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Whispers in the mangroves: Unveiling the silent impact of potential toxic metals (PTMs) on Indian Sundarbans fungi

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ABSTRACT

This study investigates sediment samples from the Indian Sundarbans' mangrove habitat, where most samples were alkaline and hypersaline, except for one acidic sample. Elemental analysis revealed poor sediment quality, with elevated Enrichment Factors (2.20–9.7), Geo-accumulation indices (−2.19–1.19), Contamination Factors (0.61–3.18), and Pollution Load Indices (1.04–1.32). Toxic metal ions, including Pb, Cu, Ni, Cd, Zn, and Cr, were identified as key contributors to compromised sediment quality. These metals inhibit crucial sediment enzymes, such as CMC-cellulase, β-glucosidase, aryl sulfatase, urease, and phosphatases, essential for nutrient cycling and organic matter decomposition. A negative correlation was found between heavy metals and biodiversity, as indicated by the Shannon index, and a similar trend was observed with fungal load. The study highlights the adverse effects of persistent trace metals on the fungal community, potentially disrupting the mangrove ecosystem and suggests using manglicolous fungi as biological indicators of environmental health.

1. Introduction

Mangroves, serve as vital ecosystems with immense ecological importance, renowned for their rich biodiversity and high productivity (Charrua et al., 2020). These ecosystems play an essential role in stabilizing coastlines, filtering water, and contributing significantly to the global carbon cycle. However, mangroves are highly dynamic environments, subject to frequent fluctuations in salinity, nutrient levels, and water availability, which they must constantly adapt to in order to survive (Subramanian et al., 2023). Their most fundamental role lies in stabilizing coastlines, filtering water, and sustaining both the food chain and the carbon cycle (Mistri and Das, 2020). Among the world's most remarkable mangrove ecosystems is the Indian Sundarbans, a vast and unique ecosystem located in the delta of the Bay of Bengal, in southern West Bengal (Hassan et al., 2019). Recognized as a UNESCO World Heritage site, the Sundarbans are distinguished by their rich biodiversity and unique ecological features (Chowdhury et al., 2019). The Sundarbans' mangrove forests are home to an extraordinary variety of life forms (Mahanty et al., 2019a, 2019b, 2020, 2021, 2023) from all domains, including a diverse range of halotolerant and manglicolous organisms (Chakraborty et al., 2015). In addition to fungi, the microbial community in these mangroves includes bacteria, archaea, and other microorganisms that play essential roles in nutrient cycling and contamination mitigation (Basak et al., 2015). Among these organisms, mangrove fungi, or "manglicolous fungi," are notable for their high tolerance to environmental stress and for secreting key extracellular enzymes like cellulase, amylase, pectinase, peroxidase, and ligninolytic enzymes, which facilitate the decomposition of mangrove litter (Abo Nouh et al., 2021; Bhadra et al., 2022; Mahanty et al., 2021; Mishra et al., 2019; Parte et al., 2017). These processes help regulate the inorganic content in sediments and maintain overall ecological stability (Keuskamp et al., 2015). Mangrove sediments, particularly those found in anaerobic

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environments, serve as crucial sinks for waterborne metallic ions, which settle in the sediment after tidal inundation (Ghosh et al., 2021a, 2021b; Islam et al., 2022; Mahanty et al., 2019a). Fungal enzymes are particularly effective in these sulphide-rich sediments, where they accelerate the microbial degradation of organic matter, leading to the formation of organometallic complexes (Bakshi et al., 2018). This process not only ensures the widespread distribution and accumulation of metal ions across surface sediments and biotic substrates, but also facilitates the smooth functioning of the metal biogeochemical cycle (Berde et al., 2022). Such natural processes enable the flow of metal ions through different trophic levels, ensuring the ecosystem's health and resilience (Ghosh et al., 2021a). Unfortunately, the Sundarbans' delicate balance has been disrupted by rapid industrialization upstream of the estuarine belt, which has led to the influx of metallic contaminants into the mangrove ecosystem's sediments through riverine chains (Sharma et al., 2022). Industries such as leather processing units, mining operations, thermal power plants, and various metal-based engineering sectors have become major contributors to anthropogenic contamination (de Almeida Ribeiro Carvalho et al., 2022). These contaminants include potentially toxic metals (PTMs) and hazardous radioactive materials, which pose serious threats due to their non-biodegradable nature, high bioaccumulation potential, and toxicity across the ecological pyramid (Mishra et al., 2023). The presence of these contaminants in the sediment disrupts the ecological functioning of the mangroves by interfering with key enzymatic processes within microbial populations. The accumulation of toxic metal ions, in particular, has a profound impact on fungal communities within mangroves, acting as interfering agents that hinder the synthesis and activity of extracellular enzymes (Mahanty et al., 2021). This inhibition reduces the efficiency of organic matter degradation, delays the formation of organometallic complexes, and affects the nutrient cycling necessary for ecosystem health (Islam et al., 2022). Moreover, heavy metals not only interfere with enzymatic activity but also alter the structure and composition of fungal communities (Caracciolo et al., 2021) by favouring metal-tolerant species and suppressing the growth of others (Chot and Reddy, 2022; Frac et al., 2018; Mahanty et al., 2021). Given the critical role of fungi in sustaining plant health and overall biodiversity in the Sundarbans, any disturbance in their functioning can have significant, far-reaching consequences for the ecosystem.

In our current research, the primary objective was to assess the load of PTMs in sediment samples from the mangrove habitat. We employed various indices such as the Enrichment Factor (EF), Geo-accumulation Index (Igeo), Contamination Factor (CF), and Pollution Load Index (PLI) to gauge the intensity of contamination. These indices provided a clear understanding of the levels of metal contamination in the sediment and offered insights into the potential environmental risks associated with these pollutants. Additionally, we utilized the Potential Ecological Risk Index (PERI) to assess the ecological risks posed by metal contamination in the sediment, evaluating how these contaminants might affect the biological integrity of the mangrove ecosystem. Building on these findings, the second phase of our study focused on quantifying enzymatic activities within the sediment, specifically measuring CMC-cellulase, β-glucosidase, aryl sulfatase, urease, phenol oxidase, and both alkaline and acid phosphatase activities. These enzymes, primarily released by fungi, play a critical role in maintaining the geochemical cycles of the sediment and breaking down organic matter to sustain the ecosystem's nutrient flow. By analyzing the enzymatic activity in the sediment, we aimed to understand the extent to which PTM contamination interferes with these essential biological processes. In addition to studying enzymatic activity, we also examined the fungal diversity within the mangrove sediments. Using metagenomic profiling techniques, we conducted an extensive survey of the fungal communities present, correlating this data with the observed PTM levels in the sediment. Our aim was to assess the impact of metal contamination on fungal diversity, particularly focusing on how contamination affects the health and functioning of fungal populations in the ecosystem. The correlation of enzymatic and fungal diversity data with PTM levels marks a novel approach to understanding the interactions between microbial communities and environmental contaminants.

Our research represents the first instance of correlating sediment PTM levels with fungal diversity and enzymatic activity to comprehensively assess fungal health in a mangrove ecosystem. The data obtained from this study offer valuable insights into the potential of manglicolous fungi as biological indicators of environmental health in vulnerable mangrove habitats. Given the critical role fungi play in maintaining the ecological balance of mangroves, understanding the factors that disrupt their function is essential for the long-term conservation of these ecosystems.

In conclusion, our study provides a foundational dataset that can be used to predict the effects of heavy metal contamination on microbial communities in mangrove ecosystems. The fungi within these ecosystems, particularly those adapted to the unique conditions of mangroves, serve as vital sentinels for detecting early signs of environmental stress. As industrial activities continue to threaten mangroves globally, the importance of monitoring and preserving these ecosystems becomes ever more urgent. The findings from this research underscore the need for proactive conservation measures to protect the Sundarbans' unique biodiversity and ensure the long-term health of these critical habitats.

2. Material and methods

2.1. Sediment sampling

The sampling procedure involved the collection of triplicate sediment samples (500 g) from surface (15 cm depth) sediments within the Indian Sundarbans mangrove wetland, situated on the northeastern coast of India. The samples (Fig. 1) were collected from Kalidashpur (S1: 22.16579722 N 88.91775833 E), Balir Dweep (S2:22.08613889 N 88.69986389 E), Dhonchi (S3: 21.69901111 N 88.43161389 E), Gangasagar (S4: 21.65164722 N 88.15033056 E) during the pre-monsoon period 2021–2022. The sampling locations S2 and S3 are on uninhabited islands dominated by mangrove land-cover, while S1 and S4 are on inhabited islands characterised with agricultural production. Locations S3 and S4 are located in the Hooghly estuary and are characterised by lower tidal influence and stronger influx of freshwater from the land, while locations S1 and S2 are further inland away from the BoB and exposed to stronger tidal influence (Chatterjee et al., 2013). At each station, samples were collected in triplicate from the designated depth, and each individual collection was thoroughly mixed to ensure homogeneity, resulting in the creation of a representative composite sample for subsequent analysis.

