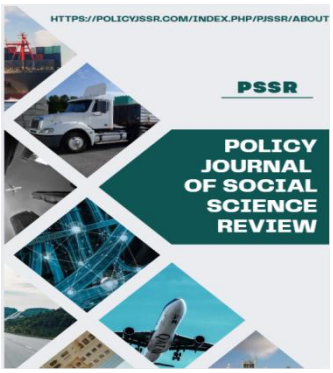
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BIODIVERSITY AND DISTRIBUTION OF FREE-LIVING PROTOZOA IN THE INDUS RIVER, SINDH, PAKISTAN

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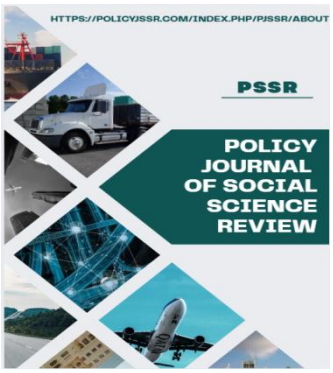
ABSTRACT

Protozoa are single cells, eukaryotes organisms that are ubiquitous. The large majority of species are there protozoan that live freely and others that live within their host organisms. They play a crucial role in the formation of a field ecosystem and they now are vital signs of pollution. They may increase the cleanliness of water improvement. The abundance of different types of protozoa in biodiversity may also vary due to multiple local variables. The composition of the environment and how it is changed. How present, absent, distributed and abundant energy is their development depends on the water bodies in their environment. How they arrive at the way that they are. Most important factors are the temperature, pH and other chemical compositions of the water bodies. Study on Protozoa occurring in the water of River Indus which are free-living. Viruses are ubiquitous due to their small size, multiple duplication and numerous cycles of growth.

Keywords: Protozoa, Fresh Water, River Indus, Sindh, Pakistan.

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Introduction

Microbes can be found in farms, water bodies, etc. Based on its salt content, all aquatic spaces are divided into two forms freshwater or marine environments. Lots of archaea and bacteria are present in both of these and they drive biogeochemical processes that impact the whole ecosystem (El-Abbassy *et al.*, 2024).

Protozoa is a sub group of Protista in the animal kingdom. They can be encountered in various environments such as fresh water, salt water and are most commonly found in streams, lakes, rivers, ponds and other water bodies. The name "Protozoa" means "first or oldest animal or organism." Protozoa are abundant and ecologically significant as they regulate the flow of nutrients and contribute to the functioning of ecosystems. They are frequently found in ponds, lakes and in the water from treatment plants. There are several groups of protozoans, which are differentiated by the way in which they move: ciliates, flagellates, amoebae and protozoans (Hettithanthri *et al.*, 2024).

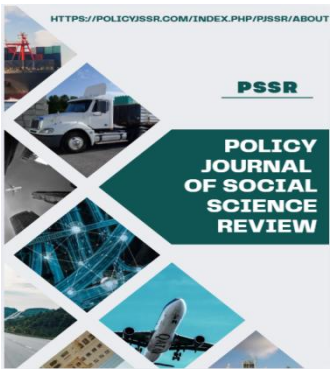
Some are large enough to only be a few micrometers in size, others are as large as a few millimeters and exhibit a wide range of shapes and functions. Protozoa are the food source for many organisms in both the water and on land. Microorganisms also function to transform organic material and put nutrients found in the environment back in the system. Occasionally protozoa are pathogenic to the human and less frequently to domestic and wild animals used for food. A wide variety of protozoa are capable of being pathogenic on the human body (Kumar *et al.*, 2024).

Only 2.5% of the total amount of water on earth is freshwater, which contains less than 1 gram per liter of salt. 68.7% of this water stays permanently frozen and 29.8% is groundwater (Landrigan *et al.*, 2023). However, freshwater ecosystems are often termed as the "blood of society" as they always played a crucial role in life and the growth of civilizations. There are communities all over the world that have developed around freshwater habitats and over half of all people live within 50 km of a river with no permanent water supply (Matoničkin Kepčija *et al.*, 2026). Most available and renewable freshwater bodies are frequently coming under extra stress due to activities such as farming, industry and home use. Thus, water contamination is caused by the emission of various anthropogenic and geogenic substances. Hence, chemical contamination of natural waters has been occurring for some time and is now perceived as a major public issue (Nzilu *et al.*, 2023).

