Multi-Disciplinary Publishing Institute Pakistan **Vol. 4, N0. 1**

Submitted: 13 MAY 2025 Accepted: 29 JUN 2025 Published: 30 JUN 2025

Original Article

An Empirical Study of the Prevalence of Free-living Protozoans from the River Indus, Sindh, Pakistan

Dr. Asma Kanwal[1], Dr. Raheela Noor Memon[2], Sadaf Fatimah[3], Dr. Sajid Siyal[4]

[1]Lecturer, Department of Zoology. Government Girls Zubaida College, Hyderabad, Sindh, Pakistan.
[2]Assistant Professor, Department of Zoology. Government Girls Zubaida College, Hyderabad, Sindh, Pakistan.
[3]Ph.D. Zoology, University of Sindh, Jamshoro, Pakistan.
[4] Lecturer, Department of Wildlife Management, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan.

Corresponding author: sajid.siyal333@gmail.com

Citation

Kanwal, A., Memon, R.N., Fatimah, S., & Siyal, S. (2025). An empirical study of the prevalence of free-living protozoans from the River Indus, Sindh, Pakistan. Open Access Public Health and Health Administration Review, 4(1), 1-8.

WEBSITE: www.mdpip.com ISSN: Print: 2959-619X
ISSN: Online: 2959-6203
PUBLISHER: MDPIP

Abstract

Protozoa are unicellular eukaryotic organisms that exist in biomes, both land and water. Out of about 200,000 currently known species of protists, over 199,000 (or about 99 %) are autotrophs or heterotrophs that occupy free-living niches, while the rest only 1 % are obligate symbionts that live inside or on a definite host organism. The research aimed to conduct an empirical study of the Prevalence of Free-living Protozoans from the River Indus, Sindh, Pakistan. In the case of a research study on the water quality of rivers in Hyderabad, Sindh, Pakistan, the polypropylene water bottles and plastic zip-lock bags were used to fill the samples of river water in the River Indus. To ensure the understanding of measurements of dissolved oxygen, free CO_2, phosphate, nitrate, and biological oxygen demand, water samples were put through several standard analytical processes. Results indicate that *Amoeba proteus* exists at high levels in every season, particularly in winter and summer. The Paramecium was most abundant during the summer. In contrast, the number of Tintinopsis became moderate all year, but they increased a bit during the monsoon. The winter and summer months saw the lowest numbers for Oxytricha, possibly because it isn't as adaptable to its surroundings. This virus remains stable most of the year, except for a small increase in the summer. These results highlight the usefulness of protozoan surveillance as a bioindicator in measuring the level of water quality, as well as give credence to the notion that there should always be periodic surveillance and analysis in determining policies guiding the preservation efforts.

Keywords: Protozoa, Monogeneans, Trematodes, Fresh-Water, Prevalence of Infection, River Indus.

Introduction

We can find microbes everywhere, from farms to water bodies. Based on its salt content, all aquatic spaces are divided into two forms: freshwater or marine environments. You will find a lot of archaea and bacteria in both of these environments, and they are responsible for biogeochemical processes that affect the entire ecosystem (Pequin, Cai, Lee, & Greer, 2022).

Protozoa are part of Protista, and a subgroup found in the animal kingdom. These microorganisms can be found in many environments, including fresh and salt water, and most often in streams, lakes, rivers, ponds, and other water bodies. The name "Protozoa" means "first or oldest animal or organism." Protozoa are both diverse and ecologically important because they help control the movement of nutrients and support how ecosystems operate. You can often catch them in ponds, lakes, and in the water used at treatment plants. The mode of movement distinguishes several types of protozoan groups: ciliates, flagellates, amoebae, and protozoans (del Carmen Nunez, Gutierrez, & Frau, 2025). Some are big enough to measure only a few micrometers, while others can reach several millimeters in size and show a wide variety of shapes and functions. Many organisms in both the water and on land rely on protozoa for food. Microorganisms also function to transform organic material and put nutrients found in the environment back in the system. Some protozoa infect humans and animals kept for food and wildlife, but these types are few. Many kinds of protozoa can cause illness in the human body (Rai *et al*., 2023).