Upon collection, approximately 500 g of sediment were collected for both metal and microbial analysis. The samples were immediately placed in sterile, rectangular polypropylene containers (1-liter capacity), with secure, tight-fitting lids to prevent contamination. To preserve microbial integrity, the samples were stored in an ice incubator during transportation, ensuring they remained at low temperatures. Once at the laboratory, they were flash-frozen in liquid nitrogen and then stored at an ultra-low temperature of −80 °C until further analysis. For metal and physicochemical parameters, a portion of the sediment samples was air-dried and then further dried in an oven, maintaining a consistent temperature of 60 °C to prevent any alteration in metal composition. After drying, the samples were ground to a fine powder and sieved through a 75 μm sieve to ensure uniform particle size. This careful preservation and preparation process, combining ice incubation, flash-freezing, oven drying, and sieving, ensures both the microbial community and sediment properties are preserved for comprehensive and accurate analyses in subsequent investigations.

2.2. Physiochemical characterisation of the sediment sample

The sediment samples underwent a series of analyses to characterize

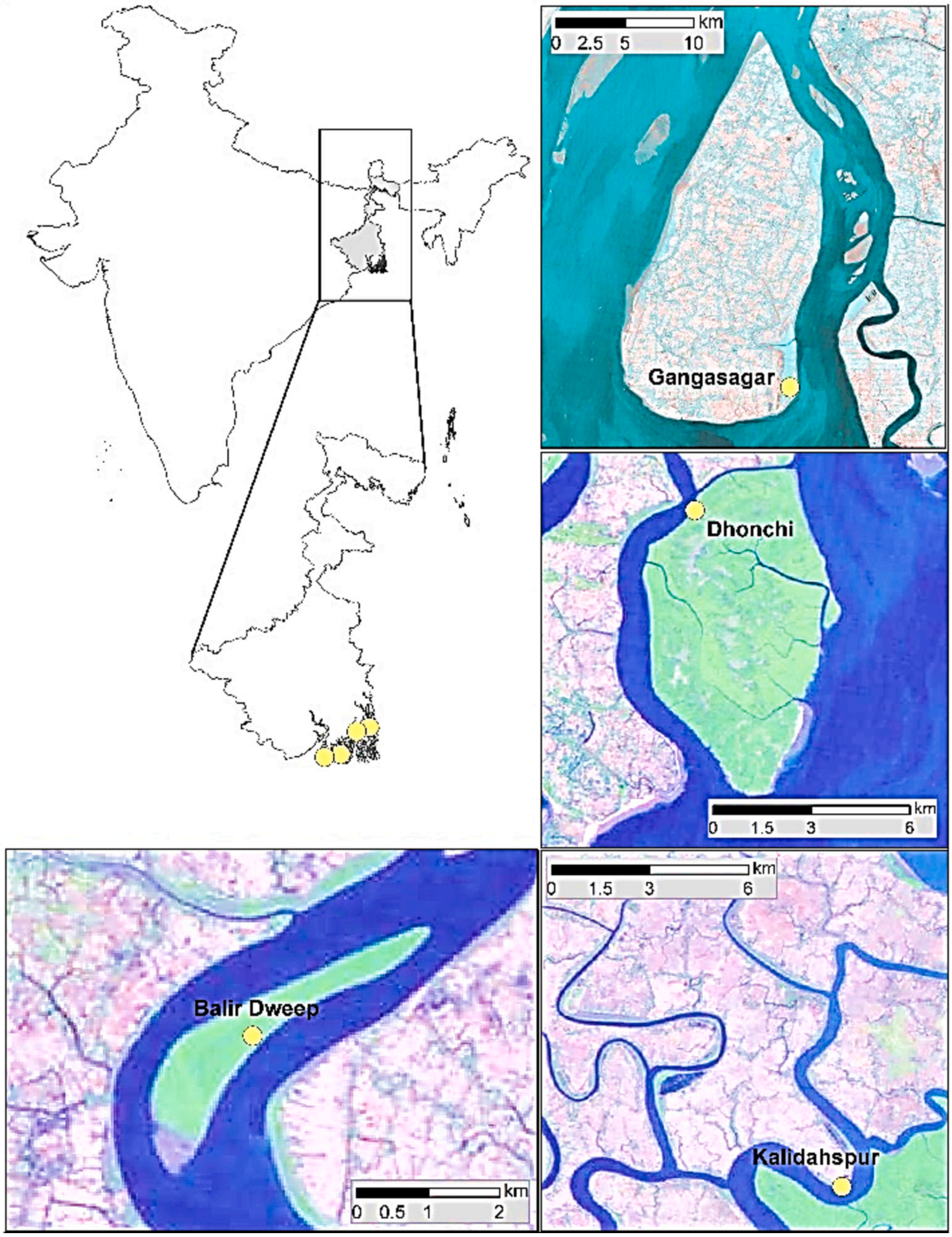

Fig. 1. Sampling sites.

their physical properties. Sediment texture was analysed following the ASTM standard method (Mozaffari et al., 2022). Prior to analysis, the samples were ground using an agate mortar and pestle to ensure uniform particle size. Sediment pH and electrical conductivity were measured using the Thermo Fisher Scientific Orion STAR A329 multi-parameter device (Sparks et al., 2018). To determine sediment salinity, Mohr's method, also known as the argentometric method, was employed (Fortuny et al., 2008). The organic carbon and organic matter content in the sediment samples was estimated using the rapid titrimetric method (Mahanty et al., 2021).

For the chemical properties, metal content analysis was conducted following a di-acid digestion process, as detailed in our previous publication (Mahanty et al., 2021). Ground sediment samples (400 mg) were digested with 8 ml of a 1:1 HNO_3 (EMSURE grade) solution in a Teflon vessel for 1 h at 200 °C, following the modified USEPA 3051 protocol. After cooling, the sediment samples were diluted to 100 ml in a cleaned volumetric flask using ultrapure water (18.2 mS cm^{-1}). The solutes were then filtered through Axiva 0.2 μm PTFE filter paper and stored at 4 °C in 50 ml polypropylene tubes. Metal analysis was performed using an inductively coupled plasma optical emission spectrometer (ICP-OES), specifically the Thermo Fisher Scientific Model iCAP 7000 Series. Plastic and glassware were meticulously cleaned with a 10 % v/v HNO_3 acid solution, followed by multiple washes with Milli-Q water and air-drying. Merck standard solution IV and IX were employed during the analysis, and a series of washing steps with Milli-Q water was carried out to ensure accuracy. The quality of the data generated by ICP-OES was verified using standard reference material (River Sediment-SRM 1645) from the National Institute of Standards and Technology (NIST). The accuracy of the analysis, expressed as percentage recovery, ranged between 97.84 % and 107.13 % for all metals (Table S1), while the precision, represented by a relative standard deviation of <5 %, was achieved for all metals. The concentration of metals was reported in mg/kg. (Fortuny et al., 2008; Mahanty et al., 2021, 2023; Mozaffari et al., 2022; Sparks et al., 2018).

In our study, we conducted an analysis of sediment quality indices to gain insights into the elemental contamination of the sediment, particularly in relation to geochemical standard background values provided in Table S2. This approach allows us to assess the influence of anthropogenic factors on specific sediment systems.

To comprehensively understand the sediment health of the system, we employed key indices, including Enrichment Factor (EF), Geo-accumulation Index (Igeo), Contamination Factor (CF), and Pollution Load Index (PLI) (as detailed in Table S3). Enrichment Factor (EF) calculations utilized Aluminium (Al) as the reference element. For Igeo calculations, geochemical background values in the Upper Continental Crust (UCC), as established by Taylor and McLennan (1985).

The CF serves as an indicator of the extent to which metals have accumulated in sediments. It is determined by comparing the metal content in the sediment to its natural baseline considering the methodology described by Bakshi et al. (2021), Chakraborty et al. (2014), Ghosh et al. (2021a), Ghosh et al. (2019), Mahanty et al. (2021) and expressed as:

$$CF = Cs/Cb \tag{1}$$

C_s is the concentration of the metal in the sediment and C_b background concentration of the metal in the sediment. Here, the natural background represents the metal's typical concentration in sediments without human interference. In the absence of established background values for Indian estuarine systems, the upper continental crust (UCC) concentrations (Taylor and McLennan, 1985) were adopted as a reliable proxy for these natural levels. This approach provides a reference point for assessing anthropogenic impact on sediment contamination. Furthermore, we assessed the Potential Ecological Risk Index (PERI) for specific metals in the mangrove habitat (Guo et al., 2015; Hakanson, 1980). This comprehensive suite of indices provides a nuanced understanding of the ecological impact of metal contamination in the sediment, allowing for a more holistic assessment of environmental health and potential risks associated with anthropogenic influences.

$$Er = Tr \times CF \tag{2}$$

$$PERI = \sum_{i=1}^{m} ER_i \tag{3}$$

where, Er is the risk factor of individual metal, CF is contamination factor; Tr is the Toxic Response Factor (TRF) of Zn, Cr, Cu, Pb and Cd ions (Hakanson, 1980) and m is the no. of toxic metal ions.