Protozoa are single-celled eukaryotes that play an important role in almost all aquatic environments. They are responsible for feeding on bacteria and decomposing organic matter, and performing a

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significant role in nutrient cycling and maintaining ecological balance. Free-living protozoa can also be valuable biological indicators since the diversity, abundance and distribution of the protozoa in water bodies can be correlated with the physical and chemical state of the water body. Indus is one of the major freshwater systems of Pakistan, particularly in Sindh, providing agricultural services, fisheries, source of drinking water and biodiversity. But natural change and man-made factors like domestic waste, farm runoff, industrial waste and seasonal fluctuations constantly influence the water quality of rivers. These changes can directly affect the distribution and diversity of the free-living protozoa in the river. Several studies have been carried out on water quality and aquatic organisms of Pakistan, but very little information is available on free-living protozoan communities of Indus River in Sindh. There is a scarcity of specific information on their occurrence, diversity and distribution, which limits understanding of the ecological health of this vital river system.

So, the present study entitled "Distribution and Diversity of Free-Living Protozoa in the Indus River, Sindh, Pakistan" was designed to study the presence, abundance and diversity of the free-living protozoa in the water of Indus River. This study will help in providing baseline data on the biodiversity of protozoans and may also help in evaluation of aquatic environments of Indus River.

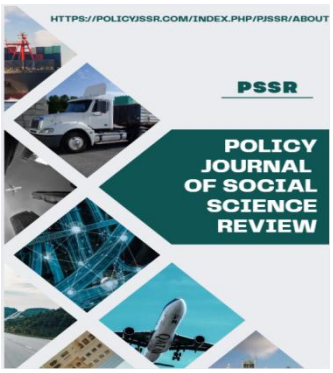
Literature Review

Protozoa are single celled, eukaryotic organisms found in freshwater, marine water, soil and moist environments. The free-living protozoa are classified as amoebae, flagellates, and ciliates in an aquatic ecosystem. They are ecologically significant as they are consumers of bacteria, algae and small organic particles and as an important component of the microbial food chain. Finlay and Esteban (1998) found that the freshwater protozoa are one of the most important grazers on the microbial communities and participate in nutrient cycling and ecosystem balance.

Free-living protozoa are important components of freshwater biodiversity. They are not distributed randomly because they grow and live in response to environmental variables like temperature, pH, dissolved oxygen, nutrients, organic matter, turbidity and salinity. Protozoan communities may be influenced in abundance and diversity by alterations in these factors. Cruaud *et al.* (2019) noted a seasonal dynamics on the composition of protist communities in freshwater and that several parameters such as water temperature and nitrogen concentration may have an impact on the structure of the community. Thus, the distribution study of protozoan could contribute towards the

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explanation of the ecological changes taking place in Freshwater bodies.

Protozoans are also regarded as valuable biological indicators of water quality. Their short life cycles, high fertility and their responses to environmental changes make them appropriate organisms for the measurement of pollution and ecological stress. Madoni (2005) stated that ciliated protozoan communities are suitable for saprobic water quality evaluation in particular water bodies of fresh water with organic pollution. Likewise, Xu, Yong, and Xu (2016) found that the presence of functional groups among planktonic ciliates was correlated with water quality parameters and that they might serve as indicators to water quality assessment.

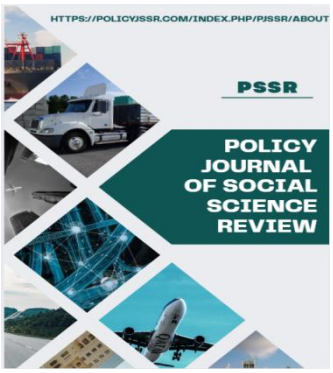
The use of protozoa as bio indicator has been further supported by recent studies. In the Nile River, El-Tohamy *et al.* (2024) reported that protozoan communities were found to be highly correlated with water quality parameters including dissolved oxygen, phosphate, ammonia, nitrite, total dissolved salts and total organic carbon. They found their study demonstrated the protozoan level of abundance, biomass, and species composition may indicate water quality changes. This suggests protozoa could be utilized to study biodiversity and to measure the ecological status of river systems.

