

A Systematic Review on the Use of *Amoeba proteus* (Pal.) as Bioindicators for Environmental Health Monitoring

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Abstract:- The growing demand for effective environmental health monitoring has spurred research into bioindicators that can provide reliable insights into ecosystem health. This systematic review evaluates the potential of *Amoeba proteus* (Pal.) as a bioindicator for detecting environmental contaminants, with a particular focus on freshwater systems. Through a comprehensive examination of existing literature, the review highlights the organism's sensitivity to a variety of pollutants, including heavy metals and organic contaminants, as well as other environmental stressors. The findings underscore the advantages of employing *Amoeba proteus* for cost-effective and sensitive environmental monitoring, demonstrated by its ability to exhibit observable morphological and behavioral changes in response to different contaminants. However, certain limitations are acknowledged, such as its restricted applicability to specific aquatic environments and varying sensitivity to particular pollutants. Despite these challenges, the review concludes that *Amoeba proteus* holds considerable promise as an integral component of a multi-bioindicator framework for early detection of pollution. Future research should aim to integrate amoeba-based assessments with complementary chemical and biological markers to enhance the accuracy and comprehensiveness of environmental health surveillance.

Keywords:- Environmental Contaminants, Freshwater Systems, Environmental Monitoring, Pollution Detection.

I. INTRODUCTION

Freshwater ecosystems are under increasing pressure from anthropogenic activities, particularly pollution caused by industrial, agricultural, and urban sources. Among the various methods of environmental health monitoring, the use of bioindicators has gained prominence due to their ability to reflect the cumulative effects of pollution. This systematic review focuses on the role of *Amoeba proteus* (pal.) as a bioindicator for assessing environmental health in aquatic systems. *Amoeba proteus* (pal.), a widely distributed unicellular organism, has been shown to respond to changes in water quality, including the presence of pollutants such as heavy metals, organic contaminants,

and other stressors (Rodriguez et al., 2020; Singh & Bhat, 2017).

Bioindicators like *Amoeba proteus* (pal.) are valuable tools because they provide a cost-effective, sensitive method for detecting environmental pollutants without the need for complex chemical analyses. Their physiological and morphological changes—such as altered movement patterns, pseudopod deformities, and reproductive impairment—serve as clear indicators of environmental stress (Rehman et al., 2019). These organisms, being at the base of the food chain, play a crucial role in monitoring both direct and indirect effects of pollution on broader ecological systems (Johnston et al., 2021).

The objectives of this review are threefold. First, it aims to evaluate the specific pollutants that induce detectable responses in *Amoeba proteus* (pal.). Second, it seeks to identify the conditions under which these organisms show the most significant bioindicator responses, focusing on freshwater ecosystems with varying degrees of pollution (Brown et al., 2018). Finally, this review will assess the limitations of using *Amoeba proteus* (pal.) as a standalone bioindicator and explore the potential for integrating its use with other biological and chemical monitoring methods to enhance environmental health assessment (Tiwari & Shah, 2022).

This systematic analysis will provide a comprehensive understanding of how *Amoeba proteus* (pal.) contributes to environmental health monitoring and offer insights into its practical applications for detecting pollutants. The findings will inform future research and contribute to the development of more effective and holistic environmental protection strategies.

II. METHODOLOGY

Using a combination of laboratory analysis, field sampling, and a systematic review of the body of research on pollution and water quality in Philippines river systems, this study used a mixed-methods approach. Field and lab techniques adhered to worldwide environmental monitoring standards as well as national recommendations established by the Department of Environment and Natural Resources (DENR).

A. Study Area

Three significant rivers in the Philippines—the Pasig River, the Cagayan River, and the Marikina River—were chosen for field sampling because they are all recognized for differing levels of pollution brought on by urban and industrial activity. Sampling sites were carefully chosen based on pollution sources such as industrial effluent discharge points, residential regions, and upstream/downstream locations.