In our assessment of sediment quality, we employed Sediment Quality Guidelines (SQG) outlined in Tables S4 and S5. These guidelines encompass two key ranges: Effect Range Low (ERL) to Effect Range Median (ERM), and Threshold Effect Level (TEL) to Probable Effect Level (PEL). This categorization methodology, established by Long and MacDonald (1998) and further detailed by Macdonald et al. (1996), allows us to evaluate the toxicity and risk levels associated with sediment contamination.

The concentrations of metals within the range defined by the TEL and ERM values serve as indicators of ecological risk. Specifically, when metal concentrations fall within this range, it suggests a lower ecological risk. Conversely, concentrations surpassing this range indicate a higher ecological risk. This approach (Long et al., 1995), provides a systematic framework for interpreting the potential environmental impact of metal concentrations in sediments, facilitating a nuanced understanding of the ecological risks associated with the studied environment.

2.3. Metabarcoding analysis of the sediment samples

2.3.1. DNA extraction and metabarcoding library preparation

DNA was extracted from 250 mg of mangrove sediment (collected in triplicate from sampling point and pooled together) using DNeasy Power SoilPro Kits (Qiagen, USA), and its concentration was measured with a Qubit Fluorimeter (V.3.0). The Qubit analysis revealed concentrations ranging from 2 to 6 ng/μl for all sediment samples, resulting in a total eluted DNA concentration of 40–50 ng. Evaluation of DNA purity through photometric measurements of 260/280 nm and 260/230 nm ratios indicated values exceeding 1.8 for all samples, indicating suitable purity for subsequent PCR targeting the ITS (Internal Transcribed Spacer) region of the fungal 18S RNA genome. PCR amplification of the ITS1-ITS2 inter-transcribed region (White et al., 1990) was carried out using the QIAGEN UCP Multiplex PCR Master Mix (USA) with ITS1 Forward primer (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS2 Reverse primer (5′-GCTGCGTTCTTCATCGATGC-3′) at a concentration of 0.25 μM each, and 5 ng of template DNA. The cycling conditions included an initial activation step at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 10 s, annealing at 50 °C for 20 s, and extension at 72 °C for 20 s. A final extension step at 72 °C for 10 min ensured complete amplification of the target region. Post-PCR, the 18S amplicons were purified using a bead-based method to eliminate residual primers, nucleotides, and contaminants. Visualization of PCR products on a 2 % agarose gel was followed by gel purification to remove non-specific amplifications. Subsequently, 5 ng of the purified PCR product was subjected to library preparation using the NEBNext Ultra DNA Library Preparation Kit. The prepared library underwent quantification and quality assessment using the Agilent 2200 TapeStation system. Finally, sequencing of the library was performed on the Illumina HiSeq 2500 platform, ensuring accurate sequencing of the ITS1-ITS2 inter-transcribed region for downstream analysis.

2.3.2. Bioinformatic analysis

Bioinformatic analysis was performed on a Linux based server at Bournemouth University and RStudio (v 4.1.3). FastQC (v 0.11.8) (Andrews, 2019) and MultiQC (Ewels et al., 2016) was used to analyse the quality of raw sequences. Sequences were filtered using Cutadapt (v 3.7) (Martin, 2011) to only include those that contained forward and reverse primer sequences across both read pairs for each fragment and primer regions were identified removed from each fragment for the remaining sequences. Approximately 99 % (533808) of the reads from Dhonchi sample site did not pass through the Cutadapt filters, signalling that a significant amount of reads did not contain the taxa specific primers. This could be a result of non-specific amplification that were erroneously sequenced or potential manipulation of raw files.

The sequences were denoised (sequence quality control; trimming, filtering and removal of chimeras) using the DADA2 pipeline (v 1.16) (Callahan et al., 2016) in Rstudio. Default parameters of DADA2 were used unless otherwise stated. After manual examination of the read quality profile from the FastQC results, forward reads were trimmed at 230 bp using the 'filterAndTrim' function in the 'DADA2' package, while the reverse reads were trimmed at 200 bp. After denoising an amplicon sequence variant (ASV) by sample table was produced. The denoised ASVs were used for taxonomic assignment using the 'assignTaxonomy' provided within the 'DADA2' package. This function uses a naïve Bayesian classifier algorithm to assign taxonomy to each ASVs using the UNITE reference database (general FASTA release for eukaryotes, ver. 9) (Abarenkov et al., 2024).

2.3.3. Statistical analysis of metabarcoding data

Rarefaction curves were computed and visualised using 'vegan' (v 2.6-2) (Oksanen et al., 2022) and 'ggplot2' (v 3.3.6) (Wickham, 2016) packages in RStudio. The dataset was rarefied to even depth at 100 reads prior to alpha diversity analysis to ensure even sampling (standardises library size across samples) throughout the dataset which is absent in amplicon sequence data (Weiss et al., 2017) using function 'rarefy_even_depth' available in 'phyloseq' package (v 1.38.0) (Ewels et al., 2016; McMurdie and Holmes, 2013) on RStudio. The resulting boxplots were visualised in 'ggplot2'. Samples with read counts <100 were not included hence the exclusion of Dhonchi sample site in alpha diversity analysis.

For subsequent analysis, ASV counts were transformed to proportional data and used in downstream analysis. Taxa with abundance <0.00001 were removed from the dataset as a default as stated in the phyloseq workflow (McMurdie and Holmes, 2013). The sequence data are available at NCBI read repository under the accession number PRJNA918540.

2.4. Enzyme analysis of the sediment samples

Enzymatic activity in the sediment, primarily driven by fungi, represents a fundamental component of ecological processes within various ecosystems (Mahanty et al., 2021). Fungi release a spectrum of enzymes into the sediment, each serving a specific function in the breakdown and transformation of organic matter. Monitoring these enzymatic activities provides insights into the health and functioning of sediment ecosystems and their response to environmental changes or disturbances (Frac et al., 2018). In our study CMC-cellulase, β-glucosidase, aryl sulfatase, urease activity, phenol oxidase, alkaline and acid phosphatase activities were assessed in the sediment samples of the mangrove habitat. To assess CMC-cellulase activity, 1 g of fresh sample underwent incubation with toluene, followed by reaction with 0.5 % carboxy methyl cellulose (CMC) in acetate buffer (50 mM, pH 5.5). After a 48-hour incubation at 30 °C, CMC-cellulase activity was determined using the Somogyi–Nelson method (Deng and Tabatabai, 1994). Controls were meticulously designed to subtract native sediment reducing sugars and any trace amounts produced by the chemical hydrolysis of CMC during incubation. The resulting CMC-cellulase units were estimated as mg of glucose kg^{-1} h^{-1}.

Subsequently, alkaline and acid phosphatase activities of sediment samples were assessed. Using 1.5 g of fresh sediment, the samples were reacted with 0.05 M para-nitrophenyl phosphate (pNPP) (Verchot and Borelli, 2005). This process released yellow-coloured para-nitrophenol (pNP) from the substrate, and the color intensity was measured at 410 nm. Enzyme activity units were calculated based on mmol of pNP released kg^{-1} h^{-1}.

To assay β-glucosidase activity, 1.5 g of fresh sample was dissolved in 0.05 M acetate buffer (pH 5). The slurry was then reacted with pNP-β-D-glucopyranoside (0.005 M), and after 2 h of incubation, 1 N NaOH was added to the clear supernatant (Verchot and Borelli, 2005). The color intensity was measured at 410 nm, and the units were calculated as mmol of pNP released kg^{-1} h^{-1}.

Aryl sulfatase activity (Whalen and Warman, 1996) was determined by incubating 0.5 g of air-dried sample in toluene at 20 °C for 1 h, followed by reaction with 0.5 M acetate buffer at pH 5.8 and 0.05 M para-nitrophenyl sulphate at 37 °C for 1 h. Activity was determined by spectrophotometry at 400 nm, with enzyme units evaluated as mg of pNP released kg^{-1} h^{-1}.

Urease activity (Kandeler and Gerber, 1988) was assayed by dissolving 2.5 g of fresh sample in 0.08 M urea solution, and after 2 h of incubation, Na salicylate/NaOH and 0.1 % Na dichloroisocyanurate were added to the clear filtrate. Absorbance was measured at 690 nm after 30 min of incubation, and the units of urease were formulated as mg of ammonia–nitrogen released kg^{-1} h^{-1}.

Considering the prevalent oxygen constraints and anoxic conditions in mangrove environments, phenol oxidase activity (Bach et al., 2013; Gallo et al., 2004) was used as an indicator. One gram of fresh sediment was dissolved in bicarbonate buffer (pH 8), creating a sediment slurry mixed with 8 mM L-DOPA, incubated for 3 h at 20 °C. The supernatant was measured for absorbance at 475 nm, and phenol oxidase activity units were calculated as mmol of dopachrome released kg^{-1} h^{-1}.

All spectrophotometric analyses were meticulously executed using a SmartSpec Plus spectrophotometer (Bio-Rad, California, USA). These enzyme assays provide a detailed insight into the microbial functional potential and dynamics within the Sundarbans mangrove wetland sediments.