Indus is one of the key freshwater systems in Pakistan. It is useful for agriculture, fisheries, drinking water supply, and various aquatic organisms. It flows through the vital regions of Guddu, Sukkur, Kotri, Hyderabad and the Indus delta in Sindh. But, the quality of the water flowing in the Indus River is influenced by a number of natural and anthropogenic factors. They are agricultural runoff, domestic sewage, industrial waste, seasonal variation and rising urbanization. The physical, chemical and heavy metal parameters of Indus River and its tributaries in Sindh were studied by Ahmad *et al.* (2021) and some parameters like turbidity, COD, hardness and some heavy metal were found above limits. Aquatic microorganisms such as free-living protozoa can be directly affected by these changes in water quality.

The Pakistan Council of Research in Water Resources also indicated the high population growth, urbanization; industrialization, farming activities, and untreated wastewater discharge are the major threats to surface water bodies in Pakistan (PCRWR, 2022). The report also revealed that the total dissolved solids, chloride, sodium adsorption ratio and dissolved oxygen, in the lower Indus sites, vary throughout the year. Protozoa are sensitive to these physicochemical changes and therefore information on diversity and distribution of protozoa can be used to assess the ecological state of Indus River.

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Very few studies have been done on the free-living protozoa of Indus River particularly in Sindh. Kanwal, Memon, Fatimah and Siyal (2025) have performed an empirical study on prevalence of free living protozoans from River Indus, Sindh, Pakistan. They observed protozoa like *Amoeba proteus*, Paramecium, Tintinopsis, *Stentor coeruleus*, Blepharisma, Amphileptus, Oxytricha, Glaucoma, Euplotes and Coleps. They found that the prevalence of protozoans varied seasonally with *Amoeba proteus* being more abundant in different seasons and Paramecium being more abundant in summer. Although this study gives good baseline data, further in-depth research is desired on the distribution, diversity and relationship of protozoa to the different physiochemical parameters at different locations of Indus River.

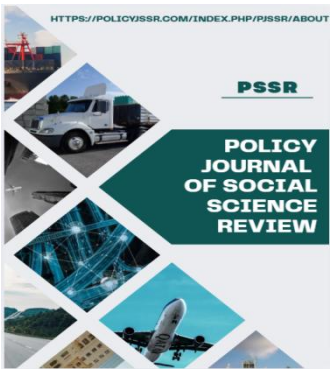
In sum, the literature reviewed indicates that free-living protozoa play a significant role in the freshwater environment through their role in microbial food webs, nutrient cycling, and biological monitoring. Their abundance and diversity depends on various environmental parameters like water temperature, pH, dissolved oxygen, nutrients, organic matter, and pollution load. The study of protozoan communities, in relation to water quality and seasonal variation in the context of the Indus River in Sindh is clearly required. Hence, the present study "Distribution and Diversity of Free-Living Protozoa in the Indus River, Sindh, Pakistan" is of significance as it will be able to give baseline information regarding the biodiversity of protozoa and will serve as a tool for the assessment of the ecological health of this great freshwater system.

Materials and Methods

The water samples were obtained from River Indus, Sindh, in water bottles and zip lock bags each month between 2025 and 2026, as part of this study (Octavianna *et al.*, 2026). APHA used standard methods to measure various physico-chemical aspects such as dissolved oxygen; free CO2, phosphate, nitrate and biological oxygen demand, in the water sample. In most cases, water collected contains floating plants, debris and leaves and many free-living protozoa are phototrophic and feed on bacteria, algae, small particles and/or other protozoa. Samples were taken in the morning because at higher temperatures the number of protozoa diminishes. Samples were taken to the laboratory, stained and then examined under the microscope to determine their shape, and identify them. At the beginning, water samples were visualized by placing some water onto a slide and a cover slip was placed on top to prevent motion, air currents and the water from evaporating. Many of the protozoa are difficult to identify because they are mobile and have locomotory organs. If we don't wish to have any additional movement, we add a

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small amount of methylcellulose (10%) to the water droplet adjacent to the living organisms, making them move slower – without being killed or exploded (Verma *et al.*, 2026).