In the Pakistani context, the most probable sources contributing to waterborne diseases in drinking water are the incorporation of municipal sewage and industrial wastewater at different points of distribution, gaps in the areas of water disinfection, and consistent monitoring of water quality at treatment plants. Pakistan National Conservation Strategy documents that about 40 percent of communicable diseases in Pakistan are water-related. The endemic infections are typhoid, giardiasis, intestinal worms, diarrheal infections, cryptosporidium, and gastroenteritis. The extreme proportion of deaths due to water-related diarrhea among infants is 60%, which is the highest number in Asia, in accordance with the report by the International Union for Conservation of Nature (IUCN). A major portion of the general population of both rural and urban parts of the country in Pakistan is exposed to microbial pollution. Microbial pollution may occur as a result of contamination of drinking-water supply lines, infiltration of sewerage into drinking-water supply, etc (Daud *et al*., 2017).

Human health is compromised by environmental determinants of health, which include pathogens, bacteria, and various dangerous minerals and organic compounds that are contained in unsafe drinking water. A large percentage of the population of emerging countries is faced with negative health consequences due to microbial contamination of drinkable water. In the context of developing countries, child deaths related to contaminated drinking water amount to about 5 million children. This is a similar situation that is compounded by the rate of high population growth, which will overload the current infrastructure and destabilize the control of water quality. It has been empirically shown that in Pakistan alone, 30% of all the morbidities and 40 % of all deaths can be associated with poor quality of water. One of the main diseases transmitted by water, i.e., diarrhea, is the leading cause of infant and pediatric deaths, and one out of five citizens suffers from contamination-related illness annually (Bhutto, Sanjrani, Khaskheli, & Chandio, 2019).

The ciliates are an extensive phylum of unicellular microscopic eukaryotes propelled by hair-like processes- cilia. They have unique features, which are why they are such a dominant, extensive phylum that appears just about everywhere in the water, shows a truncated life cycle with high reproduction rates, and can respond to environmental changes quickly. The ciliates fill most available eukaryotic ecological niches of the biosphere and are found in a variety of microhabitats wherever enough water and compatible nutrients exist. Ciliates exist in polar to equatorial latitudes, hot springs, sea ice, in litter of tropical forests, and in the soil of deserts, too. Evidence supported by empirical research proves that these organisms are part and parcel of various microhabitats. Ciliophora is an old phylum: it dates back tens of millions of years. There are some ecosystems in which ciliates are particularly important due to their position at the top of a food chain (in microbial food chains) and their role (or rather part of their role) as a key part of the microbial loop. They are also highly sensitive biosentinal organisms which allow biomonitoring of the aquatic

systems with an array of anthropogenic disturbances at spatial scales which range between streams, reservoirs, and lakes. Ciliate protists have been revealed to exhibit various ecological functions: they contribute to the synthesis of both the degradation of organic matter and phosphorus- and nitrogen-based recycling, respond to a broad range of concentrations of pollutants, and exhibit high taxonomic variability, allowing them to achieve high population density within a rather short time frame (Di Lorenzo *et al*., 2023). However, their spatial arrangement and geographical distribution are poorly studied in most regions of the Earth, and this poses a shortcoming towards any further research to examine ciliate biogeography. Most of the current literature on Ciliata remains restricted to northern European learning conditions and the adjacent surroundings, which creates an evident vacuum regarding the African and Eurasian continents. In recent taxonomic revisions, more than 1100 genera and about 8000 species are recorded, but the authenticity of these numbers is doubtful. Some researchers argue that the actual biodiversity exceeds the existing evaluation, which is not more than 10 % of the total diversity in the group. To rectify this shortfall, the current study was conducted using fence-collection techniques to describe and explain the total number of ciliate species found in the river Tigris, Baghdad, Iraq, and this brings about a stronger picture of the diversity, besides the ecology of the taxon in this community (Kadhim, 2021).