2.5. Isolation of culturable fungal isolates from sediment samples

The sediment samples collected from various sampling locations were handled with extreme care to ensure a sterile environment for fungal isolation. Aseptic pooling of these samples was conducted inside a biosafety cabinet (Class II B2), minimizing the risk of contamination during the procedure. Subsequently, serial dilutions were performed, reaching up to a 10^{-2} dilution. The dilution matrix, sourced directly from the sampling site (water samples in the intertidal zone), was first centrifuged at 10,000 rpm to remove larger particulates, followed by filtration of the supernatant using a 0.2-micron membrane filter to eliminate any smaller debris. To further ensure sterility, the entire matrix was autoclaved at 15 Psi and 121 °C for 45 min, guaranteeing that all potential contaminants were eradicated (Mahanty et al., 2021). This meticulous preparation of the samples was crucial in maintaining the integrity of the fungal isolation process.

Inoculation of the prepared culture media was carried out under sterile conditions. Modified Potato Dextrose Agar (PDA) was used, enriched with a sterilized diluting matrix to mimic the natural sediment environment, and supplemented with 100 μg l^{-1} of ampicillin to inhibit bacterial growth, thus favouring the growth of fungal species. A 100 μl aliquot from the serially diluted sediment samples was evenly spread onto the plates. These plates were then incubated in a Biobase BJPX-S48 incubator, maintained at 30 ± 2 °C for 72 h, allowing optimal growth conditions for fungal spores to germinate and form visible colonies.

After the incubation period, fungal colonies were carefully examined, and distinct isolates were selected for further study. These isolates were meticulously separated using pure culture techniques to ensure no cross-contamination between different species. Each fungal colony was sub-cultured onto fresh media in two distinct sets. The first set was stored at 4 ± 1 °C, where the isolates could be preserved for extended periods without losing their viability. This long-term storage allowed the preservation of a diverse fungal library, which could be used in future ecological studies or for reference purposes in subsequent research efforts.

The second set of isolates was reserved for experimental purposes, as outlined in our previous works Mahanty et al. (2020, 2021). This systematic approach ensures the maintenance of fungal isolates for both archival purposes and ongoing experimental investigations.

2.6. Examining the relationship between environmental factors and the total fungal load of the sediments

Principal component analysis (PCA) was performed in RStudio to assess the relationship between environmental factors (pH, salinity, organic matter content, and electrical conductivity), PTMsand total fungal load in sediment. The analysis utilized the ‘prcomp’ function in base R, and results were visualised using ‘biplot’. Detailed code can be found in the supplementary section of the manuscript.

3. Results and discussion

3.1. Sediment quality

The detailed sediment characteristics illustrated in Fig. 2 are vital indicators for assessing mangrove ecosystem health. They reflect natural dynamics and the complex interplay of Hender physical, chemical, and human factors (Mattone et al., 2022). Our research found significant spatial variation in these characteristics across sampling locations in the study region, contributing to the variability in the concentration of heavy metals in the sediment.

In the specific context of the Indian Sundarbans intertidal zone, the sediment generally showcases a mildly alkaline nature, a consequence of diurnal tidal inundation and the prevalence of the carbon dioxide-carbonate system in this unique ecological setting (Burman et al., 2019; Henderson et al., 2021). The recorded pH values in our study ranging

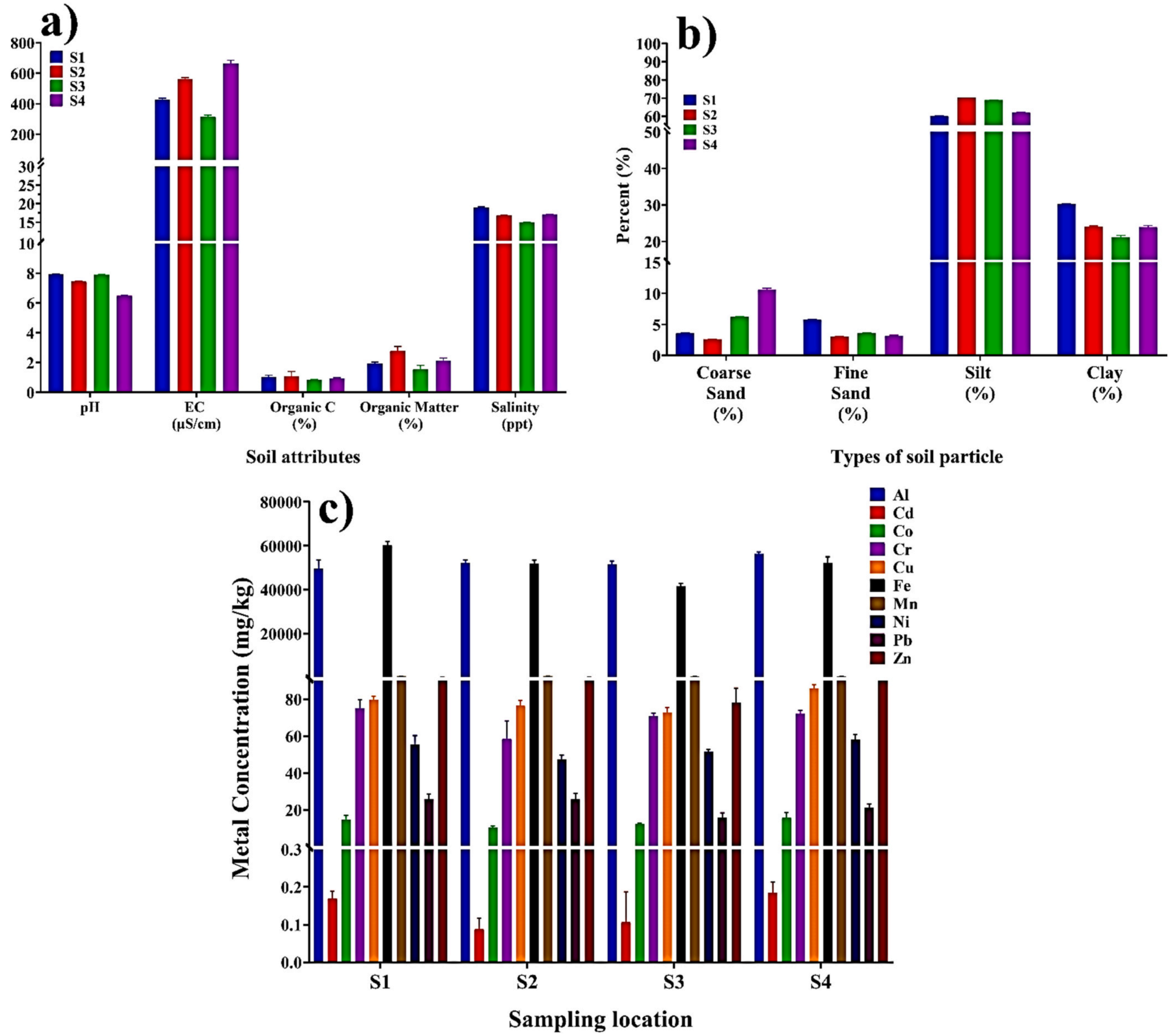

Fig. 2. General characterisation of the soil a) Physio chemical characterisation of the soil b) Soil texture c) concentration of the metals (mg/kg) in the sediment.

from 6.5 to 7.9 indicate an alkaline environment, with the exception of S4 (Gangasagar) which exhibited mildly acidic characteristic. This is potentially due to extensive agricultural activities on this largest and most populous island in the Indian Sundarbans (Mahanty et al., 2021). The use of certain ammonium-based nitrogen fertilizers in this region is known to contribute to sediment acidification (Li et al., 2019; Wu et al., 2023), which, importantly, affects the abundance and solubility of heavy metals in the sediment (Kicińska et al., 2022).

The observed salinity levels between 14.93 and 18.92 ppt are a testament to the brackish water ecosystem characterizing the Sundarbans region (Khan et al., 2020). It could also be an indicator of the degree of the estuarine mixing and the time of the year as the highest salinity levels are generally observed in the pre-monsoon period (Chowdhury et al., 2023), aligning with our sample collection dates in March and April of year 2022.

The electrical conductivity (EC) values within the range of 315 to 663 µS cm^{-1}, along with the oxidation-reduction potential (ORP) values spanning from 192 to 225, shed light on potential microbial activities. These activities, encompassing denitrification, methanogenesis, and sulphate complex reduction, contribute to the transition of sediment conditions from aerobic to anaerobic (Du Laing et al., 2009).

