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Comparative Analysis of Soil Protozoa and some Physio-chemical parameters of Effluent-Inundated Soil and Unaffected Agricultural Soils of Chhattisgarh

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ABSTRACT

Soil serves as the primary edaphic component of the Earth, providing a habitat for plants and various soil microorganisms such as fungi, bacteria, and protozoa. Although protozoa constitute only a small portion of the microfauna in terms of biomass, their functional importance is significantly greater. The number and variety of protozoa may be influenced by the quality of soil. The present study aimed to compare the diversity of soil protozoa between agricultural field irrigated with natural water as well as tube-well water and agricultural field irrigated with Bhilai Steel Plant effluent in Durg District. Soil samples were collected from two sites: a rice -cultivated field in Kotni village (Site -1) and a Bhilai Steel Plant effluent-inundated rice cultivated field near Sirsa village (Site -2). Physico-chemical parameters and protozoan diversity were analyzed using standard methods. Site -2 exhibited higher protozoan diversity with 23 species in comparison to site -1 with 16 species. Quantitative analyses were conducted to determine the density, percentage frequency and abundance, along with the importance value index (IVI). The Sorenson index was applied to evaluate the similarity of the protozoan species across both study sites. The Simpson and Shannon-Weiner indices were used to evaluate the diversity of the protozoan communities. This study highlighted the potential use of protozoan communities as bioindicators of industrial pollution and soil health. These findings contribute to the understanding of the impact of industrial effluents on soil ecosystems and may contribute a significance role in future environmental monitoring and management strategies.

INTRODUCTION

Protozoa are small eukaryotic organisms that inhabit aquatic and terrestrial environments and consume organic material, bacteria, fungi, and other protists. The soil protozoans play critical roles in nutrient cycling, soil fertility, and microbial food webs. According to Clarholm (1985), soil protozoa are essential for the mineralization of nutrients, facilitating their availability to plants and other soil organisms. They are classified into four groups: flagellates, amoebae, ciliates and testate amoebae on the basis of their morphology, taxonomy and ecology.

Soil microorganisms such as protozoa, fungi and bacteria play a crucial role in maintaining soil health and fertility by regulating microbial populations and other ecosystem services (Park *et al.*, 2024 and Tahat *et al.*, 2020). These microorganisms contribute to nutrient cycling, carbon storage and plant growth, which are essential for sustainable agriculture (Park *et al.*, 2024). Different farming practices such as organic farming and conservation agriculture have been shown to affect soil biodiversity and microbial communities (Mamabolo *et al.*, 2024 and Ostandie *et al.*, 2021). Understanding the diversity of soil protozoa in industrial effluent inundated soils is essential for assessing the ecological impact of industrial activities on cultivated fields. Previous studies have documented the presence of various soil protozoan species in different agricultural and polluted environments, highlighting their adaptability and ecological significance. Microscopic organisms including ciliates, flagellates and amoebae contribute to soil food webs by grazing on bacteria and fungi, thereby influencing nutrient turnover and soil structure. The study of soil protozoa in areas affected by industrial effluents can provide valuable insights into the resilience of soil ecosystems and potential bioremediation strategies. Furthermore, understanding the community composition and dynamics of soil protozoa in these affected soils may help in developing effective management practices for sustainable agriculture in regions facing industrial pollution challenges.

However, there is limited information on the specific diversity and abundance of soil protozoa in soils inundated with industrial effluents, particularly in cultivated fields (Du *et al.*, 2024). Despite the potential implications for soil health and crop productivity, the impact of industrial effluents on soil protozoa communities in agricultural settings remains understudied. Investigating the diversity of soil protozoa in such environments can provide insights into the resilience of soil ecosystems and inform sustainable agricultural practices.