Results

Samples of Water Analysis

The physico-chemical aspects and free-living protozoans are studied in the water of River Indus near Hyderabad, Sindh. (Figure 1): shows that the data represents studies of water analysis. If there is no oxygen dissolved in the water, then nothing can survive below the water. The May and June DO of 2025-2025 was lower (3.9 mg/L and 3.4 mg/L, respectively), whereas the DO for the next three months (October-December) was better. In May higher CO2 levels are due to increased biological activity which leads to increased CO2. The most phosphate recorded was in August at 2.0 mg/L, but in March, the lowest reading was 0.4 mg/L. BOD level in the course of the study was measured between 2.1 mg/l and 2.9 mg/l. The BOD results are in good range for most of the moderate water quality. The range for nitrate was between 7.5 mg/L to 8.5 mg/L throughout the study. This is a typical and regular concentration for fresh water (El-Abbassy *et al.*, 2023).

For every month during the 2024-2025 periods, an investigation into protozoan was carried out. Ten different marine species have visited the study area as shown in (Tab.1) and (Figure.2). *Amoeba proteus* could be found at high levels in every season, but particularly in the winter and summer. However, Paramecium was most abundant during the summer and is one of the many species found in Lake George. Stable growth was present when *Stentor coeruleus* was present and maximum abundances were observed during the summer. In contrast, the number of Tintinopsis became moderate all year, but they increased a bit during monsoon. Oxytricha numbers were lowest in the winter and summer, perhaps due to a lack of adaptability to its environment. This virus remains stable most of the year, except for a small increase in the summer.

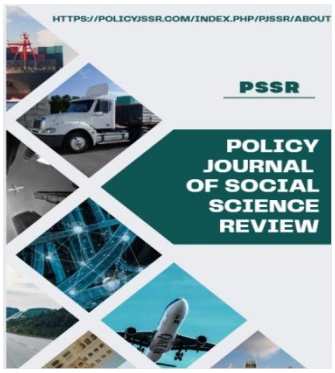
Table 1:

Physico-chemical analysis of water

Protozoa	Monsoon	Winter	Summer
Euplotes	20	13	18
Stentor coeruleus	19	12	14
Tintinopsis	18	11	18
Amphileptus sp	11	10	16