Moreover, the total organic carbon (% OC) levels ranging from 0.83 to 1.09 suggest anthropogenic influences such as agricultural and domestic waste dumping, industrial runoff, contributing to sediment conditions characterised by reduced oxygen levels and anaerobicity (Banerjee et al., 2012). The organic carbon content values obtained in our study area are lower than those reported (2.78 %) in intertidal sediments of diverse tidal and estuarine habitats along the southeastern coast of India (Banerjee et al., 2012). The distribution of organic carbon is generally influenced by sediment physicochemical properties, including texture, pH, grain size, clay mineralogy, and microbial activity, all within the context of local and regional geomorphic and geophysical factors. The lowest % OC in our study was recorded in S3 (Dhonchi) and S4 (Gangasagar), situated in the Hooghly estuary. This could be attributed to robust microbial activity and dynamic tidal forces in this location, indicating a limited adsorption capacity of organic carbon to quartz grains (Bakshi et al., 2018). Grain size analysis stands as a valuable scientific tool for discerning the depositional environment of a region, as underscored by (Kumar and Ramanathan, 2015). In the sediment samples of the Indian Sundarbans, significant variation in grain size composition is evident, with a consistent order of silt > clay > sand (Fig. 2). Within the sediment of the reclaimed islands of the Indian Sundarbans, the sand fraction ranged from 5.7 % to 13.8 %. Notably, there is a pronounced dominance of silt (%) in sampling locations, with percentages ranging between 70.22 % and 60.23 %. This disparity suggests the prevalence of moderate hydrodynamic energy in the estuarine water near the reclaimed islands of the Indian Sundarbans, aligning with the conclusions drawn by Kumar and Ramanathan (2015) and Massolo et al. (2012).

Furthermore, the % of clay fractions in our sampling locations ranged from 21.23 % to 30.25 %. The categorization of sediment under varying magnitudes of hydrodynamic energy conditions consistently indicates a trend of sediment being dominated by silt, substantiating the information presented in Fig. 2. Greater clay content can facilitate increased adsorption of heavy metals in the sediment. Examining the sediment sample through elemental analysis offers valuable information regarding the presence of metallic toxic pollutants within the mangrove habitat. The analysis demonstrated high accuracy, with elemental data showing a recovery range of 97.84 % to 107.13 % and a precision of <2 % for the relative standard deviation of all metals in the Standard Reference Material (SRM), as detailed in Table S1.

The elemental analysis of the sediment unveils variable concentrations of toxic metal ions (Co, Cd, Cr, Pb, Cu, Ni and Zn), with distinct levels observed at different sampling locations. The variation in metal distribution across the sampled locations suggests an irregularity in deposition and erosion rates within this region. The concentration of metals at sites is influenced by various geomorphological factors, including regional siltation, the intermixing of river and sea water leading to coagulation and sedimentation, tidal hydrodynamics, grain size, and others (Zhang and Wang, 2020). In addition to natural sources, external influences such as weathering processes from upstream and the decomposition of organic content play a significant role in shaping the pattern of metal distribution and accumulation (Okrusch and Frimmel, 2020). The comprehensive understanding of these diverse sources contributes to the complex dynamics of metal distribution and accumulation in the studied area.

Prevalence of toxic metals like Co, Cr, Ni, Pb and Zn could be observed in all the sediment samples. Sediments S1 (Kalidahspur) and S4 (Gangasagar) exhibit significantly higher concentrations of Cd compared to S2 (Balir Dweep) and S3 (Dhonchi). Additionally, the concentration of nickel is notably elevated in S4 (Gangasagar) compared to the other sediment samples. The variation of metal ions in the Indian Sundarbans could be observed in a range between 49,556–56,267 mg/kg for Al, 0.8–0.18 for Cd, 10.38–15.65 for Co, 58.66–75.08 for Cr, 72.74–85.83 for Cu, 41,665.67–60,304.33 for Fe, 543.41–634.98 for Mn, 47.34–58.11 for Ni, 15.77–25.87 for Pb and 78.11–98.26 for Zn. Elevated concentrations of iron (Fe) observed across all sampled locations are a common occurrence, et al. 2021 likely attributable to anoxic conditions resulting from the presence of organic content and sulfides (Thomas and Fernandez, 1997). This phenomenon may also be influenced by the textural and mineralogical characteristics of estuarine and/or marine sediments (Ramanathan et al., 1999) and further compounded by the existence of basaltic trappean rocks and laterites (Kumar Sarkar et al., 2004).

The origin of zinc (Zn) may be linked to numerous small and medium industries along the estuary banks, as well as fungicides from agricultural runoff (Khan et al., 2014; Kumar Sarkar et al., 2004). Metals like chromium (Cr), lead (Pb), and nickel (Ni) could be associated with dye use, artificial colors, vehicular emissions, low-grade fuel, petrochemical industries, and oil combustion (Rajendran et al., 2022). Cadmium (Cd) and copper (Cu) may originate from agricultural runoff, domestic and industrial discharge, shipping activities the mismanagement of battery disposal (Chowdhury et al., 2023; Kumar and Ramanathan, 2015). Similar to findings by Kumari et al. (2023), the highest level of Cd and Cu in our study sites were found in the Hooghly estuary (Gangasagar, S4), which is an international shipping zone with the third most populous metropolitan city of India (Kolkata, 14.3 million people, 2011 census) located further upstream.

The Pearson correlation analysis revealed significant interrelationships among the metals Table S6, implying a potential common origin for these metals. The significant correlation between Al with Cu and Ni, Cd with Co, Cr, Cu, Fe and Ni, Co with Cr, Cu, Mn and Ni, Cr with Ni, Cu with Fe, Ni, Pb, Zn, Fe with Ni, Pb and Zn, Mn with Pb and Zn and finally Pb with Zn could be observed. The correlations observed among metals in these studies imply a shared source, underscoring the complex and multifaceted nature of contamination (Bastami et al., 2014). The identification of contamination sources, encompassing tourism, boating, human settlements, and agricultural practices, combined with the release of industrial waste and diverse pollutants, highlights the intricate nature of anthropogenic influences on the riverine environment (Bashir et al., 2020).

In our investigation, we employed a variety of sediment quality indices (specifically EF, Igeo, CF, and PLI) Tables S2 and S3 to discern between the accumulation of potentially harmful elements arising from human-induced factors and those stemming from natural processes like weathering and erosion (Fig. 3).

The Enrichment Factor (EF) was calculated to quantify the extent of human-induced alterations in this intricate estuarine region by normalizing the concentrations of the examined metal against a reference element with low variability in occurrence, such as Mn, Ti, Al, and Fe (Reimann and De Caritat, 2000; Schiff and Weisberg, 1999). In our investigation, Al was selected as the reference element for EF. The EF was found to be in a range between 2.6 and 5.2 for Cd, 1.7 and 2.5 for Co, 1.9 and 2.6 for Cr, 8.4 and 9.7 for Cu, 2.9 and 4.5 for Fe, 2.2 and 2.6 for Mn, 3.1 and 3.8 for Ni, 2.6 and 4.6 for Pb, 3.2 and 3.9 for Zn for all the sampling stations which is higher than 1.5. This suggests that a notable proportion of the trace metals is indeed derived from non-crustal materials or processes unrelated to natural weathering, such as point and non-point pollution, as well as biotic influences.

The Igeo value was computed to assess the extent of contamination in sediments, categorized according to the seven enrichment classes Igeo value was range between −2.19 and 1.18. According to Muller (1969) classification, PTMs like Al, Cd, Co, Cr, Fe, Mn, Ni, Pb, and Zn were assigned to Class 0, indicating no pollution in the intertidal sediments of the mangrove habitats of Indian Sundarbans, except for Cd at site S1 (Kalidahspur) and S4 (Gangasagar). The Igeo values for Cu were categorized as Class II in all the sites suggesting moderate pollution of Cu in the intertidal mangrove sediments. This study emphasizes that the accumulation of PTMs (Al, Cd, Co, Cr, Fe, Mn, Ni, Pb, and Zn) in the mangrove sediments does not inherently have a geogenic origin but is likely attributed to local or upstream human-induced developmental and industrial activities. Conversely, the increase in Cu levels in the intertidal mangrove sediments may be associated with natural geological processes, such as the chemical weathering of source rocks (Achyuthan et al., 2002), in conjunction with anthropogenic influences upstream, including industrial discharges (Banerjee et al., 2012).

The assessment of contamination in the intertidal mangrove sediments involved the calculation of the Contamination Factor (Cf) following (Hakanson, 1980) methodology. Across different sites of Indian Sundarbans, Cf values for PTMs varied between 0.79 and 3.1. According to different classes of contamination factor, Co, Cr, and Mn fell into Class I, indicating a low level of sediment contamination. Cd, Fe, Ni, Pb and Zn with Cf values in Class II, indicated a moderate level of sediment contamination for all the sites. Cu, with Cf values categorized as Class III, suggested significant sediment contamination in all the sites. This study underscores the varying degrees of sediment contamination in the intertidal sediments of Indian Sundarbans. However, if PTMs enter biogeochemical cycles, they may impose adverse effects on local flora and fauna. Bioaccumulation of PTMs in aquatic life forms and sediment-dwelling organisms could pose potential risks to living beings (Bjerregaard et al., 2022).

