BOD, COD and anions (Ca, Mg, Fe, Mn, NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{2-}, F and Cl^-). The water samples' concentrations of most of the criteria under investigation were higher than the WHO's allowable level.

Khound *et al.* (2012) provided an in-depth analysis of the surface water quality in the Jia-Bharali River basin. The following pattern may be seen in the principal ion contents: Ca > Na > Mg > K, while the composition of the anion follows the pattern HCO_3 > Cl > SO_4 > PO_4 > NO_3. The spatial and temporal variability of the physicochemical parameters found in this research (within the acceptable limitations set by the WHO) might be utilized in the future as baseline data to monitor and control any changes that occur because of changes in land use.

Jena *et al.* (2013) assessed the physical and chemical properties of the Kharoon River, such as its turbidity, pH, EC, total dissolved solids, total suspended solids, conductivity, dissolved oxygen, biological oxygen demand, chloride, total alkalinity, hardness, sodium, potassium, calcium, magnesium, and sulfate concentrations. The consequences of human activity have caused various measures to go up, yet these increases stayed within the WHO's allowed bounds.

Yadav, *et al.* (2013) assessed the water quality of the Satak reservoir in Madhya Pradesh, India, employing physico-chemical parameters (including chloride, total hardness, alkalinity, temperature, and pH) and determined that the reservoir was classified as a mesotrophic water body, slightly inclined towards eutrophication.

Rauf, et al. (2019) did research measuring amounts of heavy metals (cadmium and chromium) in the gills, kidneys, liver, skin, muscles, and scales of three fish species from the river Ravi: *Catla catla, Labeo rohita,* and *Cirrhina mrigala*. Compared to *Catla catla*, which showed substantially higher levels, *Labeo rohita* and *Cirrhina mrigala* exhibited lower levels of metals. Baloki Headworks had the highest amounts of metal contamination, perhaps because there were more effluent from both sewage and industrial water.

Butu and Bichi, (2013) investigated the spatial variation in the concentration of various heavy metals in the Galma Dam, Nigeria, concluding that elements sourced from weathering activities are predominantly found in the upper sections of the dam, while elements from anthropogenic sources, including copper, chromium, lead, cadmium, and zinc, are more prevalent in the lower section. Studies done in the past on *C. batrachus* contributed significantly to our understanding of its taxonomic status, distribution pattern, habitat range, physiological characteristics and eating habits.

According to Wokoma (2022), out of the seven species of *Clarias* that may be found on the Indian subcontinent, *C. batrachus* is the one that is found the most often.

Lidia Robaina, *et al.* (2019), conducted research on the pollutant poisoning of *C. batrachus* that occurred via the use of culture Pond water. Mukhopadhyay, *et al*. (1986) performed the research necessary to establish a correlation between the enzymatic activities of *C. batrachus* and its dietary routines.

In their 2016 study, Malakootian *et al.* investigated how the amount of times *C. batrachus* fed affected the species' overall development as well as its dispersion.

Kumar (2018) looked at the impact that the toxicity of sodium arsenite has on the hematological profile. Tripathi (2025) performed an analysis on the breeding pattern of *C. batrachus* when it was subjected to intense farming.

E.F. Fanta, (2003) conducted an exhaustive investigation of the extra respiratory organs that are present in *Clarias batrachus*. Sharma, *et al.* (2017) investigated the effects of extreme salinity on the growth, biochemical composition and rate of survivorship of *C. batrachus*. The screening and use of autochthonous probiotics to produce native *Clarias batrachus* is now receiving a significant amount of attention all over the globe. However, there are several publications available on the use of probiotics in *C. gariepinus*, although our information on *C. batrachus* is still rather restricted. Research on the use of probiotics to produce *Clarias* species using a variety of techniques has been encouraged because of the importance of achieving sustainable aquaculture.

It is common for probiotic isolates to release extracellular enzymes (such as amylases, proteases and lipases) or growth factors (such as vitamins, siderophores, fatty acids and amino acids), which may increase fish nutrition by more effectively digesting indigestible meal components. Supplementing the diet with Lactobacillus acidophilus led to increased growth, survival and utilization efficiency in *C. gariepinus*, according to Ebrahimi *et al.* (2020). Bacillus licheniformis, a bacterial strain with the capacity to synthesize enzymes, was isolated from an adult *C. batrachus* by kumar and colleagues (2015).

During the Mahakumbh celebration in 1989, Srivastava *et al.* (2010) investigated the water eminence of the Ganga River near Phaphamau (Allahabad). The findings led researchers to the conclusion that mass bathing had a major impact on the river's water quality. This was because the biological oxygen demand, chemical oxygen demand, and fecal coliforms all went up during bathing time. The investigation shows that the water shouldn't be utilized for drinking or bathing. Sharma *et al. (*2006) performed research to determine the impact of mass bathing during the Ardhkumbh festival on the water quality state of the river Ganga. According to the findings of the research, the water should not be used for either drinking or bathing reasons. The detection of faecal coliforms in water is indicative of the possible existence of pathogenic bacteria, the presence of which may lead to illnesses that are transmitted by water (P.K. Talwar, 2015). During bathing, it was discovered that the amount of dissolved oxygen was within the acceptable range, but the values of BOD and COD were determined to be higher than the maximum acceptable level. A Patra *et al.* (2023) conducted research to determine the effect that human activity has had on the water quality of the Ganga River.

At the sewage mixing point on the river Rapti, Agrahari and Kushwaha, (2012) found that there was a drop in pH, DO and carbonate alkalinity while there was an increase in electrical conductivity, TDS, free CO_2, bicarbonate alkalinity, total, Ca and Mg hardness, chloride, nitrate, phosphate, sulphate, BOD and COD. They also found that there was an increase in BOD and COD. On the other h and, these values progressively altered as one moved downstream and by the time they reached the Indian requirements, they were inside the acceptable range, suggesting that the river water is suitable for the development of aquatic life. Tanushri Deb Nath and Shaharior, (2014) investigated how pollutants affect the water quality of the Sutlej River in the Nangal area of Punjab. The study found that the river's water was moderately polluted because pollutants were dumped there often, silted and muddy water flowed in from the mountains, and the water's physical and chemical properties changed. The main research showed that the water was not safe for people to drink, but it could be used to breed wild animals, in the fishing industry, and for irrigation in agriculture (Baka S. et al, 2014).

Saikia and Das, (2018) According to the published research, there has been very little investigation into the water quality in the northeastern part of India, with most of the data coming from Assam. Research has been done on the river Kolong's water quality. Singh, *et al.* (2021) examined physiochemical parameters including pH, temperature, dissolved oxygen, total hardness, total alkalinity, biochemical oxygen demand, total dissolved solids, total suspended solids, and chloride in the Haora River in Agartala during the immersion of the Durga idol. They also looked at heavy elements including chromium, lead, cadmium, and arsenic in the river. They looked into the water studies that have been done on the effects of mining coal and limestone on the water quality in Meghalaya. For example, studies have shown that the water quality of the Lukha River is affected by human activities like mining coal and limestone and making cement in the area where the river flows. Research has also been conducted on the effects of coal and limestone mining on water quality in Meghalaya. looked at the physical and chemical qualities of the water in Kaziranga National Park's wetlands, which are in Assam.

Katherine, *et al.* (2016) When trying to bridge the gap between behavior and physiology, fishes are an especially good creature to employ as a model organism. Fish make up the most species of any order of vertebrates, with over 25,000 different species. They also have an unparalleled variety of life cycle patterns, breeding systems, sensory systems and environmental requirements. As a result, fish provide an almost infinite testing ground for either research focusing on a single species or comparative investigations into the connections between behaviors and physiology. The connection of an organism with its environment results in internal changes, which in turn impact on behavior, eating and reproduction. This seasonal regulation of many biological activities is brought about by the interaction of the organism with external stimuli.

Boeuf and Bail, (1999) described the primary source of natural light, that has been categorized as a determining element. to maintain a level of control over the growth performance of various species. When it penetrates the top layer of the aquatic environment, the radiant energy that comes from the sun is selectively absorbed and then dispersed. According to the atoms, molecules and particles, the local weather conditions and the amount of radiant energy that has been reflected by the water's surface, 10% of the energy is lost due to reflection.

Aberoumad, *et al.* (2010) has been observed that the amount of light that fishes get, both directly and indirectly, has a significant role in their life. The periodicity of life is connected to most of the basic rhythms that can be found in nature, whether they are diurnal or seasonal. According to Agrawal, *et al.* (2010), the activity levels of many kinds of animals, including fish, follow a 24-hour cycle. The amount of light present has a considerable impact on many elements of fish life, including their behavior, notably the diurnal activities they engage in, as well as on many others. Researchers Ackerman *et al.* (2020) concluded that light has an impact on metabolism, development, behavior and other aspects of fish.

Paulo *et al.* (2006) noted that fish with nocturnal eating habits like catfish or species that are aggressive may enhance feed intake while held in darkness, but those fish with diurnal feeding habits may develop better on longer photoperiods. In recent years long photoperiod regimes have been used in Atlantic Salmon (*Salmon salar*) rainbow trout (*Oncorhynchus mykiss*), Atlantic halibut (*Hippoglossus*), Sea bream (Sparus aurata) and sea base (Dicentrichus labrax) to enhance growth and or to delay sexual maturity on silver cat fish, *Rhamdia quelen, Sktichney* and on channel cat fish *Ictalurus puntatus*, on African cat fish *Clarias gariepineus*. Alteration of photoperiodic cycles has a measurable effect on eating behavior. Masopust, (2008) studied fisheries to biologically assess the physiological response to environmental stress. This information is particularly useful when comparing studies of various fish species that live in diverse environments. Changes in fishes blood parameters that occur on a cyclical basis have been documented by many workers. It has been said that the hematological profiles of blood may give significant information on the internal environment of the organism.

Chapter 3

RESEARCH METHODOLOGY

Throughout tropical Asia, the omnivorous freshwater fish *Clarias batrachus* is a prized delicacy. Its resilience and rapid growth rate make it a popular cultivation plant. It is a plankton-phagous and pelagophile species. It consumes unicellular algae as a larva. It is an economically important pan fish, cultured in fresh water as well as in brackish water Ponds in tropical countries. *C. batrachus* is a highly cultivable species and contributes to the provision of animal protein in India. It readily adapts to laboratory conditions, as it is easily available, inexpensive and suitable for field studies.

All the materials and methods have followed the national and international standards/regulations of National Fisheries Development Board (NFDB), International organization for standardization (ISO), FAO (Food and agricultural organization) and American public health association (APHA).

3.1 Sampling Ponds

The current research investigates the water quality of five historically significant sacred Ponds (kunds) in Varanasi, Uttar Pradesh. Details of these selected Ponds are provided in Table 3.1. Water samples were collected using sterile one-liter PTFE (polytetrafluoroethylene) containers to ensure sample integrity during transport and analysis. This approach enables a precise assessment of the physicochemical parameters influencing the ecological and cultural value of these ancient water bodies.

Table 3.1 Detailed description of five ancient sacred Ponds (kunds) in Varanasi city along with the respective point and non-point sources of pollution

Sampling Site		Site Description	
Name	**Picture**		
Suryakund Pond **Latitude:** 25°18'43.2"N **Longitude**: 83°00'09.3"E		• Pale green • Ritual activity, ritual materials damping. Devotees bathing. Heaps of garbage. • Idol immersion	There is significant anthropogenic pressure on it as a result of the dense urban settlements nearby.
Pishach Mochan Latitude: 25°19'18.7"N Longitude: 82°59'43.6″E		• Pishach mochan Pond gives salvation to wandering souls. • Ritual activity, ritual materials dumping. Devotees bathing. Heaps of garbage. • Idol immersion • Ceremonial activity -Pind Daan & Tripindi Sradh	
Pushkar Pond Latitude: 25°17'09.0"N Longitude: 83°00'16.0"E		• Oldest historical Ponds • Holds social significance, and is presently the subject of a legal dispute • Heaps of shredded plastic bags and clay cups • Idol immersion, devotees and animal bathing, washing clothes	
Ishwar Gangi Pond Latitude: 25°19'31.5"N Longitude: 83°00'30.3"E		• Devotees gather here to immerse their loved ones ashes in the water in the firm belief that doing so will aid the dead in their salvation. • Occasional practice of bathing, washing clothes, throwing organic waste, etc. in the form of flowers.	
Kurukshetra Pond Latitude: 25°17'23.8"N Longitude: 83°00'07.8″E		On religious and social occasions, there may be the occasional practice of bathing. Washing clothes, throwing organic waste, etc. in the form of flowers.	

3.2 Methodology

The following headings provide an explanation of the methods taken for the current investigation:

3.2.1 Preliminary survey

To take water samples, extensive research was done to determine the true condition of ancient religious Ponds. To regulate the problems, they had with the Pond's water, devotees and locals were queried.

3.2.2 Sampling and preservation

Water samples were collected using standard methods for the examination of water and wastewater (APHA, 2001 and IS 10500: 2012). Pre-monsoon (May), monsoon (August) and post-monsoon (November) seasons of the Pond were sampled in 2021 and 2022 respectively. At each sampling location, Pond water samples were taken in triplicate. In pre-acidic cleaned PTFE bottles with a volume of 1000 ml, water samples were collected. Water samples were placed in bottles that were then labeled and carefully sealed.

Samples for biological oxygen demand, chlorophyll-a and bacteriological studies were preserved at a temperature of < 4 ^{o}C in the ice box. Once samples reached the lab, samples were placed in the refrigerator. Samples for chemical oxygen demand, nitrate, phosphate, chloride, and sulphate were preserved at pH < 2 by the addition of concentrated H_2SO_4. For metals, samples were acidified with conc HNO_3 to a pH lower than 2 for its preservation. As promptly as possible, ideally within 24 hours, samples were brought to the lab.

Table 3.2: Preservation techniques of water samples for chemical analysis (APHA-AWWA-WEF, 23rd Edition, 2017)

Sr. No	Tests/Properties Measurements'	Recommended Sample volume (ml)	Type of Container	Preservation	Allowable holding time
1	Temperature	100	PTFE/Glass	Determined at site	N/A
2	pH Value	100	PTFE/Glass	Determined at site	N/A
3	Electrical Conductance	100	PTFE/Glass	Determined at site	N/A
4	Total Dissolved Solids (TDS)	100	PTFE/Glass	Refrigerate - 4 °C	7 days
5	Chloride	50	PTFE/Glass	Not Required	7 days
6	Sulphate	50	PTFE/Glass	Refrigerate - 4 °C	7 days
7	Nitrate	100	PTFE/Glass	Refrigerate - 4 °C, H_2SO_4 to pH<2	24 hr
8	Phosphate	50	PTFE/Glass	Refrigerate - 4 °C	24 hr
9	Elemental Analysis-Pb, Ni, Cu and Zn	200	PTFE/Glass	Refrigerate - 4 °C, HNCO3 to pH<2	6 months
10	DO	100	PTFE/Glass	Determined at site	NA
11	BOD	1000	PTFE/Glass	Refrigerate - 4 °C	24 hr
12	COD	50	PTFE/Glass	H_2SO_4 to pH>2	7 days

Preservation techniques for *Clarias batrachus* for morphometric and histopathological analysis

After collection from the Pond, fish were rinsed with $KMnO_4$ solution to prevent dermal infections, placed in well-aerated containers and transported live to the laboratory within 24 hours for Morphometric analysis.

Liver and gill tissues for histopathology were preserved in 10% neutral buffered formalin, following standard guidelines.

3.2.3 Water and Wastewater Testing

Analyses were performed following APHA-AWWA-WEF *Standard Methods* (23rd ed., 2017), with method references provided in the table below (Table-3.3).

TABLE 3.3. Water and Wastewater Testing Based on APHA-AWWA-WEF Standard Methods (23rd ed.,2017)

Sr. No.	Water Quality Parameter	Abbreviation	Unit	Analytical Method	Reference
1	Temperature	T	˚C	Portable multi-parameter	-
2	pH Value	pH	Moles/l	Portable multi-parameter	-
3	Electrical Conductance	EC	mS/cm	Portable multi-parameter	-
4	Total Dissolve Solid	TDS	Mg/l	Portable multi-parameter	-
5	Chloride	Cl^-	Mg/l	Amperometric Titration Method	APHA (23rd ed),2017
6	Sulphate	SO_4^{2-}	Mg/l	Turbidimetric Method	APHA (23rd ed),2017
7	Nitrate	NO_3^-	Mg/l	Ultraviolet Spectrophotometric Screening Method	APHA (23rd ed),2017
8	Phosphate	PO_4^{3-}	Mg/l	Stannuos Chloride Method	APHA (23rd ed),2017
9	Elemental Analysis-Pb, Ni, Cu and Zn	Pb	Mg/l	Atomic Adsorption Spectrometry	APHA (23rd ed),2017
10	DO	DO	Mg/l	Winkler's Iodometric Method	APHA (23rd ed),2017
11	BOD	BOD	Mg/l	5-Day BOD Test Method	APHA (23rd ed),2017
12	COD	COD	Mg/l	Open Reflux Method	APHA (23rd ed),2017

3.2.4 Testing on-the-spot

To stop any changes from happening during shipment, a number of factors were examined right away, including pH, temperature, electrical conductivity, total dissolved solids, and dissolved oxygen.

3.2.4.1 Temperature

The portable multi-parameter EUTECH PCSTestr 35 was used to assess the temperature of the water samples at the sampling location in Pond water. The result was expressed in °C.

3.2.4.2 pH

The EUTECH PCSTestr 35, a portable multi-parameter instrument, was used to test the pH of the water samples at the sampling site.

3.2.4.3 Electrical Conductivity

The portable multi-parameter EUTECH PCSTestr 35 was used to measure the Electrical Conductivity at the sampling location. The measurement was in mS/cm.

3.2.4.4 Total dissolved Solids

The portable multi-parameter EUTECH PCSTestr 35 was used to measure the total dissolved solids at the sampling location. The measurement was in mg/l.

3.2.4.5 Dissolved Oxygen (DO)

We used a universal dissolved oxygen meter to find out how much dissolved oxygen was in samples of pond water and reported the results in mg/l. A magnetic stirrer is put inside the incubation bottle, which is then filled with water and put in an incubator. The magnetic stirrer within the bottle keeps spinning. Then, the dissolved oxygen meter was used to take three readings: one at the bottom of the bottle, one in the center, and one at the top. The arithmetic mean of the data was used to figure out how much oxygen was dissolved in the Pond water sample.

3.2.5 Laboratory testing

Water samples were filtered via Whatman No. 42 filter paper to get rid of suspended particles. The samples physicochemical characteristics, as well as any trace heavy metals, were all analyzed.

3.2.5.1 Chloride (Cl)

A 50 mL aliquot of Pond water was carefully transferred into a porcelain evaporating dish for further analysis. (Frank *et al.*., 2000). A second dish with the same volume of distilled water was then used to compare the colours. 1 mL of potassium chromate indicator was added to each of them. After that, a standard silver nitrate solution was gradually added to the burette sample while it was being constantly stirred. A comparison with distilled water was used to ascertain this. It was noted how much silver nitrate was utilized.

Calculation

$$\text{Chloride (Cl), ppm} = \frac{\text{Volume of AgNO}_3 \text{ used x 500}}{\text{Volume of Sample}}$$

3.2.5.2 Nitrate

The sodium salicylate technique was used to determine the nitrate content. Nitrate changes into sodium paranitrosalicylate in the presence of sodium salicylate, which has a yellow colour Jean and is detectable by a spectrophotometer at a wavelength of 415 nm. The measured value of the sample is then subtracted from the measured value of the blank (distilled water). Using the calibration curve, the outcome is computed. The curve directly reveals the nitrogen concentration, given as mg N/l of sample, for a 10ml sample. Multiply the value by 4.43 to get the nitrate (NO_3).

3.2.5.3 Phosphate

We used 500 ml of distilled water to mix with 70 ml of concentrated sulfuric acid to generate 5N sulfuric acid. To produce a solution of potassium antimony tartrate, 1.3715g of potassium antimony tartrate was mixed with 500ml of distilled water. The ammonium molybdate solution was made by mixing 20g of ammonium molybdate with 500ml of distilled water. To make an ascorbic acid solution, 1.76g of ascorbic acid was dissolved in 100ml of distilled water.

To make the mixed reagent, combine 15 ml of ammonium molybdate solution, 50 ml of sulfuric acid (5 N), 5 ml of potassium antimony tartrate solution, and 30 ml of ascorbic acid solution. To generate a stock phosphate solution, 219.5 mg of KH2PO4 were mixed with 1000 ml of distilled water. From stock phosphate solutions, six different standard phosphate solutions were made: 0.02 mg/l, 0.04 mg/l, 0.06 mg/l, 0.08 mg/l, 1 mg/l, 1.2 mg/l, 1.4 mg/l, and 1.8 mg/l. A little amount of phenolphthalein was added to a 50 ml sample. A crimson color appeared after adding a drop of 5N sulfuric acid. After that, 8 ml of the combined reagent was added to the sample, the blank, and the standard solutions. Then, the spectrophotometer calibration curve, which was made using standard values, measured absorbance at 880 nm. The results were obtained using the linear equation shown below:

Calculations

y = mx + c

Where,

y = Absorbance of sample

m = Slope of line

c = Blank correction

x = Concentration of phosphate in mg

$$\text{Phosphate, ppm} = \frac{X \, X \; 1000}{\text{Volume of sample taken}}$$

3.2.5.4 Biochemical oxygen demand (BOD)

The IS: 3025 (part 44) method was used to calculate the biochemical oxygen demand. In one liter of water, 400 g of $MnSO_4.2H_2O$ was added to make a manganous sulphate solution. We made alkaline iodide sodium azide solution by mixing sodium iodide solution (500 g of sodium hydroxide and 135 g of sodium iodide in 1000 ml of distilled water) and sodium azide solution (10 g of sodium azide mixed in 40 ml of distilled water). To make a sodium thiosulfate stock solution, 25 g of sodium thiosulfate was mixed with 1000 ml of distilled water. We mixed 25 g of sodium thiosulphate with 1000 ml of distilled water to generate a stock solution. To make a starch indicator, boil 2 g of starch in distilled water. A calcium chloride solution was made by mixing 27.5 g of $CaCl_2$ with 1000 mL of distilled water. Magnesium sulphate solution was produced by mixing 22.5 g of $MgSO_4.7H_2O$ with 1000 ml of distilled water. 100 mL of distilled water was mixed with 0.15 g of ferric chloride to produce a ferric chloride solution. 8.5 g KH_2PO4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4.7\ H_2O$ and 1.7 g NH_4Cl were dissolved in 1000 ml of distilled water to prepare the phosphate buffer solution. Five mL of each solution—calcium chloride, magnesium sulfate, ferric chloride, and phosphate buffer solution—was added to 5000 mL of distilled water to make the dilution water. After adding 10 mL of sample water, a 300 mL BOD container was filled with dilution water. After that, glass stoppers were put on the samples right away, and they were kept in the BOD incubator at 20 °C for five days. Again, 10 ml of the sample was obtained and put into a 300-mL BOD container with water for dilution. A micropipette was used to add 2 ml of $MnSO_4$ solution just below the surface. A 2 ml solution of sodium azide and alkaline iodide was also added. They let the bottles sit still for a bit so that the precipitation might settle on them. Adding two ml of strong sulfuric acid dissolved the flocculants. A 20-ml sample solution from the BOD container was put in a flask and titrated against a sodium thiosulfate solution. After the yellow color had already formed, 1 mL of starch solution was added. This made the blue color emerge. Titration was done until the blue color was gone. The reading on the burette was taken. The same thing was done after five days of incubation at 20 °C in the BOD incubator with sample solutions.

Calculation

$$\text{BOD, ppm} = \frac{(\text{DO}_0\text{-DO}_5\text{-BC})\ X\ \text{Volume of diluted sample}}{\text{Volume of sample taken}}$$

Where,

DO_0 = Initial DO of diluted sample

DO_5 = DO at end of 5^{th} day

BC = Blank correction

3.2.5.5 Chemical oxygen demand (COD)

The IS: 3025 (part 58) technique was used to perform a chemical oxygen demand test. A standard potassium dichromate reagent was made by mixing 167 ml of concentrated sulfuric acid with 4.913 g of potassium dichromate and 33.3 g of mercuric sulphate. The following day, distilled water was added to the solution to bring the volume to 1000 mL. To make the sulphuric acid reagent, 5.5 g of silver sulphate crystals were added to 500 mL of concentrated sulphuric acid. Then, the crystals were allowed to dissolve in this mixture overnight totally. Dissolving 39.2 g of ferrous ammonium sulfate crystals in 1000 ml of distilled water produced a typical ferrous ammonium sulphate solution. There are 2.5 ml samples in each COD vial. Next, 1.5 ml of potassium dichromate reagent and 3.5 ml of sulfuric acid reagent were added. After properly sealing the vial, the COD digester was heated to 150 °C for two hours. One vial was also left empty. After being on ice for two hours, the vials were brought back to room temperature. After the samples were put in flasks, ferroin indicator was added to them. When ferrous ammonium sulfate was added to the sample solutions in the burette, they became reddish-brown. The amount of titrate that was utilized was recorded.

Calculation

$$\text{COD, ppm} = \frac{(\text{A - B}) \text{ X N X 8 X } 1000}{\text{Volume of sample taken}}$$

Where,

A= Volume of ferrous ammonium sulphate for blank

B= Volume of ferrous ammonium sulphate for sample

N= Normality of ferrous ammonium sulphate

V= Volume of sample

8= Milli equivalent weight of oxygen

To convert the sample size from ml to l results were multiplied with 1000ml/l.

3.2.5.6 Sulphate

The calibration curve was used to perform the turbidimetric method's examination of sulphate ions. This was produced by adding 3.0 mL of 0.1 mol/L $BaCl_2$ solution and precisely measured quantities of 0.01 mol/L sulphate solution (0.5-3.0 mL) in 50 mL volumetric flasks. Using a digital spectrophotometer (1 cm glass cell) in comparison to distilled water, the samples were diluted with distilled water before the turbidity was determined after 5 minutes of standby. The selectivity coefficients were used to assess the turbidimetric method's selectivity. These were estimated as the sulphate ion concentration to the interfering ion (CO_3^{2-} or NO_3^-) concentration ratio, which results in a 5% decrease in turbidity. Two real water samples were used for the turbidimetric method's validation: one was taken from the Pond water and the other was tap water from the laboratories. Prior to analysis, all solid contaminants were removed from the waste samples by filtering them with quantitative filter paper.

3.2.6 Data Treatment and Multivariate Statistical Techniques

Descriptive Statistics (mean, minimum, maximum, standard deviation) were used to summarize and understand the basic characteristics of the data set. Pearson Correlation was applied to examine linear relationships between physico-chemical parameters. One-Way ANOVA was conducted to determine significant differences among sampling sites. Principal Component Analysis (PCA) helped reduce data dimensionality and identify key influencing variables. Cluster Analysis was used to classify sampling sites based on similarity in water quality characteristics. All analyses were performed using IBM SPSS (v22.0) and XLSTAT 2015 integrated with Excel 2021.

3.3 Collection and Handling of *Clarias batrachus*

To collect *Clarias batrachus* from Ponds for morphometric and histopathological analysis in the laboratory, begin by selecting an accessible Pond with a healthy population of the species. Areas with dense vegetation or feeding zones are often ideal sampling locations due to their tendency to attract these fish. Before sampling, prepare the required equipment, including fine-mesh nets suitable for the Pond size, holding containers with Pond water and aeration to maintain fish health post-capture. Additionally, an anesthetic solution (such as MS-222 or clove oil) should be prepared to sedate the fish for easier handling and measurement. To capture the fish, gently deploy the net in targeted areas, moving carefully to avoid startling them. Immediately after capture, transfer each fish to the aerated holding container to reduce stress. For Morphometric measurements, place each fish in a container with the anesthetic solution until sedated, indicated by slower movements and opercular motion. Gently remove the sedated fish, lay it on a soft, wet surface to prevent desiccation and measure parameters such as total length, standard length, body depth, head length and fin lengths using a digital caliper. For histopathological analysis, euthanize the fish humanely by increasing the anesthetic concentration. Dissect the fish using sterile tools to collect tissue samples (e.g., gills, liver, kidneys, intestine) and place each in labeled vials containing 10% buffered formalin for preservation. Transport samples to the laboratory in a cooler with ice packs if necessary. In the lab, process tissue samples by embedding, sectioning and staining them for histological examination. This method ensures high-quality data collection for both Morphometric and histopathological analyses while adhering to ethical guidelines for fish handling and euthanasia.

To ensure optimal growth and minimize stress, the recommended water quality parameters, maintained during the controlled laboratory culture of *Clarias batrachus*, are summarized in table 3.4, which highlights the desirable ranges necessary for sustainable culture.

Table 3.4: Optimal Water Quality Parameters for Lab-Controlled Culture of *Clarias batrachus* (Asian Catfish)- (Zidni, II, 2019; Nath TB *et al.*, 2014)

Study Parameters	Optimal Value	Remark
Temperature, ^{O}C	18-25	A stable, warm temperature is essential for metabolic processes and growth. While *C. batrachus* can tolerate a wider range, this is the optimal for culture.
pH	7.5–8.5	A slightly alkaline pH is generally preferred for catfish culture. Fluctuations greater than 0.5 can cause stress.
Electrical Conductivity, mS/cm	0.1 – 0.14	A range within these values indicates a suitable mineral content for freshwater fish.
Total Ammonia Nitrogen mg/l	< 0.1 mg/l	Levels above 0.02 mg/l can be stressful and toxic, particularly the unionized form, NH_3 A level of <0.1 mg/l is generally recommended across aquaculture practices.
Chloride, mg/l	30	Adequate chloride levels are important for osmoregulation and reducing the effects of nitrite toxicity. It should be monitored to avoid salinity stress, especially in freshwater cultures.
Sulphate, mg/l	39–51	Values within this range support a healthy aquatic ecosystem.
Nitrate, mg/l	< 1.0	As the final product of the nitrogen cycle, nitrate is relatively non-toxic but should be monitored. Keeping levels low prevents toxicity and algae growth.
Phosphate, mg/l	0.02–0.06	Excess phosphate can lead to algal blooms, but a small amount is a vital nutrient.
TDS, mg/l	50–80	High levels of TDS may indicate excess mineral salts or dissolved organic matter.

DO, mg/l	≥ 5	While *Clarias batrachus* is an air- breathing fish, sufficient DO in the water is still vital for optimal growth and health.
BOD, mg/l	2.2–3.2	Represents the amount of oxygen needed to break down organic matter; low values indicate clean water.
COD, mg/l	Low concentrations well below BOD	While specific optimal values are not as standardized as BOD, COD should remain low to indicate a healthy system.
Lead, mg/l	Safe below 10 ppm in water, but bioaccumulates	Lead is highly toxic and bioaccumulates in the fish's tissues, especially the liver.
Nickel, mg/l	Lower toxicity compared to other heavy metals	Bioaccumulates in liver and gills, causing tissue damage at higher concentrations.
Copper, mg/l	Safe below 10 ppm in water	Copper is more toxic than lead or cadmium in short-term exposure. Toxicity increases with concentration.

3.4. Standard Method for Culturing *Clarias batrachus* (Experimental design for control):

Healthy fingerlings (post-larval stage, generally 25–60 days old) of *Clarias batrachus* were obtained from a certified hatchery. Fish were acclimatized in cemented tanks for 10–14 days under controlled conditions. Water quality parameters (temperature, pH, DO, ammonia) were monitored and maintained as per standard guidelines. Fish were fed twice daily with a formulated pellet diet (30–35% protein). Partial water exchange (30–40%) was done every 2–3 days to maintain hygiene. Stocking density was kept moderate (e.g., 5–7 fish/m²) to minimize stress and ensure uniform growth. Regular health monitoring was performed to avoid disease outbreaks. After the culture period, fish were used for Morphometric measurements and tissue sampling (liver, gill, kidney) for histopathological examination.

Fig. 3.1: Handling and sorting of collected *Clarias batrachus* during field sampling

3.4.1 Optimal Water Quality Parameters for Lab-Controlled Culture of *Clarias batrachus*

The growth, survival and overall health of *Clarias batrachus* are strongly influenced by the quality of water in which they are cultured. In laboratory-controlled systems, maintaining stable physicochemical conditions is particularly important because fluctuations can lead to stress, reduced growth performance, or increased susceptibility to diseases. Therefore, careful monitoring and regulation of water parameters such as temperature, pH, and dissolved oxygen and ammonia levels are essential for creating a conducive rearing environment. To ensure optimal growth and minimize stress, the recommended water quality parameters, maintained during the controlled laboratory culture of *Clarias batrachus,* are summarized in table 3.4, which highlights the desirable ranges necessary for sustainable culture.