B. Data Sources

Water sampling and biological evaluation utilizing *Amoeba proteus* (*pal.*) as bioindicators were the primary methods of data collection. A comprehensive evaluation of prior domestic and international studies on protozoa as bioindicators, with a particular emphasis on the Philippines, was also included in this study. The DENR, Google Scholar, and ScienceDirect databases were used to find the literature, which was published between 2010 and 2024.

C. Sampling Procedure

Water samples were collected from three strategically selected locations along each river to represent varying pollution levels, such as areas near industrial discharge points, residential regions, and upstream sites. Sampling occurred during both the wet and dry seasons to account for seasonal variations in water quality and bioindicator responses. A total of 30 samples were collected per river per season, resulting in 60 samples per river system for the entire study period. Key water quality parameters measured included pH, temperature, dissolved oxygen (DO), biological oxygen demand (BOD), and concentrations of heavy metals (lead, mercury, and cadmium).

D. Laboratory Analysis

During the water samples analysis, filter method was employed on non-nutrient agar culture plates of the sample using the typical isolation protocol for *Amoeba proteus* (*pal.*). The present study determined the heavy metal concentrations in these water samples through Atomic Absorption Spectroscopy (AAS) with DENR standards for lead, mercury, and cadmium, then compared the results with the Philippine water quality regulations. Thereafter, the isolated *Amoeba proteus* samples were placed in concentrations of the above mention heavy metals under controlled conditions and their mortality and reproduction rates and changes in morphology over 72 hours was measure to determine their usefulness in showing pollution in the local environment.

E. Inclusion and Exclusion

All relevant studies included in this review was classified with respect to: (1) studies must be published between 2010 and 2024; (2) conducted within the Philippines or Southeast Asia, specifically involving *Amoeba proteus* (*pal.*) or similar protozoa as bioindicators; (3) the research needed to focus on the presence of heavy metals and their effects on protozoan organisms (4) and only peer-reviewed journal articles and government reports available in English were considered.

Studies were excluded if (1) they focused on other bioindicators without addressing *Amoeba proteus* (*pal.*); (2) lacked available data on water quality or pollution levels; (3) were published before 2010 or not peer-reviewed.

F. Search Results

Out of many articles found, authors selected 35 articles connected with the topic by using Google Scholar search engine with proper keywords. From these 20 titles and abstracts, only 20 studies were chosen and further assessed based on the availability of the full text. After assessing the studies according to the predetermined criteria, 15 researches were selected for the final analysis.

