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Mini Review: Protozoa as Bioindicator for the Water Quality Assessment

Osama M. S. Mostafa*, Nourhan A. A. Abd El-Hady, Ahmed M. H. Nigm

Zoology Department, Faculty of Science, Ain Shams University, Cairo, Abbassia 11566, Egypt.

***Corresponding author:** osamamostafa@hotmail.com, osamamostafa@sci.asu.edu.eg

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ABSTRACT

Protozoans are unicellular, eukaryotic organisms that have typical internal structures like animal cells and perform all animal-like functions. They are essential components of aquatic ecosystems and soil biodiversity, contributing significantly to energy transfer to higher trophic levels and organic matter decomposition. They are abundantly present in a variety of environmental conditions and widely distributed, as well as being relatively sensitive to different contaminants. Protozoa are promising candidates for bioindication of water quality and soil health. The purpose of the present review was to highlight the importance of protozoa as bioindicators for water quality monitoring. The basic aim of bioindication is the use of biota as indicators of environmental conditions. Three broad categories of bioindicators can be recognized based on their aims and objectives: environmental, ecological, and biodiversity indicators. There are seven key advantages of Protista bioindicators, namely: environmental sensitivity, functional importance, distribution, size and numbers, response times, ease of analysis, and preservation potential. Concerning water pollution, protozoa seem to be an excellent tool to assess both toxicity and pollution of effluent water of treatment plants, and water quality in both freshwater and marine ecosystems.

Introduction

Contamination of the aquatic environment by pollutants has become a very serious problem in recent years. In particular, rivers and lakes, which are recipients of agrarian, urban, and industrial wastewaters containing contaminants, such as heavy metals, pesticides, and phenols, often in high concentrations. In addition, surface runoffs, industrial effluents, leachates from landfills, and contaminated groundwater are all known to contain numerous chemicals. The indiscriminate use of pesticides and heavy metal salts is detrimental to the environment, causing imbalances, especially in aquatic ecosystems **(Twagilimana *et al.*, 1998)**.

Over recent years, much research has been carried out on the toxicity of various relevant toxic compounds in a series of biotests using several test organisms. The appeal of these tests lies in their simplicity and high degree of reproducibility. However, test organisms for assessing environmental risk and impact must possess a number of desirable features: they must be eukaryotic, their biology and general responses must be well known, laboratory handling must be relatively easy and a short generation time is desirable whenever studies of long-term effects are necessary **(Nilsson, 1989)**.

Regarding water pollution, protozoa seem to be an excellent tool to assess both toxicity and pollution: they are considered as biological indicators of pollution when their presence or absence can be related to particular environmental conditions, and they are regarded as test organisms when a species or population is used to evaluate the toxicity of relevant toxic compounds **(Nicolau *et al.*, 2001)**.

Additionally, protozoa have proven to be excellent tools for assessing the occurrence of pollution during wastewater biological treatment, along with its role in the control of pollution itself through the grazing of dispersed bacteria and maintenance of a healthy trophic web in those artificial ecosystems. The protozoan community in the aeration tank of activated sludge plants remains an innovative and useful instrument to monitor biological wastewater treatment. More studies on this subject, aimed at collecting data and comparing the effects of toxicants on this community, will be of great interest and should make the protozoa a better tool in monitoring these processes **(Nicolau *et al.*, 2001)**.

What "bioindication" term means?

The use of biota as indicators of environmental condition is the basic aim of bioindication, although even within this broad definition, goals, methodology, and practice differ significantly. **McGeoch and Chown (1998)** argued that three broad categories of bioindicators can be recognized based on their aims and objectives: environmental, ecological and biodiversity indicators. Although these categories may be considered somewhat artificial, and certainly overlap, they provide a useful framework in which the diversity and differences in bioindication approaches can be considered.

Environmental indicators aim to quantitatively or semi-quantitatively indicate the value of an environmental variable often by studying the response of organism phenotype, population size, and community structure. Furthermore, this category of bioindicators also includes the bioaccumulators organisms, which accumulate pollutants to indicate environmental exposure, and environmental sentinels, organisms deliberately introduced to indicate the presence or level of pollutants.