3.5 Morphometric Analysis

To conduct a Morphometric analysis of *Clarias batrachus*, begin by sedating each fish using an anesthetic solution, such as MS-222 or clove oil, to minimize stress and ease handling (fig-3.3). Once sedated, place the fish on a soft, wet surface to prevent dehydration and ensure accurate measurements. Using a digital caliper or measuring tape, measure the specified parameters. For Total Length (TL), measure from the tip of the snout to the end of the caudal fin; for Standard Length (SL), measure from the snout to the base of the caudal fin; and for Caudal Length (CL), measure from the base to the tip of the caudal fin. Eye Diameter (ED) is taken as the horizontal distance across the eye. Pre-Dorsal Length (PDL) is measured from the snout to the origin of the dorsal fin, Pre-Anal Length (PAL) from the snout to the start of the anal fin and Pre-Pectoral Length (PPEL) from the snout to the base of the pectoral fin. Measure the length of the dorsal fin (DFL) and anal fin (AFL) from the base to the tip of the longest ray of each respective fin. Finally, Body Depth (BD) is measured as the vertical depth at the deepest part of the body, usually near the pectoral fin region. Record all measurements on a data sheet, ensuring accuracy and, if required, place the fish in a recovery tank after measurement or follow ethical guidelines for further processing. This procedure ensures precise Morphometric data collection for *Clarias batrachus*.

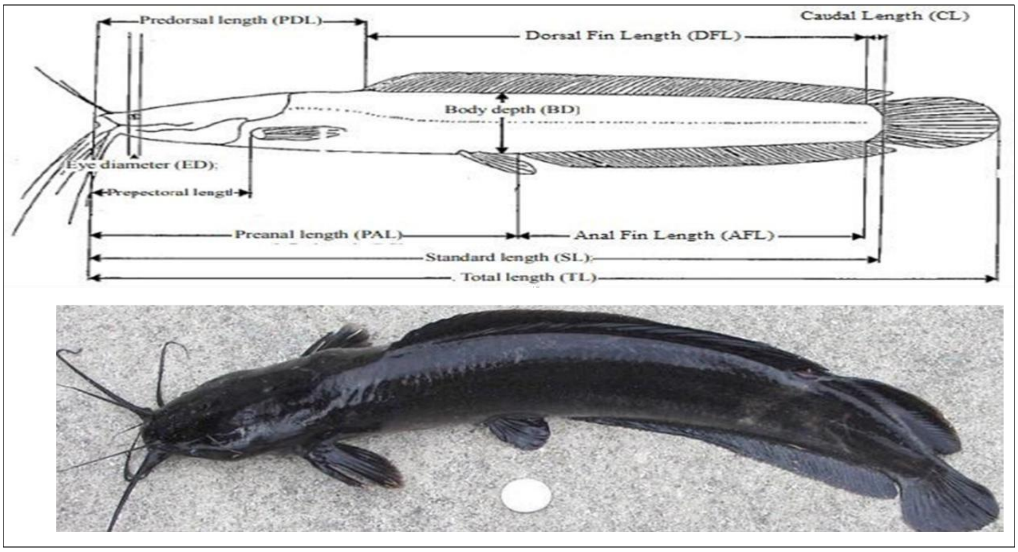


Fig.3.2: Diagram showing morphometric measurements of a *Clarias batrachus*

Fig.3.3: Field sampling location and representative freshwater fish *Clarias batrachus* specimens collected during the study

Table 3.5: Morphometric character of *Clarias batrachus* (Kumari, P *et al.* 2025; Tiwari, P., & Paul, N. , 2023)	
Total Length (TL), cm	Horizontal distance from front tip of snout to hind tip of caudal fin
Standard Length (SL)	Horizontal distance from front tip of snout to base (or articulation) of caudal fin
Caudal Length (CL)	Horizontal distance from hind margin of anal fin (or from that of dorsal fin if this extends further backwards than anal) to base of caudal fin
Eye Diameter (ED)	Horizontal diameter of eye
Pre-Dorsal Length (PDL	Horizontal distance from front tip of snout to the articulation of first dorsal-fin ray
Pre-Anal Length (PAL)	Horizontal distance from front tip of snout to the articulation of the first anal-fin ray
Pre-Pectoral Length (PPEL)	Horizontal distance from front tip of snout to the articulation of the first pectoral- fin ray.
Dorsal Fin Length (DFL)	Maximal horizontal distance measured between both ends
Anal Fin Length (AFL)	See dorsal-fin base

Body Depth (BD)	Maximum vertical depth of fish, excluding fins.

3.6 Histopathological analysis

To perform histopathological analysis of transverse sections (TS) of the kidney, liver and gills of freshly caught *Clarias batrachus* from selected Ponds, begin by immediately transferring the fish to the laboratory in a well-aerated container. Upon arrival, euthanize the fish humanely with an overdose of anesthetic solution, such as MS-222, to ensure minimal stress and preserve tissue quality. Carefully dissect the fish to expose the kidney, liver and gills, excising small pieces (approximately 5 mm) of each organ with sterile tools. Place the tissues in 10% buffered formalin for fixation, leaving them to fix for 24–48 hours at room temperature to ensure tissue structure is well-preserved. After fixation, dehydrate the samples in a graded ethanol series (70%, 80%, 90% and 100%), allowing each step to last about 30 minutes. Following dehydration, clear the tissues in xylene until transparent, then embed them in molten paraffin wax, creating a stable support block for sectioning. Using a microtome, cut thin sections (4–6 µm) from each tissue sample, floating the sections on a warm water bath to flatten them and carefully mount them onto glass slides. Once dried, deparaffinize the slides by passing them through xylene and rehydrate in graded ethanol solutions. Stain the sections with hematoxylin and eosin (H&E), which stains cell nuclei a deep blue and cytoplasm and extracellular components pink, allowing clear visualization of cellular structures. Following staining, rinse the slides, dehydrate again in graded alcohol, clear in xylene and apply a coverslip using a mounting medium. Examine the prepared slides under a light microscope at different magnifications to observe cellular organization and any histopathological changes in the kidney, liver and gill tissues, documenting findings with photomicrographs as needed (fig-3.4, fig-3.5 and fig-3.6).

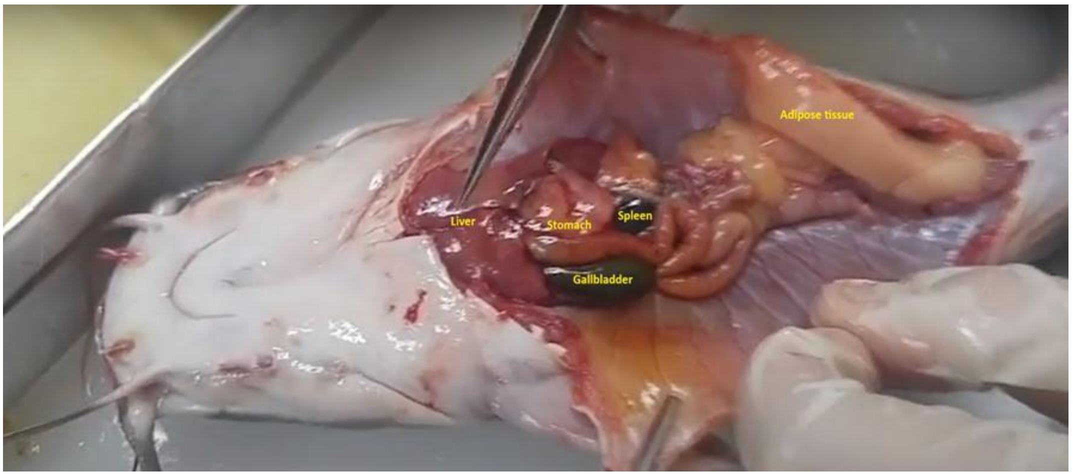


Figure 3.4: Dissected abdominal cavity of *Clarias batrachus* showing major internal organs liver, stomach, spleen, gall bladder and surrounding adipose tissue exposed during anatomical examination for histopathological analysis.

Figure 3.5: Exposed kidney of *Clarias batrachus* during dissection

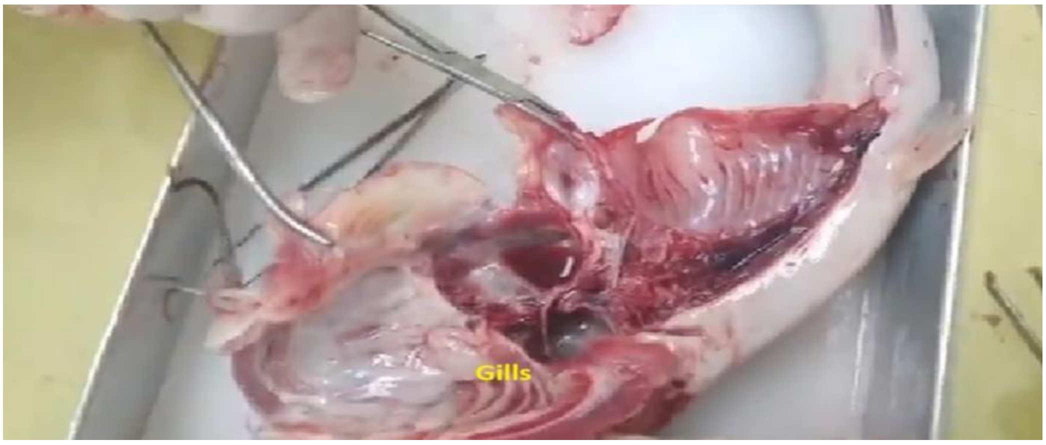


Figure 3.6: Exposed gill region of *Clarias batrachus* during dissection

Chapter 4

RESULTS AND DISCUSSION

The physical, chemical and biological characteristics of water have the potential to characterize its quality. Water may be acquired from a variety of sources, including wells, Ponds, rivers, lakes, etc., but sadly, clean, pure and safe water only exists temporarily in nature before being directly contaminated by prevailing environmental conditions and human activities. Therefore, water from the majority of sources is unfit for consumption without some type of treatment.

In the sacred Ponds of Varanasi, namely Suryakund Pond, Pishach mochan Pond, Pushkar Pond, Ishwargangi Pond, and Kurukshetra Pond, a wide range of ritual and religious activities have been identified as the major contributors to heavy metal pollution and deterioration of physical and chemical water quality parameters. During festivals such as Ganesh Chaturthi, Durga Puja, Saraswati Puja, Navratri, Kartik Purnima, and Pitra Paksha, devotees immerse idols made from Plaster of Paris (POP), clay, thermocol, synthetic resins, adhesives, and decorative paints containing metallic pigments. These materials release lead (Pb), chromium (Cr), cadmium (Cd), mercury (Hg), nickel (Ni), zinc (Zn), and copper (Cu) into water upon dissolution and sedimentation. Similarly, metallic-coated diyas (earthen lamps), incense sticks (agarbatti), camphor (kapoor), and coloured wax residues discarded after rituals contribute Pb, Cu, Zn, and Ni, while coins, brass and copper utensils, bells, ornaments, and metallic foils corrode and leach Cu, Zn, Ni, and traces of Pb into water. Offerings such as vermillion (sindoor), kumkum, turmeric (haldi), abir-gulal, and artificial colour powders often contain Pb, Cr, and Cd due to the use of synthetic dyes. Additionally, artificial and dyed flowers, plastic garlands, metallic paper decorations, coloured thermocol, and foil-wrapped sweets or coconuts introduce Zn, Cu, and Pb as they degrade in the Pond environment. Temple maintenance activities, including washing of brass idols, copper utensils, and temple floors with detergent and chemical cleansers, add further Cu, Cr, Ni, and Pb, while organic offerings such as milk, fruits, betel leaves, coconuts, rice, sweets, and food residues wrapped in aluminium or silver foils increase the organic load and nutrient content of the Ponds (Singh *et al..*, 2022; Yadav *et al..*, 2013).

These ritual and material inputs drastically alter the physical and chemical parameters of the Pond ecosystems. The presence of suspended ritual waste and decomposing organic matter leads to increased total dissolved solids (TDS), reducing light penetration and primary productivity. The dissolution of chemical paints and ashes raises electrical conductivity (EC), while burnt offerings and detergents elevate pH levels above neutrality (often >8.5). The decomposition of floral, food, and organic waste increases biological oxygen demand (BOD) and chemical oxygen demand (COD), resulting in significant depletion of dissolved oxygen (DO) that is vital for aquatic organisms. Nutrients such as phosphates, nitrates, and sulphates, released from organic offerings and detergent residues, accelerate eutrophication, leading to algal blooms and anoxic conditions. Elevated chloride and ammonium concentrations are attributed to ritual ashes, human activities, and detergent washings. The accumulation of heavy metals such as Pb, Cu, Ni, and Zn in sediments and water further enhances ecological toxicity. Continuous exposure to such polluted environments causes bioaccumulation of toxic metals in aquatic biota, particularly in the fish species *Clarias batrachus*, which shows severe histopathological alterations—including glomerular degeneration in kidney, hepatic necrosis in liver, and epithelial lifting and lamellar fusion in gills—along with biochemical and enzymatic imbalances, indicating oxidative stress and metabolic disruption (Pandey *et al.*, 2025). Water from ancient and sacred Ponds (kunds) was collected and analyzed in order to explore the variations in water quality. The results are a blatant sign of the Ponds' deteriorating health and the effects of pollution and untreated wastewater carelessly being released into drains. These variations were mostly influenced by the amount of both residential and commercial effluent that was discharged untreated, urban runoff and nearby population activities such as ritual, ceremonial, bathing, washing clothing and dumping of garbage and ritual materials on the edge of Ponds.

4.1 Descriptive Statistics

Seasonal descriptive statistics of physicochemical and trace metals of five Ponds (kunds) recorded during the years 2021 and 2022 compared with the BIS, ICMR, WHO, EPA and EU standards are presented in terms of minimum, maximum and mean with standard deviation given in Table 4.1 and Table 4.2, respectively.

Table 4.1: Descriptive Statistics of Physico-chemical and Trace metals characteristics of sampling Ponds (Kunds) Recorded during the year 2021

Study Parameters	Standard						Pre-monsoon (May 2021)				Monsoon (August 2021)				Autumn season (October-2021)				Post-monsoon (December-2021)			
	WHO	BIS	ICMR	EPA	EU	N	Mean	Min	Max	STDEV	Mean	Min	Max	STDEV	Mean	Min	Max	STDEV	Mean	Min	Max	STDEV
Temperature, ^{O}C						5	34.28	32.3	36	1.37	30	29	33	1.65	24.84	22	27.3	2.51	17.1	17	17.6	0.42
pH	6.5-9.5	6.5-8.5	7.0-8.5			5	8.10	7.2	8.8	0.61	7.92	7.8	8.1	0.12	8.11	7.6	8.6	0.5	8.25	7.8	8.72	0.46
Electrical Conductivity (mS/cm)	0.25		-		0.25	5	0.749	0.649	0.813	0.06	0.52	0.4	0.56	0.06	0.615	0.5	0.72	0.07	0.647	0.5	0.77	0.09
Chloride, mg/l		250	200			5	40	34	46	4.47	24.6	21	27	2.88	28.8	26	32	2.28	27	24	32	3
Sulphate, mg/l		200	200			5	37.2	35	40	1.92	44.2	42	46	1.79	29.2	24	32	3.35	33.2	28	37	3.27
Nitrate, mg/l		45	20			5	4.4	3.2	5.8	1.09	2.34	1.8	3.2	0.7	4.48	3.6	4.9	0.52	4.02	2.9	5.2	0.97
Phosphate, mg/l			-	25		5	0.598	0.3	0.82	0.26	0.34	0.1	0.5	0.18	0.41	0.3	0.54	0.083	0.34	0.2	0.47	0.15
TDS, mg/l		500	500			5	479.6	418	520	37.56	335	280	360	35.68	393.6	346	460	41.94	414	324	490	62.11
DO, mg/l		<6	-			5	6.24	5.4	7.2	0.74	5.8	5.2	6.9	0.79	5.056	4.2	5.6	0.61	5.22	4.4	6.32	0.84
BOD, mg/l		>2	-			5	7.264	5.6	9.2	1.34	6.29	4.8	8.5	1.42	7.356	6.5	8.32	0.89	5.9	4.7	8.64	1.62
COD, mg/l	>10		-			5	14.24	11.8	18.2	2.71	12.4	10	17	2.5	15.37	14	16.8	1.33	12	9.2	17.4	3.33
Lead, Pb, mg/l	0.01	0.01	-		0.01	5	0.007	0.004	0.012	0.004	0.02	0	0.1	0.04	0.01	0	0.01	0.003	0.01	0	0.02	0.005
Nickel, Ni, mg/l			-			5	0.007	0.003	0.009	0.003	0.01	0	0	0.003	0.007	0	0.01	0.004	0.01	0	0.01	0.003
Copper, Cu, mg/l		0.01	0			5	0.015	0.003	0.045	0.019	0.01	0	0	0.006	0.007	0	0.01	0.003	0.01	0	0.01	0.002
Zinc, Zn, mg/l						5	3.34	1.9	6.3	1.71	3.92	2.4	6.7	1.81	3.16	1.6	5.8	1.72	3.4	1.4	5.2	1.56

BIS: Bureau of Indian Standard (BIS 10500: 2012), WHO: World Health Organization (2011), ICMR: Indian Council of Medical Research, EPA: U.S. Environmental Protection Agency: EU: European Commission Standard, 1998

Table 4.2: Descriptive Statistics of Physico-chemical and Trace metals characteristics of Ponds(Kunds) Recorded during the year 2022

Study Parameters	Standard						Pre-monsoon (May-2022)				Monsoon(August-2022)				Autumn season(October-2022)				Post-monsoon(December-2022)			
	WHO	BIS	ICMR	EPA	EU	N	Mean	Min	Max	STDEV	Mean	Min	Max	STDEV	Mean	Min	Max	STDEV	Mean	Min	Max	STDEV
Temperature, ^{O}C						5	32.96	31.2	34.4	1.18	31	30.4	31.2	0.35	24.08	22.1	25.5	1.49	17.14	15.9	19.6	1.48
pH	6.5-9.5	6.5-8.5	7.0-8.5			5	8.21	8.42	7.94	8.9	0.41	7.94	7.8	8.15	0.13	7.78	7.46	8.3	0.33	8.17	7.76	8.6
Electrical Conductivity (mS/cm)	0.25		-		0.25	5	0.788	0.656	0.938	0.11	0.515	0.406	0.597	0.07	0.672	0.597	0.719	0.05	0.625	0.508	0.752	0.095
Chloride, mg/l		250	200			5	39.2	33	43	3.96	24	20	28	2.92	26.8	23	31	2.86	28.2	25	34	3.56
Sulphate, mg/l		200	200			5	36.8	34	38	1.79	44.8	41	48	2.59	31	24	34	4	31.2	28	34	2.28
Nitrate, mg/l		45	20			5	4.46	3.4	5.6	0.93	2.44	1.6	3.5	0.94	3.6	2.8	4.6	0.82	4.76	4.6	5.1	0.23
Phosphate, mg/l			-	25		5	0.586	0.29	0.81	0.25	0.364	0.18	0.5	0.15	0.328	0.16	0.48	0.13	0.436	0.36	0.6	0.09
TDS, mg/l		500	500			5	504.4	420	600	68.82	330	260	382	44.74	429.8	382	460	32.45	399.6	325	481	60.4
DO, mg/l		<6	-			5	6.292	5.2	7.3	0.75	6.002	4.9	7.1	0.78	5.36	4.2	6.4	0.8	5.012	4.56	5.36	0.38
BOD, mg/l		>2	-			5	7.42	6.3	8.6	0.96	6.96	6.2	8.2	0.85	5.53	4.52	6.82	0.99	7.02	4.96	8.64	1.49
COD, mg/l	>10		-			5	15.3	13.3	17.6	1.76	14.18	11.9	16.2	1.85	11.30	9.2	13.2	1.65	14.25	10.86	17.6	2.78
Lead, Pb, mg/l	0.01	0.01	-		0.01	5	0.003	0.003	0.01	0.004	0.009	0.001	0.022	0.008	0.0080.004		0.012	0.004	0.007	0.001	0.012	0.005
Nickel, Ni, mg/l			-			5	0.005	0.004	0.006	0.001	0.007	0.003	0.012	0.003	0.006	0.0020.012		0.005	0.005	0.003	0.008	0.002
Copper, Cu, mg/l		0.01	0			5	0.011	0.001	0.032	0.012	0.008	0.008	0.012	0.002	0.0050.001		0.012	0.006	0.008	0.001	0.014	0.005
Zinc, Zn, mg/l						5	3.3	1.9	4.4	0.89	4.24	3.2	5.8	1.18	3.52	0.8	6.2	1.98	3.12	2.2	4.2	0.88

BIS: Bureau of Indian Standard (BIS 10500: 2012), WHO: World Health Organization (2011), ICMR: Indian Council of Medical Research, EPA: U.S. Environmental Protection Agency: EU: European Commission Standard, 1998

4.1.1 Temperature:

Water temperature is a measure of heat and cold. When the water temperature is cooler, there’s more oxygen, while when it's warmer, there’s less oxygen for life in Ponds. Water temperature ranged from 32.3–36.00, 29.00–33.00, 22.00-37.30 and 17.00–17.60°C, with a mean value of 34.28±1.37, 30.00±1.65, 24.84±2.51 and 17.1±0.42 °C in 2021, whereas it ranged from 31.20–34.40, 30.40–31.20, 22.1-25.5 and 15.90–19.60 °C, with a mean value of 32.00±1.18, 31.00±0.35, 24.08±1.49 and 17.14±1.48 °C in 2022 in the Pre-monsoon, monsoon, autumn and post- monsoon seasons. In 2021, the water temperature at Ishwargangi Pond was highest in the Pre-monsoon season and lowest in the post- monsoon season at Suryakund Pond, while in 2022, the highest temperature was recorded at Pushkar Pond in the Pre-monsoon seasons and the lowest at Ishwargangi Pond in the post-monsoon season (fig-4.1). The seasonal succession of the biota is significantly influenced by temperature. Season, location and sample time are often the main factors influencing Pond water temperature differences.

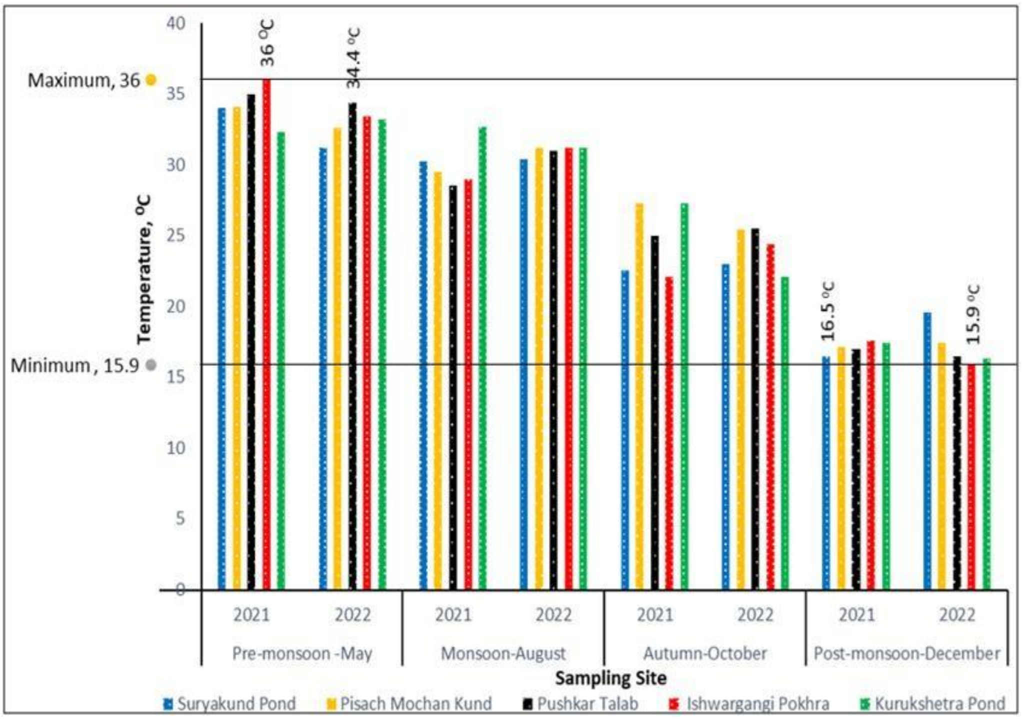


Figure 4.1 Spatio-temporal changes in water quality parameter, T^0C

4.1.2 pH

pH is an important parameter to check the quality of water. pH is a measure of whether a water body is acidic, alkaline or neutral. Acidic water contains elevated levels of toxic metals than alkaline water. Water pH ranged from 7.2–8.80, 7.80–8.10, 7.60-8.60 and 7.80–8.72, with a mean value of 8.1±0.61, 7.92±ph0.12, 8.11±0.5 and8.25±0.46 in 2021, whereas it ranged from 7.94–8.9, 8.15–7.8, 7.46-8.3 and 7.76–8.6, with a mean value of 8.42±0.41, 7.94±0.13, 7.78±0.33 and 8.17±0.42 in 2022 in the Pre-monsoon, monsoon, autumn and post-monsoon seasons respectively. It indicates that the water is naturally somewhat alkaline. In 2021, the water pH at suryakund was maximum in the pre-monsoon season and lowest in Pre-monsoon season at Ishwargangi Pond, while in 2022, the maximum pH value was recorded at Pishach Mochan in the Pre- monsoon seasons and the lowest at Pushkar Pond in the autumn season (fig-4.2). pH values in drinking water should range from 6.5 to 8.5, 7.0 to 8.5 and 6.5 to 9.5, as recommended by the BIS, ICMR and WHO, respectively. It was found that during the pre-monsoon, monsoon and post-monsoon periods, all samples were within the BIS, ICMR and WHO permissible limits. Urban runoff, leaking septic systems, waste disposal and human activities including rituals, washing, bathing, etc. are all common causes of pH changes.

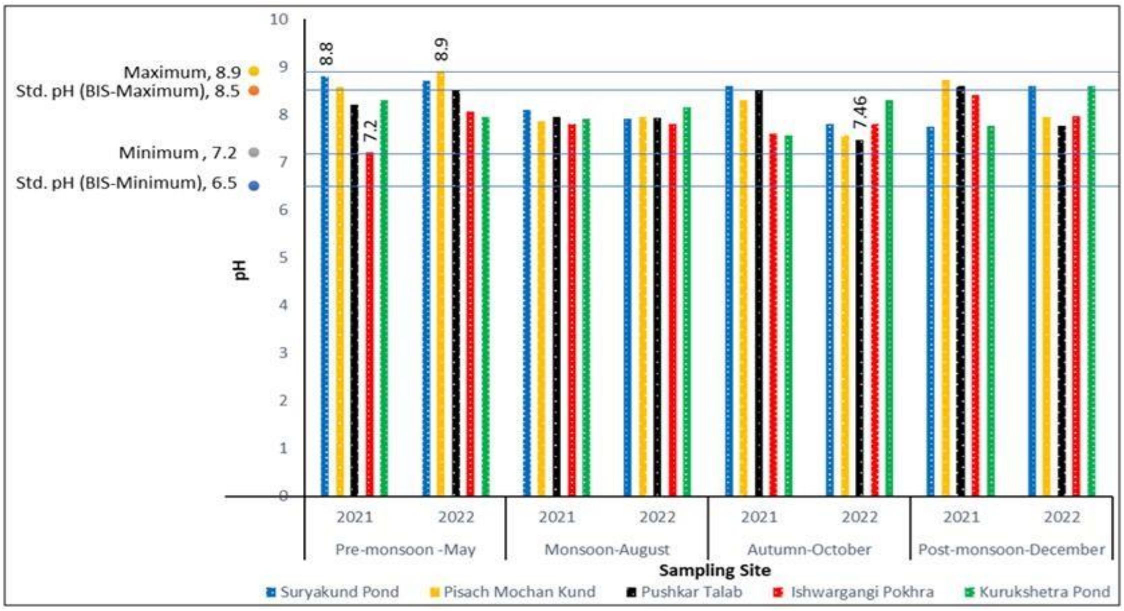


Fig. 4.2: Spatio-temporal changes in water quality parameter, pH

4.1.3 Electrical Conductivity

Electrical conductivity is a key indicator for checking water quality. It measures the impurities (dissolved substances, minerals and chemicals) in water. Its rising or falling level indicates the amount of pollution affecting water quality. An increasing level of conductivity indicates an increasing level of salinity and a decreasing level of dissolved oxygen, which can have a negative effect on the aquatic ecosystem. Its increasing level in Ponds clearly indicates the sewage leak or urban runoff which contributes to the presence of ions such as nitrate, phosphate and chloride. Water conductivity ranged from 0.649–0.813, 0.40-0.56, 0.50-0.72 and 0.5–0.77 mS/cm, with a mean value of 0.749±0.06, 0.52±0.06, 0.52±0.06 and 0.647±0.09 mS/cm in 2021, whereas it ranged from 0.656–0.938, 0.406–0.597, 0.597-0.719 and 0.508– 0.752 mS/cm, with a mean value of 0.788±0.11, 0.515±0.07,0.672±0.05 and 0.625±0.095 mS/cm in 2022 in the Pre- monsoon, monsoon, autumn and post-monsoon seasons, respectively. In 2021, the water conductivity at Suryakund Pond was highest in the Pre-monsoon season and lowest in the monsoon season at Pishach Mochan, while in 2022, the highest conductivity was recorded at Suryakund Pond in the Pre-monsoon seasons and the lowest at Pishach Mochan in the monsoon season (fig-4.3). Electrical conductivity in drinking water should not exceed 0.25 mS/cm, as recommended by the WHO and EU. It was found that during the pre-monsoon, monsoon and post-monsoon seasons, all the samples showed a higher value of conductivity than the WHO and EU permissible limits. The substances dissolved in the water, which dissociate into positively and negatively charged ions, are responsible for the water's electrical conductivity. Urban runoff and waste disposal are the main causes of higher water conductivity.

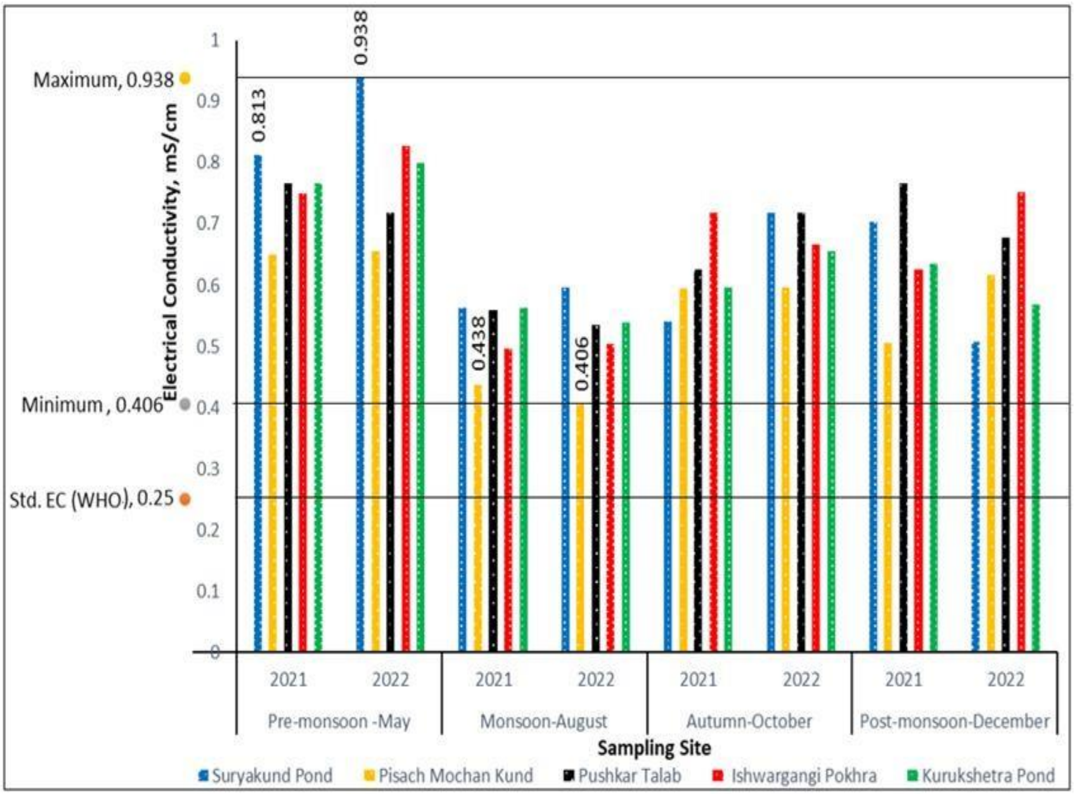


Fig. 4.3: Spatio-temporal changes in water quality parameter, EC, mS/cm

4.1.4 Chloride

Because of its gradual accumulation in the depth of Ponds over time, chloride has become an emerging concern in recent decades. Its accumulation makes denser water at lower Pond depths, which can impede Pond turnover and then the bottom of Ponds can get depleted of oxygen, which can have an impact on organisms as well as lead to nutrient release from the sediment at the bottom of Ponds Its elevated level in Pond water can be harmful to aquatic life, vegetation and the ecosystem. Chloride in Ponds water ranged from 34.00–46.00, 21.00-27.00, 26.00-32.00 and 24.00-32.00 mg/l, with a mean value of 40.00±4.47, 2 4 .60±2.88, 28.80±2.28 and 27.00±3.00 mg/l in 2021, whereas it ranged from 33.00–43.00, 20.00–28.00, 23.00-31.00 and 25.00-34.00 mg/l, with a mean value of 39.20±3, 24.00±2.92, 26.80±2.86, and 28.20±3.56 mg/l in 2022 in the Pre-monsoon, monsoon, autumn and post-monsoon seasons, respectively. In 2021, the chloride at Ishwar Gangi Pond was highest in the Pre-monsoon season and lowest in the monsoon season at Pishach Mochan and Suryakund , while in 2022, the highest chloride was recorded at Kurukshetra Pond in the Pre-monsoon seasons and the lowest at Suryakund Pond in the monsoon season. Chloride ion in drinking water should not exceed 250 mg/l and 200 mg/l, as recommended by the BIS and ICMR, respectively (fig-4.4). It was found that during the Pre-monsoon, monsoon, autumn and post-monsoon seasons, all samples were found to be within the BIS and ICMR permissible limits. Water with a high chloride content likely contains organic waste, typically of an animal origin. Urban runoff and waste disposal are the main causes of higher water chloride.