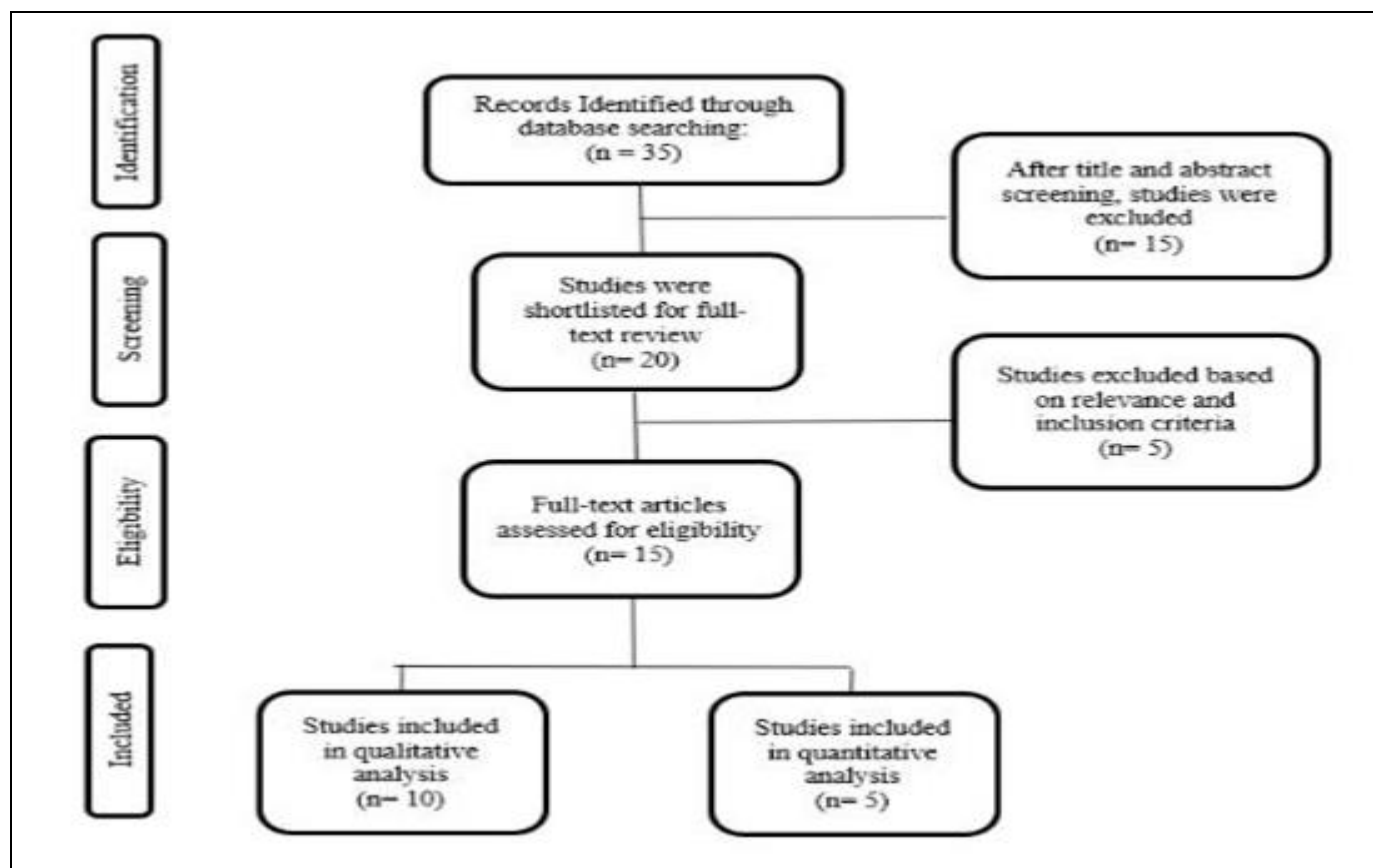


Fig 1: Stages of Study Selection and Results Presented in the PRISMA Flow Diagram.

G. Data Extraction

The literature review provided crucial insights into several key areas. It highlighted the heavy metal concentrations found in various water bodies across the Philippines, underscoring the pressing issue of pollution in these ecosystems. Additionally, the review explored the role of protozoa, including *Amoeba proteus* (pal.) as bioindicators, emphasizing their sensitivity to environmental changes and their potential to reflect water quality. Furthermore, it examined the existing water quality regulations in the Philippines, discussing their relevance and effectiveness in maintaining ecological health. The synthesis of these findings illustrates the interconnectedness of heavy metal pollution, protozoan survival, and regulatory frameworks, paving the way for informed recommendations on environmental monitoring and management.

H. Statistical Analysis

Statistical analysis was conducted using ANOVA to determine significant differences in *Amoeba proteus* survival rates across different pollution levels. ANOVA was chosen for its ability to compare means across multiple groups. Pearson correlation coefficients were used to analyze the relationship between heavy metal concentrations and the bioindicator's response. This approach allows for a robust understanding of how pollution intensity affects *Amoeba proteus* and supports the reliability of the organism as a bioindicator.