The primary objective of this study was to assess the diversity and abundance of soil protozoa in the industrial effluent inundated soils of cultivated fields and unaffected agricultural soil of the region. This study aimed to fill a critical knowledge gap by examining the diversity and abundance of soil protozoa in a unique environmental context. By focusing on industrial effluent inundated soils in cultivated fields, this research sheds light on the impact of anthropogenic activities on soil ecosystems which will be helpful in agricultural management practices and environmental conservation efforts in areas affected by industrial pollution.

Furthermore, understanding the relationship between protozoan communities and soil properties could lead to the development of novel soil remediation techniques that leverage the natural abilities of these microorganisms to break down pollutants and improve soil structure.

Study Area and Sites

In the present study, the first site was the Bhilai Steel Plant effluent inundated soil located near Sirsa village (Bhilai 3), and the second site was Kotni village, which is located near the Shivnath River has been analyzed for the types and abundance of protozoa along with the physico-chemical characteristics of the soil from both the sites between February 2024 and March 2024.

Materials and Methods

Sample Preparation

Soil samples were collected randomly from rice cultivated fields of village Kotni (Site -1) and Bhilai Steel Plant effluent-inundated rice cultivated field (Site -2) in plastic bags. Soil samples were collected in the early morning from all corners and the center, at a distance of 10 feet, and at a depth of 15 cm. Half of each soil sample was thoroughly mixed to create a composite sample, whereas the other half was stored in separate plastic bags. Soil samples were transported to the laboratory in plastic bags within 2 hours of collection for subsequent analysis. Chemical composition tests were conducted on the samples as soon as possible and protozoa observations were completed within two days.

Physico-chemical and biological analysis of soil samples

A digital pH meter was used to measure the pH of the collected soil samples and electrical conductivity was determined using a digital conductivity meter. The moisture content was assessed using the oven-drying method, and the total organic carbon was evaluated using the Walkley and Black titrimetric method (Jackson, 1973).

The protozoan species were observed using the non-flooded petri dish method (Foisner, 1987). One hundred gram of soil was mixed with distilled water in a Petri dish (Foissner, 1997a). At intervals of 24, 48, and 72 hours, a small quantity of water was extracted from the flooded Petri dish. A trinocular research microscope equipped with an image capture system was used to observe and photographed the protozoan species. The observed protozoan species were identified using different identification keys and monographs, such as Bhatia (1936), Edmondson (1959), Kudo (1966), Curds *et al.,* (1983) Foissner (1987), Berger and Foissner (1996), Berger (1999, 2006, 2008) and Lynn (2008). The number of protozoan species was determined using the Most Probable Number (MPN) dilution culturing technique (Darbyshire *et al.,* 1974).

Statistical Analysis

The density, percentage frequency, abundance and importance value index (IVI) of the protozoan species in each soil habitat were determined. The Simpson and Shannon-Weiner indices were used to evaluate the diversity of the protozoan communities. The Sorenson index (SI) was used to evaluate similarities between protozoan species inhabiting the soil environment.

Observations

The physico-chemical values of different samples and protozoan species identified in the present study are shown in Tables 1 and 2 respectively. Out of 30 protozoan species, two genera of rhizopods, two genera of actinopods, one genus of flagellates and 25 genera of ciliates were found in the present study.

Table 1: Physico-chemical properties of the soil from site-1 and site- 2.

Parameter	Site -1 (Kotni)	Site -2 (BSP Effluent Inundated Soil)
Colour	Black	Black
Texture	Loamy	Loamy
Temperature (^{0}C)	21^{0}C	23^{0}C
pH	8.00	7.85
Electrical Conductivity (ds/m)	-048	-041
Organic Carbon (%)	0.98	1.95
Organic Matter (%)	1.69	3.36
Moisture Content (%)	31.08	36.31

Table 2: List of Soil Protozoa in the soil samples collected from site-1 and site- 2.

