https://doi.org/10.48047/AFJBS.6.5.2024. 10216-10225

AFJBS

AfricanJournalofBiological Sciences

Effects of seasonal dynamics on Microalgal diversity at Sambhar Lake, India

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Article History
Volume 6, Issue 5, 2024
Received: 22 May 2024
Accepted: 03 Jun 2024
doi:*10.48047/AFJBS.6.5.2024. 10216-10225*

Abstract

The phytoplankton community in saltwater bodies is vital for sustainable development in numerous ways. The present diversity study addresses seasonal changes in the micro-flora community to detect rapid changes in available nutrients and other components compared with diversity indices in Sambhar Lake. Significant reduction in Lake body surface area and increase in algal bloom may be triggered by some climate factors, anthropogenic and industrial activities, and heavy metals contamination resulting in danger to this ecosystem. The physicochemical or biochemical changes in any aquatic environment control the change detection in the algal community regarding the dominance or loss of particular algal species and give an early warning of changes in water quality or pollution. An attempt has been made to find uninvestigated fast-growing and bio-monitoring potential algae to protect and conserve this Ramsar site from pollution and heavy metals contamination. Collected samples from Sambhar Lake generated new information on the seasonal dynamics of the phytoplankton community exploring *Dunaliella*, *Chlorella*, *Oscillatoria* and *Microcystis* algal species crucial for sustainable development.Algae (cyanobacteria, blue-green algae, and Diatoms) have been used as bio-indicator as a reliable, rapid and inexpensive way to direct measurement of water chemistry concerning aquatic organisms across several sites in a given time.

Keywords. Phytoplankton, diversity index, heavy metals, water quality, Sambhar Salt Lake.

Introduction

Salt Lakes, considered temporary or permanent water bodies, consisting of salinity of more than 3 g/L are widespread in all continents, confined to arid and semi-arid climate zones. Salt Lakes having endorheic drainage basins or periodic outflows during high water levels offer an outstanding opportunity to study past environmental conditions and changing patterns in their limnology (Demnati *et al*, 2017). The saline ecosystems vulnerable to Global warming, climate change and anthropogenic disturbances have been degraded and facing shrinking loss in the last few decades (Williams, 2002). Their ecological contributions help tomaintain a healthy environment as they are rich in unique biodiversity consisting of diverse flora and fauna. The biodiversity adapted to high salinity and temperature are highly responsive to climate change and serves as a sensitive indicator system (Bellinger and Sigee, 2015).

The diatoms and cyanobacteria occupied in saline systems evolved during the Precambrian era (approximately 2.5-3.5 billion years ago) and had a significant role in atmospheric transition (Kumar *et al*, 2019). Microalgal based bioremediation techniques are found not only suitable for detoxification of toxic heavy metals but also facilitate extraction (Nayak 2022).

The halophilic microorganism has a remarkable capability to grow and acquire effective mechanisms to protect itself against various biotic and abiotic stresses. The diversity and productivity of aquatic biota are directly or indirectly controlled by climate and human-induced changes and seasonal variations in temperature, salinity, pH and other abiotic factors (Meshramm and Dhande, 2005). The climatological and geographical variations support a variety of wetlands in India and there are 75 wetlands declared as Ramsar sites by the Ramsar Convention, which was signed in 1971, is an international framework for the conservation and wise use of wetlands with national and international cooperation (Mao *et al*, 2021). The Sambhar Lake in Rajasthan was designated as the Ramsar site (March 1990) for providing an area to thousands of seasonal migratory water birds and is also famous for producing 9% of total salt production in India. The ecological importance of Sambhar Lakefor avifauna makes this wetland a suitable site for including in Ramsar sites under the convention of wetlands of International importance (Kulshreshtha2013). The greatest threat to Sambhar Lake is groundwater extraction, mushrooming private salt industries, construction of dams in the Lake area and other domestic and industrial activities that are a serious concern to the Lake's existence (Pathak and Cherekar, 2015). The toxic substances presented in industrial and domestic waste from the surroundings contaminate the water and deteriorate the Lake water quality and disturb the community structure by ruining the food web.Previous researches on Sambhar Salt Lake have only focused on birds, halo-tolerant bacterial species identification, isolation and characterization, limnology, paleoclimatology for sensor calibration andvalidation and on extremophilic algal assessment. However, the effects of seasonal dynamics on algal community and core challenge of the lake’s pollution and rapid shrinkage is not addressed yet.