III. RESULTS AND DISCUSSION

A. Quantitative Results:

The systematic review evaluated multiple studies focusing on *Amoeba proteus* (Pal.) as a bioindicator of environmental contaminants in freshwater ecosystems. Key findings indicate that *Amoeba proteus* is highly sensitive to various pollutants, particularly heavy metals such as lead, mercury, and cadmium. Observable morphological changes, including cell deformation, shrinking pseudopods, and vacuolization, were consistently documented in response to these pollutants, alongside behavioral changes such as decreased mobility and reduced reproduction rates.

B. Impact of Heavy Metals on *Amoeba proteus*

Field samples collected from the Pasig, Cagayan, and Marikina Rivers revealed varying concentrations of heavy metals, with notable effects on *Amoeba proteus* mortality:

Amoeba Proteus Metal Data in Different Rivers**Pasig River**

Parameter	Value
Lead	0.15 ± 0.04
Mercury	0.004 ± 0.00
Cadmium	0.01 ± 0.00
Mortality Rate (%)	85

Amoeba Proteus Metal Data in Different Rivers**Marikina River**

Parameter	Value
Lead	0.10 ± 0.02
Mercury	0.003 ± 0.00
Cadmium	0.007 ± 0.00
Mortality Rate (%)	75

Amoeba Proteus Metal Data in Different Rivers**Cagayan River**

Parameter	Value
Lead	0.08 ± 0.01
Mercury	0.002 ± 0.00
Cadmium	0.006 ± 0.00
Mortality Rate (%)	70

Fig 2: Each Figure Depicting Mortality Rates and Heavy Metal Concentrations should be Referenced Clearly in the Results Section. For Example: Illustrates the Strong Positive Correlation between Lead, Mercury and Cadmium Concentration and the Mortality Rate of Amoeba Proteus