The overall pollution status of a specified region can be summarized

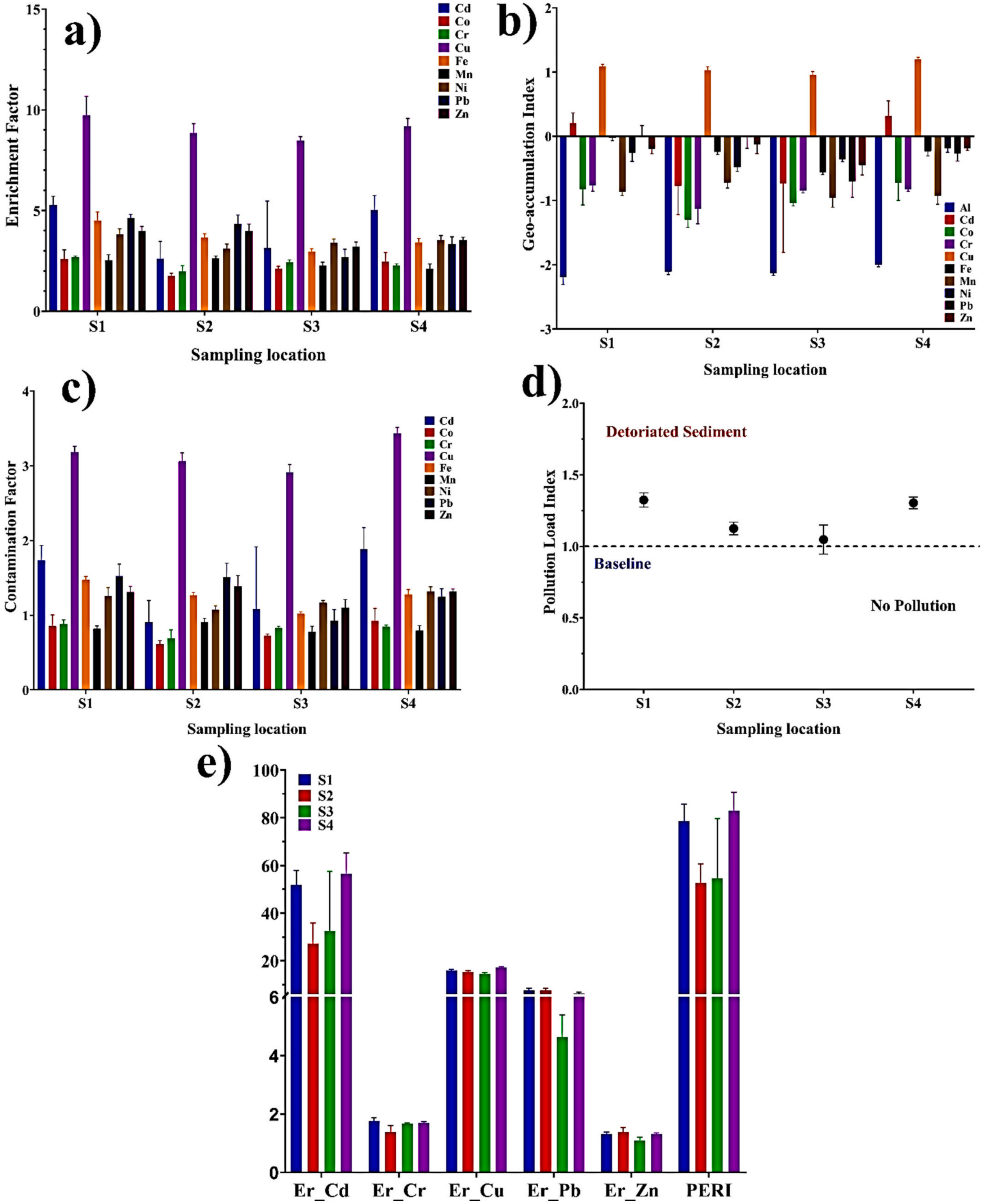

Fig. 3. Sediment quality indices: a) Enrichment factor b) Geo-accumulation Index c) Contamination factor d) Pollution load index. Ecotoxicological risk in the sediment: e) Potential risk of individual metal and potential ecological risk index in habitat sediments.

by calculating the Pollution Load Index (PLI) score for sediment, as outlined by Tomlinson et al. (1980). PLI scores were determined for four locations, ranging from 1.04 (Dhonchi, S3) to 1.32 (Kalidahspur, S1), with most values exceeding the baseline indicating a progressive deterioration in estuarine quality (Fig. 3d). The increasing order of PLI is S3 > S2 > S4 > S1. All sediment quality indices collectively point towards moderate contamination and a progressive decline in sediment quality in the study area. The most contaminated locations are identified as Gangasagar (S4) and Kalidaspur (S1), while the least contaminated site is Dhonchi (S3).

The heightened metal enrichment, including Cd, Co, Cu, Fe, Pb, and Zn, observed in most sampling sites, results from increased upstream natural weathering and unplanned rapid urban-industrial development in the studied region. The sources of contaminants can be attributed to intensive chemical weathering, industrial and domestic waste, gaseous emissions, fishery, shipping activities, and other anthropogenic interferences. Fig. 3 illustrates the potential risk of each studied metal (Er) and their comprehensive potential ecological risk index (PERI). The highest Er values for Cd (56.53), Cr (1.6), Cu (17.16) were observed at location S4 (Gangasagar), while for Pb (7.53) and Zn (1.38), it was lowest at S3 (Dhonchi). The risk category for Cd appeared to be considerable at S1 (Kalidahspur) and S4 (Gangasagar), while other potentially toxic metals (PTMs) were categorized as low risk (Tables S4 and S5).

The PERI values for sediments across the four sites exhibited a wide range from 52.75 (Balir Dweep, S2) to 82.96 (Gangasagar, S4). The risk values indicate 'moderate risk' level. The maximum degree of risk was observed at S4 (Gangasagar), falling within the 'considerable risk category,' warranting special attention. The calculated order of increasing PERI values is S4 > S1 > S3 > S2, with again locations Gangasagar (S4) and Kalidaspur (S1) having the highest risk.

Comparisons with standard threshold values (TEL-PEL and ERL-ERM) further underscore the elevated concentrations of Cr, Cu, and Ni, potentially influenced by proximity to pollution sources, sediment weathering, and the complex physicochemical parameters of the sediment affected by tidal inundation in estuarine habitats (Long et al., 1995; Long and MacDonald, 1998; Macdonald et al., 1996). The concentration of Cd was consistently higher than the TEL in all sampling locations. Similarly, the concentration of Cu exceeded both ERL and TEL values. Finally, Ni surpassed the ERM values in sampling sites S1 (Kalidahspur), S3 (Dhonchi), and S4 (Gangasagar), while aside from these, the ERL, TEL, and PEL values were exceeded at all sampling points. The observed ranges of toxic metal ion concentrations align with prior research findings (Ghosh et al., 2020; Mahanty et al., 2021; Ranjan et al., 2018; Silva Filho et al., 2011). In summary, the most polluted sites in this study, S1 (Kalidahspur) and S4 (Gangasagar), face higher anthropogenic pressure at least partly due to human habitation (both islands are inhabited). Gangasagar (S4), situated in the Hooghly estuary downstream near Kolkata, faces additional pressures through upstream contaminants. The increased sediment acidity, possibly from nitrogen-based fertilizers used in paddy rice cultivation, further impacts metal abundance and solubility at this location. In S1 (Kalidahspur), higher clay content (possibly from stronger tidal inundation) further promotes metal adsorption in the sediment.

The elevated concentrations of heavy metal ions have far-reaching implications on microbial diversity and metabolic activity. The ensuing effects within the microbial population result in the selection of species tolerant to these adverse conditions. Fungi, as one of the microbial organisms exhibiting tolerance to the polluted environment, showcase unique cellular modifications in response to metal pollution (Jiao et al., 2023).

Metal pollution in sediment can have significant effects on fungal diversity, which in turn can impact various ecological processes and functions (Jiao et al., 2023). Different metals can have distinct effects on fungi, and the overall impact depends on factors such as the type and concentration of metals, sediment characteristics, and the specific fungal species involved (Sharma et al., 2022). One of the primary responses to metal pollution is species-specific, with different fungal species exhibiting varying levels of tolerance or sensitivity to metals (Mishra et al., 2023). Some fungi may be more resistant and able to thrive in metal-contaminated environments, while others may be highly susceptible, leading to shifts in fungal community composition (de Almeida Ribeiro Carvalho et al., 2022). High concentrations of metals, such as lead, cadmium, and copper, can lead to a decrease in fungal diversity (Caracciolo et al., 2021). This decline is often attributed to the toxic effects of metals on sensitive fungal species, resulting in a reduction in their abundance. As a consequence, changes in the overall community structure of fungi within the sediment are observed (Frac et al., 2018). Metal pollution can also impair the functional diversity of sediment fungi (Chot and Reddy, 2022). Given their crucial roles in nutrient cycling and organic matter decomposition, disruptions to these functions can have cascading effects on the health and functioning of the ecosystem (Jiao et al., 2023). Mycorrhizal fungi, which form symbiotic relationships with plants for nutrient uptake, may be particularly affected, impacting plant health and growth (Chot and Reddy, 2022). Prolonged exposure to metal pollution can lead to genetic and physiological adaptations in certain fungal species. Over time, this may result in the emergence of metal-tolerant fungal populations, further influencing the dynamics of fungal communities in metal-contaminated sediments (Mahanty et al., 2021). Metal pollution often occurs alongside other environmental stressors, such as changes in pH, temperature, or organic matter content (Zhang et al., 2023a, 2023b). The combined effects of these stressors can exacerbate the impact on fungal diversity, creating a complex web of interactions that shape the sediment ecosystem (Lin et al., 2020). Changes in fungal diversity have broader ecological consequences, affecting nutrient cycling, sediment structure, and the overall stability of the ecosystem (Caracciolo et al., 2021). These effects can extend to higher trophic levels, including plants and animals, emphasizing the need for a comprehensive understanding of the interactions between metal pollution and fungal communities in sediment (Sun et al., 2022; Zhang et al., 2023a, 2023b). Monitoring and mitigating the impacts of metal pollution are crucial for maintaining biodiversity and ecosystem functionality in affected environments.