In this case, ecological indicators aim to demonstrate impacts on a broader range of organisms or ecosystem parameters. Organisms occupying the top of food web were considered reliable ecological indicators since changes in testate amoebae can be argued to indicate alterations in a wider range of species, given their position at the top of the microbial food chain.

Biodiversity indicators focus on the diversity of one group of organisms as an indicator for the broader range of organisms diversity **(McGeoch & Chown, 1998)**. This is an important topic in conservation biology where a major question is how to direct conservation efforts to maximize preservation of biodiversity; the diversity of all groups cannot be feasibly studied in all areas, hence there is a desire to identify indicator organisms as proxies for ecosystem biodiversity **(Kati *et al.*, 2004; Schulze *et al.*, 2004)**.

Why Protozoans are considered as useful bioindicators?

Payne (2013) believed that seven key features make protists particularly valuable as bioindicators:

1. Environmental sensitivity: There is much evidence for the sensitivity of protists to many types of environmental change. This sensitivity to environmental change provides the essential prerequisite to the use of protist bioindicators.

2. Functional importance and trophic position: While the environmental sensitivity of protists makes them potentially-useful environmental indicators, it is their functional importance and trophic positions which makes them potentially interesting ecological indicators.

3. Distribution: The widespread distribution of both protist groups and many individual species within those groups means that an indicator based on the abundance or phenotype of a species in one region can potentially be applied in another.

4. Size and numbers: Protists range in size from approximately 1μm diameter in the case of the diminutive marine alga Ostreococcus tauri **(Chrétiennot-Dinet *et al.* 1995)** to over 10cm in the case of the largest xenophyophores **(Lecroq *et al.* 2009)**; however, most taxa are in the range of 5– 200μm. For bioindication, this means that even a very small sample of water, soil or sediment is likely to contain more than enough abundance and diversity for meaningful community quantification reducing sampling disturbance, improving potential spatial resolution, and easing the logistical difficulties of sample movement and storage.

5. Response times: Generation times of protists are typically short. For instance, some figures reported in the literature show generation times of as little as 18 hours for the Antarctic marine diatoms **(Spies, 1987)**, seven hours for marine ciliates **(Dolan, 1991)** and freshwater heterotrophic flagellates **(Laybourn-Parry & Walton 1998)**, and four hours for the testate amoeba Phryganella acropodia **(Beyens & Meisterfeld, 2001)**. This speed of response combined with sensitivity to many forms of environmental change

indicates that protist communities are capable of rapid reorganization and may allow high-frequency biomonitoring.

6. Ease of analysis: A key advantage of protists as bioindicators is their ability to be studied relatively simply despite the difficulties involved with the microscopic identification of protists. However, the fact remains that it is possible for an experienced observer with cheap and simple methods to identify consistent morph-species in many protist groups. This is not the case for many other microorganisms. The protists have all the advantages of using microorganisms as bioindicators without requiring specialized facilities and expensive analyses.

7. Preservation potential: Many protist taxa including dinoflagellates, chrysophytes, foraminifera, diatoms and testate amoebae produce hard body parts which are resistant to decomposition. Reconstructing the community structure after hundreds, thousands, or even millions of years is feasible in favorable sedimentary environments, allowing for the qualitative or quantitative reconstruction of environmental change. More ecological research on various protist groups has been conducted recently, largely due to the growing use of protists by palaeoecologists. A useful but infrequently applied method is the simultaneous analysis of living and fossil communities to enable the placing of contemporary change in a longer-term context. Although protists may react quickly to short-term changes, the assemblages studied by palaeoecologists typically integrate changes over longer periods.

Tetrahymena pyriformis is preferred protozoans in bioassay studies

Ciliated protozoa fulfill all the requirements outlined by **Payne (2013)**. They can be ideal early-warning indicators of aquatic ecosystem deterioration **(Twagilimana *et al*., 1998)**. The trials to use ciliated protozoa as bioindicators started earlier since **Burbanck and Spoon (1967)** used sessile ciliates *Stentor* sp. and *Vorticella* sp. collected in plastic petri dishes for rapid assessment of water pollution. More than other ciliates, *Tetrahymena pyriformis* was extensively utilized in this context. *T. pyriformis* is a large (length 40- 60µm) unicellular organism **(Hill, 1972)** which is often used in bioassay studies. Since it gives a readily observable, rapid response to sub-optimum environmental conditions **(Burbanck & Spoon, 1967; Schultz *et al*., 1978)**. It occurs naturally in unpolluted fresh waters, occupying a median position in the aquatic food chain **(Carter & Cameron, 1973)**. This organism is simple to culture and can be inexpensively maintained in the laboratory. Its rapid growth rate ensures a large number of cells within the required period **(Hill, 1972)**.