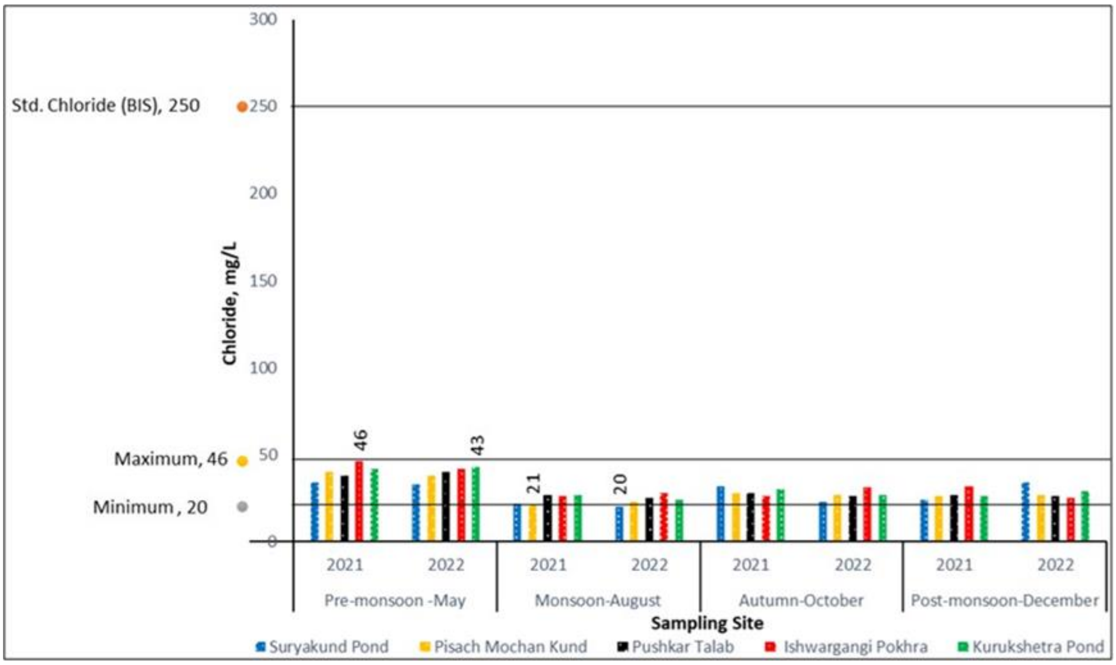


Figure 4.4 - Spatio-temporal changes in water quality parameter, Chloride, mg/l

4.1.5 Sulphate

Along with other anthropogenic contaminants, increased levels of sulphate have revealed the presence of inorganic impurities in river water. Anthropogenic activities including mining, agriculture and the burning of fossil fuels and waste produce one- third of the sulphate that ends up in the Ponds. Sulphate in Ponds water ranged from 34.00-38.00, 41.00-48.00, 24.00-34.00 and 28.00-34.00 mg/l, with a mean value of 36.80±1.79, 44.8±2.59, 44.8±2.59 and 31.2±2.28 mg/l in 2022, whereas it ranged from 35.00-40.00, 42.00-46.00, 24.00-32.00 and 28.00-37.00 mg/l, with a mean value of 37.2±1.92, 44.2-±1.79, 29.02-±3.35 and33.80±3.27 mg/l in 2021 in the Pre-monsoon , monsoon, autumn and post- monsoon seasons, respectively. In 2021, the sulphate at Iswargangi and Kurukshetra was highest in the monsoon season and lowest in the autumn season at Pishach mochan Pond, while in 2022, the highest sulphate ion was recorded at Kurukshetra Pond in the monsoon seasons and the lowest at Kurukshetra Pond in the autumn season. sulphate ion in drinking water should not exceed 200 mg/l, as recommended by the BIS and ICMR (fig-4.5). It was found that during the pre-monsoon, monsoon, autumn and post-monsoon season all samples were not found within the BIS and ICMR permissible limits. The major contributors to rising water sulphate levels in Ponds are sewage infiltration, fertilizers, synthetic detergents and others.

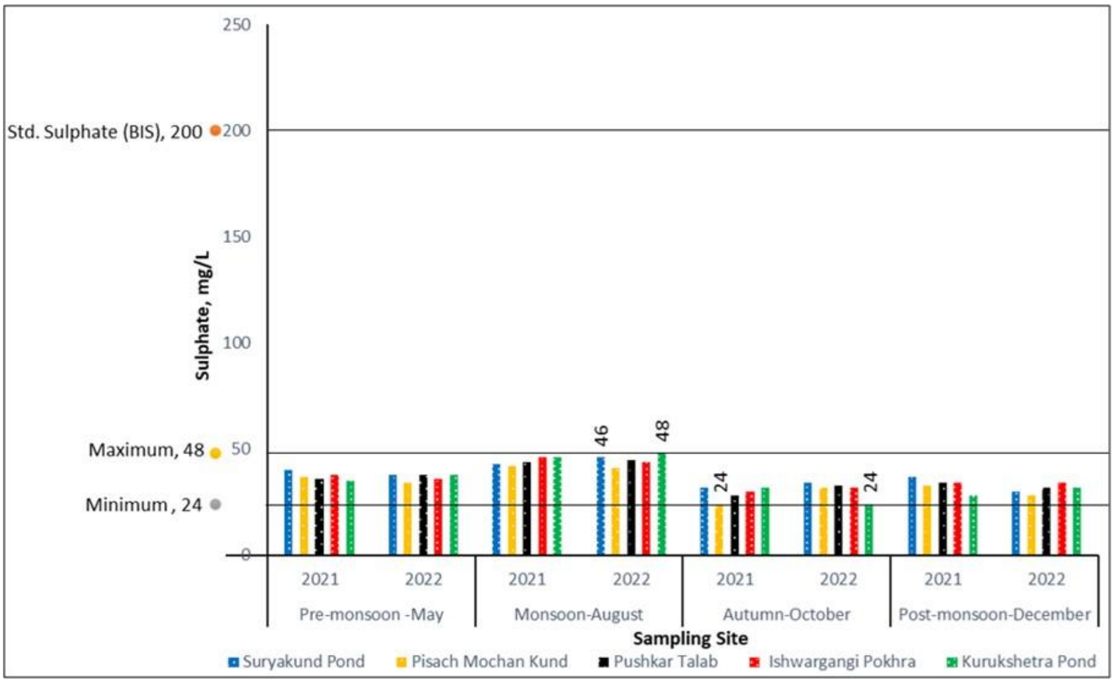


Fig. 4.5: Spatio-temporal changes in water quality parameter, Sulphate, mg/l

4.1.6 Nitrate

Because of its natural occurrence, solubility and anionic state, nitrate must be analyzed in water due to its well-known negative effects on Ponds health. Many ecological and environmental concerns, including toxic algal blooms, eutrophication of Ponds and the extinction of species in the Pond's ecosystem, can be led on by high nitrate concentrations. Nitrate in Ponds water ranged from 3.2-5.8, 1.8-3.2, 3.6-4.9 and 2.9-5.2 mg/l, with a mean value of 4.4±1.09, 2.34±0.7, 4.48±0.52 and 4.02±0.97 mg/l in 2021, whereas it ranged from 3.4-5.6, 1.6-3.5, 2.8-4.6 and4.6-5.1 mg/l, with a mean value of 4.46±0.93, 2.44±0.94, 3.6±0.82 and 4.76±0.23 mg/l in 2022 in the pre-monsoon, monsoon and post-monsoon seasons respectively. In 2021, the nitrate at Pushkar Pond was highest in the Pre-monsoon season and lowest in the monsoon season at I s hwar

Gangi Pond. Whereas, in 2022, the highest nitrate was recorded at Pushkar Pond in the Pre-monsoon seasons and the lowest at Ishwargangi Pond and Suryakund Pond in the monsoon season (fig-4.6). Nitrate ion in drinking water should not exceed 45 mg/l and 20 mg/l as recommended by the BIS and ICMR. It was found that during the pre-monsoon, monsoon and post- monsoon seasons all samples were found to be within the BIS and ICMR permissible limits. The major contributors to rising water nitrate levels in Ponds are runoff from urban areas, leaking septic systems, etc.

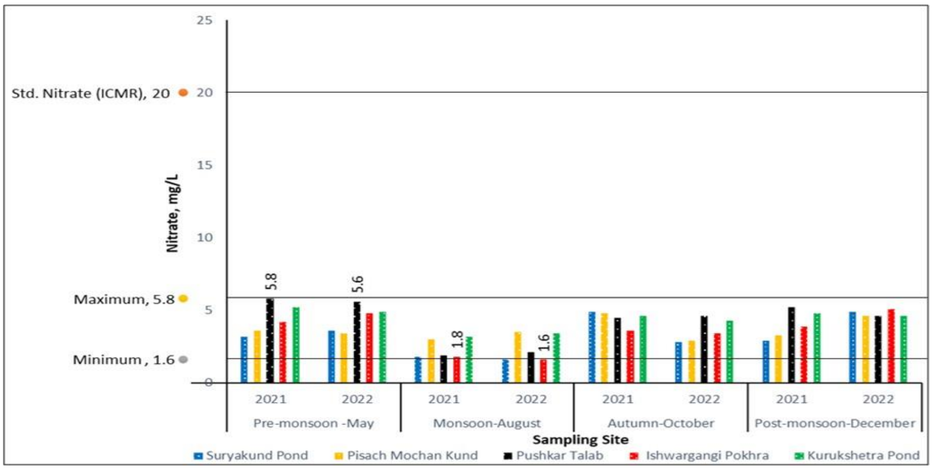


Fig. 4.6: Spatio-temporal changes in water quality parameter, Nitrate, mg/l

4.1.7 Phosphate

Phosphate is a critical nutrient for sustaining life in the aquatic ecosystem if it is present in specific proportions. A toxic cyanobacterial bloom may result in increased phosphate levels. A cyanobacterial bloom depletes oxygen levels and blocks sunlight, both of which have an adverse impact on the sustainability of aquatic life. Ponds receive too much phosphate from runoff, sewage and other decomposing matter. Phosphate in Ponds water ranged from 0.30-0.82, 0.10-0.50, 0.3 -0.54 and 0.2-0.47 mg/l, with a mean value of 0.598±0.26, 0.34±0.18, 0.41±0.08 and 0.34±0.15 mg/l in 2021, whereas it ranged from 0.29-0.81, 0.18-0.50, 0.16- 0.48 and 0.36-0.6 mg/l, with a mean value of 0.59±0.25, 0.36±0.15, 0.33±0.13 and 0.44±0.09 mg/l in 2022 in the Pre-monsoon, monsoon, autumn and post-monsoon seasons, respectively. In 2021, the Phosphate at Pushkar Pond was highest in the Pre-monsoon season and lowest in the monsoon season at Ishwargangi Pond, while in 2022, the highest Phosphate was recorded at Pishach Mochan in the Pre-monsoon seasons and the lowest at Ishwargangi Pond in the autumn season (fig-4.7). Phosphate in drinking water should not exceed 45 mg/l and 20 mg/l as recommended by the BIS and ICMR. It was found that during the pre-monsoon, monsoon standard and post-monsoon seasons, all samples were found to be within the BIS and ICMR permissible limits. The major contributors to rising water nitrate levels in Ponds are runoff from urban areas, leaking septic systems, etc.

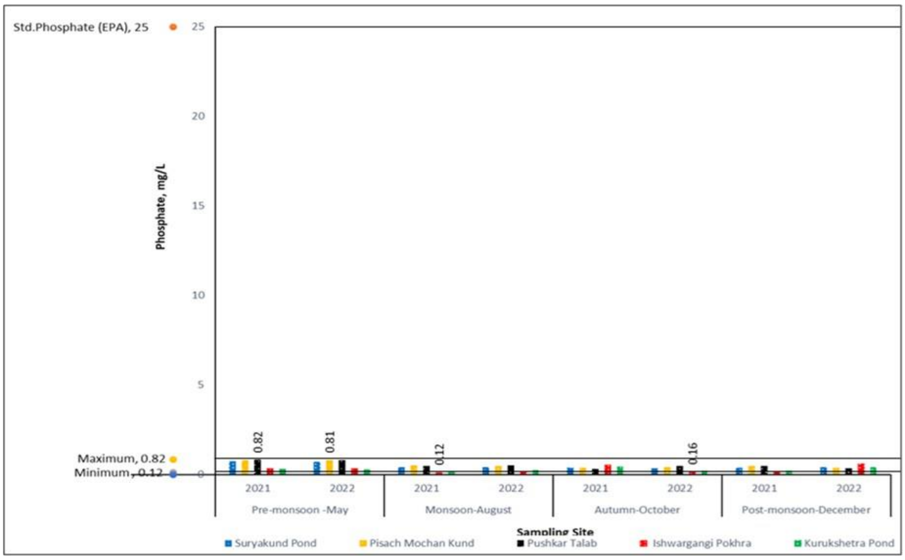


Fig.4.7: Spatio-temporal changes in water quality parameter, Phosphate, mg/l

4.1.8 Total Dissolved Solid

Total dissolved solids are generally believed to be a significant measure of surface water quality. The concentration of dissolved substances in the water is measured as total dissolved solids. Total dissolved solids primarily consist of inorganic salts, with a trace quantity of organic matter. Elevated levels of total dissolved solids frequently harm aquatic species in water bodies like Ponds. Total dissolved solid in Ponds water ranged from 418-520, 280-360, 346-460 and324-490 mg/l, with a mean value of 479.6±37.56, 335±35.68, 393.6±41.94 and414±62.11 mg/l in 2021, whereas it ranged from 420-600, 260-382, 382-460 and 325-481 mg/l, with a mean value of 504.4±68.82, 330±44.74, 429.8±32.45 and 399.6±60.4 mg/l in 2022 in the Pre-monsoon, monsoon, autumn and post-monsoon seasons respectively. In 2021, the total dissolved solid at Suryakund Pond was highest in the Pre- monsoon season and lowest in the monsoon season at Pishach Mochan, while in 2022, the highest total dissolved solid was also recorded at Suryakund Pond in the Pre-monsoon seasons and the lowest at Pishach Mochan in the monsoon season (fig- 4.8), respectively highest total dissolved solid in drinking water should not exceed 300 mg/l (upper limit -500 mg/l) as recommended by the BIS, ICMR and WHO. It was found that during the Pre-monsoon, monsoon, autumn and post-monsoon seasons, most of the samples showed a higher value of total dissolved solid than the BIS, ICMR and WHO permissible limits.

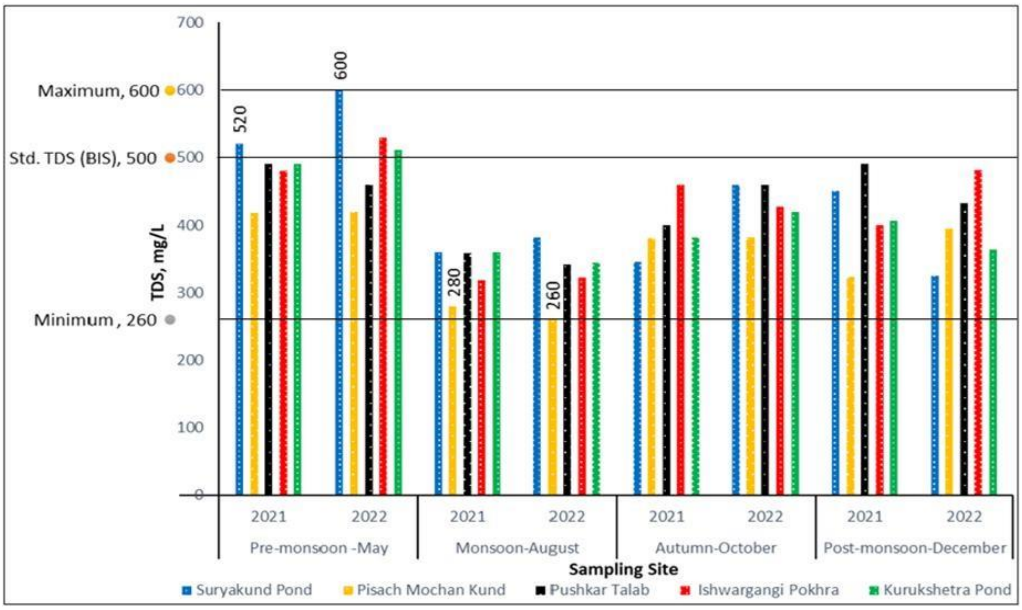


Fig.4.8: Spatio-temporal changes in water quality parameter, TDS, mg/l

4.1.9 Dissolved Oxygen

In order to survive the amount of dissolved oxygen in the water is a great indicator of the water quality. For aquatic life to be healthy, DO is necessary. Dissolved oxygen in Ponds water ranged from 5.40-7.20, 5.20-6.90, 4.20-5.60 and 4.4- 6.32 mg/l, with a mean value of 6.24±0.74, 5.80±0.79,5.05±0.61 and5.22±0.84 mg/l in 2021, whereas it ranged from 5.20-7.30, 4.90-7.10, 4.2-6.4 and 4.56-5,36 mg/l, with a mean value of 6.29±0.75, 6.00±0.78, 5.36±0.8 and 5.01±0.38 mg/l in 2022 in the Pre-monsoon, monsoon, autumn and post-monsoon seasons, respectively. In 2021, the Dissolved oxygen at Surya Kund Pond was highest in the Pre-monsoon season and lowest in the autumn season at Pishach mochan Pond whereas, in 2022, the highest Phosphate also was recorded at Surya Kund Pond in the Pre-monsoon seasons and the lowest at Ishwargangi Pond in the autumn season (fig-4.9). Dissolved oxygen in drinking water should be < 6 mg/l as recommended by the BIS. The majority of the samples had a lower level of dissolved oxygen than the BIS permissible limits during the pre-monsoon, monsoon and post-monsoon seasons.

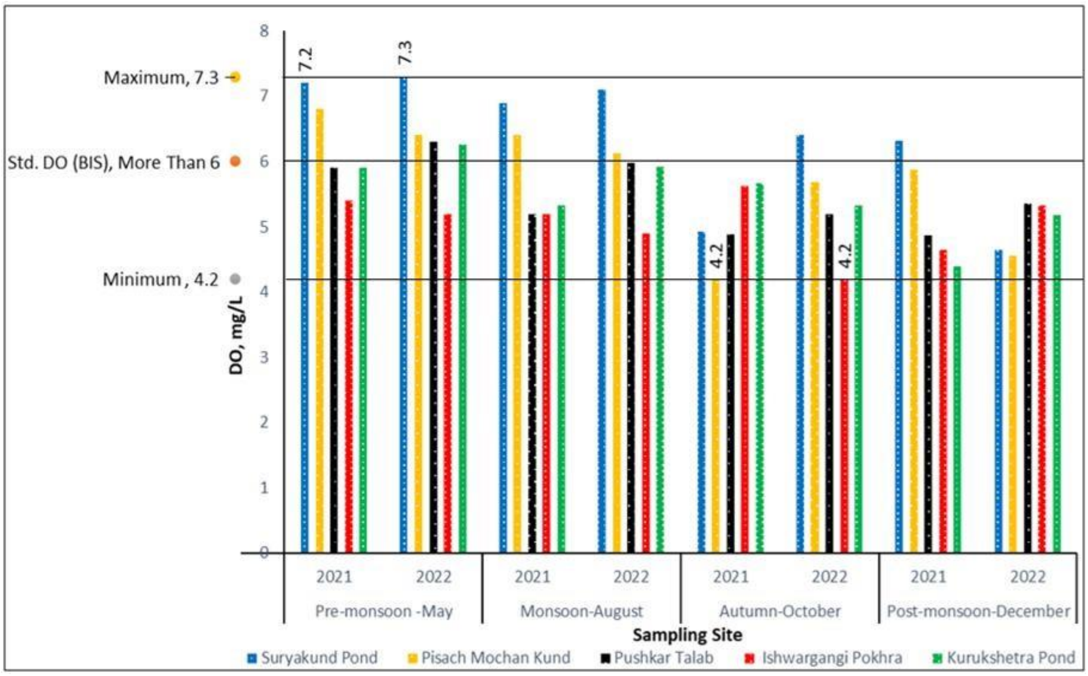


Fig.4.9: Spatio-temporal changes in water quality parameter, DO, mg/l

4.1.10 Biological Oxygen Demand

Commonly, BOD levels in water samples may be used to monitor organic waste. BOD is a parameter that shows how much molecular oxygen is needed by microorganisms to oxidize organic compounds. When a significant amount of organic waste is introduced to a river, the microbial activity of the aquatic system is increased, which causes BOD to increase and DO to decrease. Biological oxygen demand in Ponds water ranged from 5.60-9.2, 4.80-8.50, 6.5-8.32 and 4.7-8.64 mg/l, with a mean value of 7.26±1.34, 6.29±1.42, 7.36±0.89 and5.9±1.62 mg/l in 2021, whereas it ranged from 6.30-8.60, 6.20-8.20, 4.52- 6.82 and 4.96-8.64 mg/l, with a mean value of 7.42±0.96, 6.96±0.85, 5.35±0.99 and 7.2±1.49 mg/l in the Pre- monsoon, monsoon, autumn and post-monsoon seasons, respectively. In 2021, the biological oxygen demand at Kurukshetra was highest in the Pre-monsoon season and lowest in the post-monsoon season at Pishach Mochan, while in 2022, the biological oxygen demand at Kurukshetra was highest in the Post-monsoon season and lowest in the post-monsoon season at Pishach Mochan (fig-4.10). Biological oxygen demand in drinking water should be >2 mg/l as recommended by the BIS. During the pre-monsoon, monsoon and post-monsoon seasons, most of the samples showed higher levels of biological oxygen demand than the BIS acceptable limits.

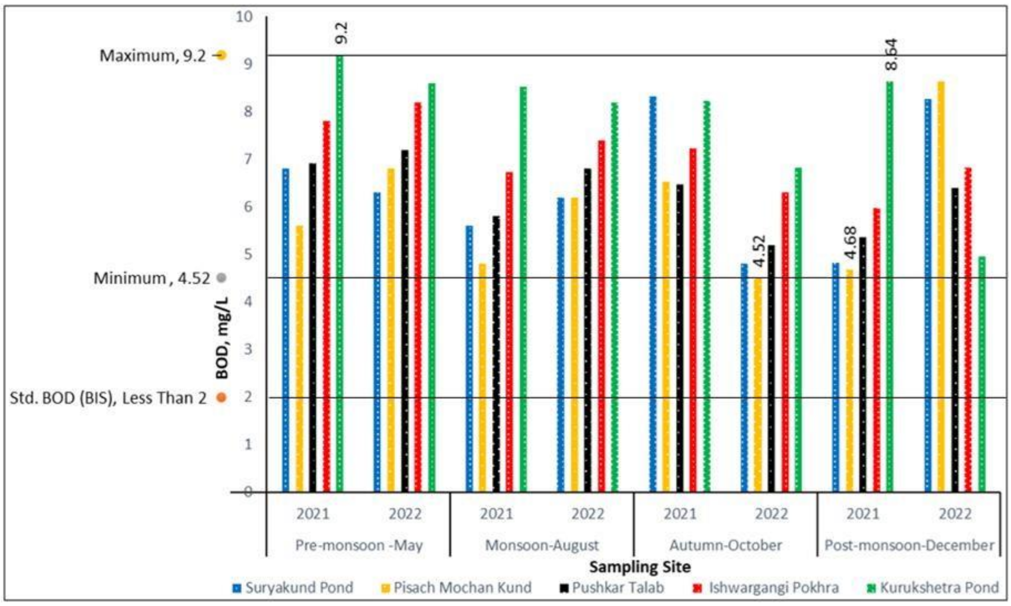


Fig.4.10: Spatio-temporal changes in water quality parameter, BOD, mg/l

4.1.11 Chemical Oxygen Demand

Commonly, the Chemical oxygen demand levels in water samples may be used to monitor organic waste. Chemical oxygen demand is a parameter that shows how much oxygen is utilized to oxidize organic compounds. The more severe the organic matter contamination by water, the greater the Chemical oxygen demand value. The most typical use of Chemical oxygen demand is to estimate the number of oxidizable contaminants present in Pond water. A high Chemical oxygen demand indicates the presence of all types of organic material, both biodegradable and non-biodegradable and subsequently the degree of pollution in the water. Chemical oxygen demand in Ponds water ranged from 11.80-18.20, 10.00-17.00, 14.00-16.80 and 9.20-17.40 mg/l, with a mean value of 14.24±2.71, 12.4±2.5, 15.37±1.33 mg/l and 12.00±3.33 mg/l in 2021, whereas it ranged from 13.30-17.60, 11.90-16.2, 9.2-132 and10.86-17.60 mg/l, with a mean value of 15.3±1.76, 14.18±1.85, 11.30±1.65 and 14.25±2.78 mg/l in 2022 in the Pre-monsoon , monsoon, autumn and post-monsoon seasons, respectively. In 2021, the Chemical oxygen demand at Kurukshetra Pond was highest in the Pre-monsoon season and lowest in the post- monsoon season at Suryakund , while in 2022, the Chemical oxygen demand was recorded highest at Ishwargangi Pond in the pre- monsoon season and Pishach Mochan in post-monsoon season and the lowest at Suryakund in the autumn season (fig-4.11). Chemical oxygen demand in drinking water should not exceed 10 mg/l as recommended by the WHO. It was found that during the Pre- monsoon, monsoon, autumn and post-monsoon seasons, most of the samples showed higher value of chemical oxygen demand than the WHO permissible limits.

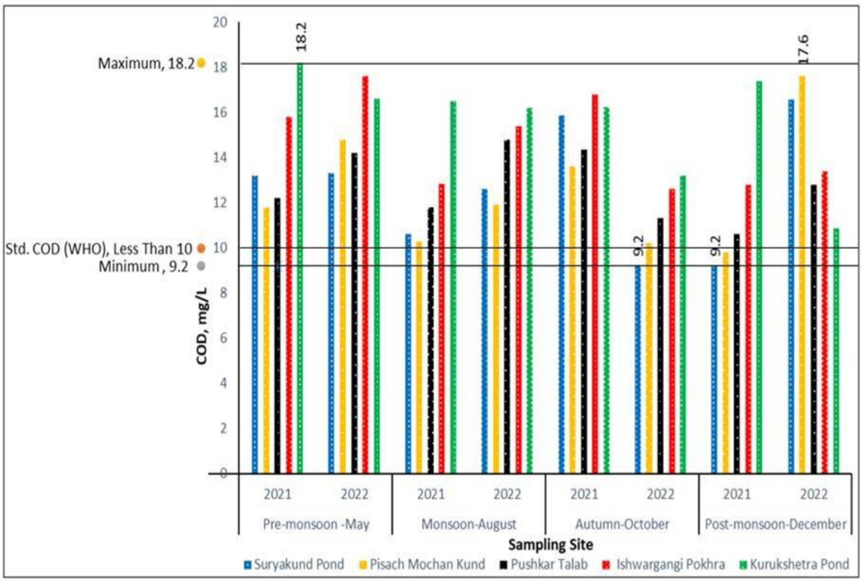


Fig.4.11: Spatio-temporal changes in water quality parameter, COD, mg/l

4.1.12 Lead (Pb)

A little quantity of lead is present almost everywhere. Human activities such as the burning of fossil fuels, mining and industrialization increase the quantity of lead in our environment. The recognized toxicity of lead to humans and other living things makes it a significant environmental pollutant. Only small number of elements falls into the category of being purely toxic, especially lead. Therefore, Lead can have a number of negative effects, including an increase in blood pressure, kidney and brain damage, sperm damage, a decrease in children's intellectual abilities and so on. Lead (Pb) in Ponds water ranged from 0.004-0.012, 0.00-0.01, 0.00-0.01 and

0.01-0.017 mg/l, with a mean value of 0.007±0.004, 0.02±0.04, 0.01±0.003 and 0.01±0.005 mg/l in 2021, whereas it arranged from 0.003-0.01, 0.001-0.022, 0.004-0.012 and0.004-0.012 mg/l, with a mean value of 0.003±0.004, 0.009±0.008, 0.008±0.004 and 0.007±0.005 mg/l in 2022 in the Pre-monsoon, monsoon, autumn and post-monsoon seasons, respectively. Plastic and rubber processing, paint, metals and alloys, batteries, cloth, waste disposal and other industries are potential sources of lead in Ponds. Lead enters the Pond along with urban runoff, which is mixed with industrial effluent and the city's sewage. In 2021, the Lead at Ishwargangi Pond was highest in the post-monsoon season and lowest in the monsoon season at Pushkar Pond. While in 2022, the highest Lead was recorded at Pishach Mochan in the monsoon seasons and the lowest at Pushkar Pond in the monsoon and Kurukshetra Pond in post-monsoon season (fig-4.12). Lead in in drinking water should not exceed 0.01 mg/l as recommended by the BIS, WHO and EU. It was found that during the Pre-monsoon, monsoon, autumn and post-monsoon seasons, some of the samples showed a higher value of lead than the BIS, WHO and EU permissible limits. It was found that during the Pre-monsoon, monsoon, autumn and post-monsoon seasons, most of the samples showed a higher value of Lead than the BIS, WHO and EU permissible limits.