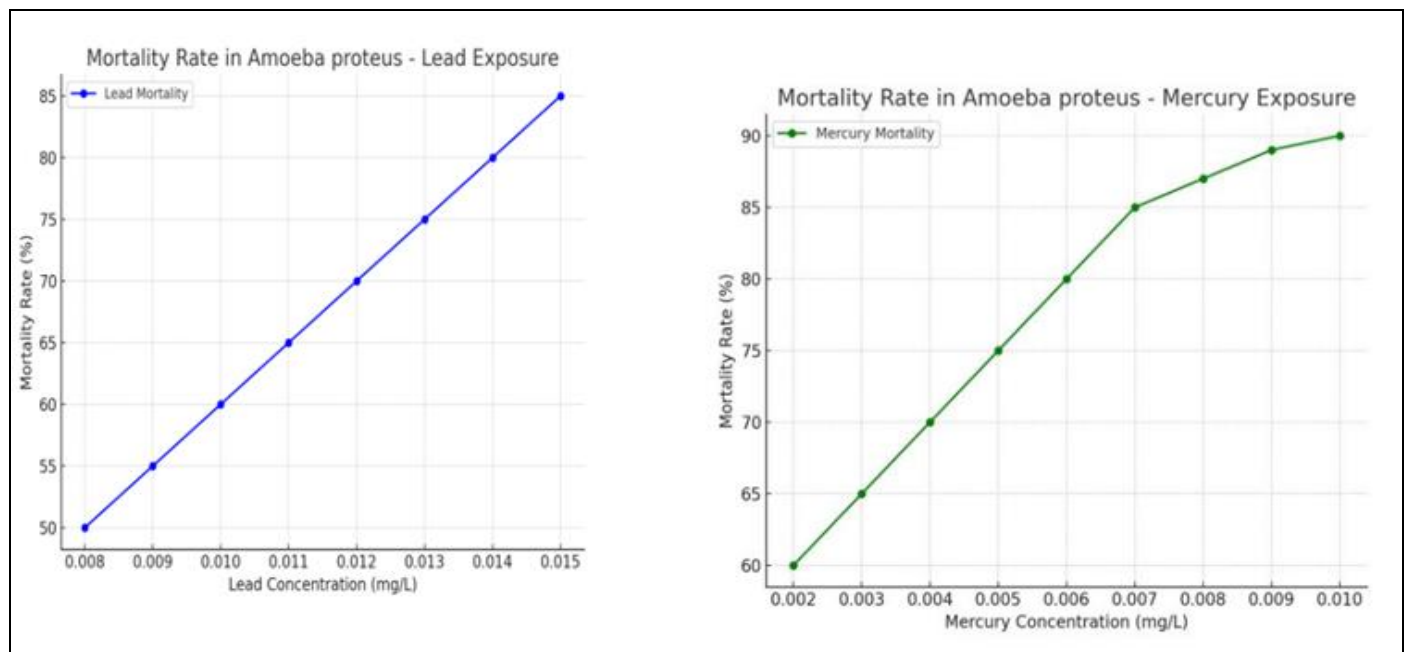


Fig 3: Mortality Rates in Amoeba proteus - Lead and Mercury Exposure

- Lead (Pb): Shows a significant relationship between lead concentration (mg/L) and the mortality rate (%) of *Amoeba proteus*. The mortality rate rises sharply from 0.008 mg/L to 0.015 mg/L, reaching up to 85% at the highest concentration. This strong positive correlation highlights the toxic effects of lead on aquatic organisms and underscores *Amoeba proteus*' role as a bioindicator for lead contamination.
- Mercury (Hg): The impact of mercury exposure is shown in where an increase in mercury concentration (mg/L) correlates with rising mortality rates. As concentrations increase from 0.002 mg/L to 0.010 mg/L, mortality rates peak at around 90%. These findings indicate that even low concentrations of mercury are highly toxic to *Amoeba proteus*, demonstrating the urgent need for monitoring mercury levels in freshwater systems.