For more than four decades, the ciliated *T. pyriformis* has been the organism of choice in analyses, evaluation of protein quality, and determination of the effects of several toxic substances. Moreover, it was the first protozoon to be axenically cultivated, i.e., in a standard medium, free from bacteria or other organisms, making it a suitable model cell system since the addition of a compound is, in principle, the only change in culture conditions **(Nicolau *et al.*, 2001)**.

Protozoa as indicator of water treatment efficiency

Ciliated protozoa are believed to be important grazers of bacteria and other microorganisms, and this activity appears to stimulate the rates of carbon and nitrogen cycling in soils **(Finlay *et al.*, 2000)**. Moreover, in some artificial ecosystems such as activated sludge wastewater treatment plants, ciliates significantly improve effluent quality **(Nicolau *et al.*, 2001)**. Furthermore, the community structure of ciliate species is an effective biological indicator of sewage plant functional conditions **(Madoni, 1994)**. Data on the toxic effects of metals on ciliate populations in activated sludge plants indicate that this microbiota has been negatively affected by the presence of the pollutants although the effect varied according to the bioavailable concentration and nature of the heavy metal **(Nederlof & Van Riemsdijk, 1995)**.

Protozoa as potential bioindicator of water quality in freshwater ecosystem

The use of the free living protozoan communities has benefited in perfectly characterizing and monitoring the prevailing environmental conditions of aquatic habitats that are typically found in marginal freshwater regions. A particular community of organism may be useful as an environmental indicator due to many reasons. Some may have sensitivity to low levels of anthropogenic contaminants, yet some others may tolerate and survive in the hardy and extreme conditions, and others may react quickly to changes in environment. Thus, they tend to become a unique biotic tool to understand the ecological status of an aquatic habitat. **Radhakrishnan and Jayaprakas (2015)** used free living protozoans as bioindicators in Vembanad Lake, Kerala, India. This lake is a biological niche of a multitude of organisms, and it is intricately woven with the lives of the resident communities of its banks. The lake has also been facing severe environmental crisis during the last 3 decades due to anthropogenic influences. Nineteen species of free-living protozoans have been identified and characterized from this lake. A total of 15 testacid rhizopods belonging to 2 orders, 6 families and 9 genera were recorded, and the ciliates of 3 orders, 3 families were recorded. Among the testaceous rhizopods, 1 species from Arcellidae family, 5 from Centropyxidae, 1 species from Nebelidae, 6 from Difflugidae belonging to the Class Lobosea and 2 species from the Class Filosea belonging to Cyphoderiidae and Euglyphidae families were identified. Some of these free-living forms have given certain insights into the prevailing ecological conditions of this lake, thus acting as perfect bioindicators. *Euglypha tuberculata* reported in the previous study is a species of wide tolerance surviving in diverse habitats. Similarly, *Cryptodifflugia oviformis* which was reported for the first time in India in the same study prefers dryer environments. Due to its small size, this species feeds mainly on bacteria and yeasts; their high abundance explains the active decomposition process in the area. The diversity of the free-living ciliates in the study area included species belonging to 3 genera; namely Euplotes, Tachysoma and Coleps, and they are pollution indicators possessing the property of heavy metal uptake. The water quality analysis, along with the heavy metal analysis, also confirmed that the lake waters are polluted with heavy metal concentrations. This study highlighted the possibility of using these dominant ciliate species for the bioremediation of aquatic pollutants in this lake. Since these freshwater-free living protozoans serve as good bioindicators reflecting the natural ecological conditions prevailing in the Vembanad Lake, they can also be effective bioremediation tools that can be applied to solve the heavy metal pollution challenge of the lake.