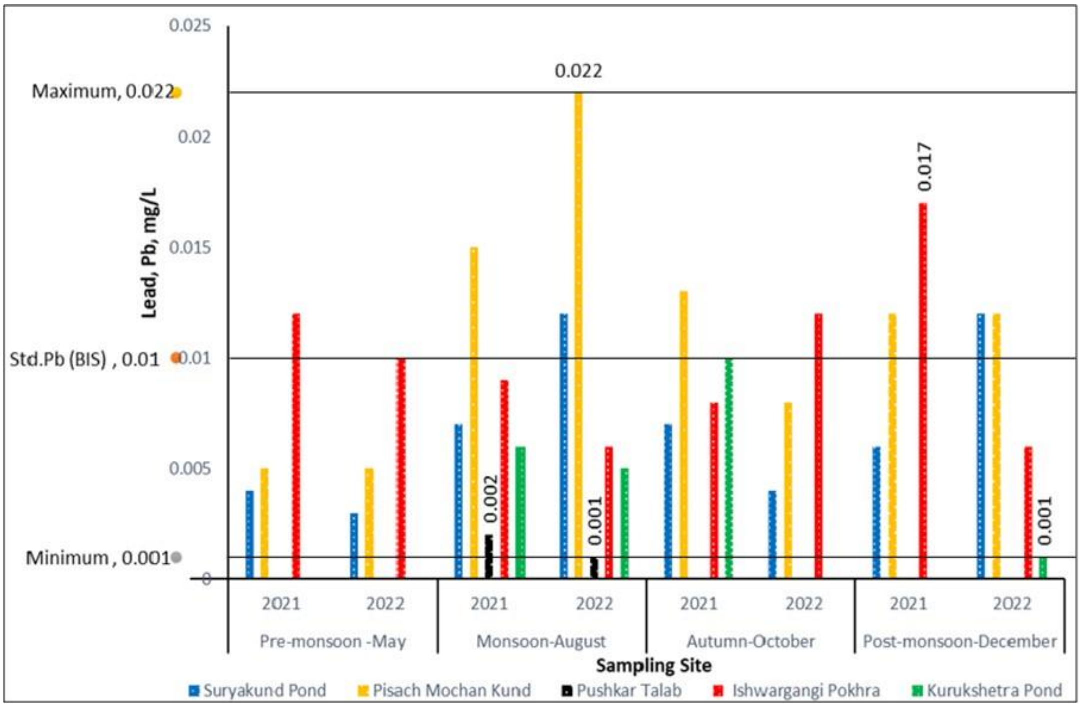


Fig.4.12: Spatio-temporal changes in water quality parameter, Lead, mg/l

4.1.13 Nickel (Ni)

Nickel pollution in Pond water can severely impact aquatic ecosystems and water quality. Elevated nickel concentrations can be toxic to fish and invertebrates, disrupting their growth, reproduction and overall health, which in turn reduces biodiversity. The metal can bioaccumulates in organisms, posing risks to predators and potentially affecting human health through contaminated fish consumption. Additionally, nickel alters the chemical composition of the water, impacting pH levels and nutrient availability, which can lead to further ecological imbalances. Nickel in Ponds water ranged from 0.003-0.009, 0.00-0.00, 0.00-0.01 and 0.01-0.01 mg/l, with a mean value of 0.007±0.003, 0.00±0.00, 0.007±0.004 and 0.01±0.003 mg/l in 2021, whereas it ranged from 0.004-0.006, 0.003-0.012, 0.002-0.012 and0.003-0.008 mg/l, with mean value of 0.005±0.001, 0.007±0.003, 0.006±0.005 and 0.005±0.002 mg/l in 2022 in the Pre-monsoon , monsoon, autumn and post-monsoon seasons, respectively. A key source of nickel in urban Ponds is storm water runoff from roads and industrial areas, carrying pollutants like vehicle emissions and industrial waste. In 2021, the nickel was highest at Pishach Mochan in autumn and ishwargangi Pond in monsoon season and lowest in the post-monsoon season at Kurukshetra Pond. While in 2022, the highest nickel was recorded at Ishwargangi Pond in the monsoon and autumn season both and the lowest at Kurukshetra Pond in the autumn season.(fig- 4.13)

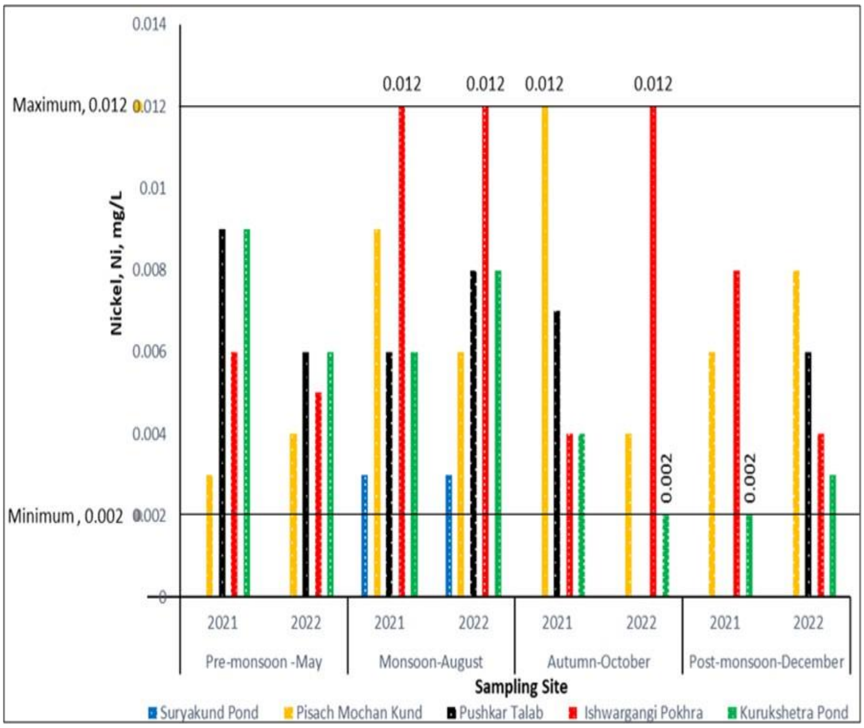


Fig.4.13: Spatio-temporal changes in water quality parameter, Nickel, mg/l

4.1.14 Copper (Cu)

Copper pollution in urban Ponds primarily stems from storm water runoff, vehicle brake pad wear and corrosion of building materials like copper pipes and roofing. Industrial discharges and leaching from agricultural activities can also contribute to elevated copper levels in these water bodies. This pollution poses significant risks to aquatic ecosystems, as copper are highly toxic to fish, invertebrates and algae, leading to disruptions in food chains and overall Pond health. Urbanization intensifies copper contamination, especially during rain events when pollutants are washed into Ponds. Copper in Ponds water ranged from 0.003- 0.045, 0.00-0.00, 0.00-0.01 and 0.00-0.01 mg/l, with a mean value of 0.015±0.019, 0.01±0.006, 0.007±0.003 and 0.01±0.002 mg/l in 2021, whereas it ranged from 0.001-0.032, 0.008-0.012, 0.001-0.012 and 0.001-0.014 mg/l, with a mean value of 0.011±0.012, 0.008±0.002, 0.005±0.006 and 0.008±0.005 mg/l in the Pre-monsoon, monsoon, autumn and post-monsoon seasons, respectively. In 2021, the copper at Suryakund Pond was highest in the Pre-monsoon season and lowest in the autumn season at Pushkar Pond. while in 2022, the highest copper was recorded at suryakund Pond in the pre-monsoon season and the lowest at Kurukshetra Pond in pre-monsoon as well as pushkar Pond in post monsoon season (fig-4.13). Copper in drinking water should not exceed 0.01 mg/l as recommended by the BIS. It was found that during the Pre- monsoon, monsoon, autumn and post-monsoon seasons, some of the samples showed a higher value of copper than the BIS permissible limits. It was found that during the Pre monsoon, autumn, most of the samples showed a higher value of copper than the BIS permissible limits.

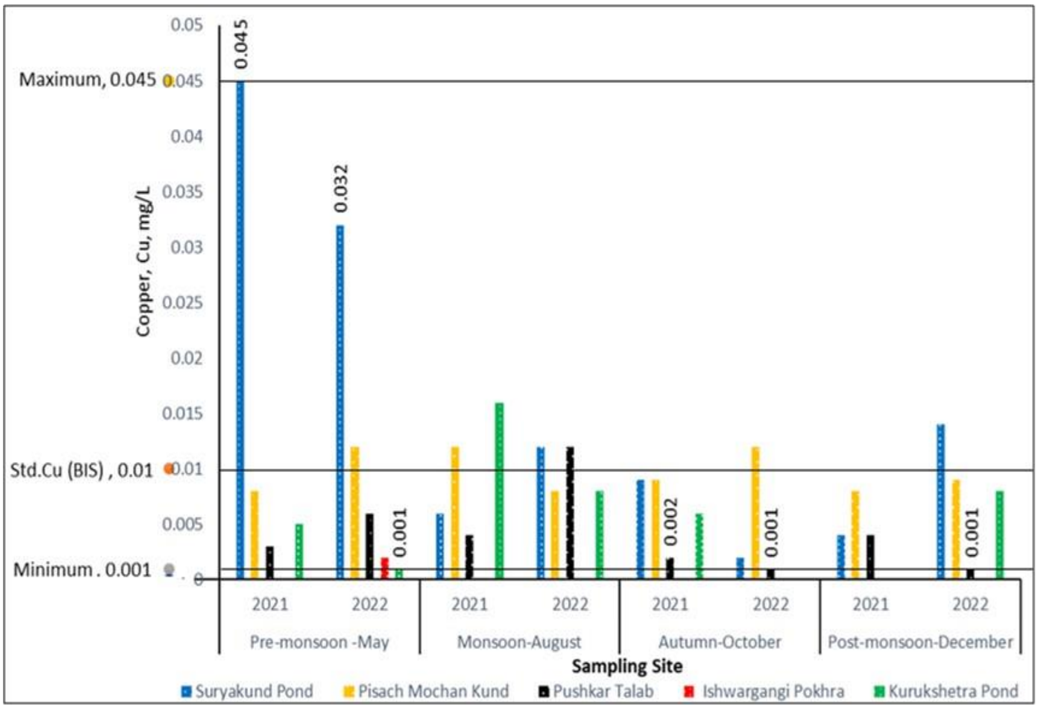


Fig.4.14. Spatio-temporal changes in water quality parameter, Copper, mg/l

4.1.15 Zinc (Zn)

Zinc pollution in urban Ponds is primarily caused by storm water runoff from roads, roofs and buildings, as well as from industrial activities and vehicle tire wear. Zinc can also enter Ponds through corrosion of galvanized metal structures and improper disposal of industrial waste. Elevated zinc levels are harmful to aquatic life, particularly affecting fish, invertebrates and plant life, disrupting Pond ecosystems. Urbanization increases the flow of zinc-laden runoff into Ponds, especially during heavy rains. Zinc in Ponds water ranged from 1.9-6.3, 2.4-6.7, 1.60-5.8 and 1.4-5.2 mg/l, with a mean value of 3.34±1.17, 3.92±1.81, 3.16±1.72 and 3.4±1.56 mg/l in 2021, whereas it ranged from 1.9-4.4, 3.2-5.8, 0.8-6.8 and 2.2-4.2 mg/l, with a mean value of 3.3±0.89, 4.24±1.18,3.52±1.98 and 3.12±0.88 mg/l in 2022 in the Pre-monsoon , monsoon, autumn and post-monsoon seasons, respectively. In 2021, the zinc at Surya kund was highest in the monsoon season and lowest in the post- monsoon season at Pushkar Pond. While in 2022, the highest zinc was recorded at Ishwargangi Pond in the autumn season and the lowest at Pushkar Pond in the autumn season (fig-4.15).

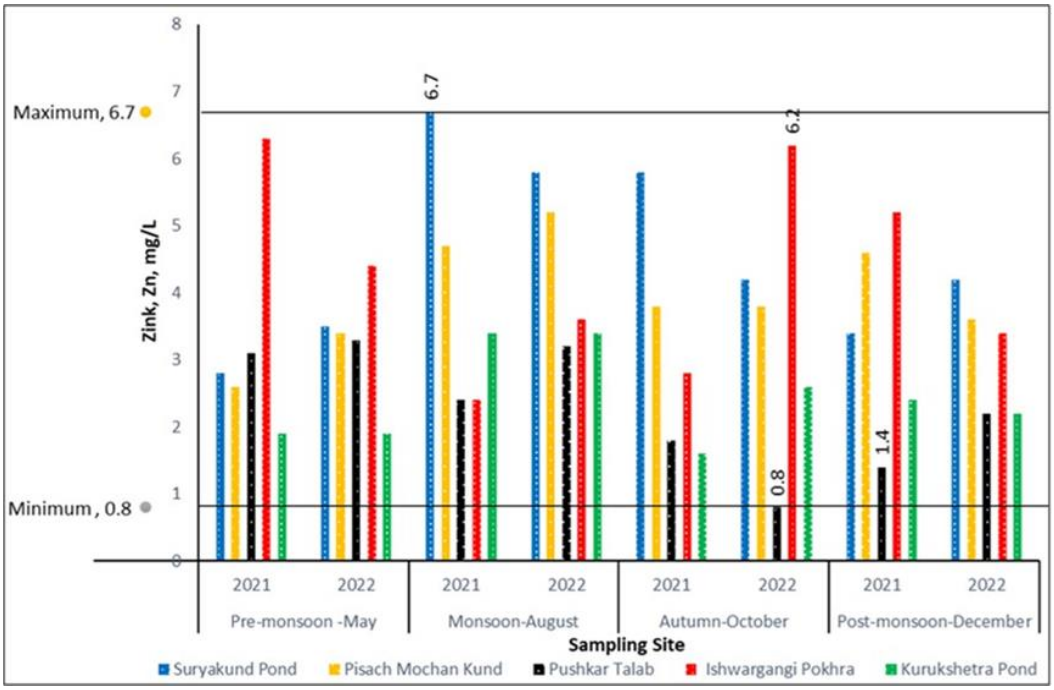


Fig.4.15. Spatio-temporal changes in water quality parameter, Zinc, mg/l

4.2 Sources Attribution of Pollution Sources

A statistical method known as principal component analysis (PCA) significantly reduces the number of variables by combining the original variables into new, uncorrelated principal components (PCs), which are linear combinations of the original variables (Singh *et al.*. 2015). With no loss of the original data, PCA is used to decipher the underlying relationships between the variables and extract the most significant data that describes the whole data set (Giripunje *et al.* 2016). In PCA, the total number of principle components (PCs) represents all potential sources of variance in the data set (Sharma *et al.*., 2015). The PCs with the highest eigenvalue have the most variation and so on, down to components with tiny or negative eigenvalues that are typically excluded from solutions. PCA with varimax rotation of standardized component loadings was employed to maximize the variance across variables under each factor and PCs with Eigen values larger than one were maintained (Singh *et al.*., 2004; Giripunje, 2016).

A biplot, a sort of statistical exploratory presentation, may be used to identify which variables are high or low, at which sampling site and at what time of sampling, as well as to get a sense of which sites have similar contaminant types and which sites have different types of contamination exposure at the maximum and minimum concentrations.

The Pre-monsoon, monsoon, autumn and post-monsoon data sets of the Ponds (kunds) for 2021 and 2022 were analyze for the principal component analysis. The number of PCs to be extracted was determined using the Kaiser's criteria (Kaiser 1960) with varimax rotation. The number of principal components with an Eigen value equal to or greater than 1 is to be acknowledged as potential sources of variation in the data set according to these criteria.

4.2.1 Pre-monsoon Season

The Pre-monsoon data for Ponds were examined and the results showed that four PCs in 2021 and four PCs in 2022 had eigenvalues larger than 1, respectively, explaining 100 % of the variance (Table 4.3 & 4.4).Their relative loading of variables on significant principal components is also tabulated (Table 4.5 & 4.6).The values of the relevant loadings, along with knowledge of the main sources of pollution at particular sites, may be used to reasonably interpret these PCs. The loadings chosen for the PC interpretation have absolute values larger than 0.5 of the maximum value (Sharma *et al.*. 2015). Additionally, the loadings were categorized as "strong," "moderate” and "weak," with absolute loading values of 0.75, 0.75-0.50 and 0.5-0.30, respectively.

The PCA of Pond water samples from Varanasi highlights distinct temporal and spatial variability in water quality, with key parameters like EC, TDS, chloride, temperature and nutrient levels (e.g., nitrate, phosphate) being significant. These findings suggest that each Pond may be subject to unique environmental and anthropogenic influences, necessitating targeted water quality management strategies to address specific pollutants or seasonal fluctuations.

Variance explained by principal components, in 2021, Component 1 accounts for 42.544% of the total variance, indicating it captures the most significant variation in the dataset. Component 2 explains an additional 26.497%, bringing the cumulative variance to 69.041%. Components 3 and 4 contribute 20.214% and 10.745%, respectively, achieving a total cumulative variance of 100%. While in 2022, Component **1** has an even higher explained variance at

50.897%, suggesting an increased contribution to data variation compared to 2021.Component **2** explains 22.647%, with a cumulative variance of 73.544%.Components 3 and 4 account for 18.821% and 7.635%, respectively, again reaching a total cumulative variance of 100%.These results imply that the first two principal components in both years explain a significant portion of the data variation, with Component 1 capturing the highest variance and Component 2 contributing additional distinctions among water quality parameters.

The loadings of each variable on the principal components provide insights into the specific parameters that contribute to the variance observed across the sites in each year. In Pre-monsoon 2021, Component 1 Shows high positive loadings for TDS (0.914), electrical conductivity (EC) (0.884), chloride (0.821) and pH (0.864), indicating that these parameters contribute significantly to the primary component. This suggests a pattern of high ion concentration (TDS and EC) associated with higher chloride levels and slightly alkaline conditions. Component 2, Primarily driven by temperature (0.914) and phosphate (0.821). The strong association of these two variables with Component 2 may reflect seasonal thermal effects and nutrient concentration dynamics in Pre-monsoon. Component 3, Strongly associated with nitrate (-0.825) and sulphate (-0.875), with both having negative loadings, suggesting that these ions may have an inverse relationship or a distinct pollution source compared to other parameters. Component 4, Primarily reflects dissolved oxygen (DO) (0.825) and copper (0.753), possibly indicating an association with oxygenation levels and trace metal concentrations. In the pre-monsoon season of 2022, Component 1 exhibited high loadings for TDS (0.942), EC (0.984), pH (-

0.894), and phosphate (-0.924), indicating strong positive associations of TDS and EC, while pH and phosphate showed negative associations. This component might indicate increased ionic content linked with changes in pH and nutrient balance. Component 2, Temperature (0.828) and copper (0.758) are major contributors, similar to 2021, suggesting a link between seasonal temperature variation and metal concentration. Component 3 Highlights nitrate (0.822) and sulphate (0.846) as in 2021 but with positive loadings, indicating shifts in their seasonal or spatial distribution. Component 4, DO (0.825) and zinc (0.723) have high loadings, indicating possible associations between oxygen levels and trace metals in the water (Kavitha, *et al.* 2012).

The biplot charts for 2021 and 2022 illustrate the distribution of water quality parameters across different Ponds. In 2021, Pushkar Pond and Kurukshetra Pond appear close to parameters like EC, TDS and chloride, suggesting higher dissolved solids in these locations. Conversely, Suryakund Pond is closer to nitrate and sulphate, indicating distinct nutrient dynamics at this site. While in 2022, Pushkar Pond and Pishach Mochan Pond show proximity to phosphate, copper and pH, suggesting elevated nutrient levels and metal content. Suryakund Pond and Ishwargangi Pond align with temperature and sulphate, reflecting possible thermal or chemical influences specific to these Ponds.

The PCA results reveal several key trends:

The dominance of EC, TDS and chloride in Component 1 across both years indicates that ion concentration and dissolved solids are primary factors influencing Pond water quality. This might be attributed to the natural mineral composition of the water or anthropogenic pollution sources (table-4.3 and table- 4.4).

The strong loading of temperature on Component 2 in both years emphasizes its role in shaping seasonal water quality dynamics, likely influencing biological activity and other temperature-sensitive parameters.

Parameters like phosphate, nitrate and metals such as copper and zinc are variably loaded across components, pointing to specific sources or environmental conditions at each Pond that impact nutrient and trace metal levels. This pattern may reflect agricultural runoff, urban wastewater, or site-specific geochemical characteristics.

The association of DO with BOD and COD highlights the interplay between dissolved oxygen levels and organic matter, which could be indicative of the Pond's health and pollution load.

Table 4.3 The PCA axes explaining complete variance of the data (Pre-monsoon 2021)

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	6.382	42.544	42.544	6.382	42.544	42.544	4.322	28.814	28.814
2	3.975	26.497	69.041	3.975	26.497	69.041	4.208	28.057	56.870
3	3.032	20.214	89.255	3.032	20.214	89.255	3.715	24.770	81.640
4	1.612	10.745	100.000	1.612	10.745	100.000	2.754	18.360	100.000

Extraction Method: Principal Component Analysis.

Table 4.4 The PCA axes explaining complete variance of the data (Pre-monsoon 2022)

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	7.635	50.897	50.897	7.635	50.897	50.897	5.909	39.393	39.393
2	3.397	22.647	73.544	3.397	22.647	73.544	3.620	24.134	63.527
3	2.823	18.821	92.365	2.823	18.821	92.365	2.981	19.871	83.398
4	1.145	7.635	100.000	1.145	7.635	100.000	2.490	16.602	100.000

Extraction Method: Principal Component Analysis.

Table 4.5 Loading of variables on significant principal component of surface water data of Ponds (Pre-monsoon 2021)

	Component			
	1	2	3	4
Temperature, ^{0}C	-.012	.914	-.405	.033
pH	.334	-.864	-.374	.047
Electrical Conductivity (mS/cm)	.031	-.024	.170	.985
Chloride, mg/l	-.338	.575	.605	-.434
Sulphate, mg/l	.851	.314	-.254	.334
Nitrate, mg/l	-.980	-.075	.057	.176
Phosphate, mg/l	.140	-.242	-.960	-.035
TDS, mg/l	.041	-.029	.166	.985
DO, mg/l	.712	-.556	-.428	-.002
BOD, mg/l	-.380	-.053	.841	.381
COD, mg/l	-.191	-.014	.967	.166
Lead, Pb, mg/l	.441	.851	.183	-.219
Nickel, Ni, mg/l	-.959	.033	.283	.001
Copper, Cu, mg/l	.764	-.337	-.212	.507
Zinc, Zn, mg/l	.053	.991	.118	.036

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

Table 4.6 Loading of variables on significant principal component of surface water data of Ponds (Pre-monsoon 2022)

	Component			
	1	2	3	4
Temperature, 0C	.317	-.463	.828	.010
pH	-.894	-.299	-.321	.089
Electrical Conductivity (mS/cm)	.043	.984	-.170	.045
Chloride, mg/l	.826	-.357	.432	-.055
Sulphate, mg/l	-.020	.701	.486	-.521
Nitrate, mg/l	.349	.027	.926	-.137
Phosphate, mg/l	-.942	-.334	.003	.020
TDS, mg/l	.044	.983	-.171	.046
DO, mg/l	-.708	.288	-.349	-.542
BOD, mg/l	.954	-.052	.276	-.106
COD, mg/l	.951	-.109	.072	.279
Lead, Pb, mg/l	.270	.004	-.278	.922
Nickel, Ni, mg/l	.572	-.556	.588	-.135
Copper, Cu, mg/l	-.736	.486	-.472	-.004
Zinc, Zn, mg/l	-.234	.092	.061	.966

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

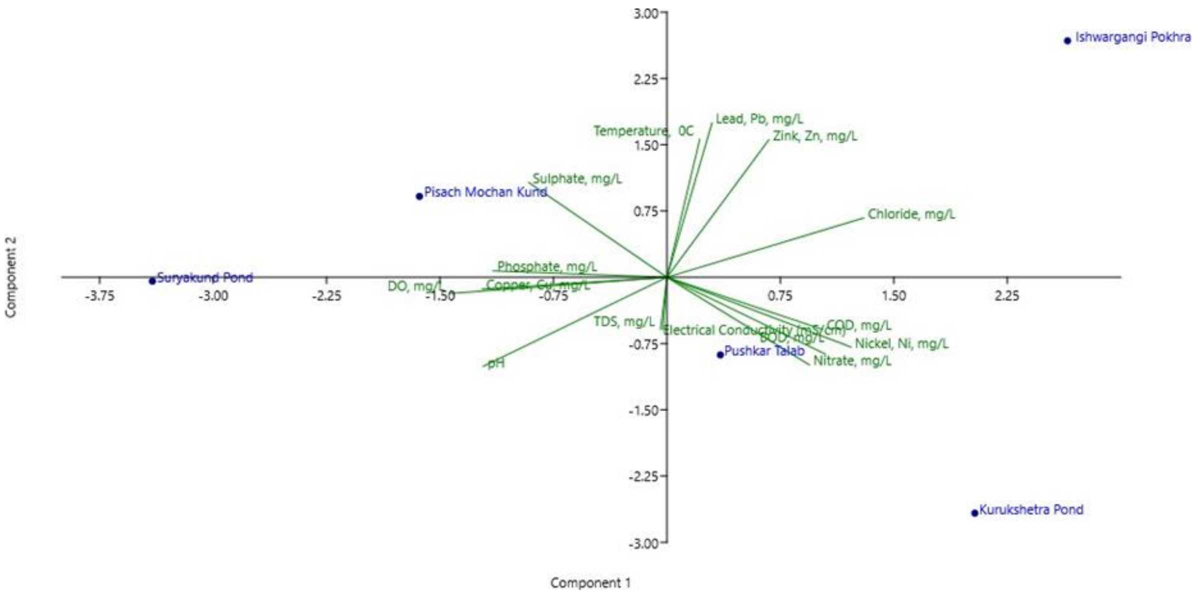


Fig.4.16. Result of principal component analysis (PCA) for the site and surface water data of Ponds (Pre-monsoon 2021)

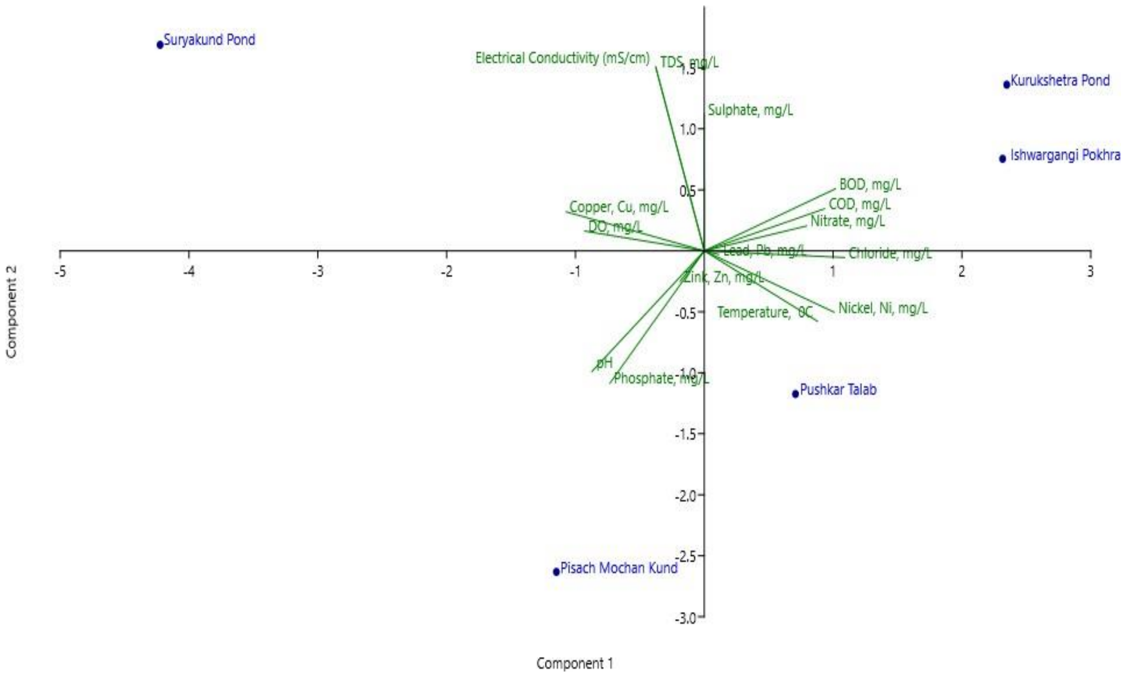


Fig. 4.17. Result of principal component analysis (PCA) for the site and surface water data of Ponds (Pre-monsoon 2022)

4.2.2 Monsoon Season

The monsoon season PCA reveals that water quality in Varanasi's Ponds is heavily influenced by ionic content (EC, TDS, and chloride), nutrient levels (nitrate and phosphate) and organic pollution (COD and sulphate). The influence of trace metals further suggests specific anthropogenic impacts. These findings indicate the need for targeted monitoring and management of ionic and nutrient pollution sources during the monsoon season to maintain water quality and ecosystem health in the region.

The findings of PCA for the Pond water data for the respective monsoon season produced four principal components in 2021 and four in 2022, which, respectively, explained 100 percent and100 percent of the total variance (Tables 4.7 and 4.8) and their corresPonding loadings of variables on important principal components are also tabulated (Tables 4.9 and 4.10).

Variance Explained by Principal Components, In Monsoon 2021, Component 1 accounts for 41.814% of the total variance, capturing a substantial portion of the data variation. Component 2 adds 29.992%, bringing the cumulative variance to 71.806%. Components 3 and 4 explain 19.879% and 8.315%, respectively, achieving a total cumulative variance of 100%. While in Monsoon 2022, Component 1 has an increased explained variance of 45.838%, indicating it captures the highest portion of variability. Component 2 explains an additional 30.645%, with a cumulative variance of 76.482%. Components 3 and 4 account for 16.527% and 8.891%, respectively, reaching a cumulative variance of 100%.

These results indicate that, in both years, the first two components account for a major portion of the variance, particularly in 2022. This suggests that the primary drivers of water quality variation are consistent and prominent during the monsoon season.

The loadings of each variable on the principal components reveal which parameters contribute most to each component. In the monsoon season of 2021, Component 1 exhibited strong positive loadings for electrical conductivity (0.934), TDS (0.919), chloride (0.882), and pH (0.873). This suggests a high influence of ionic strength and dissolved salts on water quality, likely due to increased runoff or dilution from rainwater. Component 2, High positive loading for temperature (0.562) and strong negative loading for nitrate (-0.963) and phosphate (-0.973), indicating an inverse relationship between nutrient concentrations and temperature. Component 3, Loadings are high for sulphate (0.822) and COD (0.745), suggesting an association between sulphate levels and organic matter. Component 4, Zinc has a high loading (0.990), indicating that trace metals may contribute to site-specific or seasonal variation in water quality. In m**onsoon 2022,** Component 1, High positive loadings for electrical conductivity (0.996), TDS (0.992) and pH (-0.836), with chloride also having a high loading (0.833).

This pattern is consistent with 2021, reinforcing the role of dissolved ions as a primary factor in water quality during monsoon. Component 2, High negative loadings for nitrate (-0.936) and phosphate (- 0.913), again suggesting nutrient variations inversely associated with other water quality factors. Component 3, Sulphate (0.828) and COD (0.859) contribute significantly, similar to 2021, indicating organic pollution and sulfate concentration as associated parameters. Component 4,Lead and trace metals, such as nickel, display significant loadings, highlighting the role of metal contamination as an additional factor.

The biplot charts for monsoon 2021 and 2022 illustrate the distribution of water quality parameters across different Ponds. In 2021, Kurukshetra Pond and Suryakund Pond appear to be associated with parameters like EC, TDS and chloride, indicating high ionic content. Pishach mochan Pond is closer to nitrate and phosphate, suggesting higher nutrient concentration. In 2022, similar patterns are observed. Pushkar Pond is associated with high levels of TDS, EC and chloride, while Pishach mochan Pond shows an association with phosphate and nitrate, reinforcing the observation of nutrient dynamics at this site.

The PCA analysis for the monsoon season highlights the following key findings:

- The dominance of parameters like EC, TDS and chloride in Component 1 across both years indicates that ionic concentration is a major factor influencing water quality during the monsoon. This may

  be due to runoff, which carries various dissolved salts from surrounding areas into the Ponds.
- High loadings of nitrate and phosphate, particularly in Component 2 with negative correlations, indicate that nutrient levels are distinct from other water quality parameters. This could be due to localized sources of nutrients, such as agricultural runoff or effluents, leading to variable

nutrient loads in Ponds.

- Components 3 and 4, associated with COD and sulfate, suggest that organic pollution and sulfate levels are connected. This may be due to the presence of organic pollutants, which contribute to COD and the presence of sulfate-reducing bacteria during monsoon when there is organic matter accumulation.

- Loadings for metals like zinc and nickel in 2021 and 2022 indicate site-specific variations, which may be linked to pollution sources, such as industrial runoff or urban waste, affecting the Ponds differently.

Table 4.7 The PCA axes explaining complete variance of the data (Monsoon-2021)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.272	41.814	41.814	6.272	41.814	41.814	4.068	27.119	27.119
2	4.499	29.992	71.806	4.499	29.992	71.806	3.904	26.028	53.148
3	2.982	19.879	91.685	2.982	19.879	91.685	3.759	25.059	78.206
4	1.247	8.315	100.000	1.247	8.315	100.000	3.269	21.794	100.000

- Extraction Method: Principal Component Analysis.

Table 4.8 The PCA axes explaining complete variance of the data (Monsoon-2022)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.876	45.838	45.838	6.876	45.838	45.838	5.089	33.929	33.929
2	4.597	30.645	76.482	4.597	30.645	76.482	4.532	30.213	64.143
3	2.479	16.527	93.009	2.479	16.527	93.009	2.856	19.037	83.179
4	1.049	6.991	100.000	1.049	6.991	100.000	2.523	16.821	100.000

Extraction Method: Principal Component Analysis.