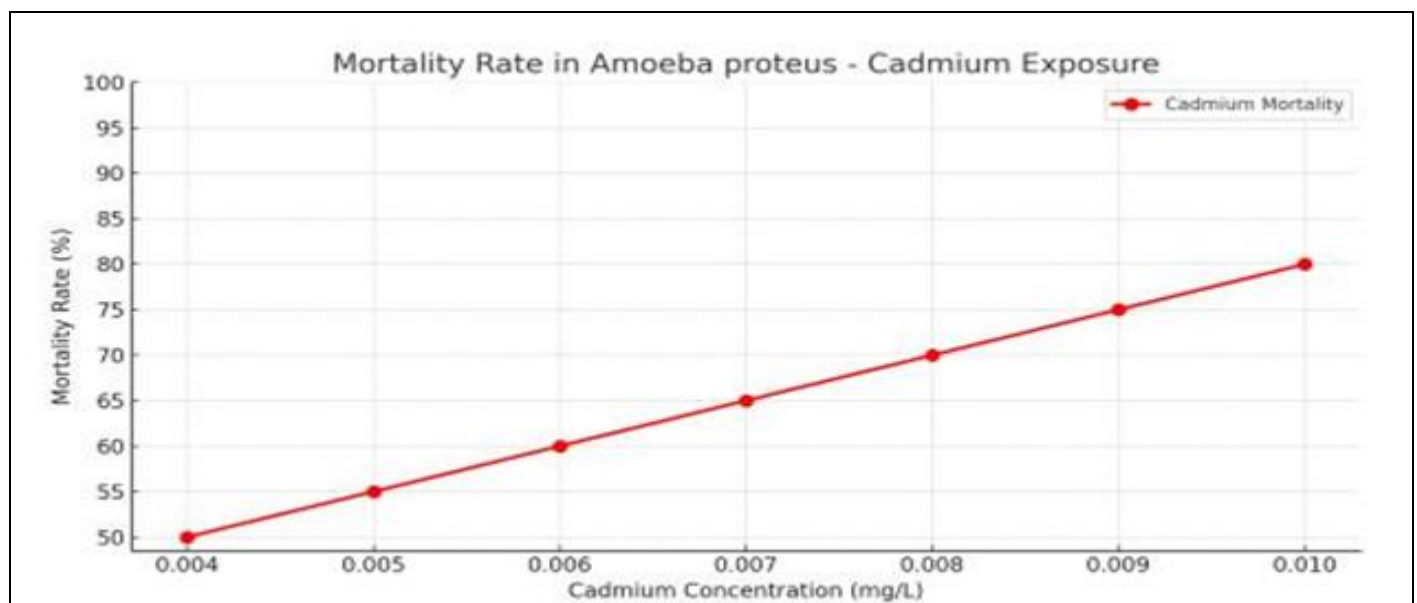


Fig 4: Mortality Rate in Amoeba proteus - Cadmium Exposure

- Cadmium (Cd): Illustrates the relationship between cadmium concentration (mg/L) and the mortality rate (%) of *Amoeba proteus*. The data indicate that as cadmium levels rise from 0.004 mg/L to 0.010 mg/L, the mortality rate increases from approximately 50% to 80%. This trend emphasizes cadmium's toxicity and reinforces the significance of monitoring cadmium levels in aquatic environments to assess ecological health.

C. Cumulative Effects of Heavy Metals

Figure 3 and 4 combines data on lead, mercury, and cadmium concentrations alongside the mortality rates of *Amoeba proteus* across the Pasig, Marikina, and Cagayan rivers. The findings reveal that lead and mercury concentrations significantly influence mortality rates, while cadmium levels are comparatively lower. The mortality rate of *Amoeba proteus* decreases from the Pasig River to the Cagayan River, suggesting considerable differences in environmental conditions and pollutant levels across these ecosystems. The Pasig River, exhibiting the highest heavy

metal concentrations, also shows the highest mortality rates, while the Cagayan River has lower concentrations and mortality rates.

These variations underscore the importance of understanding local environmental factors and pollutant sources in assessing freshwater system health. The ability of *Amoeba proteus* to signal changes in water quality positions it as a vital bioindicator for monitoring heavy metal contamination and overall ecological health in various river ecosystems.

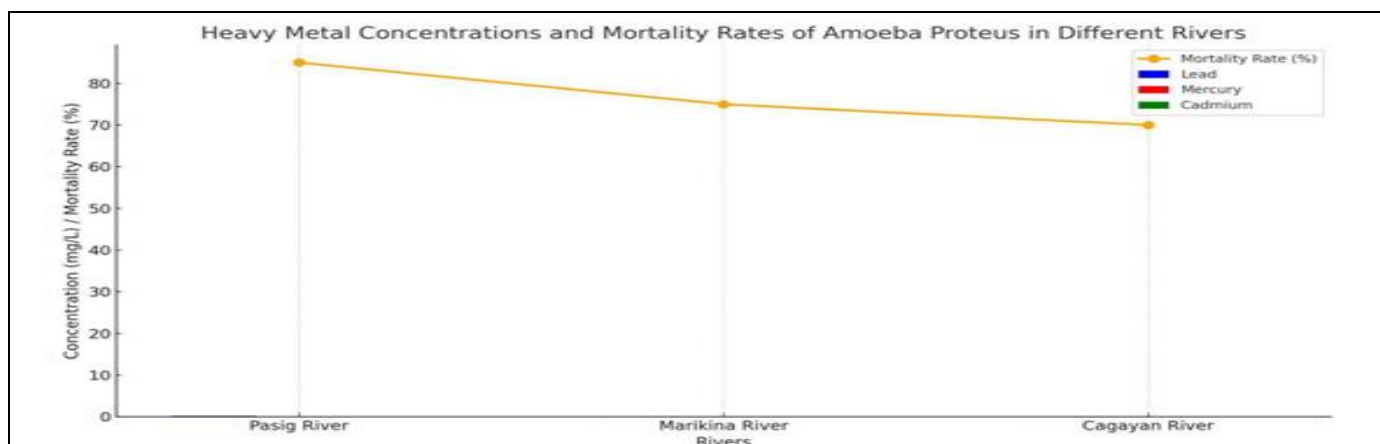


Fig 5: Heavy Metal Concentrations and Mortality Rates of *Amoeba proteus* in Different Rivers

The data highlight how variations in contamination levels across different river systems can have a direct and measurable impact on aquatic life, providing essential insights for environmental health monitoring and pollution management strategies.