Literature Review

Most freshwater has less than 1 gram of salt per liter and covers only 2.5% of the total water on Earth. 68.7% of this water stays permanently frozen, and 29.8% is groundwater (Santos, Machovsky-Capuska, & Andrades, 2021). Still, freshwater ecosystems are commonly called the “blood of society” because they have always been essential for life and for the development of civilizations. Many communities globally have built around freshwater ecosystems, and more than half of the world lives not far from a permanent river (Malone & Newton, 2020). Most available and renewable freshwater bodies are frequently coming under extra stress due to activities such as farming, industry, and home use. Consequently, water pollution occurs when many types of anthropogenic and geogenic compounds are released. Therefore, people now see chemical pollution of natural waters as a big public concern, after it has been going on for some time (Patel *et al.*, 2019). A major biological group denoted by large morphological and life-history diversity is protozoan parasites. There is a wide scheme of classification in this category that allows accurate identification on a generic and species level. Under the same scheme, four existing genera, which include Ichthyobodo nectar (Henneguy, 1883), Ichthyophthirius multifiliis (Fouquet, 1876), Trichodina sp. (Ehrenberg 1831), and Trichophyra and Hexamita (Uzmann & Jesse, 1963), have been recognized as leading aquaculture production pathogens. The growing tendency to go into an aquaculture business is usually limited by the high costs of handling compared to the small growth yield levels recorded in farmed fish populations, especially during the outbreak of a disease. The protozoan family is a major group of pathogens of freshwater fish, responsible for morbidity and mortality, especially *ichtyobodiasis*, *coccidiosis*, *ichtyopthiaris*, and *trichodiniasis*. The conditions may be linked with mortalities of close to 100 percent. The recent trends in an increase in fish intake have increased the popularity of such parasitic diseases, which has led to a vast exploration of the causative agents and the follow-up health-management measures as a result (Shafiq *et al*., 2023). The distribution, density, and morphology of ciliate populations inhabiting free-living populations in aquatic environments demonstrate that there are considerable correlations with biotic, physicochemical, and mechanical characteristics of the milieu. There has been a set of studies of free-living ciliate protozoa in freshwater that have been done to document species diversity (Qurratulayen, Ranjit, Yasmeen, & GD, 2025).

The empirical evidence shows that most of the species of aquatic free-living protozoa tend to have a worldwide distribution; the populations can be found uniformly in a definite habitat wherever that habitat occurs throughout the world. As an example, microalgae growing on peat soils and penguin guano in Antarctica are morphologically indistinguishable from microalgae on moorland peat soils and cow parts in Britain. The prevalence of the protozoa has two great consequences. First, it allows creating identification keys of superior generic applicability to all geographic regions. Second, due to the relatively smaller diversity of protozoa when compared to that of, e.g., insects, the total worldwide protozoan species is relatively narrow too. Modern estimates point to the likelihood that at any given moment, a significant proportion of local protozoan species richness is either fairly common or cryptic (i.e., latent), waiting for an opportunity to grow and multiply (Ketelaars, Wagenvoort, Peters, Wunderer, & Hijnen, 2023).

Materials and Methods

The water samples were obtained from the River Indus, Hyderabad, Sindh, in water bottles and zip lock bags each month between 2023 and 2024, as part of this study (Mishra & Kumar, 2021). The water sample was measured using standard processes in measuring different physico-chemical parameters like dissolved oxygen, free CO2, phosphate, nitrate, and biological oxygen demand. In most cases, the collected water contains plants, debris, or leaves submerged since many free-living protozoa are phototrophic and feed on bacteria, algae, small particles, or even other protozoa such as themselves. Samples were taken in the morning because at higher temperatures the number of protozoa diminishes. The samples went to the laboratory, were stained, and then were inspected under the microscope to see their form 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. Since many protozoa can swim fast in water and use locomotory organs, they are hard to identify. When we want to avoid extra movement, we add a small amount of methylcellulose (10%) to the water drop next to the living organisms, slowing them down without causing immediate harm or bursting (Basavraj & Chandrakant, 2015).