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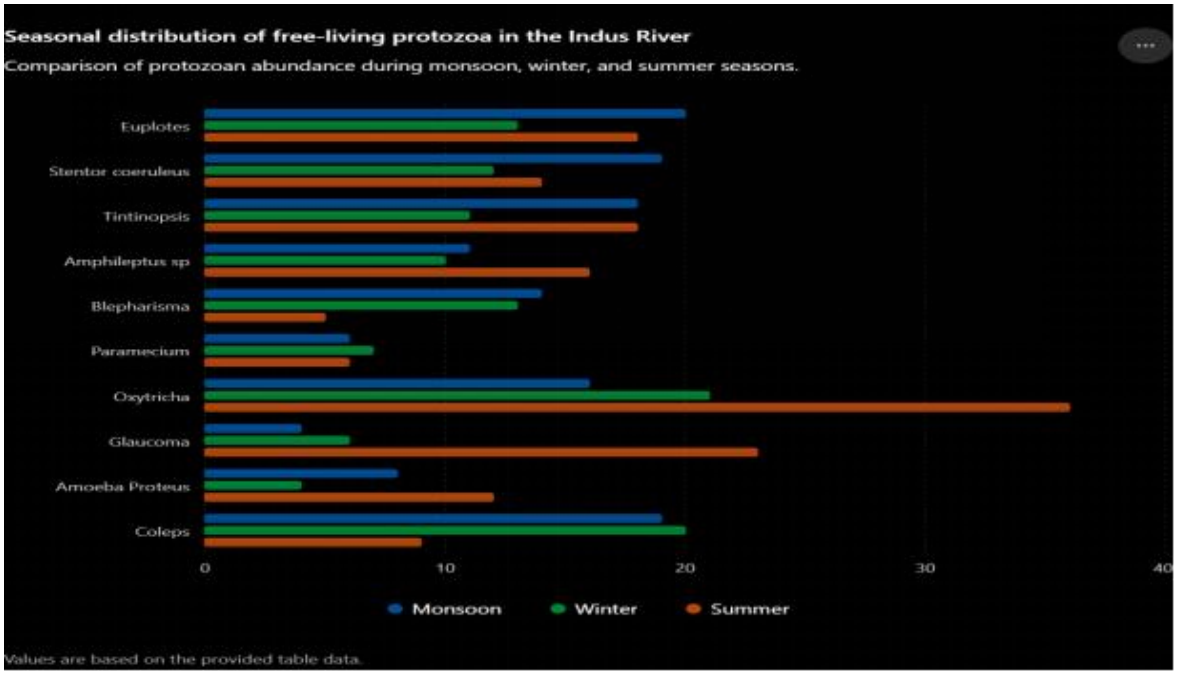
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Blepharisma	14	13	5
Paramecium	6	7	6
Oxytricha	16	21	36
Glaucoma	4	6	23
Amoeba Proteus	8	4	12
Coleps	19	20	9

Fig 2: Seasonal prevalence of protozoa during the year 2025-2026

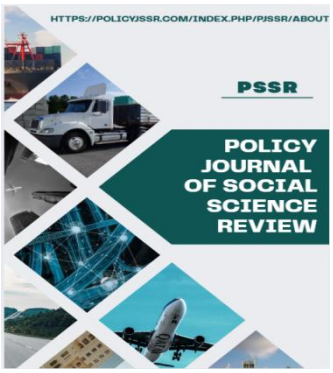


Discussion

In the present investigation, distribution and diversity of free-living protozoa of water of Indus River, Sindh, Pakistan has been studied. Ten protozoan groups were observed, namely, Euplotes, *Stentor coeruleus*, Tintinopsis, *Amphileptus sp.*, Blepharisma, Paramecium, Oxytricha, Glaucoma, *Amoeba proteus* and Coleps. These protozoans show that the Indus River is suitable microhabitats for various groups of free-living protozoans. Free-living protozoans were also reported from the River Indus by Kanwal *et al.* (2025) with

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respect to seasonal variations and physicochemical parameters of river water.

The total abundance of the protozoa was different in the three seasons. Summer had the highest total abundance, monsoon had the next highest and winter had the lowest abundance. From this point of difference between the seasons, one can assume that environmental factors like temperature, dissolved oxygen, free carbon dioxide, nutrients, phosphate, nitrate and biological oxygen demand (BOD) may have an impact on the distribution of protozoa and on their growth. High sensitivity to environmental changes is a consequence of the short life cycle and rapid reproduction of protozoa. Thus, small changes in water quality can have an impact on their abundance and community structure (Madoni, 2005; Xu *et al.*, 2016).

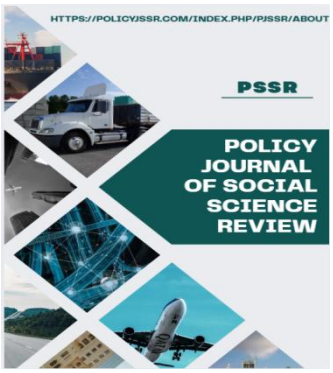
Oxytricha was the most abundant protozoan in all seasons, particularly in the summer. It grew up from values during monsoon and winter to a higher value in summer. This may reflect that summer conditions were favorable for the growth of Oxytricha in terms of temperature, food availability and organic matter. The organisms like ciliates Oxytricha are common in freshwaters and may be sensitive to fluctuations in nutrient levels and bacterial population. Finlay and Esteban (1998) found that the freshwater protozoa are important components of the microbial food webs, which are feeding on bacteria and small organic particles. Thus, the presence of a high abundance of Oxytricha can indicate enhanced supply of microbial food in the warmer months.