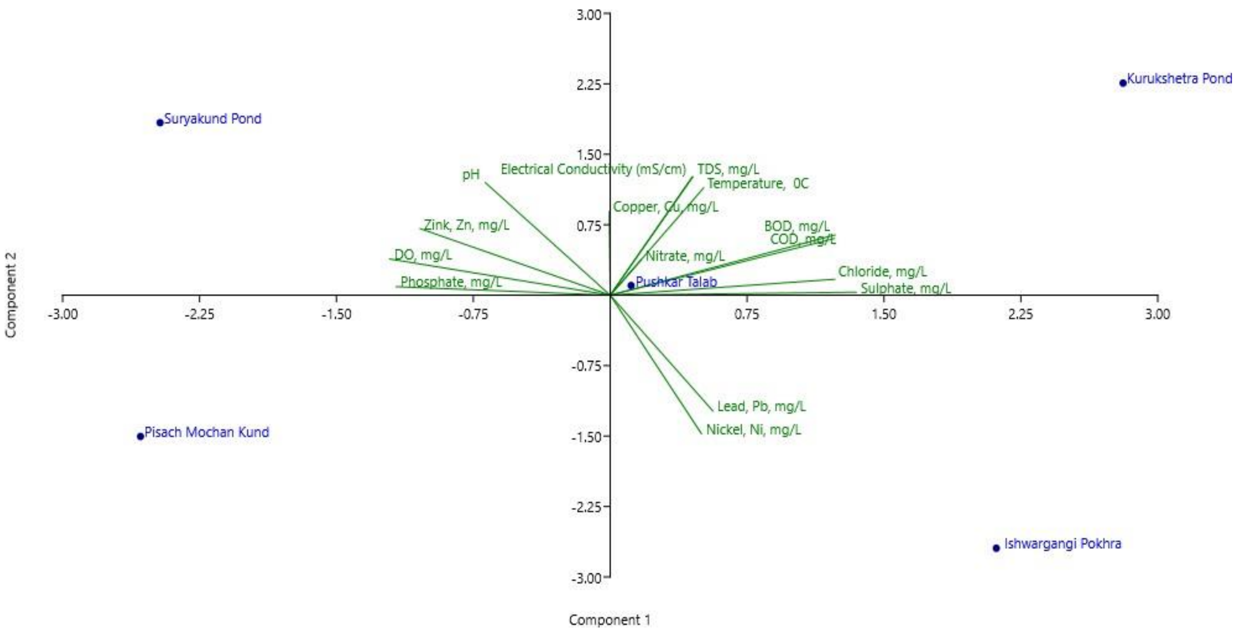


Fig.4.18: Result of principal component analysis (PCA) for the site and surface water data of Ponds (Monsoon-2021)

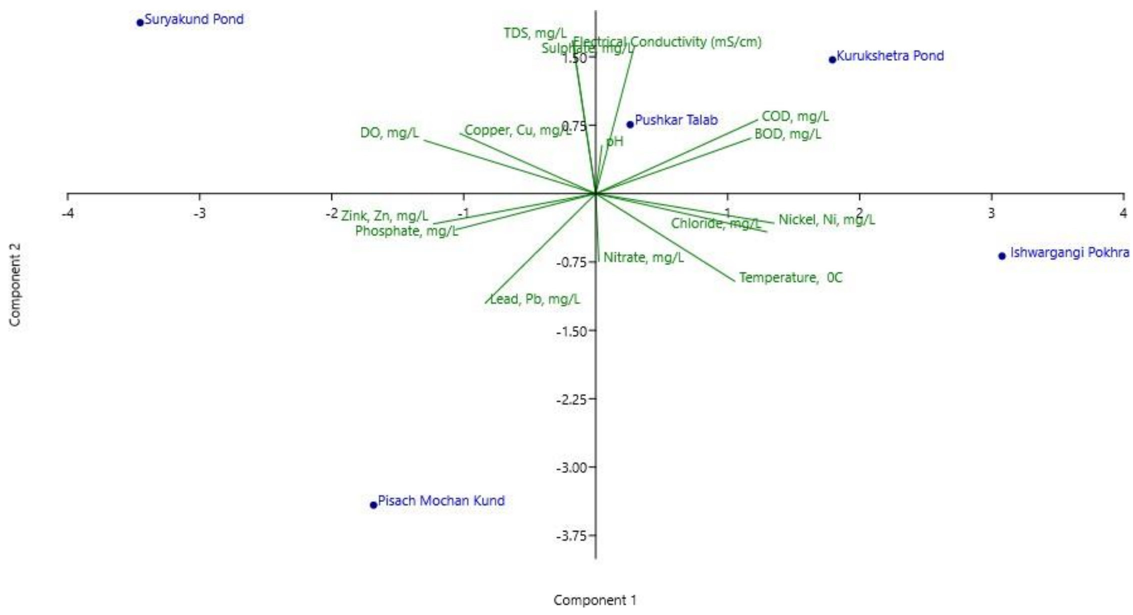


Fig.4.19: Result of principal component analysis (PCA) for the site and surface water data of Ponds (Monsoon-2022)

4.2.3 Autumn season

The PCA analysis in autumn season provides insights into the changing water quality parameters over the two years. The shift from a dominance of electrical parameters to the inclusion of pH and phosphate in PC1 suggests a change in the contributing factors affecting the Ponds' water chemistry. The emergence of metals as significant in **PC4** in 2022 may call for targeted investigations to identify potential sources of contamination.

The PCA analysis for Pond water data in the autumn season identified four principal components in both 2021 and 2022, explaining 100% of the total variance in each year (Tables 4.7 and 4.8). In 2021The first four principal components (PCs) explain 100% of the variance in the dataset.PC1 accounts for 44.831% of the variance, indicating that a significant portion of the variation in the data is represented by this component.PC2 contributes 31.753% to the cumulative variance, making up a total of 76.584% when combined with

PC1.PC3 and PC4 add an additional 13.548% and 9.868%, respectively, culminating in a cumulative variance of 100%.PC1 shows significant positive loadings for electrical conductivity (0.997), chloride (0.995) and TDS (0.986), indicating that these parameters strongly influence this component.PC2 is influenced positively by phosphate (0.987) and nitrate (0.917), suggesting their importance in explaining variance for this component.PC3 has strong negative loadings for COD (-0.854) and DO (- 0.791), indicating a correlation between these parameters.PC4 reflects significant contributions from pH (0.491) and temperature (0.770).

The biplot for 2021 shows the clustering of variables around the different principal components. High-loading parameters such as electrical conductivity, chloride and TDS align closely with PC1, signifying their combined effect in explaining the data variation.PC2 groups variables like phosphate and nitrate, showing their joint influence in the dataset. Parameters such as DO and COD are aligned with PC3, indicating their negative correlation with other components.

In 2022, the first four components explain 100% of the variance.PC1 accounts for 39.204% of the variance, slightly lower than in 2021.PC2 contributes 30.775%, leading to a combined variance of 69.979% when summed with PC1.PC3 adds 15.783% and PC4 accounts for 14.283%, completing the 100% explained variance.PC1 in 2022 features strong positive loadings for pH (0.952), phosphate (0.941) and COD (0.914).PC2 shows significant loadings for chloride (0.896), sulphate (0.829) and TDS (0.735).PC3 is associated with temperature (0.857) and BOD (0.840), highlighting their combined variance.PC4 is influenced by metal concentrations such as copper (0.814) and zinc (0.780).

In the 2022 biplot illustrates different relationships compared to 2021. Variables like pH and phosphate are prominently aligned with PC1, showing a shift in the major contributing parameters.PC2 highlights the influence of chloride, sulphate and TDS. Metals like copper and zinc contribute significantly to PC4, indicating changes in metal concentration among Ponds over time.

The PCA results for 2021 and 2022 indicate shifts in the dominant water quality parameters over the two years. While PC1 in 2021 was heavily influenced by electrical conductivity, chloride and TDS, in 2022, PC1 shifted towards pH and phosphate, suggesting possible environmental or anthropogenic changes influencing water chemistry. The increased impact of COD and DO in 2021 suggests that organic pollution and oxygenation status played significant roles in defining the water quality, whereas in 2022, these factors were more distributed among other components.

The high positive loadings of phosphate and nitrate in PC2 (2021) and their reduced influence in 2022 imply potential changes in nutrient loading, possibly due to agricultural runoff or waste discharge. The role of metals like copper and zinc in PC4 for 2022 highlights potential contamination issues, which were less significant in 2021. This could indicate increased industrial or urban impact.

The positioning of Ponds on the biplots reflects their similarity or difference based on the principal components. Ponds closely grouped together share similar water quality characteristics, while those further apart indicate varied influences. For instance, the alignment of variables such as electrical conductivity and TDS with PC1 in 2021 may point to Ponds with higher salinity or dissolved solids concentration, potentially linked to pollution sources.

Table 4.9 The PCA axes explaining complete variance of the data (Autumn season- 2021)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.725	44.831	44.831	6.725	44.831	44.831	6.174	41.159	41.159
2	4.763	31.753	76.584	4.763	31.753	76.584	5.025	33.501	74.660
3	2.032	13.548	90.132	2.032	13.548	90.132	1.919	12.793	87.453
4	1.480	9.868	100.000	1.480	9.868	100.000	1.882	12.547	100.000

Extraction Method: Principal Component Analysis.

Table 4.10 The PCA axes explaining complete variance of the data (Autumn season-2022)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.881	39.204	39.204	5.881	39.204	39.204	4.436	29.571	29.571
2	4.616	30.775	69.979	4.616	30.775	69.979	4.162	27.746	57.317
3	2.361	15.738	85.717	2.361	15.738	85.717	3.453	23.017	80.334
4	2.142	14.283	100.000	2.142	14.283	100.000	2.950	19.666	100.000

Extraction Method: Principal Component Analysis.

Table 4.11 Loading of variables on significant principal component of surface water data of Ponds (Autumn season- 2021)

	Component			
	1	2	3	4
Temperature, 0C	.380	-.420	.294	-.770
pH	.575	-.437	-.487	.491
Electrical Conductivity (mS/cm)	-.997	-.010	-.008	-.082
Chloride, mg/l	.893	.428	-.026	.139
Sulphate, mg/l	.078	.987	-.141	.021
Nitrate, mg/l	.978	-.205	.001	-.035
Phosphate, mg/l	-.696	.506	.487	.150
TDS, mg/l	-.996	-.010	-.009	-.084
DO, mg/l	-.453	.843	-.018	-.289
BOD, mg/l	.376	.891	.243	.077
COD, mg/l	-.374	.912	.138	.094
Lead, Pb, mg/l	.110	-.080	.991	-.015
Nickel, Ni, mg/l	-.100	-.916	.200	-.333
Copper, Cu, mg/l	.873	-.143	.436	.164
Zinc, Zn, mg/l	.444	.024	.219	.869

Extraction Method: Principal Component Analysis.

4YRotation Method: Varimax with Kaiser Normalization.

Table 4.12 Loading of variables on significant principal component surface water data of Ponds (Autumn season-2022)

	Component			
	1	2	3	4
Temperature, 0C	.246	.944	-.057	-.213
pH	.072	-.997	-.005	-.003
Electrical Conductivity (mS/cm)	-.160	.066	-.171	.970
Chloride, mg/l	.925	-.020	.289	-.247
Sulphate, mg/l	-.257	.829	.390	.309
Nitrate, mg/l	.487	-.136	-.821	.266
Phosphate, mg/l	-.513	.700	-.494	-.057
TDS, mg/l	-.158	.062	-.171	.971
DO, mg/l	-.992	-.027	-.122	-.033
BOD, mg/l	.655	-.736	-.087	.150
COD, mg/l	.830	-.505	-.234	-.030
Lead, Pb, mg/l	.330	.251	.873	-.258
Nickel, Ni, mg/l	.728	.000	.668	-.154
Copper, Cu, mg/l	-.355	.439	.111	-.818
Zinc, Zn, mg/l	.178	-.144	.970	-.083

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

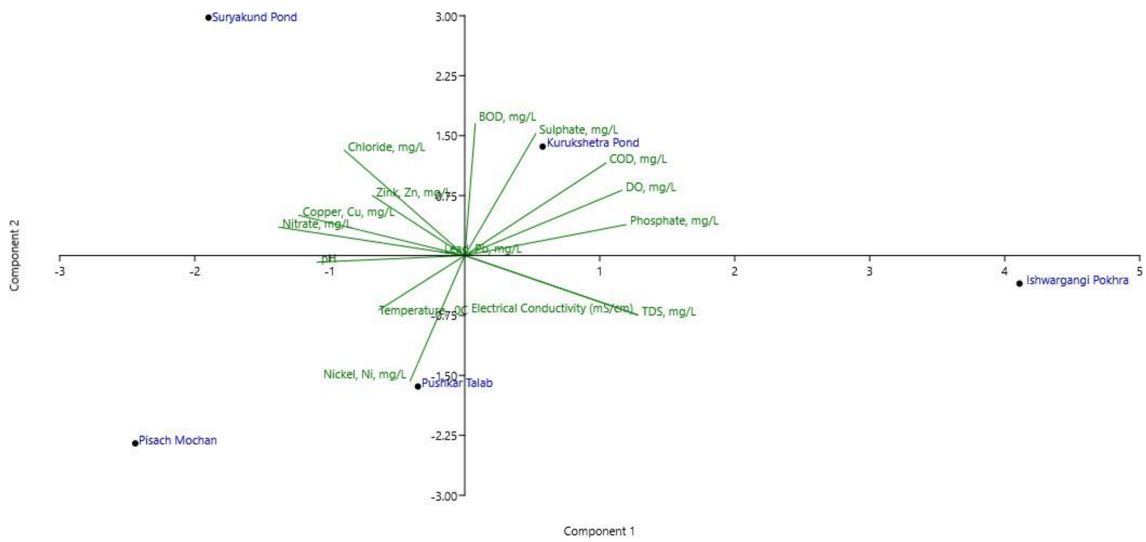


Fig.4.20.Result of principal component analysis (PCA) for the site and surface water data of Ponds (Autumn -2021)

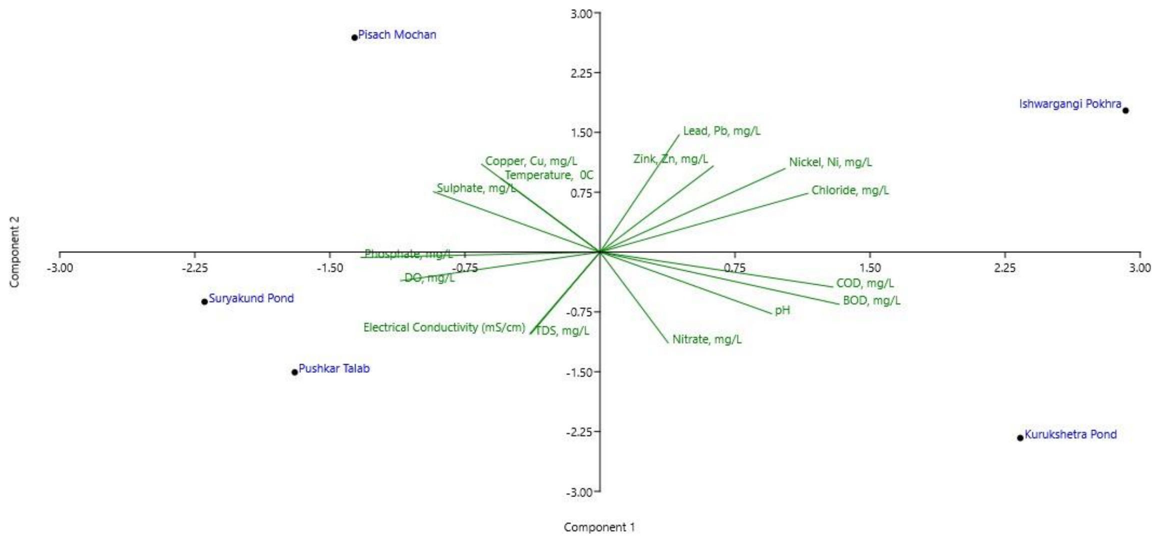


Fig.4.21.Result of principal component analysis (PCA) for the site and surface water data of Ponds (Autumn -2022)

4.2.4 Post-Monsoon Season

The PCA analysis of post-monsoon water quality data for 2021 and 2022 reveals a dynamic change in influencing factors. While PC1 in 2021 was driven by inorganic factors (sulphate and phosphate), 2022 saw a shift to a dominance by organic pollution indicators. The emergence of metals in PC3 for 2022 signals potential anthropogenic contamination.

The Principal Component Analysis (PCA) of Pond water data for the post-monsoon season identified four principal components (PCs) in both 2021 and 2022, each accounting for 100% of the total variance, indicating that these components captured all significant variations in water quality parameters across the study years. The first four components explain 100% of the variance in the dataset. In 2021, PC1 explains 40.452% of the total variance, suggesting that it captures the most critical factors influencing water quality during the post-monsoon period in 2021.PC2 contributes an additional 34.827%, with PC1 and PC2 combined explaining 75.279% of the cumulative variance.PC3 and PC**4** account for 9.154% and 11.770%, respectively, completing the variance at 100%.

PC1 has strong positive loadings for sulphate (0.969), phosphate (0.408) and temperature (-0.678). This indicates that these parameters were primary influencers in the first principal component.PC2 has high positive loadings for electrical conductivity (0.985) and nitrate (0.924), showing their significance in explaining this component's variance.PC3 shows negative loadings for COD (-0.833) and BOD (-0.820), suggesting these parameters were inversely related to others.PC4 includes pH (-0.912) and DO (-0.887) as major contributors, indicating these were important for explaining additional variance in the dataset.

The 2021 biplot shows clustering and relationships between variables. Parameters like electrical conductivity and nitrate are aligned with PC2, illustrating their joint influence. Sulphate and phosphate align with PC1, highlighting their importance. COD and BOD are positioned with PC3, reflecting their distinct contribution and inverse relationship with certain other parameters.

In 2022, the first three components explain almost all the variance, with PC1 contributing 54.243%, PC2 adding 23.268% and PC3 19.547%, together explaining 97.058% of the variance.PC4 accounts for a minimal 2.942%, which may indicate a marginal influence by factors represented by this component.

PC1 has strong positive loadings for COD (0.993), BOD (0.854) and DO (-0.854). This suggests that organic pollution indicators were primary influences in PC1 for 2022.PC2 is heavily loaded by electrical conductivity (0.891) and pH (0.936), indicating their combined impact on the second principal component.PC3 shows substantial loadings for metals, with copper (0.807) and zinc (0.884) being notable contributors, suggesting the relevance of metal contamination during this period.PC4 has only minimal loading contributions and might represent less impactful or specific localized factors.

The biplot for 2022 displays distinct clusters and relationships among parameters. COD and BOD are clustered with PC1, confirming their dominant roles as organic pollution indicators. PC2 encompasses variables such as electrical conductivity and ph. The metals (copper and zinc) are aligned with PC3, emphasizing their specific influence on this component.

PC1 in 2021 highlights the importance of sulphate and phosphate, suggesting nutrient input potentially due to agricultural runoff. In 2022, PC1 shifts to focus more on organic pollution indicators like COD and BOD, indicating an increase in organic load. PC2 in both years remains influenced by electrical conductivity, but in 2022, it also emphasizes pH, hinting at potential changes in water chemistry or buffering capacity. Metals such as copper and zinc become more significant in PC3 during 2022, implying potential industrial or urban runoff sources impacting the Ponds post-monsoon. The alignment of these metals in 2022 suggests that the influence of contamination from anthropogenic activities increased compared to 2021.

The variation in loadings between the two years shows the potential impact of different factors on the water quality across the post-monsoon period. In 2021, the influence was more related to mineral content (e.g., sulphate and phosphate), whereas in 2022, organic indicators and metals gained importance. This shift could be due to differences in rainfall, runoff patterns, or changes in l and use between 2021 and 2022.

The biplots help in visualizing how Ponds differ based on the principal components. Ponds clustered together share similar water quality traits, while those positioned separately are influenced by different factors. For instance, Ponds with higher COD and BOD concentrations are influenced more by PC1 in 2022, potentially indicating sites with more organic pollution.

Table 4.13 The PCA axes explaining complete variance of the data (post-monsoon-2021)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.068	40.452	40.452	6.068	40.452	40.452	4.970	33.134	33.134
2	5.224	34.827	75.279	5.224	34.827	75.279	4.128	27.521	60.655
3	1.943	12.951	88.230	1.943	12.951	88.230	3.613	24.085	84.740
4	1.765	11.770	100.000	1.765	11.770	100.000	2.289	15.260	100.000

Extraction Method: Principal Component Analysis.

Table 4.14 The PCA axes explaining complete variance of the data (post-monsoon-2022)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	C umulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	8.136	54.243	54.243	8.136	54.243	54.243	5.843	38.956	38.956
2	3.490	23.268	77.511	3.490	23.268	77.511	5.540	36.937	75.893
3	2.932	19.547	97.058	2.932	19.547	97.058	3.175	21.165	97.058
4	.441	2.942	100.000						

Extraction Method: Principal Component Analysis.

Table 4.15 Loading of variables on significant principal component of surface water data of Ponds (post-monsoon-2021)

	Component			
	1	2	3	4
Temperature, 0C	-.678	.276	.680	.027
pH	.087	.160	.351	.918
Electrical Conductivity (mS/cm)	.109	-.985	-.026	-.132
Chloride, mg/l	-.156	.056	.983	.074
Sulphate, mg/l	.969	-.209	.109	.075
Nitrate, mg/l	-.755	-.586	.128	.266
Phosphate, mg/l	.408	-.154	-.604	.667
TDS, mg/l	.113	-.985	-.025	-.131
DO, mg/l	.827	.261	-.497	.033
BOD, mg/l	-.877	-.054	.011	-.476
COD, mg/l	-.891	.031	.123	-.436
Lead, Pb, mg/l	.420	.631	.652	.020
Nickel, Ni, mg/l	-.045	.713	.690	.119
Copper, Cu, mg/l	.468	.311	-.499	.659
Zinc, Zn, mg/l	.364	.774	.503	-.126

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

Table 4.16 Loading of variables on significant principal component of surface water data of Ponds (post-monsoon-2022)

	Component		
	1	2	3
Temperature, 0C	.659	.678	-.077
pH	-.096	.952	.099
Electrical Conductivity (mS/cm)	-.226	-.891	.393
Chloride, mg/l	.292	.943	-.038
Sulphate, mg/l	-.722	-.201	.652
Nitrate, mg/l	.203	.011	.977
Phosphate, mg/l	-.074	-.203	.936
TDS, mg/l	-.224	-.892	.392
DO, mg/l	-.854	-.385	.292
BOD, mg/l	.988	-.050	-.009
COD, mg/l	.993	.018	-.101
Lead, Pb, mg/l	.957	.210	.125
Nickel, Ni, mg/l	.112	-.839	-.500
Copper, Cu, mg/l	.496	.809	-.277
Zinc, Zn, mg/l	.884	.253	.393

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

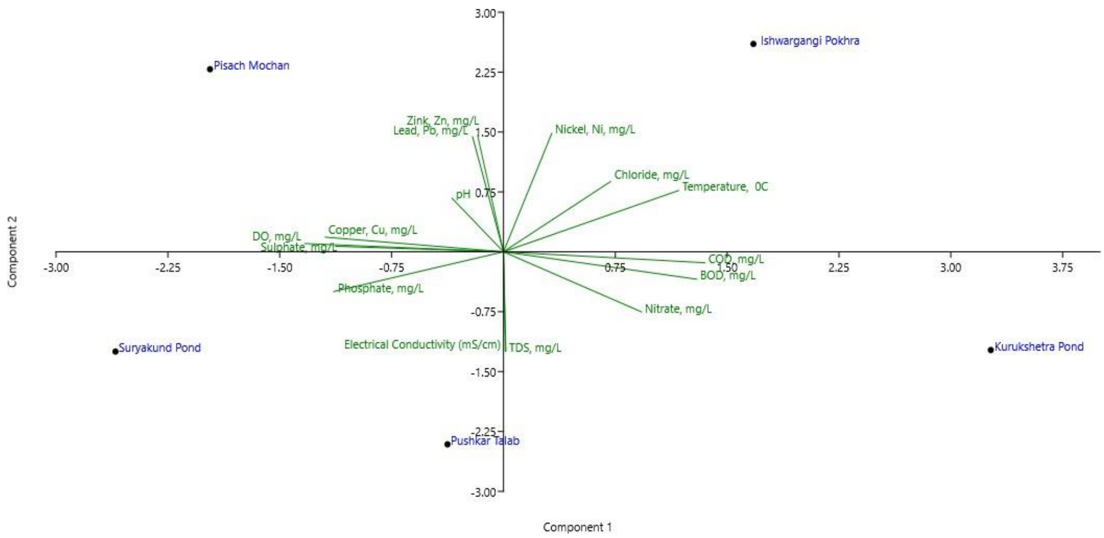


Fig.4.22. Result of principal component analysis (PCA) for the site and surface water data of Ponds (post-monsoon -2021)

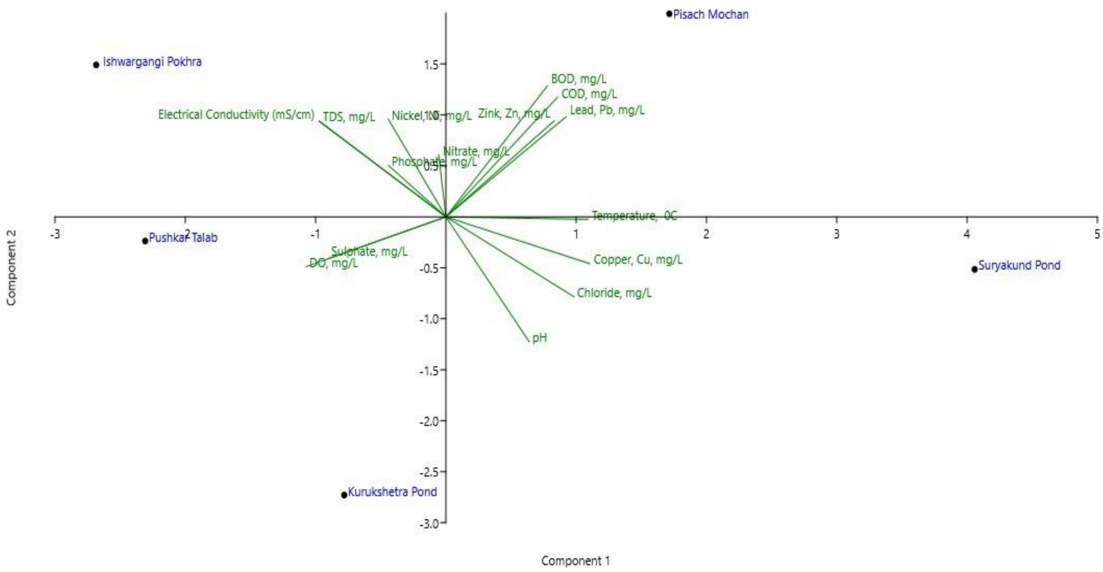


Fig.4.23 Result of principal component analysis (PCA) for the site and surface water data of Ponds (post-monsoon -2022)

4.3 Clustering of Similar Monitoring Sampling Site

The dendrogram represents the hierarchical clustering of five water sampling sites in Varanasi based on water quality parameters collected in 2021 and 2022. The statistical grouping of the five sampling Ponds was performed using Ward's linkage method and Euclidean distance measure., which aims to minimize the variance within clusters as it merges groups. The dendrogram helps identify how similar or dissimilar the water quality parameters of these sites are, allowing for a comprehensive comparison. This type of analysis is critical for identifying priority areas for water quality monitoring and management, allowing local authorities to implement site-specific strategies for pollution control and conservation efforts.

Analysis of the Dendrogram-Pre-Monsoon Season

May 2021 during the pre-monsoon season-The dendrogram divides the five sampling sites into two main clusters Cluster 1 (Contains the sites of Pushkar Pond, Kurukshetra Pond and Ishwargangi Pond.) and **Cluster 2**(Includes Suryakund Pond and Pishach Mochan Pond). The horizontal axis represents the rescaled distance between clusters, indicating how similar or different the water quality parameters of the sites are. The shorter the horizontal lines, the more similar the clusters or sites. The sites in Cluster 1 are grouped closely together, which indicates that these locations have similar water quality characteristics. Pushkar Pond and Kurukshetra Pond are more similar to each other, as they form a sub-cluster before joining with Ishwargangi Pond. This suggests that the levels of temperature, pH, electrical conductivity and other measured parameters in these three sites are relatively close to each other, indicating comparable water quality. Suryakund Pond and Pishach Mochan form the second main cluster. The fact that these two sites are separated from Cluster 1 by a longer distance indicates that their water quality parameters differ significantly from those in Cluster 1. The relatively short distance within Cluster 2 suggests that these two sites have similar water characteristics but are different enough from the other three sites to be grouped separately.

The clustering suggests that the water quality at Pushkar Pond, Kurukshetra Pond and Ishwargangi Pond is influenced by similar environmental or anthropogenic factors, making their water characteristics relatively homogeneous. These sites might share common pollution sources or natural features that lead to these similarities. On the other h and, Suryakund Pond and Pishach Mochan, which form Cluster 2, exhibit distinct water quality profiles. This could be due to different types of surrounding l and use, human activities, or environmental conditions affecting these Ponds. The differences between Cluster 1 and Cluster 2 could stem from varying levels of human activity, such as industrial discharge, agricultural runoff, or local waste disposal practices. Differences in depth, water flow, or the presence of aquatic vegetation could also contribute to variations in water quality among these sites. The rescaled distance axis helps in understanding the magnitude of dissimilarity between clusters. A larger distance between clusters indicates a significant difference in water quality characteristics. In this dendrogram, Cluster 1 and Cluster 2 are separated by a notable rescaled distance, signifying distinct water quality profiles.

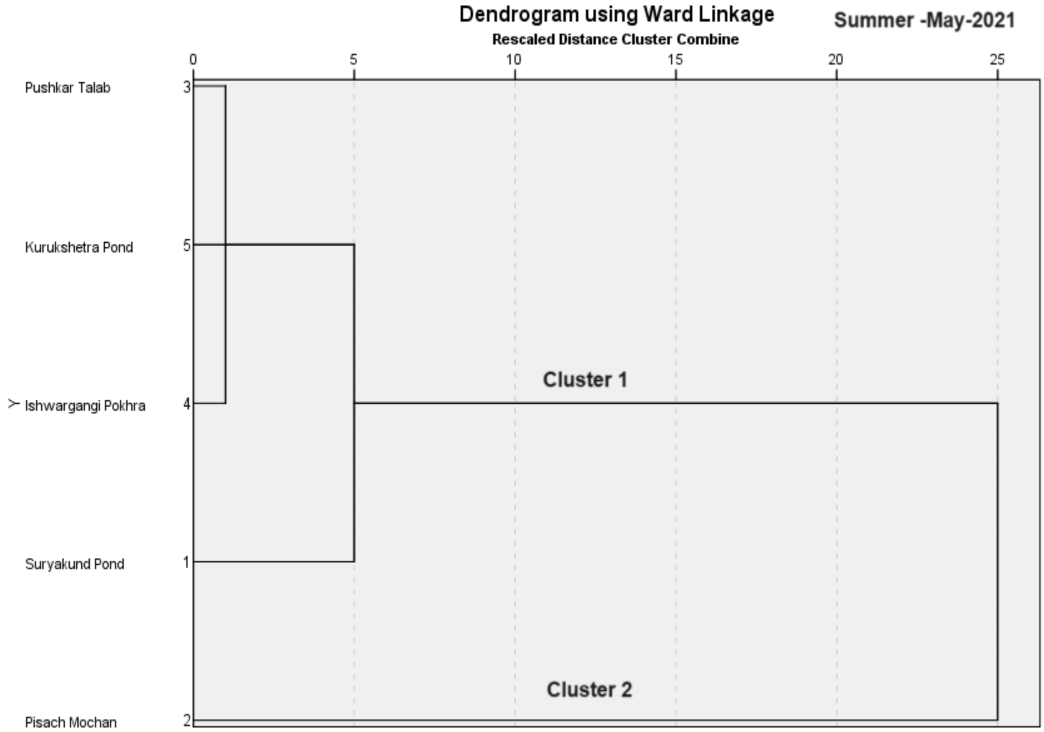


Fig.4.24. Dendrogram depicting the cluster of sample Ponds in relation to Pre-monsoon data 2021 (linkage method: Ward's method, distance measure: Euclidean)

May 2022 during the Pre-monsoon season-The dendrogram divides the five sampling sites into two main clusters. Cluster 1 (Contains the sites of Ishwargangi Pond and Kurukshetra Pond) and Cluster **2**(Includes Suryakund Pond, Pishach Mochan and Pushkar Pond). Cluster 1Contains the sites of Ishwargangi Pond and Kurukshetra Pond, indicates that these two sites had similar water quality characteristics in May 2022. This is a change from the 2021 dendrogram, where Kurukshetra Pond was closely associated with Pushkar Pond. The shift in clustering implies a change in water quality parameters over the year, suggesting potential alterations in pollution levels or environmental conditions at these sites. Cluster 2 Includes Suryakund Pond, Pishach Mochan and Pushkar Pond. Pushkar Pond joined this cluster in 2022, which marks a significant change from its 2021 association with Kurukshetra pond and Ishwargangi Pond. This change could indicate shifts in water quality at Pushkar

Pond, aligning its characteristics more closely with those of Suryakund Pond and Pishach Mochan in 2022.