Results & Discussion

The physico-chemical conditions and the occurrence of free-living protozoans in the River Indus, water at the localities of Hyderabad, Sindh, are studied. The number of studies on water analysis is displayed in Figure 1. Nothing will be able to survive under the surface of the water without dissolved oxygen. DO levels in May and June of 2023-2024 were lower (3.9 mg/L and 3.4 mg/L), but in subsequent months, October to December, the levels improved. Rising CO2 concentrations in May can be explained by enhanced biological processes, resulting in more CO2. The highest phosphate recorded was in August at 2.0 mg/L, but in March, the lowest reading was 0.4 mg/L. The Biological Oxygen Demand was studied marked between 2.1 and 2.9 mg/L. The results of the BOD are in an acceptable range, in most cases for moderate water quality. The study had a range of nitrates of 7.5 mg/L to 8.5 mg/L. It is a typical and steady magnitude of the freshwater systems (Agarwal, Rashmi Pathrikar, Mohd Mohsin, & Vidya Pradhan, 2014). For every month during the 2023-2024 period, an investigation into protozoans was carried out. The study area has been visited by 10 various marine species, reflected in Table 1 and Figure 2. *Amoeba proteus* could be found at high levels in every season, but particularly in the winter and summer. But Paramecium was most abundant during the summer, putting it among the many species in Lake George. There was stable growth when Stentor coeruleus was present, and the greatest counts were seen in the summer. In contrast, the number of Tintinopsis became moderate all year, but they increased a bit during the monsoon. The winter and summer months saw the lowest numbers for Oxytricha, possibly because it isn't as adaptable to its surroundings. This virus remains stable most of the year, except for a small increase in the summer.

Figure 1

Physico-Chemical Analysis of Water 2023-2024

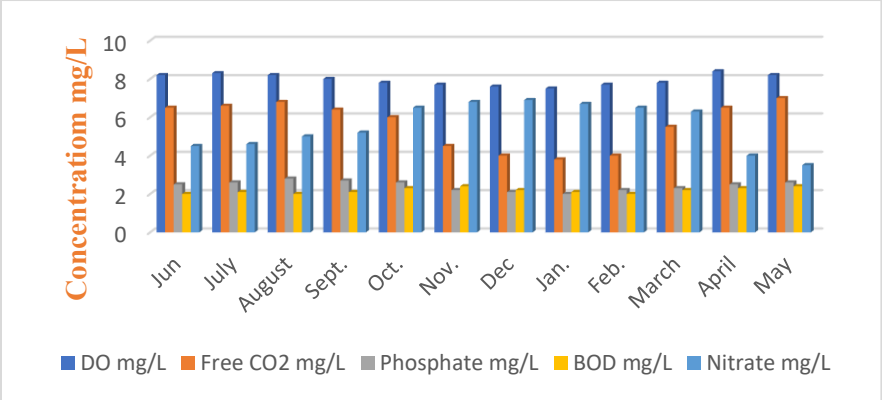
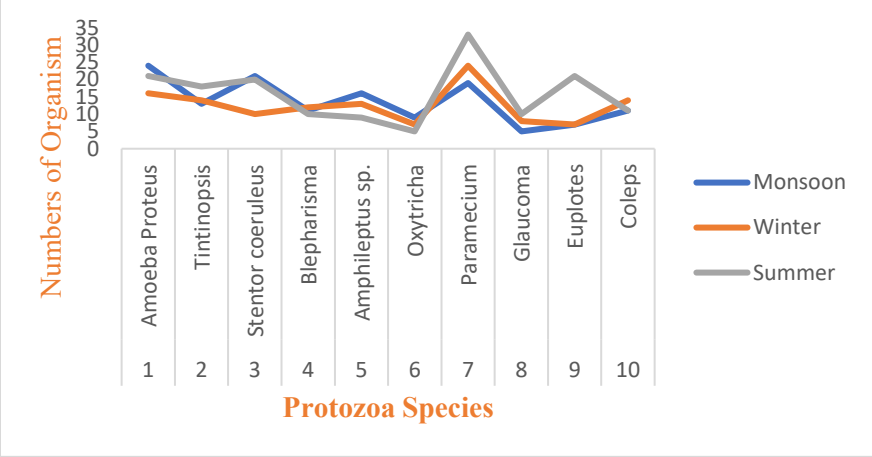