SN.	Taxa	Site -1 (Kotni)	Site -2 (BSP Effluent Inundated Soil)
1.	*Amoeba* sp.	+	+
2.	*Saccamoeba* sp.	+	-
3.	*Actinophrys* sp.	+	+
4.	*Actinospherium* sp.	-	+
5.	*Paranema trichophorum*	+	+
6.	*Vorticella* sp.	+	+
7.	*Euplotes* sp.	+	+
8.	*Oxitricha*	-	+
9.	*Cyclogramma trichocystis*	-	+
10.	*Anatoliocirrus* sp.	-	+
11.	*Notohymena* sp.	-	+
12.	*Pseudomicrothorax agilis*	-	+
13.	*Sterkiella* sp.	-	+
14.	*Paruroleptus magnificus*	+	+
15.	*Dileptus* sp.	+	+
16.	*Halteria* sp.	+	+
17.	*Holosticha pullaster*	-	+
18.	*Paramoecium* sp.	-	+
19.	*Arcuospathidium muscorum*	-	+
20.	*Tachysoma pellionella*	-	+
21.	*Cultellothrix coemeterii*	-	+
22.	*Tilina* sp.	+	-

23.	*Colpoda* sp.	+	+
24.	*Exocolpoda* sp.	+	-
25.	*Holophrya simplex*	-	+
26.	*Spirozona* sp.	-	+
27.	*Furgasonia blochmani*	+	-
28.	*Drapanomonos revoluta*	+	-
29.	*Amphisiella* sp.	+	-
30.	*Myxophylum* sp.	+	-
	Total	**16**	**23**

Results and Discussion

The study involved analysis of composite soil samples from two distinct sites and to compare their physico-chemical properties. The soil texture was found loamy in both the sites. The pH value of site -1 was found 8, while site -2 had a slightly lower pH value of 7.85. The studies by Shaaban *et al.,* 2019 and Xiong *et al.,* 2024 has revealed that soil pH can significantly influence nutrient availability and microbial activity. The electrical conductivity recorded -048 and -041 in site-1 and site-2 respectively. Total organic carbon % was found in site -1 with a value of 0.98 while in site-2 total organic carbon % was found 1.95. The organic matter % was found in site -1 with the value of 1.69 and in site -2 it was found with a value of 3.36. A positive correlation was observed between the moisture content and organic matter percentage in the soil samples. The higher total organic carbon % found in site -2 (1.95%) supports the hypothesis that this site has a higher organic matter content. Contrary to expectations, the higher pH value did not correspond to a higher organic matter content in site -1. A higher moisture content was found in site -2 with a value of 36.31 and a lower moisture content was found in site -1 with A value of 31.08.

This study significantly contributes to the field by providing detailed insights into the soil characteristics of different sites, which can aid targeted soil management. These findings have practical implications for agricultural practices, particularly for optimizing soil conditions for crop growth. The novelty of this study lies in its comprehensive analysis of multiple physicochemical parameters across industrial effluent-inundated and agricultural soils, along with their associated protozoan communities.

The physical characteristics of the soil have a substantial influence on the composition and structure of the protozoan communities. In the current investigation, we identified 30 protozoan species belonging to 11 classes, 18 orders, 21 families, and 30 genera. Ciliate species were ubiquitous and were being present at both sampling locations. Several species, including *Amoeba* sp., *Actinophrys* sp., *Vorticella* sp., *Halteria* sp., *Euplotes* sp., *Paruroleptus magnificus*, *Dileptus* sp., *Paranema trichophorum* and *Colpoda* sp. were consistently observed at both study sites.

The highest protozoan diversity was recorded at site -2, which harbored 23 species, whereas site -1 exhibited the lowest diversity with only 16 resident species. This variation in species richness may be attributed to the differences in soil properties between the two sites. Notably, organic matter and carbon content indirectly influence soil protozoan populations by affecting bacterial communities that serve as their primary food source (Ekelund *et al.,* 2002).