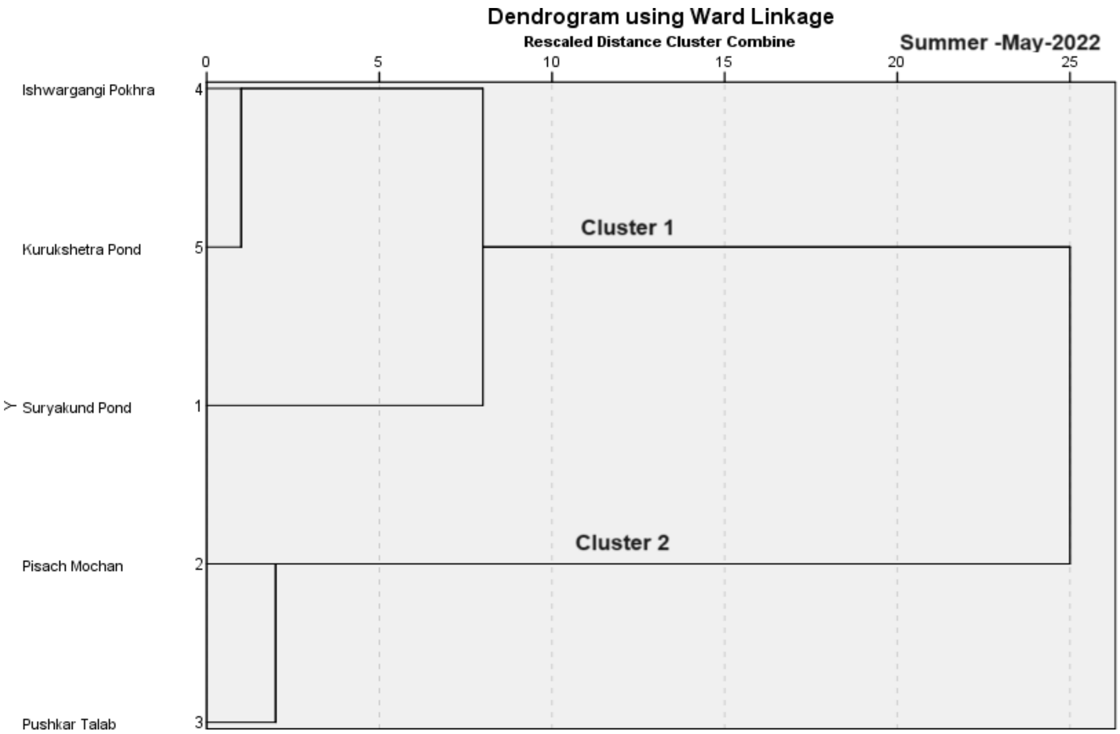


Fig.4.25. Dendrogram depicting the cluster of sample Ponds in relation to Pre-monsoon data 2022 (linkage method: Ward's method, distance measure: Euclidean)

The 2021 and 2022 Pre-monsoon season dendrograms show that some sites, such as Suryakund Pond and Pishach Mochan, remain consistently clustered together, suggesting stability in their water quality characteristics. The movement of Pushkar Pond from Cluster 1 in 2021 to Cluster 2 in 2022 indicates changes in its water quality, possibly due to varying pollution levels, environmental factors, or seasonal impacts. Changes in water quality may be influenced by human activities such as industrial discharge, domestic waste, or agricultural runoff. The shift of Pushkar Pond from one cluster to another suggests a potential increase in human impact or changes in waste management practices. Climatic variations, changes in water flow, or ecological dynamics like the growth of aquatic vegetation could also contribute to the observed changes in clustering. Identifying sites with similar water quality can help authorities target specific areas for pollution control measures. For instance, the consistent grouping of Suryakund Pond and Pishach Mochan indicates that they may require joint monitoring and management efforts. The observed changes between the years underscore the importance of continuous monitoring to detect trends and address emerging water quality issues promptly.

Analysis of the Dendrogram-Monsoon season-

August 2021 during the monsoon season- Cluster 1Contains the sites of Suryakund Pond, Pushkar Pond and Kurukshetra Pond. These sites form a primary cluster, indicating similar water quality parameters. Suryakund Pond and Pushkar Pond are more closely related as they form a sub-cluster before joining with Kurukshetra Pond. The grouping suggests that these sites may share similar environmental conditions or sources of pollutants during the monsoon season, leading to comparable water quality profiles. Cluster 2 Includes Pishach Mochan and Ishwargangi Pond. This cluster shows a separate group from Cluster 1, with a significant rescaled distance indicating distinct water quality characteristics compared to Cluster 1. The similar water quality between Pishach Mochan and Ishwargangi Pond suggests shared influences such as localized sources of pollution or specific natural features affecting these Ponds during the monsoon.

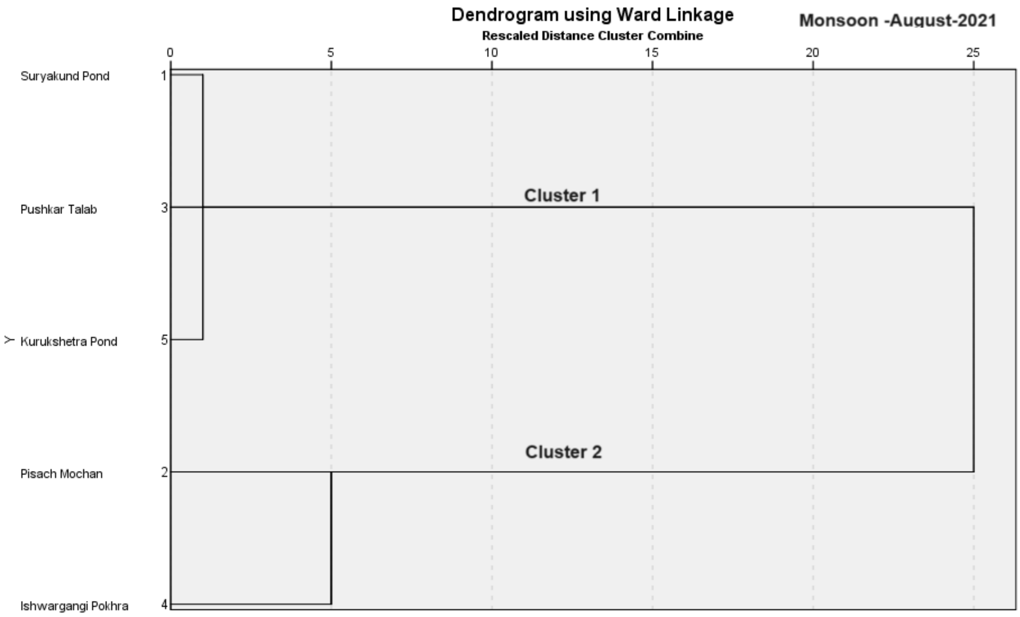


Fig.4.26 Dendrogram depicting the cluster of sample Ponds in relation to monsoon data 2021 (linkage method: Ward's method, distance measure: Euclidean)

August 2022 during the monsoon season- Cluster 1Contains the sites of Pushkar Pond, Kurukshetra Pond and Ishwargangi Pond. In 2022, these sites form a single cluster, indicating that their water quality parameters became more similar compared to the previous year. Pushkar Pond and Kurukshetra Pond are especially close, suggesting a stronger similarity between them. The shift in clustering may indicate changes in water quality due to variations in pollution sources or environmental impacts between 2021 and 2022.Cluster2IncludesSuryakund Pond and Pishach Mochan. These sites form a separate cluster, indicating distinct water quality characteristics compared to those in Cluster 1. The short distance within the cluster suggests similar water conditions between the two sites. The consistent clustering of Surya Kund Pond and Pishach Mochan, despite being separate from the other Ponds, highlights the possibility of shared water quality influences such as specific local activities or natural conditions during the monsoon.

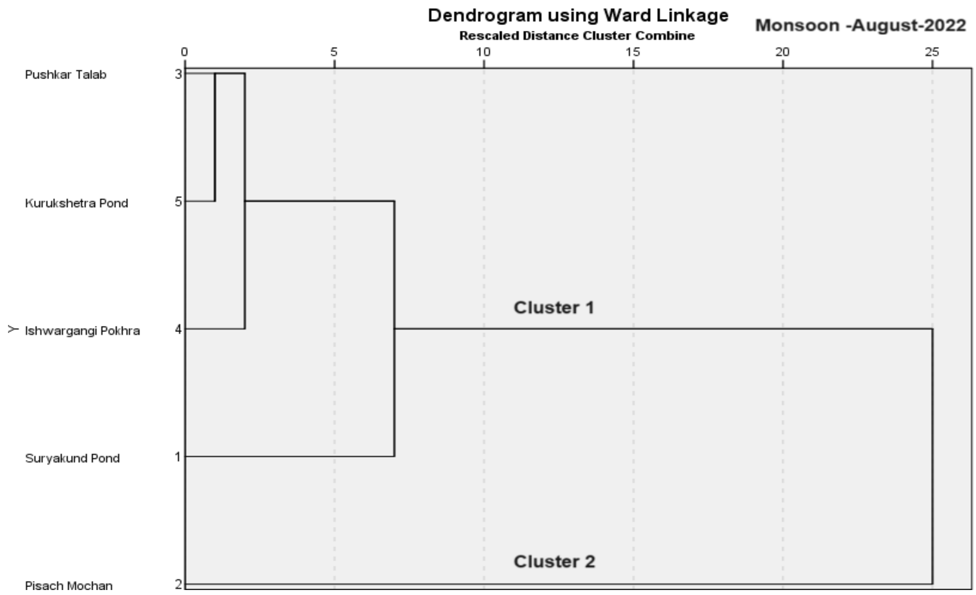


Fig.4.27. Dendrogram depicting the cluster of sample Ponds in relation to monsoon data 2022 (linkage method: Ward's method, distance measure: Euclidean)

In 2021, Suryakund Pond and Pushkar Pond were part of Cluster 1, indicating shared water quality attributes. However, by 2022, Suryakund Pond shifted to Cluster 2 alongside Pishach Mochan, suggesting a notable change in water quality parameters. Ishwargangi Pond moved from being grouped with Pishach Mochan in 2021 to joining Pushkar Pond and Kurukshetra Pond in 2022, indicating a shift in its water quality profile.

Changes in the clustering of sites could be influenced by human activities, such as changes in wastewater discharge, agricultural runoff, or other local developments affecting water quality. The monsoon season is characterized by increased water flow, dilution of pollutants and changes in the input of nutrients and other materials into the water bodies. These natural factors can affect clustering by altering the water chemistry. The changes observed in clustering between 2021 and 2022 emphasize the need for continuous monitoring to detect trends and understand the dynamics of water quality in response to seasonal variations and anthropogenic impacts. Sites that consistently form separate clusters, such as Pishach Mochan, may require targeted interventions to address specific water quality issues.

Analysis of the Dendrogram-Autumn season

October 2021 during the monsoon season- Cluster 1 consists of Pishach Mochan, Kurukshetra Pond and Pushkar Pond. This grouping suggests that these three sites had similar water quality characteristics during this time period.

Cluster 2 contains Suryakund Pond and Ishwargangi Pond. This grouping implies that these two Ponds exhibited a similar pattern in terms of the measured water quality parameters, distinct from those in Cluster 1.

The distance between the clusters (measured on the horizontal axis) shows that Cluster 1 and Cluster 2 are relatively far apart, indicating significant differences in water quality characteristics between the groups in the autumn of 2021.

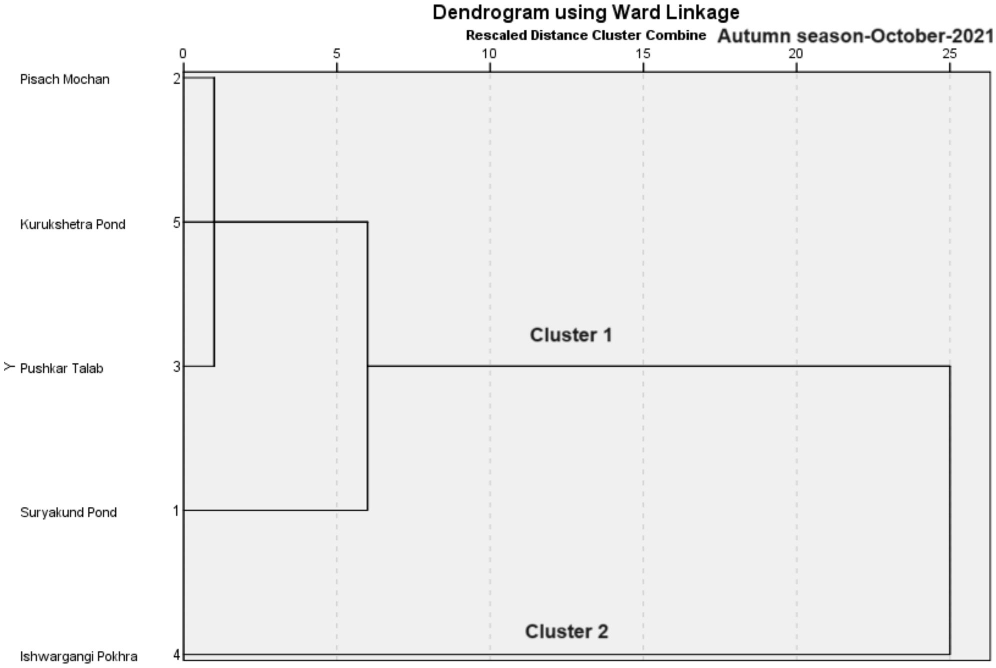


Fig.4.28. Dendrogram depicting the cluster of sample Ponds in relation to autumn season data 2021 (linkage method: Ward's method, distance measure: Euclidean)

October 2022 during the monsoon season- Cluster 1 now includes Ishwargangi Pond, Kurukshetra Pond andPishach Mochan, which differs from the 2021 clustering. This change indicates that these sites had similar water quality parameters in 2022, forming a different grouping than in the previous year. Cluster 2 is composed of Suryakund Pond and Pushkar Pond, which shows a shift in grouping as Pushkar Pond was part of Cluster 1 in 2021.

The distances between clusters indicate substantial differences in water quality characteristics between Cluster 1 and Cluster 2 in 2022, similar to the pattern observed in 2021. However, the composition of the clusters has changed, suggesting variations in water quality dynamics at these sites over the two years.

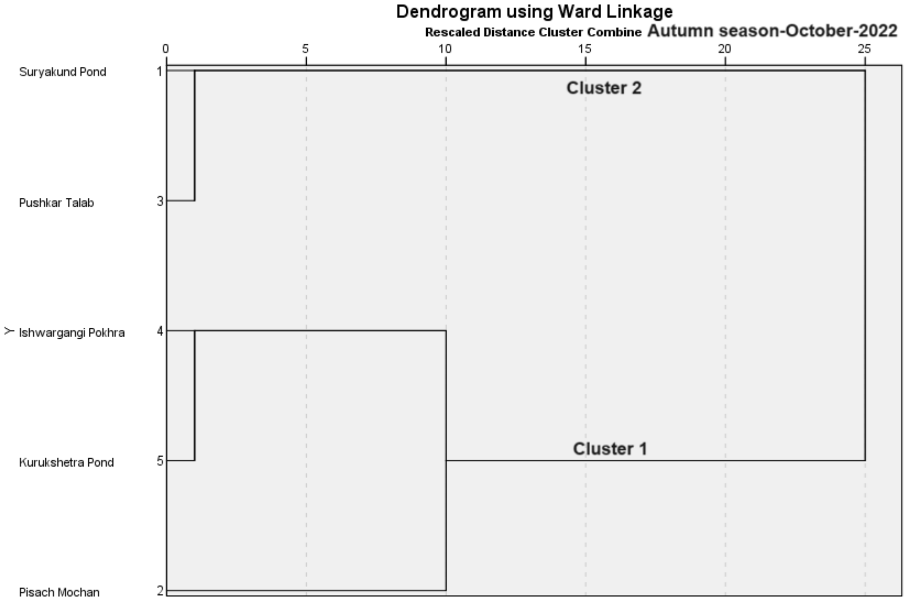


Fig.4.29. Dendrogram depicting the cluster of sample Ponds in relation to autumn season data 2022 (linkage method: Ward's method, distance measure: Euclidean)

The dendrograms reveal that there were changes in water quality characteristics between autumn season in 2021 and 2022. Some sites that were grouped together in 2021 did not form the same clusters in 2022 in same season, indicating temporal variability in water quality, potentially due to seasonal or anthropogenic influences. Each cluster represents sites with similar water quality parameters. For example, in 2021, Pishach Mochan, Kurukshetra Pond and Pushkar Pond were grouped together, while in 2022, Ishwargangi Pond, Kurukshetra Pond and Pishach Mochan formed a cluster. This shift suggests that factors affecting water quality—such as pollution sources, rainfall patterns, or seasonal runoff—may vary between sites and across years. Changes in cluster composition between 2021 and 2022 could reflect differences in environmental or human-induced factors, like changes in land use, industrial discharge, or agricultural runoff affecting certain Ponds differently over time.

Specific water quality parameters (e.g., biological oxygen demand, heavy metals like lead and nickel) may have fluctuated due to such factors. Clustering can help in identifying water bodies with similar pollution levels or types of contamination. Sites grouped in the same cluster may require similar management approaches, while sites in different clusters may need targeted strategies. For instance, sites that consistently form a separate cluster with distinct characteristics might need specific interventions based on their unique water quality issues. Continued monitoring and clustering of water quality data across multiple years could further reveal trends and patterns in the water bodies' ecological health. This data-driven approach can aid in identifying long-term impacts and in the proactive management of water resources.

Analysis of the Dendrogram-post-monsoon season

December 2021 during the post-monsoon season-Cluster 1 includes Ishwargangi Pond, Kurukshetra Pond and Pishach Mochan. This clustering suggests that these sites had similar water quality characteristics in December 2021. Given the post-monsoon season, it's possible that these Ponds share common factors like runoff from surrounding areas or seasonal changes affecting nutrient and contaminant levels. Cluster 2 is composed of Suryakund Pond and Pushkar Pond. The separation of these two Ponds into a different cluster suggests distinct water quality characteristics, potentially due to unique environmental influences or localized anthropogenic impacts.

The relatively large rescaled distance separating Cluster 1 and Cluster 2 suggests significant variation in water quality parameters between the two clusters, which could be due to factors such as differences in water source, pollution levels, or biological activity.

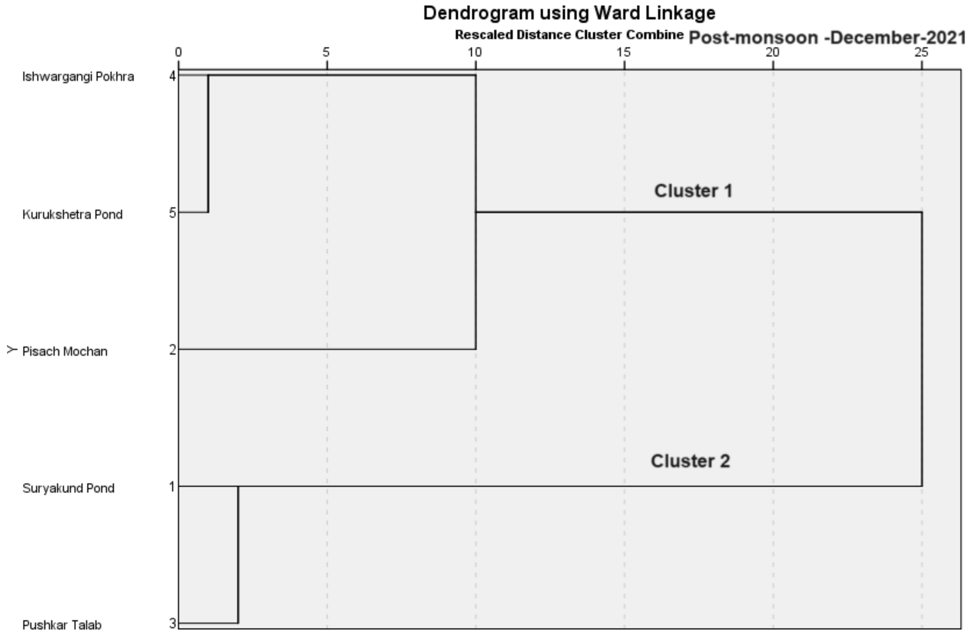


Fig.4.30. Dendrogram depicting the cluster of sample Ponds in relation to post-monsoon season data 2021 (linkage method: Ward's method, distance measure: Euclidean)

December 2022 during the post-monsoon season-Cluster 1 for 2022 consists of Pishach Mochan, Kurukshetra Pond and Suryakund Pond. Compared to 2021, Suryakund Pond has shifted from Cluster 2 to Cluster 1, indicating a change in its water quality characteristics that now aligns it more closely with Pishach Mochan and Kurukshetra Pond. Cluster 2 includes Pushkar Pond and Ishwargangi Pond, which suggests that these two sites exhibited similar water quality patterns in December 2022. This represents a shift from 2021, where Ishwargangi Pond was part of Cluster 1.

The altered grouping of Suryakund Pond and Ishwargangi Pond between the two years may indicate that their water quality characteristics were impacted differently over time, potentially due to seasonal shifts, changes in pollutant sources, or recovery processes after the monsoon season.

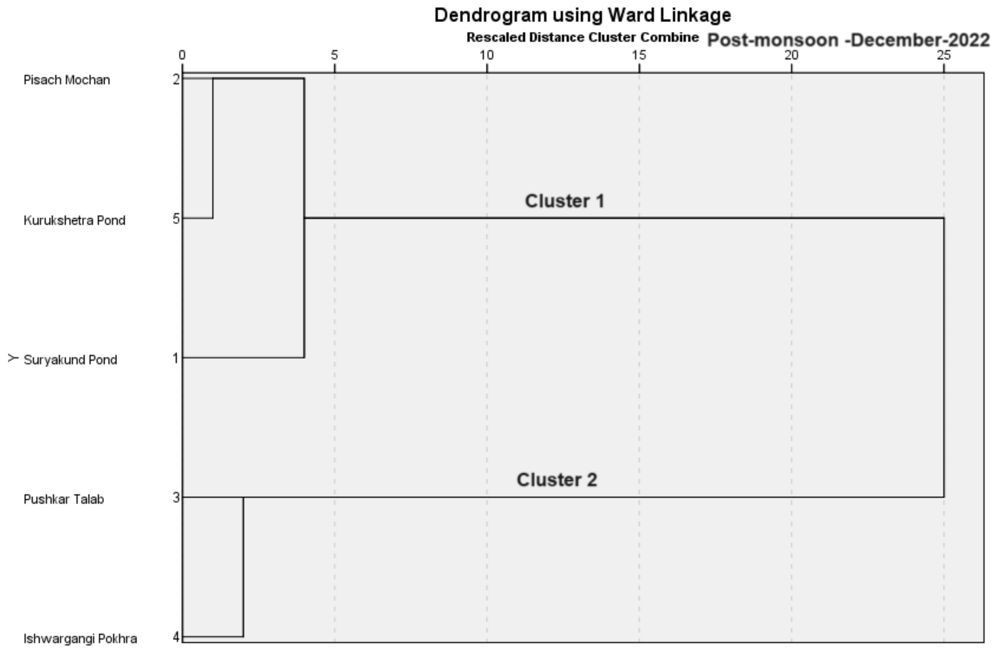


Fig.4.31. Dendrogram depicting the cluster of sample Ponds in relation to post-monsoon season data 2022 (linkage method: Ward's method, distance measure: Euclidean)

The dendrograms reveal differences in clustering patterns between post-monsoon 2021 and 2022, suggesting that the post-monsoon season has varying effects on water quality across years. Post-monsoon runoff, sediment resuspension and influx of nutrients or contaminants can significantly impact the water bodies' quality. Seasonal dynamics in nutrient loading and sedimentation, commonly seen in monsoon-affected regions, could explain some of the clustering variations.

The differences in site clustering between post-monsoon season 2021 and 2022 reflect changes in specific water quality parameters. For example, Suryakund Pond’s shift from Cluster 2 in 2021 to Cluster 1 in 2022 suggests that it became more similar to Pishach Mochan and Kurukshetra Pond in terms of water quality. Conversely, Ishwargangi Pond's movement from Cluster 1 to Cluster 2 implies a change that made it more similar to Pushkar Pond. These shifts could be influenced by various factors, including changes in human activity, natural processes, or water management practices affecting specific Ponds.

Clustering water bodies by similar water quality characteristics helps in identifying sites that may require specific management approaches. For instance, Pushkar Pond and Ishwargangi Pond might need similar management interventions in 2022 to address potential shared issues, while Pishach Mochan, Kurukshetra Pond and Suryakund Pond could benefit from a different set of measures. These water bodies, located in Varanasi, are likely impacted by nearby human activities such as religious rituals, agriculture and urban runoff, especially during and after the monsoon season. Heavy metal contamination, nutrient loading and organic pollutants are common in such settings and could account for some of the water quality differences. The rescaled distance in the dendrograms shows distinct separations, suggesting varying degrees of human impact or natural buffering across these sites. The variations observed across two years highlight the importance of continuous water quality monitoring. Such data can help detect emerging patterns or potential shifts in water quality over time, which is valuable for resource management and pollution control. Clustering analysis can also be instrumental in developing adaptive water quality management strategies based on current data trends.

Pearson Correlation (Sample Size, N=40):

This study analyzed water quality in five selected Ponds in Varanasi (Suryakund Pond, Pishach Mochan, Pushkar Pond, Ishwargangi Pond and Kurukshetra Pond) across four seasons in 2021 and 2022, focusing on several parameters like temperature, pH, electrical conductivity (EC), chloride, sulfate, nitrate, phosphate, total dissolved solids (TDS), dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD) and trace metals. Pearson correlation analysis (N=40) was conducted to examine relationships between these parameters, identifying correlations significant at both the 0.01 and 0.05 levels (two-tailed).

Significant Correlations at the 0.01 Level ($p < 0.01$)

1. **Temperature** showed significant positive correlations with:
   - Chloride ($r = 0.465$), indicating that higher temperatures may enhance chloride concentrations.
   - Sulfate ($r = 0.562$), suggesting sulfate levels increase with temperature.
   - DO ($r = 0.488$), possibly reflecting increased biological activity at higher temperatures.
2. **Electrical Conductivity (EC)** was strongly associated with:
   - Chloride ($r = 0.527$) and nitrate ($r = 0.428$), highlighting that higher ion concentrations increase conductivity.
   - TDS ($r = 0.981$), indicating that TDS is a primary contributor to EC.
3. **Chloride** showed strong relationships with:
   - Nitrate ($r = 0.494$) and TDS ($r = 0.529$), suggesting that chloride levels may be related to other dissolved solids in the water.
   - BOD ($r = 0.446$) and COD ($r = 0.441$), indicating that higher chloride

levels might correlate with higher organic pollution levels.

4. **Nitrate demonstrated**:
    - A significant positive correlation with chloride (r = 0.494) and EC (r = 0.428), supporting the association between nitrate and dissolved solids.
    - A negative correlation with sulfate (r = -0.629), which may indicate inverse behavior between these ions in the Ponds.
    - **Phosphate** was positively correlated with:pH (r = 0.404) and DO (r = 0.567), suggesting that higher pH and oxygen levels are associated with elevated phosphate.
    - Copper (r = 0.446), indicating a relationship between nutrient levels and metal concentrations.
5. **Dissolved Oxygen (DO)** showed a strong positive relationship with:
    - Phosphate (r = 0.567) and sulfate (r = 0.495), suggesting that DO levels influence nutrient dynamics.
    - Copper (r = 0.511), possibly due to increased biological oxygen demand in areas with higher copper levels.
6. **COD and BOD** had a very strong correlation (r = 0.957), indicating that COD is closely related to organic matter levels, as measured by BOD.

Significant Correlations at the 0.05 Level ($p < 0.05$)

1. **TDS** showed a moderate positive correlation with phosphate (r = 0.313), suggesting that phosphate contributes to total dissolved solids.
2. **Nitrate** showed a negative correlation with DO (r = -0.344) and a positive correlation with BOD (r = 0.375), implying that nitrate may contribute to oxygen depletion and organic matter levels.

3. **Lead** displayed negative relationships with:
    - EC (r = -0.363) and nitrate (r = -0.347), suggesting that lead concentrations are lower in waters with high ion concentrations.
    - TDS (r = -0.363), indicating an inverse relationship between lead and overall dissolved solids.
4. **Nickel** exhibited moderate negative correlations with EC (r = -0.334), phosphate (r = -0.333), TDS (r = -0.334) and DO (r = -0.335), suggesting that nickel levels may decrease as these parameters increase.

These correlation results highlight complex interactions between the physical, chemical and biological characteristics of the Pond water. For instance, the strong positive correlations between TDS, EC and chloride suggest that dissolved ions are major contributors to conductivity, while the positive associations of BOD and COD with chloride indicate that chloride levels might relate to organic pollution. Temperature's positive correlation with parameters like sulphate and DO suggests that seasonal temperature changes could influence water chemistry, potentially by affecting biological processes.

The inverse correlations, such as the negative relationship between nitrate and sulphate or lead with EC and TDS, could indicate competitive processes or differing sources for these ions. Similarly, the moderate negative correlations between nickel and several parameters, such as EC and DO, suggest that nickel concentrations may be reduced in environments with higher ionic content or oxygenation. These correlations provide insight into the interdependence of water quality parameters, highlighting possible areas for targeted water quality management. For example, the significant variation in lead and nickel with other parameters may require specific monitoring and mitigation strategies to control metal contamination in these Ponds.

Table 4.17: Pearson's Correlation Analysis of Pond Water Parameters (N = 40), Collected in 2021 and 2022															
	Temperature	**pH**	**EC**	**Chloride**	**Sulphate**	**Nitrate**	**Phosphate**	**TDS**	**DO**	**BOD**	**COD**	**Lead**	**Nickel**	**Copper**	**Zinc**
Temperature	1														
pH	-.021	1													
EC	.117	.091	1												
Chloride	**.465****	.242	**.527****	1											
Sulphate	**.562****	-.145	-.224	-.122	1										
Nitrate	-.201	.227	**.428****	**.494****	**-.629****	1									
Phosphate	.276	**.404****	.311	.250	.011	.183	1								
TDS	.118	.092	**.981****	**.529****	-.225	**.427****	**.313***	1							
DO	**.488****	.173	.226	.040	**.495****	**-.344***	**.567****	.227	1						
BOD	.256	-.047	.129	**.446****	-.021	**.375***	-.236	.128	-.257	1					
COD	.227	-.053	.129	**.441****	-.086	**.341***	-.220	.129	-.297	**.957****	1				
Lead	.050	-.167	**-.363***	-.152	.264	**-.347***	-.303	**-.363***	-.114	-.027	-.064	1			
Nickel	.268	-.209	**-.334***	.054	.195	-.135	**-.333***	-.334*	**-.335***	.188	.201	.374*	1		
Copper	.284	**.462****	.136	.032	.218	-.167	**.446****	.136	**.511****	-.031	-.034	-.101	**-.313***	1	
Zinc	.113	-.054	-.262	-.063	.195	**-.322***	-.120	-.263	.087	-.083	-.114	.150	.118	.049	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

4.3 ONE-WAY ANOVA: The study investigated water quality across five Ponds in Varanasi—Suryakund Pond, Pishach Mochan, Pushkar Pond, Ishwargangi Pond and Kurukshetra Pond—over multiple seasons in 2021 and 2022. Using one-way ANOVA, analyses were conducted to evaluate whether there were statistically significant differences in water quality parameters by year, site and season. The key findings from these analyses are discussed below:

4.3.1 Year-wise Comparison (2021 vs. 2022)

A one-way ANOVA was performed to examine variations in water quality parameters between the years 2021 and 2022. The results showed that for most parameters—including temperature, pH, electrical conductivity, chloride, sulfate, nitrate, phosphate, total dissolved solids (TDS), dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), nickel, copper and zinc—the p-values exceeded 0.5. This suggests no statistically significant differences between the two years for these parameters, implying that they remained relatively stable over the time frame.

However, an exception was observed with lead (Pb), where the p-value was less than 0.5, indicating a significant difference in lead concentrations between 2021 and 2022. This may be due to changes in external factors influencing lead levels, such as industrial or agricultural runoff, local pollution sources, or environmental factors affecting the bioavailability or mobility of lead. The significant variation in lead levels warrants further investigation to determine potential contamination sources and implement measures to prevent further pollution.

4.3.2 Site-wise Comparison (2021 and 2022)

When comparing water quality parameters across different sites (Ponds) within 2021 and 2022, the one-way ANOVA results indicated statistically significant differences for most parameters, with p-values less than 0.5. This included parameters like pH, electrical conductivity, chloride, nitrate, phosphate, TDS, DO, BOD, COD, lead, nickel, copper and zinc, suggesting notable spatial variability in water quality among the Ponds.

The site-wise differences likely reflect the distinct environmental characteristics and varying levels of anthropogenic influence at each Pond. Factors such as local land use, runoff patterns, proximity to urban or agricultural areas and natural variations in Pond morphology may contribute to these disparities. Notably, temperature and sulfate did not show significant differences across sites, with p-values above 0.5. This suggests that these parameters may be more uniformly distributed across the Ponds, potentially due to similar seasonal or atmospheric influences across the region.

4.3.3 Season-wise Comparison (2021 and 2022)

The seasonal analysis of water quality parameters revealed significant variations across different times of the year (Pre-monsoon, monsoon, autumn and post-monsoon) in both 2021 and 2022. The one-way ANOVA showed p-values below 0.5 for most parameters, including temperature, pH, electrical conductivity, chloride, sulfate, nitrate, phosphate, TDS, DO, BOD, COD, lead, nickel and copper. This finding indicates substantial seasonal variability, likely due to the influence of climate and seasonal patterns on the Ponds water chemistry.

For example, monsoon rains could dilute certain ion concentrations, while increased runoff during this season might lead to spikes in nutrient or metal concentrations. Similarly, temperature and dissolved oxygen may show variations due to seasonal changes in atmospheric temperature and water mixing patterns. Interestingly, zinc was the only parameter with a p-value above 0.5, indicating that its concentration remained relatively stable across seasons. This stability may reflect the low mobility or reactivity of zinc within the Ponds, or a minimal seasonal influence on zinc concentrations compared to other parameters.