3.2. *Sequencing results*

A total of 1,156,947 reads were produced targeting the internal transcribed spacer (ITS) region and 543,615 reads passed the quality filter. After primer removal and chimera checking a total of 332,995 reads ranging from 16,951 to 179,186 reads per sample, were retained and represented the final dataset used for taxonomic assignment and statistical analysis. Rarefaction curve showed large variation in the total number of ASVs between the samples where they reached saturation. However, for samples from S3 (Dhonchi) and S4 (Gangasagar) the curves suggest that an increased in sequencing depth would detect additional taxa. The sediment samples were aimed at characterizing fungal diversity at each sample location. Alpha diversity estimates of Chao1 and Shannon Wiener Index depicted higher values for S2 (Balir Dweep) when compared against S4 (Gangasagar) and S1 (Kalidahspur) sampling sites (Fig. 4). As the data was rarefied to a minimum depth of 100 reads, S3 sampling site (Dhonchi) was excluded from alpha diversity estimates and downstream analyses.

After taxonomic assignment and removal of low reads ASVs, 1558 ASVs remained ranging from 230 bp to 359 bp (Fig. S1). Further elimination of unassigned fungal taxa (reads that were assigned to kingdom level, that mainly comprised uncultured and environmental fungi) led to a total of 437 ASVs that were used in downstream analysis. Across all sites a large proportion of ASVs detected in the sediment samples were attributed to taxa from phyla Ascomycota, Basidiomycota and Chytridiomycota (Fig. 5).

Chytridiomycota was the most dominant phyla in sites S2 (Balir Dweep, 57.9 %) and S4 (Gangasagar, 62.2 %), while taxa from order

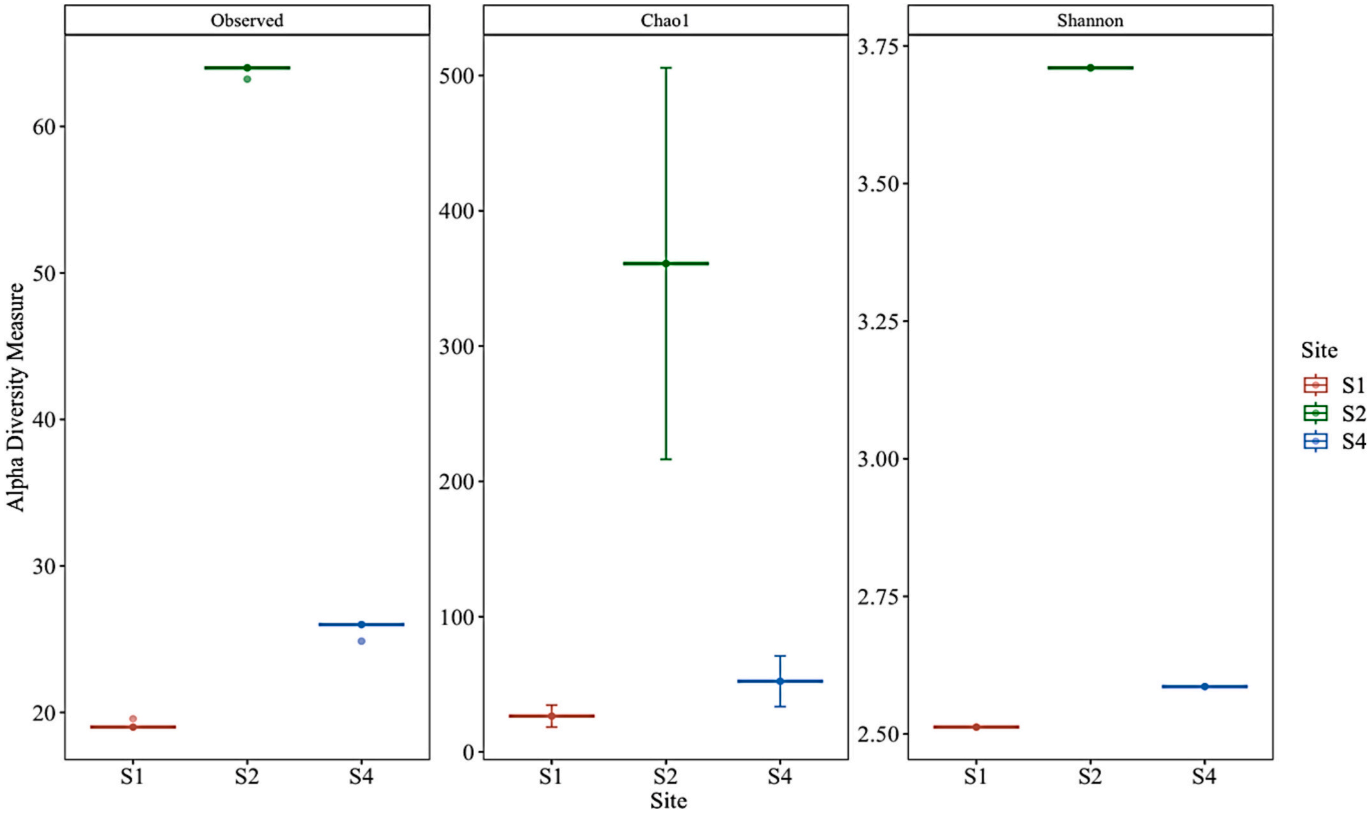

Fig. 4. Diversity measure of the fungal community in three different sites using metabarcoding results from the ITS region. Alpha diversity was measured by Chao1 and Shannon indices.

Pleosporales was dominant in S1 (Kalidahspur, Fig. 5). Hypocreales was the second most dominant taxa in S2 (Balir Dweep, 10.2 %) whereas Agaricales was prevalent in S4 (Gangasagar, 11.1 %) and S1 (Kalidahspur, 13.0 %) (Table S7).

To identify the effects of metal concentration on fungal diversity, alpha diversity calculated using Shannon index was plotted against several metal concentrations including organic carbon (Fig. 6). No clear relationships between diversity and metal concentrations were found.

3.3. Enzyme analysis of the sediment samples

The impact of heavy metal contamination on sediment enzymes in the Indian Sundarbans, a critical ecological region, is a subject of concern for environmental scientists. Heavy metals, including lead, cadmium, and mercury, have the potential to adversely affect sediment enzyme activity, disrupting essential processes such as nutrient cycling and organic matter decomposition (Mahanty et al., 2021). These metals can inhibit enzyme activity either directly, by binding to active sites, or indirectly, by influencing the microbial communities responsible for enzyme production (Karaca et al., 2010; Sharma et al., 2020; Xian et al., 2015). Additionally, alterations in sediment pH due to heavy metal presence can further impact enzyme activity, especially those enzymes sensitive to pH changes (Aponte et al., 2020). The Sundarbans, known for its unique biodiversity, is susceptible to the accumulation of heavy metals, posing potential long-term consequences for sediment health and ecosystem dynamics. To understand the specific effects in this region, sediment enzymes like CMC-cellulase, alkaline and acid phosphatase, β-glucosidase, aryl sulfatase, urease, and phenol oxidase were analysed. CMC-cellulase of concentration 15–30 units (U) was observed for all the sampling station, alkaline phosphatase of 0.04–0.15 U, acid phosphatase of 0.04–0.14 U, β-glucosidase of 0.008–0.014 U, aryl sulfatase of 26–50 U, urease of 27–45 U, and phenol oxidase of 0.6–1.5 was also observed for all the sampling points (Fig. 7a–b).

A Pearson Correlation analysis was done between the PTMs, physiochemical parameter of the sediment and enzyme analysis of the sediment and it was observed that was measured between the values. The positive significant correlation ($p < 0.05$) could be observed between Fe, Mn, Pb, Zn, OC and Salinity with CMC-cellulase, between Fe, Mn, Pb, Zn, OC and Salinity with alkaline and acid phosphatase, between Fe, OC and Salinity with aryl sulfatase, between Cd, Fe, Cu, Pb, Zn, OC and Salinity with urease, and whereas between Fe, Pb, Zn, OC and Salinity with phenol oxidase Table S8.

Organic matter and the sediment texture play a crucial role in binding enzyme proteins, providing protection against adverse environmental conditions. Additionally, it is noteworthy that certain heavy metals serve as activators for numerous enzymes when present in low concentrations.