The Sambhar salt Lake is an intermittent salt Lake, with varying physicochemical properties depending on the season and site (Divyansh and Raj, 2019). The saline and alkaline water from Sambhar Lake is channeled into shallow solar pans, where the salinity ranges from 5.0 to 30.0 (%w/v, NaCl). Halophiles are found in saline environments, including deep sea, coastal to salt mines salinity ranging from 0.2-50% but only extreme halophiles grow where salinity is found 20-30 % or more. Salinity makes the ecosystem favorable for the growth of extreme halophilic biodiversity. Microalgae assay technique is a method used to assess the eco-toxicology of water samples and the impact of environmental factors on algae growth(Pore and Dhulap2023).These microorganisms play an essential role in an aquatic ecosystem as cyanobacteria are primary producers that convert solar energy to bio-products and serve food for zooplankton, flamingos and other birds (Sharma 2021).

The collected brine samples indicate the dominance of algal diversity in winter with green color that turns pink in summer. The algal bloom and other extremely haloalkaliphilic archaebacteria are responsible for the brine’scolour change. Diatoms, blue-green algae and green algae are significant pollution tolerant species such as *Chlorella*, *Oscillatoria*, *Scenedesmus*, *Navicula* and *Chlamydomonas*, etc. (Palmer 1969). Although studieson the application of these halophilic microalgae as food, feed, cosmetics, medicines and biofuel have been reported, the diversity analysis with season variation is to be analyzed yet. Detecting change in a phytoplankton occurrence may indicate a change in water chemistry which can help to substitute management practices of a particular aquatic ecosystem. Thus algae as a bio-indicator can serve early warning signals against pollution in a particular water body and can be a potential candidate to monitor water quality and changes in nutrient availability.

Kolenati (1848) first noted the freshwater organism's potential to reflect changes in environmental conditions. Some algal species have been isolated from the salt pans, but no study for the presented microalgae with seasonal changes has been performed. The algal species occurrence and abundance differ with seasons and exhibit a relation between algal growth and N:P (nitrogen phosphorus atomic ratio) (Rajput *et al*, 2022). When there is enough light and nutrients, particularly nitrogen and phosphorus, algae can grow and reproduce rapidly (Misra*et al*, 2020). The algal diversity, richness and evenness varies with changing salinity, pH, nutrient status and other physicochemical parameters. Tiwari *et al*, 2020 developed a mathematical model to investigate the use of Phoslock in reducing phosphate bio-availability in water. They considered phosphorus, detritus, algae, and Phoslock as dynamic variables. It was assumed that the introduction rate of Phoslock would be proportional to the concentration of phosphorus in the lake.In addition to identifying the most efficient water quality monitoring, fast-growing saline algal species appeared throughout the year, and the seasonal variation of phytoplankton of the Sambhar salt Lake was studied.

Materials and methodology

Study area, sampling and analysis:

The Sambhar Lake located in Rajasthan state is the largest inland salt Lake in India and was designated as the Ramsar site in 1990 for its international importance and providing wintering area for flamingoes and other migratory birds. The Lake is spread in 3 districts (Jaipur, Nagaur and Ajmer) occupies an area of 230 km^2 area is famous for its biological importance and salt production. It is surrounded by Aravali hills and fed by two major rivers Mendha and Rupangarh (Bhatt and Sharma, 2015). Samples for the microalgal study were collected from brines of Devyani Kyars with salinity ranging from 10.0-30.0 (% w/v, NaCl) and high pH (between 8-11). The microalgal diversity and color of brines change with seasons therefore the samples were collected during three different seasons (summer, rainy and winter) throughout the year (2020-2021).

The maximum area of Sambhar Salt Lake (SSL) is used for salt production by evaporation method so, four sampling sites were selected for sample collection enriched with algae throughout the year (all seasons of the year 2020 and 2021) to access the microalgal diversity. pH and temperature were measured onsite. Samples were stored in prewashed and pre-sterilized bottles in all seasons (summer, winter and rain). The physicochemical analysis of collected samples was performed at the laboratory. Bottles were labelled and stored at $4^{O}C$ for phytoplankton study. The Microscopic identification and counting of presented algae were done (Ewebiyi*et al*, 2015).