Table 4.18 ONEWAY ANOVA by Year Wise-2021 and 2022						
		Sum of Squares	df	Mean Square	F	Sig. (p-value)
Temperature, 0C	Between Groups	.676	1	0.676	0.015	.902
	Within Groups	1661.13	38	43.714		
	Total	1661.81	39			
pH	Between Groups	.018	1	.018	.100	.754
	Within Groups	6.714	38	.177		
	Total	6.732	39			
Electrical Conductivity (mS/cm)	Between Groups	.003	1	.003	.195	.661
	Within Groups	.514	38	.014		
	Total	.517	39			
Chloride, mg/l	Between Groups	3.025	1	3.025	.067	.797
	Within Groups	1714.750	38	45.125		
	Total	1717.775	39			
Sulphate, mg/l	Between Groups	.000	1	.000	.000	1.000
	Within Groups	1483.900	38	39.050		
	Total	1483.900	39			
Nitrate, mg/l	Between Groups	.000	1	.000	.000	.989
	Within Groups	52.683	38	1.386		
	Total	52.684	39			
Phosphate, mg/l	Between Groups	.001	1	.001	.018	.895
	Within Groups	1.377	38	.036		
	Total	1.378	39			
TDS, mg/l	Between Groups	1071.225	1	1071.225	.193	.663
	Within Groups	210549.750	38	5540.783		
	Total	211620.975	39			
DO, mg/l	Between Groups	.076	1	.076	.109	.743
	Within Groups	26.352	38	.693		
	Total	26.428	39			
BOD, mg/l	Between Groups	.008	1	.008	.005	.945
	Within Groups	66.319	38	1.745		
	Total	66.327	39			
COD, mg/l	Between Groups	.689	1	.689	.102	.751
	Within Groups	255.981	38	6.736		
	Total	256.670	39			
Lead, Pb, mg/l	Between Groups	.000	1	.000	1.096	.302
	Within Groups	.008	38	.000		
	Total	.008	39			
Nickel, Ni, mg/l	Between Groups	.000	1	.000	.146	.705
	Within Groups	.001	38	.000		
	Total	.001	39			
Copper, Cu, mg/l	Between Groups	.000	1	.000	.000	.986
	Within Groups	.003	38	.000		
	Total	.003	39			
Zinc, Zn, mg/l	Between Groups	.081	1	.081	.039	.845
	Within Groups	79.559	38	2.094		
	Total	79.640	39			

Table 4.19 ONEWAY ANOVA by Site Wise-2021 and 2022						
		Sum of Squares	df	Mean Square	F	Sig. (p-value)
Temperature, 0C	Between Groups	4.117	4	1.029	.022	.999
	Within Groups	1657.697	35	47.363		
	Total	1661.815	39			
pH	Between Groups	.991	4	.248	1.510	.220
	Within Groups	5.741	35	.164		
	Total	6.732	39			
Electrical Conductivity (mS/cm)	Between Groups	.076	4	.019	1.512	.220
	Within Groups	.441	35	.013		
	Total	.517	39			
Chloride, mg/l	Between Groups	92.900	4	23.225	.500	.736
	Within Groups	1624.875	35	46.425		
	Total	1717.775	39			
Sulphate, mg/l	Between Groups	62.150	4	15.537	.382	.820
	Within Groups	1421.750	35	40.621		
	Total	1483.900	39			
Nitrate, mg/l	Between Groups	8.013	4	2.003	1.569	.204
	Within Groups	44.671	35	1.276		
	Total	52.684	39			
Phosphate, mg/l	Between Groups	.467	4	.117	4.482	.005
	Within Groups	.911	35	.026		
	Total	1.378	39			
TDS, mg/l	Between Groups	30759.350	4	7689.838	1.488	.227
	Within Groups	180861.625	35	5167.475		
	Total	211620.975	39			
DO, mg/l	Between Groups	7.224	4	1.806	3.291	.022
	Within Groups	19.204	35	.549		
	Total	26.428	39			
BOD, mg/l	Between Groups	18.990	4	4.748	3.510	.016
	Within Groups	47.336	35	1.352		
	Total	66.327	39			
COD, mg/l	Between Groups	66.244	4	16.561	3.044	.030
	Within Groups	190.426	35	5.441		
	Total	256.670	39			
Lead, Pb, mg/l	Between Groups	.002	4	.000	2.826	.039
	Within Groups	.006	35	.000		
	Total	.008	39			
Nickel, Ni, mg/l	Between Groups	.000	4	.000	6.635	.000
	Within Groups	.000	35	.000		
	Total	.001	39			
Copper, Cu, mg/l	Between Groups	.001	4	.000	4.934	.003
	Within Groups	.002	35	.000		
	Total	.003	39			
Zinc, Zn, mg/l	Between Groups	36.743	4	9.186	7.495	.000
	Within Groups	42.898	35	1.226		
	Total	79.640	39			

Table 4.20 ONEWAY ANOVA by Season Wise-2021 and 2022						
		Sum of Squares	df	Mean Square	F	Sig. (p-value)
Temperature, ^{0}C	Between Groups	1585.505	3	528.502	249.326	.000
	Within Groups	76.310	36	2.120		
	Total	1661.815	39			
pH	Between Groups	1.116	3	.372	2.383	.085
	Within Groups	5.617	36	.156		
	Total	6.732	39			
Electrical Conductivity (mS/cm)	Between Groups	.309	3	.103	17.884	.000
	Within Groups	.208	36	.006		
	Total	.517	39			
Chloride, mg/l	Between Groups	1351.275	3	450.425	44.244	.000
	Within Groups	366.500	36	10.181		
	Total	1717.775	39			
Sulphate, mg/l	Between Groups	1224.900	3	408.300	56.752	.000
	Within Groups	259.000	36	7.194		
	Total	1483.900	39			
Nitrate, mg/l	Between Groups	27.901	3	9.300	13.510	.000
	Within Groups	24.783	36	.688		
	Total	52.684	39			
Phosphate, mg/l	Between Groups	.380	3	.127	4.574	.008
	Within Groups	.998	36	.028		
	Total	1.378	39			
TDS, mg/l	Between Groups	127254.875	3	42418.292	18.100	.000
	Within Groups	84366.100	36	2343.503		
	Total	211620.975	39			
DO, mg/l	Between Groups	9.221	3	3.074	6.431	.001
	Within Groups	17.206	36	.478		
	Total	26.428	39			
BOD, mg/l	Between Groups	5.427	3	1.809	1.069	.374
	Within Groups	60.900	36	1.692		
	Total	66.327	39			
COD, mg/l	Between Groups	17.737	3	5.912	.891	.455
	Within Groups	238.933	36	6.637		
	Total	256.670	39			
Lead, Pb, mg/l	Between Groups	.001	3	.000	1.616	.203
	Within Groups	.007	36	.000		
	Total	.008	39			
Nickel, Ni, mg/l	Between Groups	.000	3	.000	1.898	.147
	Within Groups	.000	36	.000		
	Total	.001	39			
Copper, Cu, mg/l	Between Groups	.000	3	.000	1.487	.234
	Within Groups	.003	36	.000		
	Total	.003	39			
Zinc, Zn, mg/l	Between Groups	4.520	3	1.507	.722	.545
	Within Groups	75.120	36	2.087		
	Total	79.640	39			

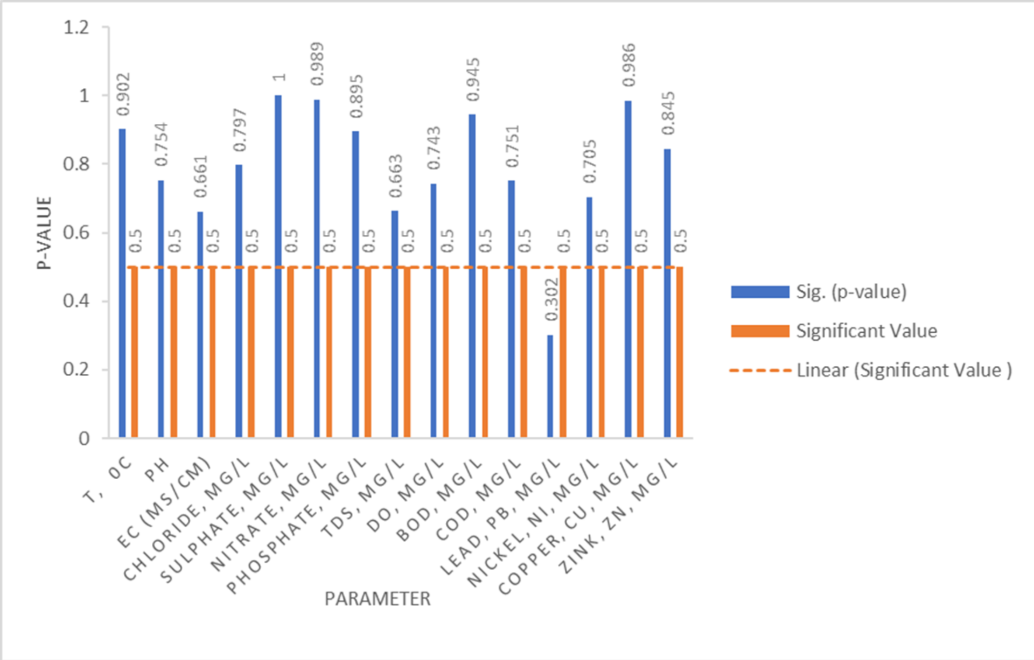


Fig 4.32 - ONEWAY ANOVA by year wise- 2021 and 2022

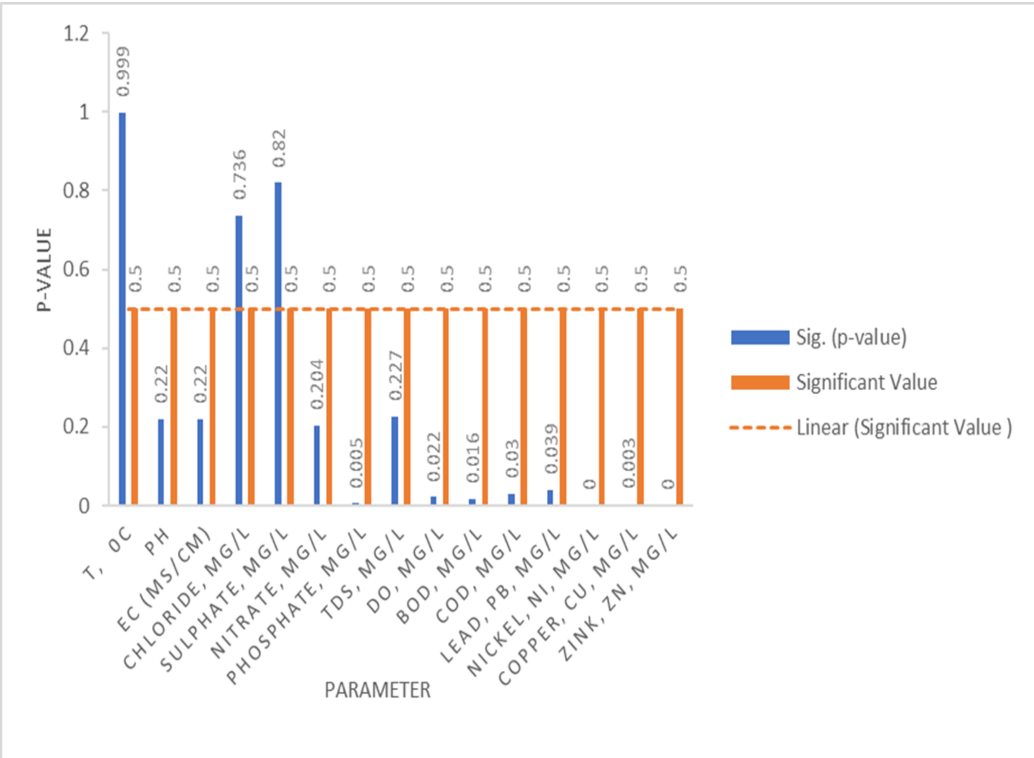


Fig.4.33. ONEWAY ANOVA by site wise- 2021 and 2022

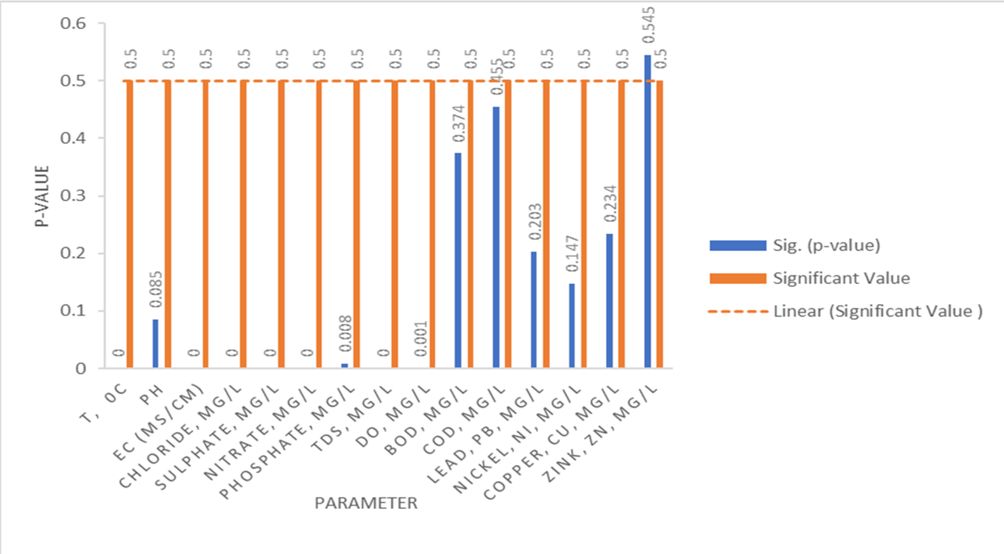


Fig.4.34. ONEWAY ANOVA by Season wise- 2021 and 2022

4.4 Morphometric character of *Clarias batrachus*

The Tables-4.23, show multiple morphometric characters, including Total Length (TL), Standard Length (SL), Caudal Length (CL) and others for *Clarias batrachus*, sampled from five Ponds in Varanasi (Suryakund Pond, Pishach Mochan, Pushkar Pond, Ishwar Gangi Pond and Kurukshetra Pond).The data is divided into seasons (Pre-monsoon , monsoon, autumn and post-monsoon) for two years, 2021 and 2022.Each location and season show variability in the morphometric measurements, which are presented as mean, minimum, maximum and standard deviation values for each parameter.

Total Length (TL) and Standard Length (SL) values vary significantly across Ponds and seasons. For example, in the Kurukshetra Pond, where heavy metal levels (such as Lead and Copper) are generally higher, the average Total Length (Mean = 17.88 cm) and Standard Length (Mean = 15.62 cm) are slightly lower compared to other Ponds with lower metal concentrations. This suggests that elevated heavy metal levels may inhibit growth, as higher concentrations can lead to toxic effects, limiting energy available for growth due to the physiological stress from detoxification processes. Suryakund Pond shows relatively stable **Caudal Length**, CL (Mean = 2.28 cm) with minor fluctuations in heavy metal content, especially Copper and Zinc. Pishach Mochan exhibits a slightly lower mean Caudal Length (Mean = 2.06 cm) in environments with relatively stable heavy metal levels, suggesting less adverse impact on caudal growth. Kurukshetra Pond, with somewhat higher Lead concentrations, shows a slightly higher variability in Caudal Length, suggesting that increased lead content may lead to inconsistent growth. Increased Lead and Copper levels might be inhibiting caudal growth, as higher variability and reduced mean values are seen in Ponds with elevated levels of these metals. **Eye Diameter (ED)** shows relatively low variability across seasons and locations (STDEV generally low, around 0.01– 0.05). This parameter might be less sensitive to heavy metal exposure than body length parameters. However, subtle differences could indicate environmental stress affecting sensory organ development. Further microscopic or histological analysis would be required to confirm this. **Pre-Dorsal Length (PDL) and Pre- Anal Length (PAL)** show significant variability across different Ponds and seasons. In Ishwar Gangi Pond, during the monsoon season, where heavy metal levels are elevated, Pre-Dorsal Length and Pre-Anal Length values are generally higher. This may indicate a compensatory growth effect or variation in growth patterns in response to changing environmental conditions, including metal toxicity. Kurukshetra Pond has a higher mean **Pre-Pectoral Length, PPEL** (Mean = 2.52 cm) with moderate variability (STDEV = 1.57). Elevated levels of Copper and Zinc, especially in post-monsoon, could lead to compensatory growth mechanisms. Pushkar Pond and Pishach Mochan have lower mean Pre-Pectoral Length values (Mean = 1.56 and 1.53 cm, respectively), which may suggest limited growth potential in environments with moderate heavy metal concentrations. Moderate heavy metal concentrations may lead to reduced Pre- Pectoral Length, while Ponds with higher metal content show increased variability in this parameter, indicating possible stress responses affecting consistent growth. In Suryakund Pond, the mean **Dorsal Fin Length (DFL)** is relatively stable at 11 cm, while Kurukshetra Pond shows higher variability (STDEV = 3.76). This Pond also has elevated levels of Lead and Copper in certain seasons, which could lead to inconsistent dorsal fin development. Ishwargangi Pond shows a mean Dorsal Fin Length of 8.72 cm, possibly reflecting the impact of lower heavy metal exposure on fin growth. Heavy metals like Lead and Copper appear to influence fin development, as evidenced by increased variability in Dorsal Fin Length in Ponds with higher concentrations of these metals. This may be due to impaired nutrient assimilation or direct toxicity effects on fin tissues. Suryakund Pond shows a mean **Anal Fin Length (AFL)** of 8.44 cm with moderate variability, while Pishach Mochan and Pushkar Pond show lower Anal Fin Length values (mean = 8.18 and 7.44 cm, respectively). Kurukshetra Pond displays higher variability (STDEV = 2.97), which may reflect the increased heavy metal levels, particularly during the post-monsoon period. Higher heavy metal levels might lead to increased stress and variability in AFL. This fluctuation suggests that while anal fin growth is somewhat resilient, extreme environmental stress from heavy metals may still influence its development. **Body Depth (BD)** measurements are notably variable across sites, potentially reflecting differing nutritional availability or stress responses due to pollutants. In Ponds with higher heavy metal levels, fish may exhibit altered body depth due to stunted growth or physiological adaptations to metal stress.

Seasonal and Pond-Specific Observations: Seasonal fluctuations in morphometric data may correspond with seasonal changes in metal concentrations. In the monsoon season, heavy metal concentrations are often diluted due to increased water volume, which might relieve some stress on fish and promote growth. Conversely, in the post-monsoon season, heavy metal concentrations might increase due to sedimentation, which can adversely affect fish growth parameters.

Impact of Heavy Metals on Fish Morphometry: Higher concentrations of heavy metals, particularly Lead and Copper, seem to correlate with reduced growth in *Clarias batrachus*. This aligns with studies showing that heavy metals impair protein synthesis and energy allocation, diverting resources away from growth. Parameters such as Total Length, Standard Length and Body Depth show notable variability across different metal concentrations and seasons, supporting the hypothesis that metal stress influences morphological development. Seasonal changes in heavy metal concentrations affect the growth patterns of *Clarias batrachus*. During monsoon, lower concentrations support better growth, while post-monsoon, higher concentrations may hinder it. This study highlights a clear relationship between heavy metal concentrations in aquatic environments and the morphometric characteristics of *Clarias batrachus* (Beatas *et al.*2010).

Table 4.21: Morphometric character of *Clarias batrachus*	
Total Length (TL), cm	Horizontal distance from front tip of snout to hind tip of caudal fin
St andard Length (SL), cm	Horizontal distance from front tip of snout to base (or articulation) of caudal fin
Caudal Length (CL),cm	Horizontal distance from hind margin of anal fin (or from that of dorsal fin if this extends further backwards than anal) to base of caudal fin
Eye Diameter (ED),cm	Horizontal diameter of eye
Pre-Dorsal Length (PDL),cm	Horizontal distance from front tip of snout to the articulation of first dorsal-fin ray
Pre-Anal Length (PAL),cm	Horizontal distance from front tip of snout to the articulation of the first anal-fin ray
Pre-Pectoral Length (PPEL),cm	Horizontal distance from front tip of snout to the articulation of the first pectoral-fin ray.
Dorsal Fin Length (DFL),cm	Maximal horizontal distance measured between both ends
Anal Fin Length (AFL),cm	See dorsal-fin base
Body Depth (BD),cm	Maximum vertical depth of fish, excluding fins

Table 4.22: Mean, Minimum, Maximum and Standard Deviation of different morphometric character of *Clarias batrachus*

	Control: 30-Day-	Suryakund Pond				Pishach Mochan Pond				Pushkar Pond				Ishwargangi Pond				Kurukshetra Pond			
		Mean	Min	Max	STDEV	Mean	Min	Max	STDEV	Mean	Min	Max	STDEV	Mean	Min	Max	STDEV	Mean	Min	Max	STDEV
Total Length (TL), cm	18.05	21.24	12	32.4	8.11	16.38	8.4	28.3	7.31	18.72	12.6	32.2	7.81	16.04	10.4	22.6	5.25	17.88	11.6	30.4	7.43
St andard Length (SL),cm	15.89	18.96	9.8	30.2	8.15	14.32	6.2	26.4	7.41	16.66	10.4	30	7.74	13.92	8.6	20.2	5.11	15.62	9.2	28.1	7.43
Caudal Length (CL),cm	2.16	2.28	2.2	2.4	0.1	2.06	1.9	2.2	0.11	2.06	1.8	2.2	0.19	2.12	1.8	2.4	0.24	2.26	2	2.4	0.17
Eye Diameter (ED),cm	0.26	0.29	0.2	0.4	0.08	0.22	0.2	0.3	0.04	0.28	0.2	0.4	0.08	0.24	0.2	0.3	0.05	0.3	0.2	0.4	0.1
Pre-Dorsal Length (PDL), cm	5.62	7.08	3.2	13.2	4.14	4.92	2.2	10.2	3.09	5.92	2.3	13.5	4.4	4.56	2.8	7.4	2.09	5.6	3.2	11.2	3.25
Pre-Anal Length (PAL),cm	6.71	8.26	4.4	14.4	4.15	6.1	3.4	11.3	3.04	6.88	3.2	14.6	4.5	5.62	3.7	8.4	2.12	6.68	4.3	12.3	3.28
Pre-Pectoral Length (PPEL),cm	2.64	3.24	1.4	6.1	1.9	2.56	1.4	5.2	1.53	2.66	1.2	6.2	2.04	2.22	1.2	3.9	1.24	2.52	1.2	5.1	1.57
Dorsal Fin Length (DFL),cm	9.58	11	6.8	16.2	3.7	8.6	5.2	14.2	3.34	9.84	7.2	16.3	3.75	8.72	6.4	11.2	2.3	9.76	6.8	16.2	3.76
Anal Fin Length (AFL),cm	7.19	8.44	4.8	12.8	3.18	6.52	3.3	11.3	2.94	7.44	5.1	12.8	3.09	6.46	4.1	9.4	2.24	7.1	4.6	12.1	2.97
Body Depth (BD),cm	2.26	2.74	1.5	4.5	1.18	2.12	1.1	3.5	0.92	2.3	1.5	4	0.98	1.96	1.3	2.8	0.65	2.2	1.4	3.8	0.95

*Healthy fingerlings of *Clarias batrachus* (post-larval stage, generally 25–60 days old) were obtained from a certified hatchery.

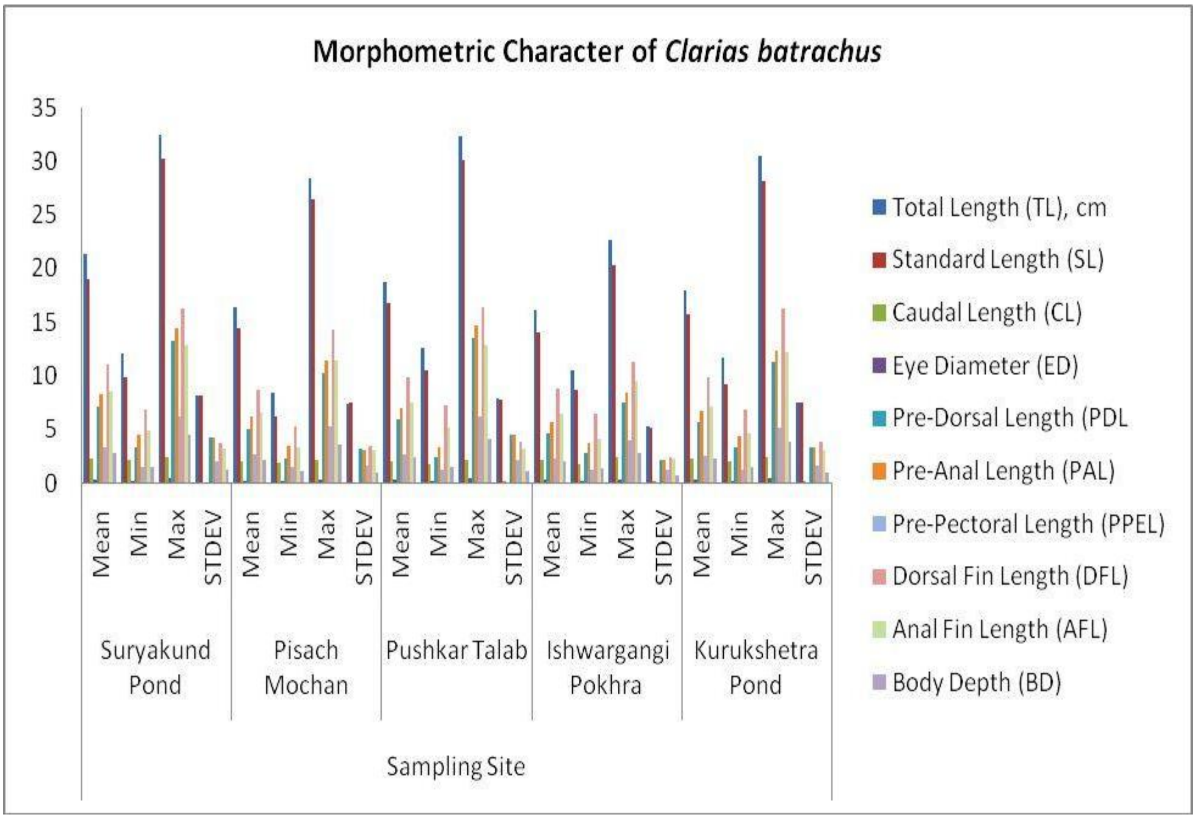


Fig.4.35. Morphometric character of *Clarias batrachus*

4.5 Histopathological Findings in Liver *Clarias batrachus* from Different Ponds

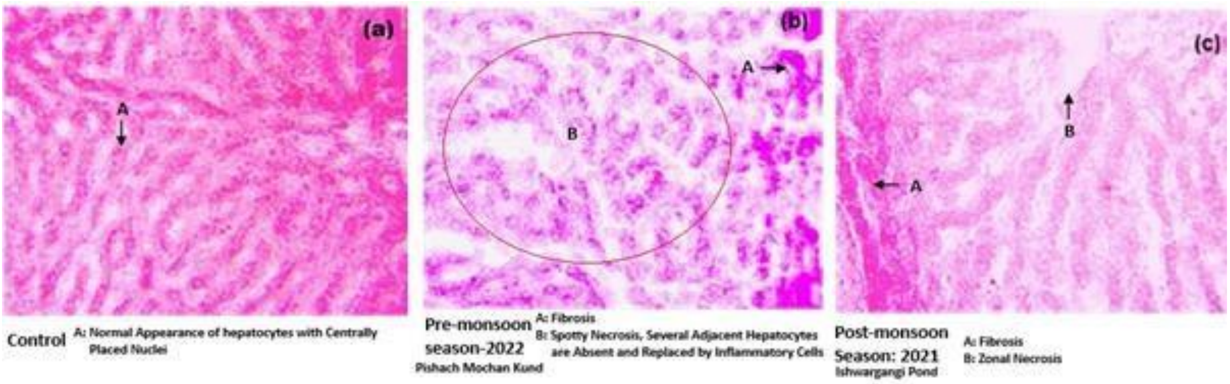


Fig.4.36: Photomicrographs of Liver TS of *Clarias batrachus* showing (a) control group and (b) and (c) exposure to Pond water

Histopathological Changes in Liver Tissues Across the Seasons:

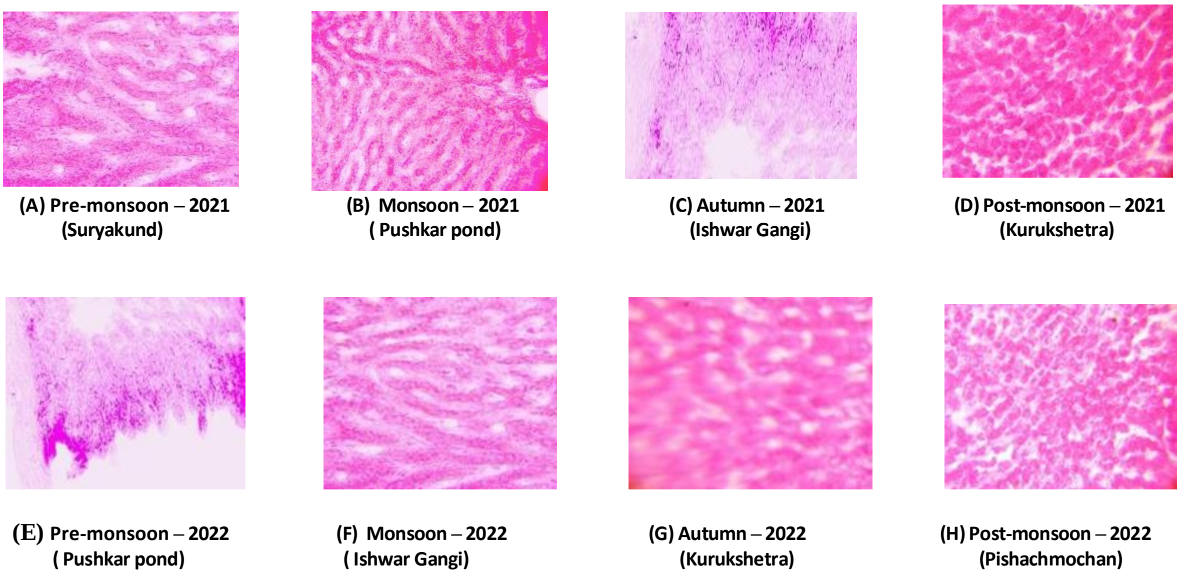


Fig.4.37: Normal appearance of hepatocytes cell, no abnormality found in these seasons in different ponds (A-H)

Observations and Analysis of TS of Liver

The liver histopathology of *Clarias batrachus* across seasons highlights significant damage due to heavy metal exposure, with observable structural changes such as vacuolization, fibrosis, inflammation and necrosis. These findings emphasize the need for heavy metal monitoring and pollution control in these Ponds to protect aquatic life. The seasonal variations in liver pathology indicate that while certain seasons allow partial recovery, the cumulative effects of heavy metals pose a serious threat to fish health and the ecological balance of these aquatic systems.

Based on the histopathological images of the liver transverse sections of *Clarias batrachus*, Figure 4.34, can analyze the impact of heavy metal concentrations from different Ponds in Varanasi across seasons (Pre-monsoon, Monsoon, Autumn and Post-Monsoon of 2021 and 2022). The liver, being a primary detoxification organ in fish, often accumulates pollutants like heavy metals, which can lead to observable histopathological changes.

Heavy Metal Concentrations:

Seasonal variations in heavy metal concentrations are noticeable, with certain metals like Zinc and Copper showing significant levels across multiple seasons and sites. Lead and Nickel, known for their toxicity, also vary but tend to reach higher levels during specific seasons (monsoon and post-monsoon). The liver tissues' condition reflects the potential impact of these metal concentrations, as heavy metals can induce oxidative stress, cellular damage and inflammation.

Histopathological Changes in Liver Tissues Across Seasons

Pre-monsoon Season (2022): Liver tissues in the Pre-monsoon in Pishachmochan pond images exhibit signs of cellular disruption and vacuolization (formation of empty spaces within cells), indicating stress on hepatocytes (liver cells). The higher temperatures in Pre-monsoon could enhance metabolic rates, leading to increased metal absorption and subsequent oxidative damage in liver tissues. Observed histopathological changes like hepatocyte hypertrophy (cell enlargement) and nuclear changes might result from the detoxification of metals like Lead and Copper, which tend to peak in warmer seasons.

Monsoon and Autumn Season (2021 and 2022): The liver section shows normal appearance of hepatocytes cells, no abnormality found in these seasons.

Post-Monsoon Season (2021): In Post-monsoon season at Ishwargangi pond, the fish liver sections show persistent signs of inflammation and degeneration, particularly around the central veins and hepatic cells. High Nickel and Zinc levels during this season might be contributing to prolonged oxidative stress, causing structural changes such as sinusoidal dilation (widening of liver capillaries) and hepatocyte disorganization. The presence of metal-induced cellular disruptions highlights the impact of residual pollutants after monsoon washout, as liver tissues struggle to manage the detoxification burden.

Impact of Heavy Metals on Liver Morphology

The liver is central to detoxifying contaminants, but metals like Lead, Nickel, Copper and Zinc can interfere with its functioning, especially when they accumulate to toxic levels. **Lead** and **Nickel** are particularly damaging, as they can induce lipid peroxidation (oxidative degradation of lipids) in cell membranes, resulting in hepatocyte necrosis and apoptosis (programmed cell death). **Copper**, while essential in trace amounts, becomes toxic at high concentrations and can impair mitochondrial function, affect cellular energy production and lead to hepatocyte swelling and cell death. **Zinc**, though less toxic than Lead or Copper, can still disrupt enzyme functions within the liver when present in high concentrations, leading to chronic inflammatory responses.