Quantitative analysis of species diversity revealed that site -2 exhibited higher richness and evenness, as evidenced by the Shannon-Wiener index value of 3.08 while site -1 exhibited lower richness and evenness, with a Shannon-Wiener index value of 2.75. The Simpson index, which measures dominance, was higher in site -1 (0.07) in comparison to site -2 (0.05). These diversity indices collectively indicated that site -1 possesses the lowest species diversity, while site -2 boasts the highest species diversity among the studied locations. Analysis of Important Value Index (IVI) in site -1 revealed that *Euplotes* sp. exhibited the highest value at 25.28, whereas *Amphisiella* sp. exhibited the lowest IVI at 11.78. Similarly, in site -2, *Actinophrys* sp. displayed the maximum IVI of 20.85, while *Paruroleptus magnificus* showed the minimum IVI of 9.02.

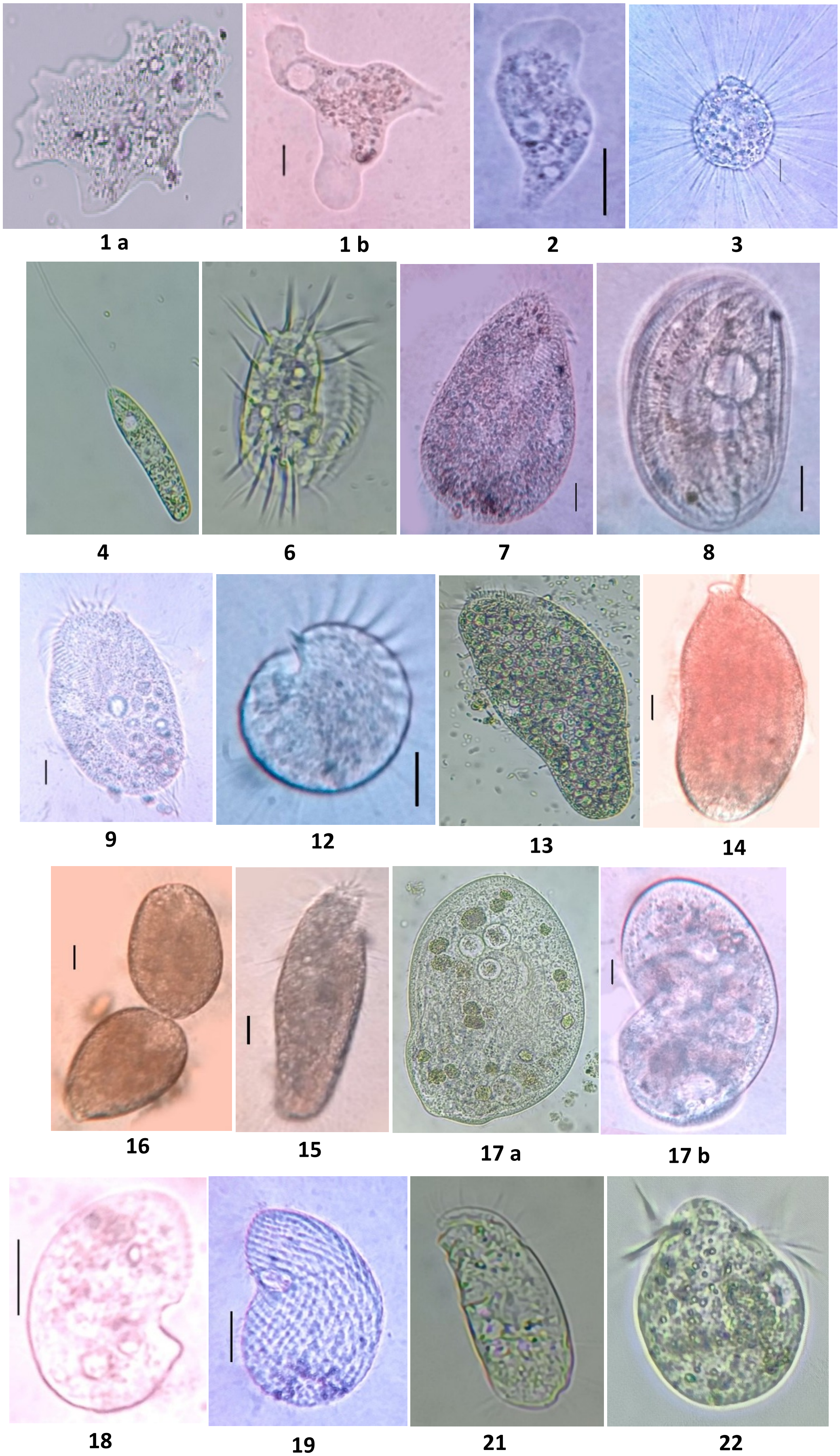
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Fig.1. microphotographs of Soil Protozoa in soil samples collected from site-1 and site- 2. 1. *Amoeba* sp.; 2. *Saccamoeba* sp.; 3. *Actinophrys* sp.; 4. *Paranema trichophorum;* 5. a &b *Vorticella* sp.; 6. *Euplotes* sp.; 7. *Anatoliocirrus* sp.; 8. *Pseudomicrothorax* sp.; 9. *Sterkiella* sp.; 10. *Paruroleptus magnificus*; 11. *Dileptus* sp.; 12. *Halteria* sp.; 13. *Pramecium* sp.; 14. *Arcuospathidium muscorum*; 15. *Tachysoma pellionella*; 16. *Cultellothrix coemeterri*; 17. *Tilina* sp.; 18. *Colpoda* sp.; 19. *Exocolpoda* sp.; 20. *Furgasonia blochmanni;* 21. *Drepanomonas revoluta*; 22. *Myxophylum* sp..