Table 1
Physico-Chemical Analysis of Water

Sr. No.	Protozoa	Monsoon	Winter	Summer
1	Amoeba Proteus	24	16	21
2	Tintinopsis	13	14	18
3	Stentor coeruleus	21	10	20
4	Blepharisma	11	12	10
5	Amphileptus sp.	16	13	9
6	Oxytricha	9	7	5
7	Paramecium	19	24	33
8	Glaucoma	5	8	10
9	Euplotes	7	7	21
10	Coleus	11	14	11

Figure 2
Seasonal Prevalence of Protozoa During the Year 2023-2024



Conclusion

The research aimed to conduct an empirical study of the Prevalence of Free-living Protozoans from the River Indus, Sindh, Pakistan. In the case of a research study on the water quality of rivers in Hyderabad, Sindh, Pakistan, the polypropylene water bottles and plastic zip-lock bags were used to fill the samples of river water in the River Indus. To ensure the understanding of measurements of dissolved oxygen, free CO_2, phosphate, nitrate, and biological oxygen demand, water samples were put through several standard analytical processes. Results indicate that *Amoeba proteus* exists at high levels in every season, particularly in winter and summer. The Paramecium was most abundant during the summer. In contrast, the number of Tintinopsis became moderate all year, but they increased a bit during the monsoon. The winter and summer months saw the lowest numbers for Oxytricha, possibly because it isn't as adaptable to its surroundings. This virus remains stable most of the year, except for a small increase in the summer. These results highlight the usefulness of protozoan surveillance as a bioindicator in measuring the level of water quality, as well as give credence to the notion that there should always be periodic surveillance and analysis in determining policies guiding the preservation efforts. This work identifies the various protozoa found in the River Indus and shows how their prevalence fluctuates with the changing levels of dissolved oxygen, CO2, and nutrients in the water. Most of the

samples included *Amoeba proteus* and Paramecium, which followed their seasonal trends. The research shows the importance of protozoa in assessing how clean local water bodies are and reminds us that we need to regularly check them to guide conservation plans.

Limitations and Future Directions of the Study

The research sample was taken in some of the sampling locations along the River Indus in Sindh Province, although that is not what represents the full ecological variation of the river system-nor does it extend to the rest of Pakistan. The current study aimed at determining protozoan prevalence only and did not perform a thorough examination of the physicochemical parameters, i.e., nutrient concentration, heavy metals, and pH, that were likely to affect the distribution of protozoa. There has never been a proper representation of the free-living protozoans in river ecosystems, and this is largely due to the insufficiency of sampling intensity and frequency to note all the diversity or population behavior of the microorganisms. The study was conducted on the water of the river Indus/Sindh, to get a better understanding; in the future, researchers could conduct the study on the Lakes as well as other small rivers in the country.

Declarations

Ethical Approval and Consent to Participate: This study strictly adhered to the Declaration of Helsinki and relevant national and institutional ethical guidelines. Informed consent was not required, as secondary data available on websites was obtained for analysis. All procedures performed in this study were by the ethical standards of the Helsinki Declaration.

Consent for Publication: Not Applicable

Availability of Data and Materials: Data for this study will be made available upon request from the corresponding author.

Competing Interest: The authors declare no competing interest.

Funding: Not Applicable

Authors' Contribution: All authors have equal contributions.

Acknowledgement: We authors are highly thankful to colleagues and fellows for their cooperation in conducting this study.