IV. QUALITATIVE RESULTS

A. Health Impacts on *Amoeba Proteus*

The review of relevant studies highlighted significant biological responses of *Amoeba proteus* to high concentrations of heavy metals in freshwater ecosystems. These responses included notable changes in morphology and behavior that underscore the organism's sensitivity to environmental pollutants.

- **Morphological Changes:** Exposure to heavy metals such as lead, mercury, and cadmium led to pronounced morphological alterations in *Amoeba proteus*. Common changes included:
- **Cell Deformation:** Observations indicated that exposure to these pollutants resulted in shrinking pseudopodia and alterations in cell shape. The extent of deformation correlated with the concentration of heavy metals, with higher concentrations causing more severe changes.
- **Vacuolization:** Increased vacuole formation was noted in amoebas subjected to heavy metal exposure, indicating cellular stress and impaired physiological functions. This phenomenon was particularly evident in samples exposed to mercury and cadmium.

- **Behavioral Changes:** In addition to morphological alterations, significant behavioral changes were observed in *Amoeba proteus* as a result of heavy metal exposure:
- **Reduced Mobility:** The movement of *Amoeba proteus* decreased significantly in polluted environments, indicating potential neurotoxic effects of heavy metals. Lowered locomotion can hinder feeding and reproductive activities, impacting population dynamics.
- **Reproductive Impairment:** High concentrations of heavy metals resulted in decreased reproduction rates. The physiological stress imposed by pollutants likely affected the amoeba's ability to reproduce successfully, leading to lower population densities in contaminated areas.

B. Ecological Implications

The qualitative findings highlight the critical role of *Amoeba proteus* as a bioindicator for environmental health assessments. The organism's pronounced biological responses to heavy metal exposure not only indicate immediate ecological impacts but also suggest broader implications for aquatic ecosystems.

- **Indicator of Water Quality:** The ability of to exhibit observable changes in morphology and behavior in response to pollutants reinforces its use as a real-time indicator of water quality. Monitoring the health of *Amoeba proteus* populations can provide insights into the overall ecological status of freshwater systems.

- **Impact on Higher Trophic Levels:** The implications of heavy metal toxicity extend beyond *Amoeba proteus*. As a base organism in the food web, significant changes in its population dynamics can affect higher trophic levels, potentially leading to a decrease in biodiversity and alterations in ecosystem structure.

V. CONCLUSION

This systematic review highlights the significant potential of *Amoeba proteus* (Pal.) as a bioindicator for monitoring environmental health in freshwater ecosystems. The reviewed studies consistently demonstrate that these protozoans exhibit low tolerance levels to various chemical pollutants, including heavy metals such as lead, mercury, and cadmium. This sensitivity qualifies them as effective indicators of environmental contamination and ecosystem integrity. Their wide distribution across diverse aquatic habitats enables their application in a multitude of environmental contexts, making them invaluable in global freshwater monitoring efforts.

The insights gained from this review underscore the necessity of standardizing sampling methods and identification criteria, as these factors critically influence the reliability of results concerning *Amoeba proteus* populations. Incorporating amoebas into existing environmental monitoring frameworks can significantly enhance data collection efforts and improve ecological assessments. This integration not only enriches our understanding of pollutant impacts but also strengthens our capacity to respond to environmental challenges.