15 ml out of 1000 ml water samples were centrifuged at 3000 rpm at room temperature. The visible portion was placed on a glass slide and observed under a light microscope. To avoid drying and crystallization glycerin was added to the sample. The samples were examined and images were taken on 40 X magnification. Morphological features color, cell character, motility, and reproductive and physical structure were used to identify the presented algal species. Identification of algal species was performed using Bellinger and Sigee, 2015 key (Siangbood and Ramanujan, 2014).

Diversity study:

Mathematical modeling is a powerful tool that helps us better understand the interplay between different species, allowing us to preserve biodiversity and ecosystems (Sarkar *et al*, 2020).In general, the diversity study of an aquatic ecosystem can be understood using diversity indexes. Samples were collected and algal genera were counted to study the diversity of presented algae at different sites. In the present study Shannon's abundance index (H), Simpson’s dominance index (Ds), Pielou’s species evenness index (J) and Marglef’s species richness index (D) were used to analyze the species diversity. The following formula were used to analyze the algal diversity:

Shannon’s species abundance index

$$(H) = \sum(pi) \ln (pi),$$

Simpsons’s species dominance index

$$(Ds) = \sum Pi2,$$

Pielou’s species evenness index

$$(J) = H/H\ max,$$

Marglef's species richness index

$$(D) = S\text{-}1/\ \text{In}\ N$$

Results

To analyze the diversity of halophilic microalgae the water samples were collected from brines of the Lake area in three different seasons (summer, rainy and winter 2020- 2021). A variety of colors indicated the dominance of algal diversity during the winter season (green and orange color) and bacterial diversity was dominant (pink color) in summer depending on pH and salt concentrations. The microscopic observation revealed the presence of different green algae and cyanobacteria having a rod, spiral, oval and filamentous-shaped morphology. A correlation between microalgal diversity and population density with regards to the chemical composition of water was observed. The algal growth was controlled by the Nitrogen and Phosphorus ratio and the Phosphorus was a limiting factor (Zhou *et al*, 2011).

The Nitrogen and Phosphorus emphasized the growth of algal species *Dunaliella* and *Oscillatoria* in the summer season at all sites while the number of *Chlorella*, *Oocystis*, *Nostoc*, *Microcystis*, *Spirulina*, *Phormidium*, *Navicula*, *Anabaena* and *Euhalothece* were reported different in presence of Nitrogen and Phosphorus ratio. The growth of *Dunaliella*, *Chlorella*, *Microcystis*, *Spirulina*, *Oscillatoria*, *Nostoc*, *Phormidium*, *Euhalothece* and *Anabaena* algae was heightened by the availability of Nitrogen and Phosphorus content in the rainy season at all collection sites while *Scenedesmus*, *Oocystis* and *Navicula* species were not present in any sample. The presence of maximum Phosphorus and Nitrogen enhanced the growth of *Dunaliella*, *Chlorella*, *Oocystis*, *Spirulina*, *Oscillatoria* and *Microcystis* algal species in the winter season. The number of *Scenedesmus*, *Nostoc*, *Phormidium*, *Euhalothece*, *Anabaena* and *Navicula* algae species were different at all four sites (table 1). The algal species of *Dunaliella*, *Chlorella* and *Oscillatoria* were capable to survive in all seasons, adaptable to salinity, temperature and other parameters which are required to maximize their growth and reaction mechanism and allow for their existence in saline medium (Talebi*et al*, 2013, Sankaranarayanan*et al*, 2021).

Table 1. Distribution of presented Microalgal in different sites in all seasons (August 2020 to September 2021)

Class	**Summer**					**Rain**				**Winter**			
	Algal species	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Chlorophyceae	*Dunaliella*	+	+	+	+	+	+	+	+	+	+	+	+
	Chlorella	+	+		+	+	+	+	+	+	+	+	+
	Oocystis	+		+						+	+	+	+
	Scenedesmus											+	+
Cyanophyceae	*Spirulina*	+			+	+	+	+	+	+	+	+	+
	Microcystis	+		+	+	+	+	+	+	+	+	+	+
	Oscillatoria	+	+	+	+	+	+	+	+	+	+	+	+
	Nostoc		+		+	+	+	+	+	+		+	+
	Phormidium		+	+		+	+	+	+	+			+
	Euhalothece	+		+	+	+	+	+	+			+	+
	Anabaena		+	+		+	+	+	+		+	+	+
Bascillariophyceae	*Navicula*				+					+		+	+

Note: '+' denotes presence of particular algal species.