There was also a definite rise in glaucoma in the summer season as compared to monsoon and winter. This could be connected with increased biological activity, organic matter decomposition and bacterial prey availability during warm conditions. El-Tohamy *et al.* (2024) demonstrated that there may be strong relationships between protozoan abundance and biomass and water quality parameters (dissolved oxygen, phosphate, ammonia, nitrite, total dissolved salts, and total organic carbon). Thus, the increased numbers of some protozoans in summer of the present study may also be an indication that some changes in nutrient availability and organic load of the river water occurred.

Certain protozoans, such as Euplotes, Tintinopsis, *Stentor coeruleus*, and Coleps, were very abundant in all seasons. The occurrence of Euplotes was relatively stable in monsoon, winter and summer suggesting that it may tolerate a wider range of environmental conditions. Tintinopsis was equally abundant in both monsoon and summer but reduced in winter and *Stentor coeruleus* was more abundant in monsoon as compared to winter and summer. Coleps abundance increased in monsoon and winter and

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decreased in summer. The patterns show that there is variable response to seasonal changes by the various species of protozoans. Cruaud *et al.* (2019) also found that seasonal fluctuations in temperature, nutrients and microbial interactions could create strong seasonal dynamics of the freshwater protist community.

Therefore, the population of Paramecium was found to be low in all seasons, indicating that it was less dominant in the samples under study than the other protozoa. Likewise, *Amoeba proteus* also exhibited moderate abundance, which was higher during the summer than winter. The low or moderate abundance of amoebae and ciliates is possibly explained by competition, food supply and/or the effects of unfavorable physicochemical conditions, as these groups are bacteria, organic matter and microhabitat dependent. These patterns should be used with caution, however, in the absence of detailed statistical correlation between protozoan concentration and water-quality parameters.

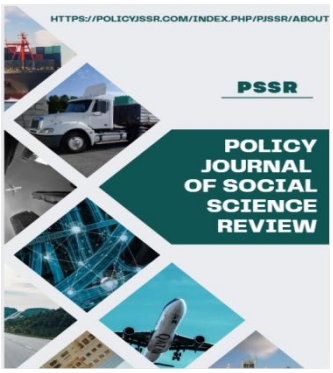
The results of this study reflect the concept of using free-living protozoa as indicators of the quality of flowing waters. Protozoan communities are important because they are sensitive to changes in the environment and are indicative of the biological health of aquatic systems. Based on the ciliate community, Madoni (2005) proposed that the saprobic system of water quality can be evaluated and Xu *et al.* (2016) indicated that functional groups of planktonic ciliates can serve as bio indicators of water quality. In line with that, El-Tohamy *et al.* (2024) reported that the protozoan community in the Nile River was a suitable tool to evaluate water quality. Under such a scenario, information on the distribution and diversity of protozoa in Indus River can be used as a tool for evaluating its ecological status.

Indus River is under various natural and anthropogenic stresses such as seasonal flow fluctuation, agriculture runoff, domestic and industrial effluents. The Indus River and its tributaries of Sindh are the rivers that are affected by the variation in the physicochemical parameter and heavy metals were reported by Ahmad *et al.*, 2021. This could impact aquatic microorganisms, such as protozoa. So the occurrence of protozoa in the present study and seasonal variations may be correlated with the water changes in river system.

The study concludes that the Indus River contains a wide variety of free living protozoa. Environmental conditions may also be important in determining protozoan communities, as the highest numbers of protozoans were found during the summer. The high numbers of Oxytricha and the greater abundance of Glaucoma in the warmer months suggest that some protozoans may be highly sensitive to variations in temperature and

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nutrient supply. The presence of various groups of protozoans in all seasons also indicates that the River Indus offers favorable living conditions for the free-living protozoa. To understand the water quality and environmental changes in the Indus River it is recommended to monitor the protozoan diversity regularly as well as physicochemical parameters.