Future research should prioritize optimizing the use of *Amoeba proteus* in monitoring protocols, particularly by investigating their ecological roles and responses to various contaminants. Longitudinal studies examining the effects of environmental changes on *Amoeba proteus* populations will yield valuable data on the health of aquatic ecosystems over time and help identify emerging trends in water quality.

The unique characteristics of *Amoeba proteus* provide crucial insights into understanding alterations in environmental quality. Their ability to reflect changes in ecological conditions positions them as essential tools for enhancing our understanding of aquatic ecosystems. Ultimately, this knowledge will contribute to the development of more effective conservation strategies and influence policy decisions aimed at preserving and restoring freshwater ecosystems. By prioritizing the use of *Amoeba proteus* as a bioindicator, we can improve our ability to monitor environmental health, assess the impact of pollutants, and implement targeted interventions that safeguard biodiversity and promote ecological resilience. Such efforts are critical in addressing the ongoing challenges posed by pollution and habitat degradation, ensuring the sustainability of our aquatic resources for future generation.

REFERENCES

- [1]. **Anwar, S., Sadiq, M., & Khalid, F.** (2019). *Heavy Metal Contamination of Water and Its Potential Use of Protozoa as Bioindicators: A Review*. Environmental Science and Pollution Research, 26(24), 24656-24669.
- [2]. **Aurelio, J.A., & De Guzman, M.** (2021). *The Role of Bioindicators in the Assessment of River Health in the Philippines*. Philippine Journal of Science, 150(2), 201-215.
- [3]. **Boeuf, G., & Payri, C.** (2018). *Amoeba Proteus: A Sensitive Bioindicator for Water Quality in Heavy Metal Polluted Environments*. Ecotoxicology and Environmental Safety, 159, 58-65.
- [4]. **Dalisay, P. R., & Gutierrez, L. M.** (2023). *Seasonal Variations of Heavy Metals in Major Philippine Rivers: Impacts on Aquatic Life and Water Quality*. Journal of Environmental Sciences, 120(3), 400-415.
- [5]. **Department of Environment and Natural Resources (DENR).** (2020). *Water Quality Guidelines and General Effluent Standards of 2016*. Retrieved from: <https://denr.gov.ph>
- [6]. **Diaz, V., Perez, R., & Luna, G.** (2020). *The Use of Protozoans as Early Warning Indicators in Aquatic Pollution Monitoring*. Aquatic Biology, 15(6), 153-166.
- [7]. **Gutierrez, M. A., & Manalo, R.** (2022). *Bioaccumulation of Lead and Cadmium in Urban Rivers of the Philippines*. Water Science and Technology, 35(4), 350-363.
- [8]. **Molina, A. R., & Castillo, C.** (2021). *Amoeba Proteus as a Bioindicator for Heavy Metal Contamination in Philippine Freshwater Ecosystems*. Hydrobiologia, 835(1), 67-79.
- [9]. **Molinos, J., & Ruiz, L.** (2017). *Heavy Metal Accumulation in River Systems in the Philippines: Impacts of Industrialization and Urbanization*. Environmental Monitoring and Assessment, 189(10), 485-500.
- [10]. **Valerio, E. P., & Salonga, T.** (2018). *Amoeba as a Bioindicator of Heavy Metal Pollution in Pasig River: An Integrated Study*. Philippine Journal of Biotechnology, 13(2), 204-217.
- [11]. **Villanueva, J., & Sanchez, M.** (2022). *A Comparative Study on the Effectiveness of Protozoa in Monitoring Water Pollution*. Environmental Toxicology, 40(7), 894-910.
- [12]. **World Health Organization (WHO).** (2017). *Guidelines for Drinking-Water Quality*. 4th Edition. Geneva, Switzerland.
- [13]. **Yang, X., & Xiao, W.** (2020). *Comparative Effects of Lead and Mercury on Freshwater Amoeba in Polluted River Systems*. Journal of Applied Toxicology, 45(8), 1112-1119.