The maximum algal biomass was reported in the rainy season followed by the winter season. The mean biomass in summer was 6.96 in rainy season 25.03 and 16.9 in winter. *Nostoc* (35%) was reported maximum in the rainy season and *Phormidium* and *Chlorella* (2%) was minimum(fig 1 a). *Microcystis* (35%) showed maximum percentage abundance and *Chlorella* (1%) was lowest in the summer season (fig. 1 b). The percentage abundance of *Dunaliella* (31%) species was maximum and *Navicula* (1%) was minimum in the winter season (fig. 1 c).Salt production via evaporation method covers the maximum area of Sambhar salt Lake so, less area provides water habitat for different microalgae and cyanobacteria. Despite it, four sites (salt pans near pump house and Jhapok dam) were selected for diversity study. The average occurrence of halophiles at site 1 was relatively equal to site 4 (48.33 and 48), minimum at site 2(37.33) and maximum at site 3(52) throughout the year.

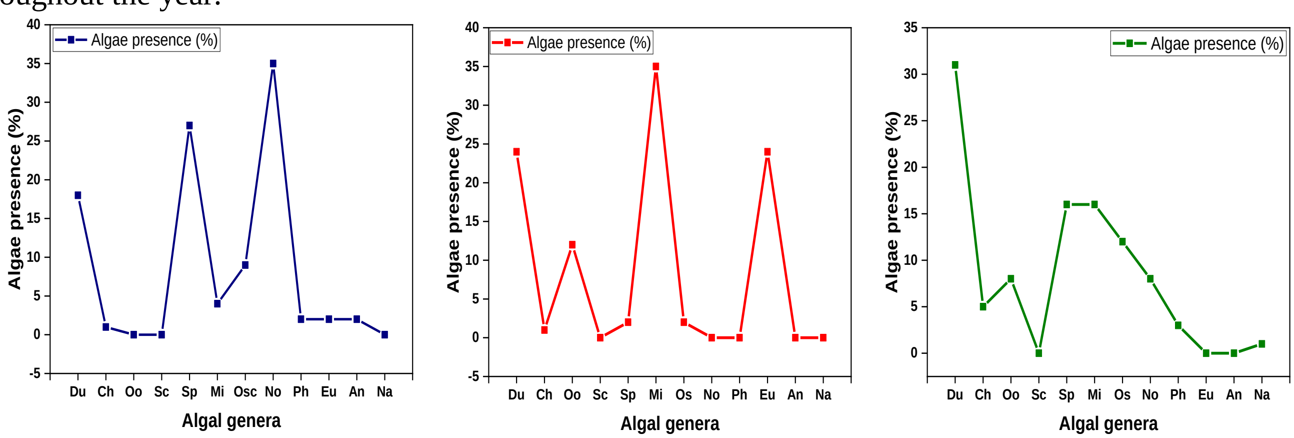

Figure 1 (a): Percentage abundance of algal species in rainy season, (b) in summer season, (c) in winter season.

The diversity index of the phytoplankton community was calculated for different algal genera at selected sites. Shannon's diversity index (H) was highest for *Microcystis* (0.886) followed by *Dunaliella* (0.725) and lowest for *Scenedesmus* (0.026). It was similar for *Oscillatoria* and *Euhalothece*. The Simpson's dominance index (Ds) ranged from 0.001 to 0.23. However, the highest value for Simpson's index was observed for *Dunaliella* sp. followed by *Nostoc* and the lowest for *Navicula*. In contrast, the microalgae *Microcystis* showed a maximum Pielou (J) index value and it was minimum for *Scenedesmus* algalspecies (fig. 2).The diversity indexes for seasonal variation were calculated and variations in values were observed. Shannon's diversity index ranged between 1.7 to 1.9, highest for summer and lowest for the winter season. Although it was minimum for average for the rainy season the appearance of microflora was maximum during this period.