The Sorenson similarity index (SSI) is a measure of species similarity between two sampling sites, with values ranging from zero (no similarity) to one (complete similarity) (Alizadeh *et al.*, 2020). In this study, the observed Sorenson similarity index (SSI) was 0.46 indicates a moderate level of similarity between protozoan species. This finding suggests that whether there exists some overlap in species composition between the two sites, there are also substantial differences are present.

A positive correlation was found between the level of industrial effluent and protozoan species richness. This suggests that industrial effluents may create unique niches or conditions that support a diverse array of protozoan species. Further investigation into the specific components of industrial effluents that promote protozoan diversity could provide valuable insights into the microbial ecology and adaptation mechanisms. Moreover, understanding the long-term effects of increased protozoan diversity on soil health and ecosystem functioning could have important implications for environmental management and restoration strategies.

CONCLUSION

In the present study the results showed a high richness of protozoan species in the soil affected by the industrial effluent. This study also revealed the correlations between the physico-chemical properties of the soil samples and the observed protozoan diversity. Interestingly, certain protozoan species with specific physico-chemical characteristics were found to be more abundant in soil samples fed with industrial effluent, suggesting potential bioindicators for industrial pollution. These findings contribute to our understanding of how industrial effluents affect soil ecosystems and may inform future environmental monitoring strategies.

There was a notable increase in protozoan species richness in the soil inundated with industrial effluent compared to the site-1 with unaffected soil. The higher value of species richness in contaminated soil suggests that certain protozoan species may thrive under altered environmental conditions, possibly because of reduced competition or increased nutrient availability.

A comparison between effluent-inundated and non-inundated soils revealed significant differences in protozoan diversity. This study opens new avenues for investigating interactions between soil contaminants and microbial communities.

In Contrast to previous studies (Yuan et al., 2024), our results indicated the higher protozoa diversity in effluent-affected soils. These finding challenge the conventional assumptions about the impact of industrial pollution on microbial communities and also raises questions regarding the adaptive capabilities of protozoa in response to environmental stressors.

An exhaustive study requires to explore the functional roles of these protozoan communities in contaminated soils may provide insight into their potential for bioremediation and ecosystem restoration.

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