Protozoa as potential bioindicator of water quality in marine ecosystem

Rakshit *et al.* (2017) investigated the viability of a prospective bioindicator based on functional groups of microzooplankton tintinnids for bioassessments of the status of marine water quality during southwest monsoon (June to September) along the coastal waters of Kalpakkam, India in the period of 2012– 2015. The work highlighted the following features (1) tintinnid community, composed of 28 species belonging to 11 genera and 9 families, revealed significant differences among the four study sites; (2) the maximum numerical abundance and species diversity of tintinnid were recorded toward the Bay of Bengal, whereas the minimum abundance and diversity were encountered at the backwater sites; (3) multivariate analyses, biota-environment, and canonical analysis of principal coordinates revealed that nitrate and phosphate were the potential causative factors for tintinnid distribution. Based on the results, the afore-mentioned authors suggest that tintinnids may be used as a potential bioindicator of water quality status in marine ecosystem.

In their study, **Kazmi *et al.* (2022)** determined redundancy levels in situations of varying water quality by conducting a baseline study of periphytic protozoan communities in the coastal waters of the Yellow Sea and northern China over one year. Four sampling, locations were used to gather samples along a pollutant gradient. To compare with biotic factors, measurements were made of environmental variables, including salinity, chemical oxygen demand, and concentrations of dissolved oxygen, soluble reactive phosphates, ammonium nitrogen, and nitrate nitrogen. According to their research, periphytic protozoan communities could be valuable bioindicators of the quality of marine water.

The drawbacks of utilizing protozoans as bioindicators and how to overcome them

According to **Nicolau *et al.* (2001)**, two of the main disadvantages of utilizing protozoa for wastewater treatment diagnosis are the length of time required and the requirement for competent professionals in protozoology. Currently, image analysis is a well-established addition to electronic and optical microscopy. It enables automatic, non-subjective routine classification and measurement of microorganisms. Image analysis has become a standard practice in cellular technology research due to the exponential rise in computing power and the subsequent decrease in cost. In this respect, a program was developed to assess digitized photos of protozoa semi-automatically. It will always be necessary, therefore, for an experienced team to verify the accuracy and viability of the findings and address issues that computer technology is unable to address such as the distinction between related species.

Conclusion

Protozoa have proven to be excellent tools for assessing the occurrence of pollution during wastewater biological treatment, along with its role in the control of pollution itself. Protists make up a substantial proportion of all life on Earth, with huge numbers and vast genetic and phenotypic diversity in almost all habitats. For water pollution, protozoa seem to be an excellent tool to assess both toxicity and pollution. The protozoan community in the aeration tank of activated sludge plants remains an innovative and useful instrument to monitor biological wastewater treatment. Hence, protozoa are considered good bioindicators for the assessment of water quality. It is worthnoting that, the drawbacks of utilizing protozoa as bioindicators can be overcome by employing image analysis technology.

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Arabic Summary

البروتوزوا هي كائنات وحيدة الخلية حقيقية النواة وتؤدي جميع الوظائف الحيوانية. إنها مكون أساسي للنظم الإيكولوجية المائية والتنوع البيولوجي للتربة، وتساهم بشكل كبير في نقل الطاقة إلى المستويات الاعلى وتلعب دوراً هاماً في تحلل المواد العضوية. عالمية الإنتشار، شديدة التنوع، تزدهر في مختلف الظروف البيئية، وهي حساسة نسبيًا للملوثات المختلفة. تشكل البروتوزوا مرشحًا واعدًا للدلالة الحيوية على جودة المياه وصحة التربة. الهدف من هذا البحث المرجعي هو تسليط الضوء على أهمية البروتوزوا كمؤشرات بيولوجية لرصد جودة المياه، وتعرف المؤشرات البيولوجية بــ "استخدام الكائنات الحية كمؤشرات للظروف البيئية" وتنقسم الى ثلاث فئات: المؤشرات البيئية و المؤشرات الإيكولوجية و مؤشرات التنوع البيولوجي وتلعب الاوليات دوراً هاماً في الفئات الثلاث. هناك سبع مزايا رئيسية للمؤشرات الحيوية للاوليات وهي: الحساسية البيئية ، الأهمية الوظيفية، التوزيع، الحجم، الاستجابة، سهولة الفحص وإمكانية الحفظ. فيما يتعلق بتلوث المياه ثبت أن البروتوزوا أداة ممتازة لتقييم كل من سمية وتلوث المياه الناتجة من محطات المعالجة، وكذلك جودة كل من المياه العذبة ومياه البحار والمحيطات.