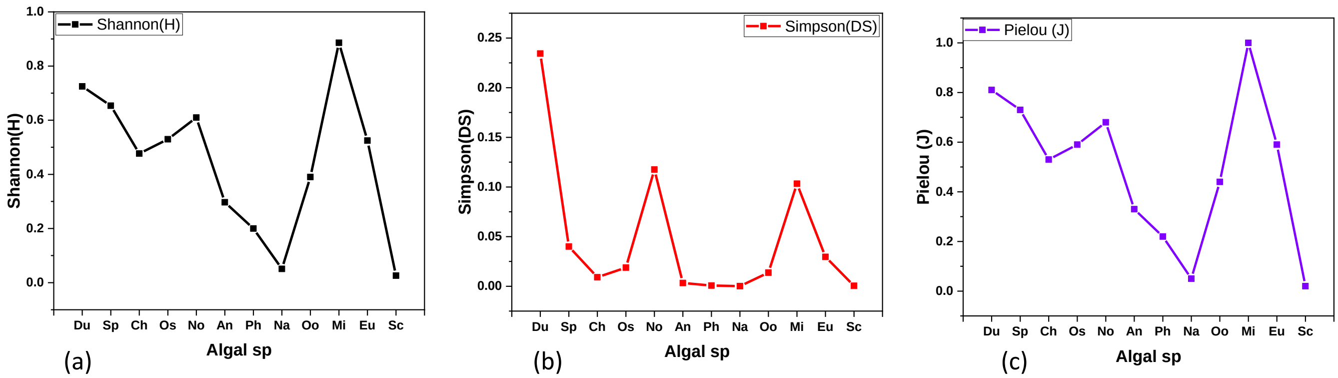

Figure 2: Value of different diversity indexes for all appeared algal genera

Note: Du- *Dunaliella*, Sp-*Spirullina*, Ch- *Chlorella*, Os-*Oscillatoria*, No- *Nostoc*, An-*Anabaena*, Ph- *Phormidium*, Na-*Navicula*, Oo- *Oocystis*, Mi-*Microcystis*, Eu- *Euhalothece*, Sc-*Scenedesmus*

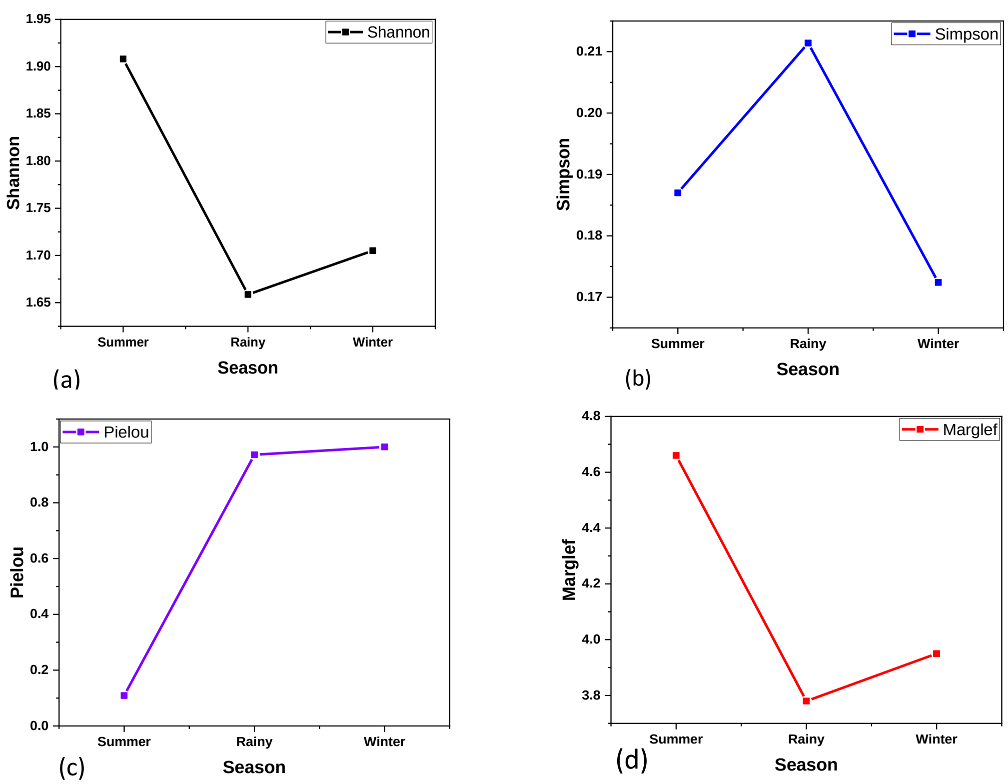

Figure 3: Value of different diversity indexes in all seasons (a) Shannon (b) Simpson (c) Pielou (d) Marglef diversity indexes

The Simpson's diversity index is calculated similarly for summer and winter and maximum for the rainy season. Pielou index indicates evenness of species occurred, ranged between 0.1 to 1, observed low for summer and almost similar for winter and rainy season. Margalef's index value was significantly different for all seasons. It was high (4.6) for summer followed by winter (3.9) and the rainy season (3.7) (fig 3).