References

Agarwal, B., Rashmi Pathrikar, R. P., Mohd Mohsin, M. M., & Vidya Pradhan, V. P. (2014). A study of physico-chemical parameters of ground water of Aland Taluka Phulmari of Aurangabad district, Maharashtra. *Advances in Applied Scientific Research*, available at: https://www.primescholars.com/abstract/a-study-of-physicochemical-parameters-of-ground-water-of-aland-talukaphulmari-of-aurangabad-district-maharashtra-89242.html

Basavraj, d. D. L., & chandrakant, d. M. L. (2015). Study of zoonotic waterborne protozoan parasites from freshwater bodies in Solapur, Maharashtra State, India. *Water Health*. 5(1):1-38.

Bhutto, S. U. A., Sanjrani, M., Khaskheli, N., & Chandio, S. (2019). Microbial Water Pollution in Pakistan. *North American Academic Research,* 2(4), 185-192.

Daud, M., Nafees, M., Ali, S., Rizwan, M., Bajwa, R. A., Shakoor, M. B., . . . Murad, W. (2017). Drinking water quality status and contamination in Pakistan. *BioMed Research International, 2017*(1), 7908183.

del Carmen Nunez, M., Gutierrez, M. F., & Frau, D. (2025). Insights into heterotrophic protozoa assemblages' spatiotemporal distribution in urban-agricultural landscape streams. *Aquatic Ecology, 59*(1), 169-183.

Di Lorenzo, T., Cabigliera, S. B., Martellini, T., Laurati, M., Chelazzi, D., Galassi, D. M. P., & Cincinelli, A. (2023). Ingestion of microplastics and textile cellulose particles by some meiofaunal taxa of an urban stream. *Chemosphere, 310*, 136830.

Kadhim, Z. Y. (2021). *New record of freshwater ciliates (Protozoa, Ciliophora) from the Tigris River in Baghdad city, Iraq.* Paper presented at the Journal of Physics: Conference Series.

Ketelaars, H. A., Wagenvoort, A. J., Peters, M. C., Wunderer, J., & Hijnen, W. A. (2023). Taxonomic diversity and biomass of the invertebrate fauna of nine drinking water treatment plants and their non-chlorinated distribution systems. *Water Research, 242*, 120269.

Malone, T. C., & Newton, A. (2020). The globalization of cultural eutrophication in the coastal ocean: causes and consequences. *Frontiers in Marine Science, 7*, 670.

Mishra, S., & Kumar, A. (2021). Estimation of physicochemical characteristics and associated metal contamination risk in the Narmada River, India. *Environmental Engineering Research, 26*(1).

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman Jr, C. U., & Mohan, D. (2019). Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. *Chemical reviews, 119*(6), 3510-3673.

Péquin, B., Cai, Q., Lee, K., & Greer, C. W. (2022). Natural attenuation of oil in marine environments: A review. *Marine Pollution Bulletin, 176*, 113464.

Qurratulayen, S., Ranjit, R., Yasmeen, S., & GD, S. (2025). Studies on Physicochemical Parameters of Kalvati Lake in Ambajogai. *Sciences (IJRSEAS), 2*(2), 1-4.

Rai, A. R., Sharma, V., Jain, D., Kaur, A., Nagar, V., Pandit, P. P., . . . Sankhla, M. S. (2023). Qualitative and quantitative analysis of freshwater protozoa from the Godavari River. *Materials Today: Proceedings, 95*, 3-10.

Santos, R. G., Machovsky-Capuska, G. E., & Andrades, R. (2021). Plastic ingestion as an evolutionary trap: Toward a holistic understanding. *Science, 373*(6550), 56-60.

Shafiq, A., Abbas, F., Hafeez-ur-Rehman, M., Khan, B. N., Aihetasham, A., Amin, I., . . . Khalil, A. A. K. (2023). Parasite diversity in a freshwater ecosystem. *Microorganisms, 11*(8), 1940.

Note: **Open Access Public Health and Health Administration Review** is recognized by the Higher Education Commission of Pakistan in the Y category.

Disclaimer/ Publisher's Note: The statements, opinions, and data contained in all publications in this journal are solely those of the individual author(s) and not of the MDPIP and/ or the editor(s). MDPIP and editor(s) disclaim responsibility for any injury to the people or property resulting from any ideas, methods, instructions, or products referred to *in the content.*