Seasonal Variations in Liver Damage

In **Pre-monsoon,** High temperatures and increased metabolic activity likely enhance metal uptake, as reflected in the observed vacuolization and hepatocyte hypertrophy. Increased Lead and Copper exposure in Pre-monsoon could drive these changes, causing oxidative damage to hepatocytes. In **monsoon**, Runoff from surrounding areas likely increases metal concentration, as seen in the inflammation and cell necrosis within liver tissues. The presence of Nickel and Lead in particular seems to correlate with signs of cellular damage and congestion in the liver. In **Autumn**, Partial regeneration is observed, but fibrosis and chronic tissue abnormalities remain. Reduced metal exposure may allow the liver some recovery, but cumulative damage persists, indicating the lasting impact of Pre-monsoon and monsoon exposure. In **Post- Monsoon**: Lingering heavy metal presence, especially Nickel and Zinc, continues to affect liver health. Observed sinusoidal dilation and disorganization of liver cells reflect the stress of ongoing exposure, highlighting the challenge of recovery despite seasonal reduction in certain metal concentrations.

Ecological and Health Implications

The liver damage in *Clarias batrachus* indicates that these Ponds are subject to persistent heavy metal pollution, likely from anthropogenic sources like agriculture, industrial runoff and urban pollution. The impairment of liver function in fish can reduce their growth, immunity and reproductive success, potentially impacting population dynamics in these water bodies. The chronic exposure to heavy metals could lead to bioaccumulation in the food web, impacting not only fish but also other organisms that consume them, including humans.

4.6 Histopathological Findings in Kidney *Clarias batrachus* from Different Ponds

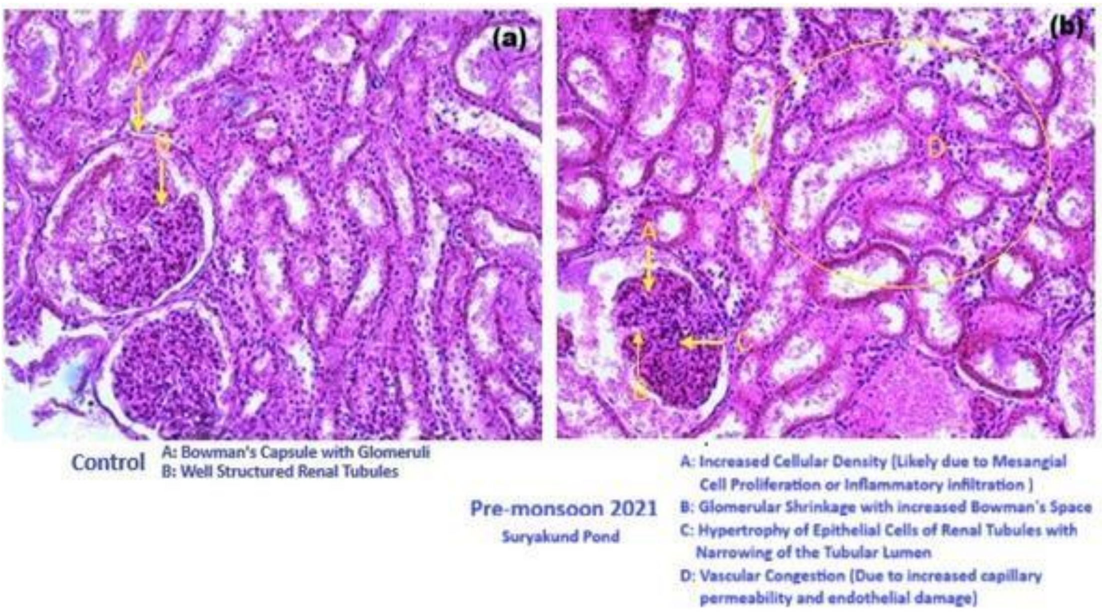


Fig.4.38. Photomicrographs of Kidneys of *Clarias batrachus* showing (a) control group and (b) exposure to Pond water

Histopathological Changes in kidney Tissues Across Seasons

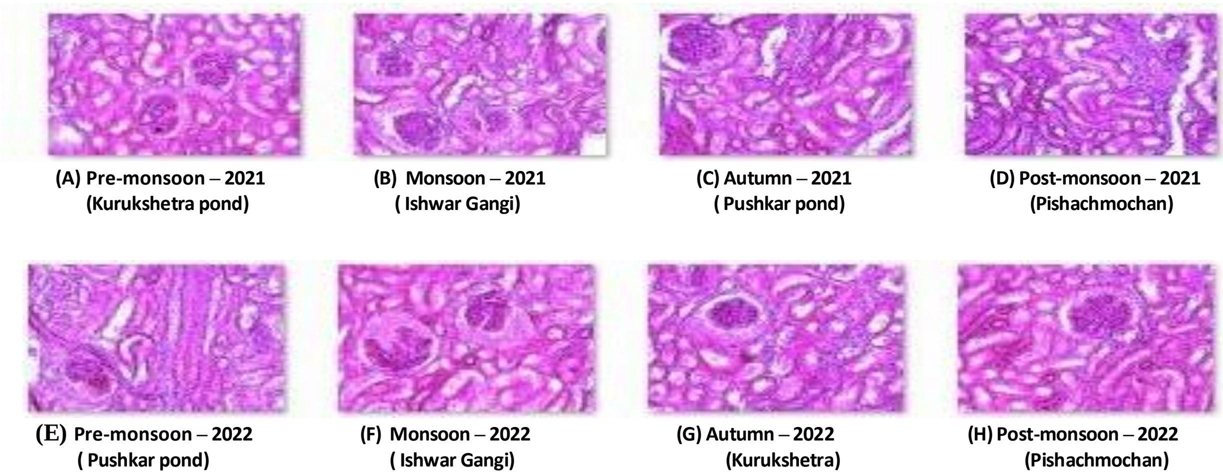


Fig.4.39. Normal appearance of Bowman capsule with glomeruli and normal structure of renal tubules, no abnormality found in these seasons (A-H)

Observations and Analysis of TS of Kidney-

Kidney pathology in *Clarias batrachus* reflects chronic heavy metal exposure, with notable histopathological changes across seasons due to varying metal concentrations. The severity of kidney damage is consistent with high metal levels, particularly during Pre-monsoon and monsoon seasons. The seasonal histopathological examination of *Clarias batrachus* kidneys reveals significant structural damage linked to heavy metal exposure. The observed tubular necrosis, fibrosis and glomerular disorganization align with elevated Lead, Nickel, Copper and Zinc levels in the Pond water, suggesting that heavy metals play a critical role in kidney pathology. The findings underscore the need for pollution control and regular monitoring to safeguard aquatic life and prevent potential human health risks from metal bioaccumulation in these ecosystems.

Based on the histopathological transverse section images of the kidney sections of *Clarias batrachus* from different Ponds in Varanasi, can analyze the impact of seasonal heavy metal contamination (Lead, Nickel, Copper and Zinc) from various sources: Suryakund Pond, Pishach Mochan, Pushkar Pond, Ishwargangi Pond and Kurukshetra Pond, across seasons (Pre-monsoon, Monsoon, Autumn and Post- Monsoon of 2021 and 2022). The kidney, as a key organ for filtration and excretion, is sensitive to toxic substances and can exhibit structural changes when exposed to heavy metals.

Heavy Metal Concentrations

Elevated concentrations of Copper (Cu) and Zinc (Zn) are also noted, with their highest values fluctuating seasonally, potentially related to runoff and anthropogenic activities like agriculture and industrial discharge. These metals, especially Lead and Nickel, are nephrotoxic (toxic to the kidney) and chronic exposure can lead to structural kidney damage, impairing function over time.

Histopathological Changes in Kidney Tissues Across Seasons

Pre-monsoon Season (2021): At Surya Kund Pond, kidney tissue in the Pre-monsoon shows significant histopathological alterations, including cellular hypertrophy, degeneration and glomerular shrinkage. These changes reflect cellular stress and possible toxic effects due to metal exposure. The high metabolic rate in warmer temperatures could amplify metal uptake, leading to increased cellular toxicity. The high concentrations of Lead and Nickel in this season are correlated with observable changes like tubular degeneration and necrosis, indicating that these metals may disrupt kidney filtration functions.

Monsoon, Autumn and Post-Monsoon Seasons (2021 and 2022): Kidney sections from these seasons shows normal bowman capsule with glomeruli and normal renal tubules structure. No abnormality shows in these seasons.

Impact of Heavy Metals on Kidney Morphology: **Lead (Pb)** and **Nickel (Ni)** are known to be nephrotoxic, leading to oxidative stress that damages cellular proteins, lipids and DNA in the kidneys. Observed glomerular shrinkage, tubular necrosis and inflammatory cell infiltration are common responses to prolonged heavy metal exposure. **Copper (Cu)**, although essential in small amounts, can induce oxidative damage at higher concentrations, leading to cellular degeneration and necrosis in renal tissues. **Zinc (Zn)**, another essential trace metal, may cause kidney toxicity when elevated, contributing to chronic structural damage over time, evident in the fibrosis and cellular disorganization in post-monsoon and autumn samples.

Seasonal Variation and Kidney Damage Patterns: In **Pre-monsoon, increased** metabolic activity and high temperatures may exacerbate heavy metal uptake, as evidenced by the hypertrophy, necrosis and glomerular degeneration seen in the images. This is consistent with the elevated levels of Lead and Nickel during this season, which directly impact kidney cells. In **Monsoon**, the influx of contaminant from runoff likely contributes to kidney congestion, inflammatory infiltration and necrosis, especially due to Nickel and Copper. This suggests that monsoon runoff increases pollutant levels in the Ponds, affecting fish health. In **Autumn**, while lower concentrations of metals allow for partial recovery, lingering fibrosis and cellular disorganization highlight the residual impact of metal exposure. Fibrosis indicates chronic stress that may permanently alter kidney function. In **post-monsoon**, persistent high metal concentrations, particularly Nickel and Zinc, continue to affect renal health. The observed tubular dilation and necrosis indicate that metal detoxification is challenging for the fish, with structural kidney damage as a result (Olfat, M.N.and El-Greisy, 2017).

Ecological and Health Implications: The structural kidney damage observed in *Clarias batrachus* across seasons suggests that these Ponds are contaminated with nephrotoxic metals, which could have a cumulative impact on fish populations by reducing survival and reproductive rates. Kidney impairment can affect fish health by disrupting osmoregulation, filtration and excretion processes, reducing their ability to withstand other environmental stressors. Bioaccumulation of these metals can also impact other organisms within the aquatic ecosystem, including humans, who might consume fish from these contaminated Ponds. This underlines the importance of monitoring and mitigating heavy metal contamination.

4.7 Histopathological Findings in Gill *Clarias batrachus* from Different Ponds

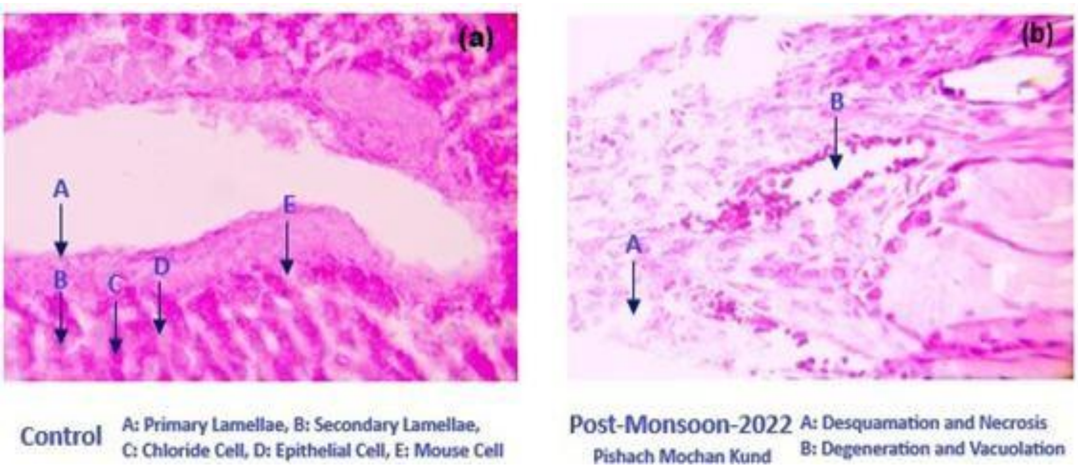
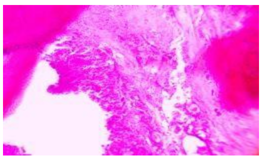
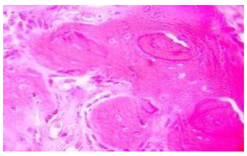
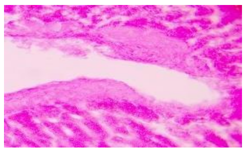


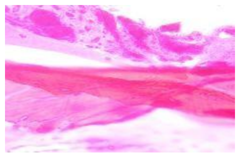
Fig.4.40. Photomicrographs of Gill TS of *Clarias batrachus* showing (a) Control group and (b) exposure to Pond water

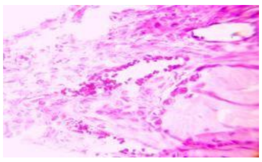
Histopathological Changes in Gill Tissues Across Seasons

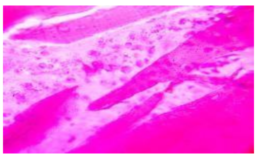

(A) Pre-monsoon – 2021 (Pushkar pond)

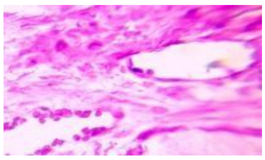

(B) Monsoon – 2021 (Ishwar Gangi)


(C) Autumn – 2021 (Kurukshetra)


(D) Post-monsoon – 2021 (Suryakund)


(E) Pre-monsoon – 2022 (suryakund)


(F) Monsoon – 2022 (Ishwar Gangi)


(G) Autumn – 2022 (Kurukshetra)

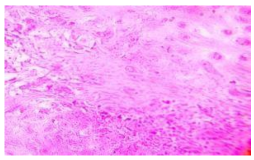

(H) Post-monsoon 2022 (pushkar pond)

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Fig.4.41.Normal appearance of primary, secondary lamellae, chloride cells and epithelial cells no abnormality found in these seasons (A-H).

Observations and Analysis of TS of Gill

The histopathological analysis of *Clarias batrachus* gills across seasons provides strong evidence of chronic exposure to heavy metals like Lead, Nickel, Copper and Zinc. The seasonal patterns of tissue damage and recovery suggest that environmental factors, (B.L.Luhovy,2007) such as rainfall and temperature, influence metal bioavailability and toxicity. Efforts to control heavy metal pollution and periodic monitoring are essential to protect aquatic life in these environments, as continued exposure may lead to severe ecological consequences.

Based on the transverse section, can analyze the histopathology of the gills of *Clarias batrachus* collected from various Ponds in Varanasi, comparing seasonal variations in tissue morphology and corresponding heavy metal concentrations (such as lead, nickel, copper and zinc). The data provides insights into the potential impacts of these heavy metals on the gill structure across different seasons in 2021 and 2022**.**

Heavy Metal Concentrations

Over the course of the seasons (Pre-monsoon, Monsoon, Autumn, Post- Monsoon for 2021 and 2022), variations are observed in the concentrations of these metals. Highest levels of specific metals vary by location, but generally, Zinc and Copper are consistently present, with slight seasonal fluctuations. High concentrations of these metals, especially Lead and Nickel, are known to be toxic to aquatic *organisms* and their accumulation in gill tissues can lead to histopathological changes, as seen in the gill transverse section images.

Histopathological Changes in Gill Tissues Across Seasons

Pre-monsoon, monsoon and autumn Seasons (2021 and 2022) : Normal appearance of primary lamellae, secondary lamellae, chloride cells and epithelial cells no abnormality found in these seasons.

Post-Monsoon Season (2022): At Pishachmochan pond, in post-monsoon season images indicate mixed results, with some tissues showing substantial inflammation and others showing relatively normal structure. High Nickel and Zinc concentrations during this period may have caused specific structural changes like epithelial cell lifting and lamellar disorganization. The histopathology indicates ongoing recovery with continued low-level stress, likely due to the residual metals in water after the monsoon season has washed away some pollutants.

Heavy Metal Impacts on Gill Morphology

The histological transvers section reveal that heavy metals, even at relatively low concentrations, can lead to visible damage in gill tissues. Lead and Nickel, in particular, are notorious for causing oxidative stress, leading to cell damage and death in fish. These metals can interfere with oxygen uptake by damaging the delicate structure of the gill filaments. Copper, while essential in trace amounts, becomes toxic at elevated levels, leading to enzymatic dysfunctions in respiratory tissues. High copper levels seen in Pre-monsoon may explain the severe tissue alterations, as copper disrupts mitochondrial function, affecting cell energy production.

Seasonal Variations in Gill Damage

Seasonal variations in gill morphology reflect both the external metal concentrations h and the physiological state of the fish. In **Pre-monsoon,** higher temperatures are likely to increase metal uptake, with subsequent tissue damage due to increased oxidative stress. In **monsoon**, increased runoff may introduce additional metals, exacerbate the damage and prompting protective responses like mucus production. In **autumn**, slight recovery may be due to reduced metal exposure, though chronic effects persist. In post-monsoon, Mixed tissue states indicate partial recovery but residual stress, as the lingering metal exposure continues to affect fish physiology.

Environmental and Ecological Implications

The observed histopathological changes suggest that these Ponds experience pollution from various sources, likely due to urban runoff, agricultural activities, or industrial discharge in the surrounding areas. The changes in gill structure could impair the respiratory and excretory functions of *Clarias batrachus,* potentially reducing their growth, reproduction and survival rates. This can impact not only the fish population but also the larger aquatic ecosystem by altering food web dynamics. Monitoring and managing metal concentrations in these Ponds are crucial to preventing long-term ecological damage.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

The interplay between urbanization, cultural preservation and ecological health is intricately woven in the context of Varanasi's ancient kunds. The assessment of trace metal pollution, particularly Pb, Ni, Cu and Zn, in these historical water bodies has unveiled concerning patterns of environmental degradation. The stark morphometric changes observed in *Clarias batrachus*, an inhabitant of these kunds, underscores the tangible repercussions of such pollution on aquatic life.

It becomes abundantly clear that these water bodies are not just repositories of religious and cultural significance but are crucial ecological hubs that require immediate attention. As urban centers continue to expand and intensify their anthropogenic footprint, holistic strategies that encompass the preservation of both cultural heritage and environmental integrity become paramount.

Furthermore, the bioaccumulation potential of these metals in the food chain accentuates a broader ecological risk, extending to predators and potentially impacting human consumers. This not only necessitates improved trace metal management in urban landscapes but also emphasizes the importance of regular ecological monitoring to detect and address emerging environmental threats promptly.

The present investigation provides a comprehensive assessment of the impact of aquatic pollution on water quality and its biological implications in five ancient and sacred Ponds (kunds) of Varanasi—Suryakund Pond, Pishach mochan Pond, Pushkar Pond, Ishwargangi Pond and Kurukshetra Pond. By integrating seasonal physicochemical characterization, trace metal analysis, multivariate statistical techniques and biological responses in *Clarias batrachus*, the study successfully fulfils its stated objectives and offers critical insights into the deteriorating ecological health of these culturally significant water bodies.

This research not only contributes valuable baseline data on the environmental status of ancient urban Ponds but also highlights the role of integrated physico-chemical, biological and statistical approaches in aquatic pollution assessment. The outcomes have significant implications for environmental management, public health and sustainable conservation of culturally important freshwater resources, and they provide a scientific foundation for future policy formulation and remediation efforts.

5.1 Objective-wise Conclusions

Objective 1:

To analyze the impact of aquatic pollution on morphometry and histopathology in *Clarias batrachus*.

- The present study conclusively demonstrates that aquatic pollution prevailing in the sacred Ponds under investigation has a profound and multifaceted impact on the biological health of *Clarias batrachus*. The deteriorated water quality, characterized by elevated organic load, reduced dissolved oxygen and the presence of trace metal contaminants, significantly influenced the morphometric, histopathological and biochemical attributes of the fish.

- Alterations observed in morphometric parameters clearly indicate stress-induced growth impairment and a decline in the overall physiological condition of *Clarias batrachus*. Reduced growth indices and changes in body proportions suggest that prolonged exposure to polluted aquatic environments adversely affects nutrient assimilation, energy allocation and normal growth patterns. These morphometric deviations serve as early indicators of environmental stress and reflect sub-lethal effects of pollution on fish populations.

- Histopathological investigations revealed extensive structural damage in vital organs of *Clarias batrachus*, confirming chronic toxic stress. Gill tissues exhibited epithelial lifting, lamellar fusion and disruption of normal architecture, which are indicative of impaired respiratory efficiency and reduced gas exchange capacity. Liver tissues showed pronounced degenerative changes, including hepatocellular necrosis, vacuolization and cellular disorganization, reflecting compromised metabolic and detoxification functions. Similarly, kidney tissues displayed glomerular shrinkage, tubular degeneration and structural disintegration, indicating impaired osmoregulatory and excretory functions. The severity of these lesions highlights the prolonged and cumulative impact of aquatic pollutants on organ integrity.
- Collectively, the integration of morphometric and histopathological evidence conclusively establishes that persistent aquatic pollution poses serious threats to the health, survival and ecological fitness of *Clarias batrachus*. The findings validate the suitability of *Clarias batrachus* as a sensitive bioindicator species for assessing aquatic pollution and underscore the urgent need for effective pollution control and conservation measures to protect aquatic life in culturally significant freshwater ecosystems.

Objective 2:

To perform a comparative analysis of morphometry and histopathology studies of *Clarias batrachus* in different water bodies.

- The comparative evaluation of morphometry and histopathological parameters of *Clarias batrachus* across the five selected sacred Ponds revealed pronounced spatial variation in biological stress responses. This variation clearly reflects differences in local environmental conditions, pollution load and intensity of anthropogenic activities associated with each Pond. The results demonstrate that fish health is strongly influenced by site- specific characteristics rather than being uniformly affected across all water bodies.

- Fish collected from Ponds subjected to higher anthropogenic pressure exhibited significantly greater morphometric deviations, indicating stress-induced growth retardation and poor physiological condition. These deviations were accompanied by severe histopathological alterations in vital organs, including extensive gill damage, hepatic degeneration and renal structural impairment. In contrast, fish from comparatively less impacted Ponds showed relatively milder structural and physiological alterations, highlighting the role of environmental quality in determining biological health.
- The severity of biological impairment was found to vary in direct proportion to site-specific pollution intensity, sources of contamination and human activities such as ritual practices, urban runoff and discharge of untreated wastewater. These observations confirm that pollution impacts on aquatic organisms are spatially heterogeneous and governed by localized environmental pressures.
- The comparative analysis conclusively establishes that aquatic pollution does not exert uniform effects across different water bodies. Instead, its impact is strongly site-dependent, emphasizing the necessity for Pond-specific monitoring, targeted management interventions and localized restoration strategies. Such an approach is essential for effective conservation of aquatic biodiversity and sustainable management of culturally significant freshwater ecosystems.

Objective 3:

To correlate aquatic pollutants with morphometrical and histopathological parameters.

- The present study establishes a strong and consistent relationship between deteriorating aquatic environmental conditions and adverse biological responses in *Clarias batrachus*. Correlation analysis clearly demonstrated that variations in physicochemical parameters and pollutant concentrations were closely associated with changes in morphometric indices, tissue architecture and biochemical profiles of the fish. These findings provide compelling evidence that aquatic pollutants are the primary drivers of biological stress in the studied Pond ecosystems.

- Elevated levels of key physicochemical parameters, including electrical conductivity, total dissolved solids, biological oxygen demand and chemical oxygen demand, showed significant associations with morphometric impairment and structural damage in fish tissues. Reduced dissolved oxygen concentrations were strongly correlated with gill pathology, particularly epithelial lifting and lamellar fusion, indicating compromised respiratory efficiency under polluted conditions. Such correlations highlight the direct influence of organic pollution and oxygen depletion on fish health.

- Trace metal contamination, especially due to metals such as lead, copper, nickel and zinc, exhibited a pronounced relationship with histopathological alterations in vital organs. Increased metal concentrations were associated with hepatic necrosis, cellular vacuolization and renal tubular degeneration, reflecting the bioaccumulative and toxic nature of these contaminants. These organ-specific damages underline the role of trace metals in inducing chronic toxicity and physiological dysfunction in aquatic organisms.

- Multivariate statistical analysis, particularly Principal Component Analysis (PCA), effectively integrated complex datasets and identified common pollution sources influencing both water quality and biological responses. The clustering of physicochemical parameters with biological stress indicators confirmed the interconnectedness of environmental degradation and fish health impairment.

- The correlation analysis conclusively demonstrates that anthropogenic pollutants play a decisive role in shaping biological responses in *Clarias batrachus*, reinforcing the importance of integrated monitoring and pollution mitigation strategies for the protection and sustainable management of freshwater ecosystems.

5.2 Major Findings of the Study

- The present study clearly establishes that the sacred Ponds of Varanasi—namely Suryakund Pond, Pishach mochan Pond, Pushkar Pond, Ishwargangi Pond and Kurukshetra Pond—are experiencing progressive deterioration of water quality due to sustained anthropogenic pressure. Physicochemical analysis revealed that key parameters such as electrical conductivity, total dissolved solids, biological oxygen demand and chemical oxygen demand frequently exceeded recommended permissible limits, indicating substantial organic and inorganic pollution load across all seasons.
- Organic pollution was identified as a major factor affecting Pond ecosystem health, as reflected by consistently elevated BOD and COD values accompanied by reduced dissolved oxygen concentrations. These conditions indicate impaired self-purification capacity of the Ponds and create physiologically stressful environments for aquatic organisms. In addition to organic contamination, trace metals such as lead, copper, nickel and zinc were detected in Pond water at levels that varied seasonally and spatially, with higher concentrations recorded particularly during pre-monsoon and monsoon seasons, suggesting contributions from surface runoff, ritual activities and urban inputs.
- Seasonal variation emerged as a critical determinant of water quality dynamics. Distinct fluctuations in physicochemical parameters and metal

concentrations were observed during pre-monsoon, monsoon, post-monsoon and winter seasons. Increased pollutant concentrations during pre-monsoon periods were associated with evaporation and reduced water volume, while monsoon season values reflected dilution effects and enhanced runoff-mediated inputs. These seasonal trends significantly influenced pollutant distribution and overall water quality status of the Ponds.

- The biological responses of *Clarias batrachus* closely mirrored the observed physicochemical degradation. Fish collected from polluted Ponds exhibited altered morphometric indices, severe histopathological damage in gill, liver and kidney tissues, and pronounced biochemical disturbances. Gill lesions such as epithelial lifting and lamellar fusion were associated with low dissolved oxygen and high organic load, while hepatic necrosis and renal tubular degeneration corresponded with elevated trace metal concentrations. These biological alterations clearly reflect the cumulative and chronic impact of physicochemical stressors present in Pond water.
- Multivariate statistical analysis, particularly Principal Component Analysis (PCA), proved highly effective in identifying dominant pollution sources and interrelationships among water quality parameters. PCA results consistently grouped electrical conductivity, total dissolved solids, chloride, BOD and COD as major contributors to water quality variation, confirming their role as key pollution indicators. The clustering of physicochemical parameters with biological stress responses further validated the strong linkage between environmental degradation and fish health impairment.
- The major findings of the study demonstrate that the ecological health of sacred Ponds in Varanasi is being adversely affected by a combination of organic pollution, trace metal contamination and seasonal variability. The integrated

assessment of water quality parameters, biological responses of *Clarias batrachus* and multivariate statistical analysis provides robust scientific evidence of pollution-induced stress in these culturally significant freshwater ecosystems.

5.3 Recommendations

Based on the physicochemical analysis, biological assessment and multivariate statistical interpretation of the studied sacred Ponds, the following recommendations are proposed for effective management and restoration of these culturally significant freshwater ecosystems:

- Regulation and scientific management of ritual waste disposal and idol immersion practices should be immediately implemented, particularly in highly impacted Ponds such as Suryakund Pond, Pishach mochan Pond and Pushkar Pond. The elevated biological oxygen demand, chemical oxygen demand and trace metal concentrations recorded in these Ponds indicate excessive organic and inorganic inputs associated with religious offerings, immersion materials and ceremonial residues. Designated immersion zones, controlled immersion schedules and post- ritual waste removal mechanisms should be enforced to minimize pollutant loading.

- Promotion and adoption of eco-friendly alternatives for religious and ceremonial activities are essential to reduce organic pollution and trace metal contamination. The use of biodegradable materials, natural dyes, clay idols free from synthetic paints and reduced use of metallic and plastic components should be actively encouraged. These measures are particularly relevant in light of the observed seasonal spikes in pollutant concentrations during pre-monsoon and monsoon periods, when runoff-mediated transport of ritual waste was evident.

- Regular and periodic monitoring of water quality parameters and aquatic biota should be mandated for all sacred Ponds, with special emphasis on electrical conductivity, total dissolved solids, dissolved oxygen, BOD, COD and trace metals such as lead, copper, nickel and zinc. Continuous biological monitoring using *Clarias batrachus* as a bioindicator species is recommended, as histopathological and biochemical responses were found to sensitively reflect cumulative pollution stress. Seasonal monitoring is crucial to capture temporal variations and assess the effectiveness of management interventions.

- Interception, diversion and treatment of urban runoff and untreated domestic wastewater entering the Ponds must be prioritized. The consistent association of high organic load and reduced dissolved oxygen with degraded fish health indicates that untreated inflows are a major pollution source. Construction of peripheral drains, sedimentation chambers and decentralized wastewater treatment units should be undertaken to prevent direct discharge into Pond ecosystems, especially during the monsoon season.

- Public awareness and community participation programs should be initiated to integrate cultural practices with environmental sustainability. Awareness campaigns highlighting the scientific findings of water quality degradation and its biological impacts can promote responsible behavior among local residents, devotees and temple authorities. Community-based stewardship programs can play a vital role in reducing pollution inputs and ensuring long-term conservation of sacred Ponds.

5.4 Scope for Future Research

The findings of the present investigation highlight several critical areas where further research is necessary to strengthen understanding of pollution dynamics and support effective restoration of sacred Pond ecosystems:

- Long-term and continuous monitoring studies should be undertaken to assess temporal trends in water quality deterioration and potential ecosystem recovery.

The present study, conducted over two consecutive years with seasonal sampling, revealed significant seasonal and spatial variations in physicochemical parameters and biological responses. Extended multi-year monitoring would help in evaluating long-term pollution trajectories, inter-annual variability and the effectiveness of implemented management and remediation measures.

- Detailed sediment quality assessment and metal bioavailability studies are required to gain deeper insight into contamination dynamics within Pond ecosystems. The detection of elevated trace metals in water samples suggests possible accumulation in bottom sediments, which can act as long-term pollutant reservoirs. Future studies should focus on sediment–water interactions, metal speciation, mobility and bioavailability to better understand their role in chronic toxicity and recontamination of the water column.

- Human health risk assessment studies related to the consumption of contaminated fish are strongly recommended. The observed accumulation of trace metals and associated histopathological damage in *Clarias batrachus* indicate potential risks to higher trophic levels, including humans. Future research should quantify metal bioaccumulation in edible tissues, estimate dietary exposure and evaluate potential health risks to local populations relying on Pond fish for consumption.

- Development of integrated Pond restoration and management models tailored to sacred urban water bodies is essential. Based on the combined evidence of organic pollution, trace metal contamination, seasonal variability and biological stress responses, future studies should focus on designing holistic management frameworks. These models should integrate scientific monitoring, pollution control technologies, sediment management, biodiversity conservation and culturally sensitive community participation to ensure sustainable restoration of sacred Ponds.

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List of Publications

1. Singh S, Singh AN, Singh R, Sharma NR. 2023. Heavy Metal Pollution in the Aquatic System and Their Toxicological Effects on *Claris Batrachus*. International Journal of Membrane Science and Technology, 10(1): 496-506. (Scopus Indexed)

2. Singh S, Singh AN, Singh R, Sharma NR. 2023. Seasonal Variation of Trace Metals in Ancient Pond and Its Impact on Morphometry of *Claris Batrachus*: A Case Study of Varanasi City. Journal of Advance Zoology, 44 (S-2):2609-2614. (Scopus Indexed)

3. Singh S, Singh R, Sharma NR. 2022. Impact Of Pollution Of Fresh Water Bodies On The Biochemical, Histological Changes And Well-Being Of *Clarias Batrachus*, Journal of Pharmaceutical Negative Results, 13(10):6465-6475. (Scopus Indexed)

4. Singh S, Singh R, Sharma NR. 2022. Extract from *Clarias Batrachus,* Fins as Environmental Benign Corrosion Inhibitor for Mild Steel in Acidic Solution. International Journal of Electrochemical Science, 17 (2022); 1452-3981. (Scopus Indexed)