1. Discussion

To evaluate the water quality status the micro-vegetation diversity of Sambhar salt Lakewas studied (August 2020- August 2021) as these genera represent versatile responses to environmental conditions and anthropogenic disturbances. The relationship between nutrient availability and phytoplankton community was studied to compile the water health status. These hypersaline aquatic sites provide the extreme environment for the luxurious growth of halophiles that making them rich in microbial diversity. High pH, salinity, low to high temperature and nutrient availability affect the growth of cyanobacteria and other algal genera (Reynolds *et al*, 2002).

To study microbial diversity Shannon's diversity index, Simpson's dominance index, Pielou evenness and Margalef's species richness index were used in all seasons throughout the year. Abiotic factors were observed responsible for the temporal and spatial distribution of halophiles (Brandao2016). The results showed the presence of Chlorophyceae, Cyanophyceae and Bacillariophyceae members. The Shannon diversity index represented the highest value in summer resulting in algal bloom appearing on the surface of the water. Richness in Phosphorus and Nitrogen in the dry season makes adaptable conditions for cyanobacteria and specific algal species to survive in extreme environments increasing the density of these algal species. The winter season provides suitable conditions for the growth of micro-flora resulting in the maximum number of algal genera appearing in this period (November to February) followed by the monsoon season. Although the Simpson’s index is related to richness and evenness, it gives more weightage to the dominance of the presented species. The maximum value of Simpson's index showed the dominance of *Dunaliella* at all sites in all seasons. The algae species *Dunaliella*, *Oscillatoria*, *Chlorella* and *Microcystis* species were observed throughout the study period at all sites can be used to access the water quality as they were reported for surviving in all seasons. It was concluded that *Dunaliella* is the most salt-tolerant microalgae capable to grow and survive in salinity range from 50%-125% (Gautam and Kapoor, 2022).

According to Eigemann *et al* (2016) phytoplankton and diatoms are used as bio-indicators worldwide to examine the water quality. The studies showed presence of commonly found green algal species of *Chlorella* and *Scenedesmus* in wastewaters in India (Tharavathi and Hosetti, 2003) but their diversity study in saltwater bodies is underreported. Our study came out with the identification of the most abundant, salt-tolerant, fast-growing algal species as a new bio-resource of feed, fuel and food which help in the environment inventory of these species and for water quality monitoring. In temperate Lakes where nutrient availability of Phosphorus and Nitrogen falls low in summer, the water surface is dominated by the algal bloom of blue-green algae (eg. *Microcystis*), and seasonal dynamics can be analyzed (Ray 2019). The saline algae can be a novel candidate for biological monitoring of changing climate factors affecting any aquatic ecosystem.

Conclusion:

The dynamics of the algal population indicated that specific algal species thrived during the winter season, resulting in an increase in their density. On the other hand, the summer season trigged the diversity of the overall phytoplankton community.The current description of the ecological ranges of the dominant species in relation to various water quality parameters offers valuable ecological insights for future use of these species, particularly when selecting suitable species for bioremediation or industrial cultivation.Overall, the study serves as a model for identifying fast-growing algal species and conducting environmental inventory in eutrophic waters.The knowledge generated is crucial for the advancement of algal-based industries for food, fuel, nutraceutical, and bioremediation purposes, which are vital for sustainable development in all nations (Ray *et al*, 2019).

Rapid industrialization and human induced activities have altered the natural components of water reservoirs that hinder infrastructure development. Bioremediation techniques (ex-situ and in-situ) including natural attenuation, Bioventing, Bioslurping and Biospargingenhance the sustainability of heavy metals contaminated sites using microorganisms (Sravya and Sangeetha, 2022).

Acknoledgement- The authors express their gratitude to theassociate editor and the reviewers whose comments and suggestions have helped the improvements in this paper.
Competing interests- The authors who contributedto this manuscript declare no competinginterests.

Author contributions-

Jyoti Saini: Sample collection, data analysis, interpretation and manuscript writing.

Dr Sonali Pandey: Conceptualization of the work, critical revision of the article and final approval of the version to be published.

Funding: None

Availability of data and materials: The datasetsanalyzed during the current study are available from the corresponding author on reasonable request.

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