Conclusion

The present study revealed the presence of various species of free-living protozoans in the water of the River Indus and emphasized the variability of the protozoans with change in environmental condition. Dissolved oxygen, carbon dioxide, nutrient availability, temperature and pH were important physicochemical factors that influenced the prevalence of protozoa. In the protozoans recorded *Amoeba proteus* and Paramecium were generally present in all the samples and their distribution and abundance exhibited marked seasonal differences. The results show the free-living protozoa are important in the aquatic ecosystems and useful biological indicators for assessing the ecological condition and cleanliness of freshwater bodies. It is thus imperative to carry regular surveys of protozoan diversity, besides the routine water quality parameters to understand the environmental changes in the River Indus. These studies can serve as knowledge base for future research, water quality management and conservation planning.

Limitations of the Study

There are certain limitations of the present study. First, the study was done at the selected sampling sites from Indus River, Sindh, Pakistan which may not be representative of the whole river Indus. Second, sampling was carried out during the seasons selected, and as such may not have captured changes in the environment on a short term scale. Third, the morphology and the microscopic appearance were mainly used in identifying protozoa which may limit the correct identification at the species level.

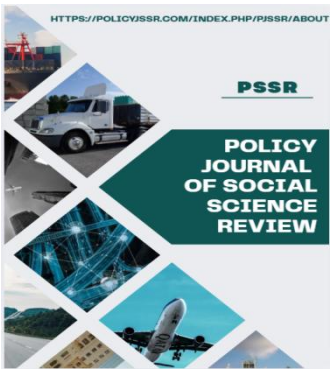
Another factor to be noted is only the selected physico-chemical parameter such as dissolved oxygen, carbon dioxide; nutrients, phosphate, nitrate and biological oxygen demand etc were considered. Other significant things such as heavy metal, pesticides, bacterial load etc. we're not investigated in detail. Additionally, there was no verification of protozoan diversity through the use of advanced molecular methods. These limitations make it relevant to consider the results as baseline information for future detailed studies of free living protozoa of Indus River.

Recommendations

Future studies are suggested to be conducted in different localities of Indus River (Sindh) in order to get an overview of the distribution and diversity of protozoan.

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Sampling should also be done throughout the year to develop a more complete understanding of the seasonal variation and long term ecological changes. In the future, microscopic and molecular methods should be used together in order to determine the correct classification of protozoans. Additional physicochemical and biological parameters like heavy metals, pesticides, turbidity, salinity, organic pollutions and bacterial contaminations should also be measured to understand their impacts on protozoan community. Regular monitoring of free-living protozoa is recommended since they may give good biological indicators of freshwater quality. Water-quality assessment of the Indus River should be on regular basis by the government agencies, environmental departments and research institutions. Ensuring proper management of domestic waste, agricultural runoff, and industrial emissions is also crucial to safeguard aquatic biodiversity and the ecological well-being of the river. The output of this research can be used as baseline data in future research and also can be used as a source of information for the conservation planning and water quality management in Indus River, Sindh, Pakistan.

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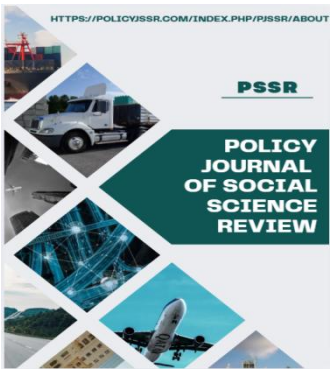
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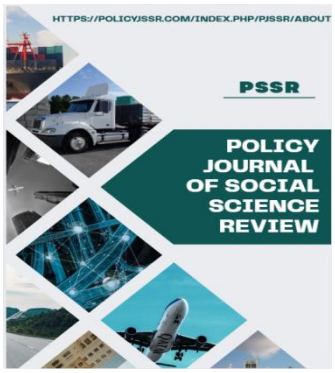
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