In contrast, Gülser and Erdoğan (2008) reported significant negative correlations of some heavy metals with arylsulfatase, acid phosphatase, and urease enzymes was observed which complements the finding of this research. The impact of heavy metals on sediment enzymes varies; for instance, Cd can inhibit dehydrogenase, catalase, and urease, while Zn only inhibits urease, and lead inhibits urease to a lesser extent than cadmium or zinc (Wyszkowska et al., 2013). These researchers demonstrated that the combined presence of cadmium, zinc, and lead results in a more pronounced inactivation of enzyme activity compared to the individual metals. The influence on sediment enzymatic activity is further contingent on hydrothermal conditions, specifically temperature distribution and rainfall, as these factors significantly affect microorganisms and the associated enzymatic activity of the sediment (Criquet et al., 2004).

Significant positive relationships were observed between the content of Fe, Mn, Pb, Zn, Cd and OC. Pb, although not highly mobile in sediments and with low availability for plants, can form various complex organic and inorganic compounds that are readily absorbed by plant roots and accumulated. Similar behaviour was noted for zinc, which is

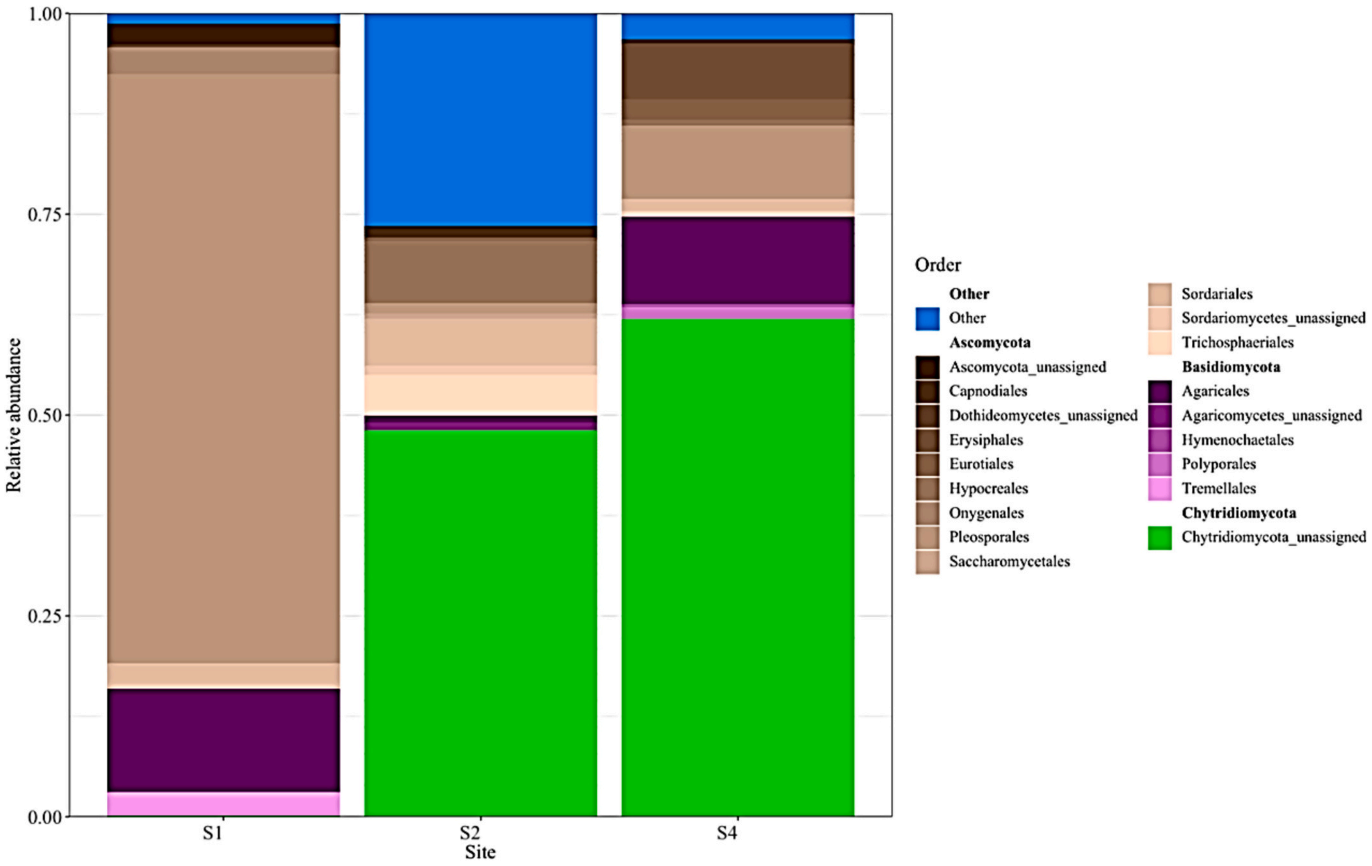

Fig. 5. Relative abundance of the fungal community from sediment samples at order level.

mobile and typically accumulates in the surface layers of mineral sediments and in humus by bonding with organic matter. Meanwhile, cadmium accumulates most abundantly in organic horizons among all metals and easily enters the food chain due to its good solubility and bioavailability (Hussain et al., 2021).

An essential factor contributing to the increased toxicity of heavy metals in sediments is pH. In the examined sediments, a statistically significant negative correlation was identified between Al, Cd, Co, Cr, Cu, and Ni content and pH. Notably, in sediments with a pH above 7.0, phytotoxicity is markedly amplified due to alkaline environment of the sediment.

The influence on sediment enzymes can indirectly manifest the effects on fungal diversity in sediment, with these interconnections being mediated by sediment physical and chemical characteristics as well as the content of heavy metals (Frac et al., 2018). Sediment enzymes, essential players in nutrient cycling and organic matter decomposition, are influenced by factors such as organic matter, pH, and moisture content (Fu et al., 2021). These parameters, in turn, create microenvironments that can favour or inhibit specific fungal species (Hernández and Hobbie, 2010). For instance, the availability of organic matter influences the substrate base for fungi, impacting their growth and enzymatic activities (Zhang et al., 2020). Additionally, sediment pH, a key determinant of enzyme activity, can selectively influence the composition of fungal communities, favouring those adapted to specific pH ranges (Abdu et al., 2016). Furthermore, the presence of heavy metals in the sediment, whether from natural sources or anthropogenic activities, can exert selective pressures on both enzymes and fungi (Kamal et al., 2010). Some fungi exhibit tolerance or resistance to certain heavy metals, influencing their abundance and diversity. Conversely, elevated heavy metal concentrations can suppress enzyme activity and alter sediment conditions, indirectly affecting fungal communities (Pérez-De-Mora et al., 2006). Therefore, the intricate relationships between sediment enzymes, fungal diversity, and sediment characteristics highlight the importance of considering multifaceted interactions to comprehend the nuanced dynamics within sediment ecosystems.

3.4. Effect of physiochemical parameters and heavy metals on fungal load of the sediment

Isolation of manglicolous fungi was done from different location of Indian Sundarban and on surface sediment indicated prevalence of 39 genus (*Alternaria* sp., *Arthrinium* sp., *Aspergillus* sp., *Aureobasidium* sp., *Candida* sp., *Cephalosporium* sp., *Chaetomium* sp., *Chrysosporium* sp., *Cladorrhinum* sp., *Cladosporium* sp., *Colletotrichum* sp., *Corynespora* sp., *Cunninghamella* sp., *Curvularia* sp., *Fusarium* sp., *Gongronella* sp., *Hansfordia* sp., *Helimenthosporium* sp., *Humicola* sp., *Khuskia* sp., *Mucor* sp., *Nigrospora* sp., *Nodulisporium* sp., *Oidiodendron* sp., *Paecilomyces* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Phialemonium* sp., *Phialophora* sp., *Phytophthora* sp., *Pithomyces* sp., *Rhizoctonia* sp., *Rhizopus* sp., *Scolecobasidium* sp., *Stachybotrys* sp., *Trichocladium* sp., *Trichoderma* sp., *Tripospermun* sp., *Verticillium* sp.). Major fungal divisions were found to be Ascomycota, Basidiomycota, Zygomycota, and Oomycota. Fungal load (CFU/g) of sediment on the surface sediment was found to be $336 \times 10^2 \pm 13.95$ from the sediment samples collected from Kalidashpur (S1), $222 \times 10^2 \pm 9.57$ from Balir dweep (S2), $179 \times 10^2 \pm 7.50$ from Dhonchi (S3) and $433 \times 10^2 \pm 19.06$ from Gangasagar (S4) *Aspergillus* sp. and *Penicillium* sp. was found to widely spread species in the entire habitat (Fig. 7c–d).

Pearson correlation analysis (Table S9) of fungal load with sediment physio chemical parameters and PTMs indicated presence negative correlation between fungal load with heavy metals indicates adverse effect of heavy metals on the fungal diversity. The impact of heavy metals and sediment physiochemical parameters on fungal diversity underscores the intricate relationships shaping sediment ecosystems

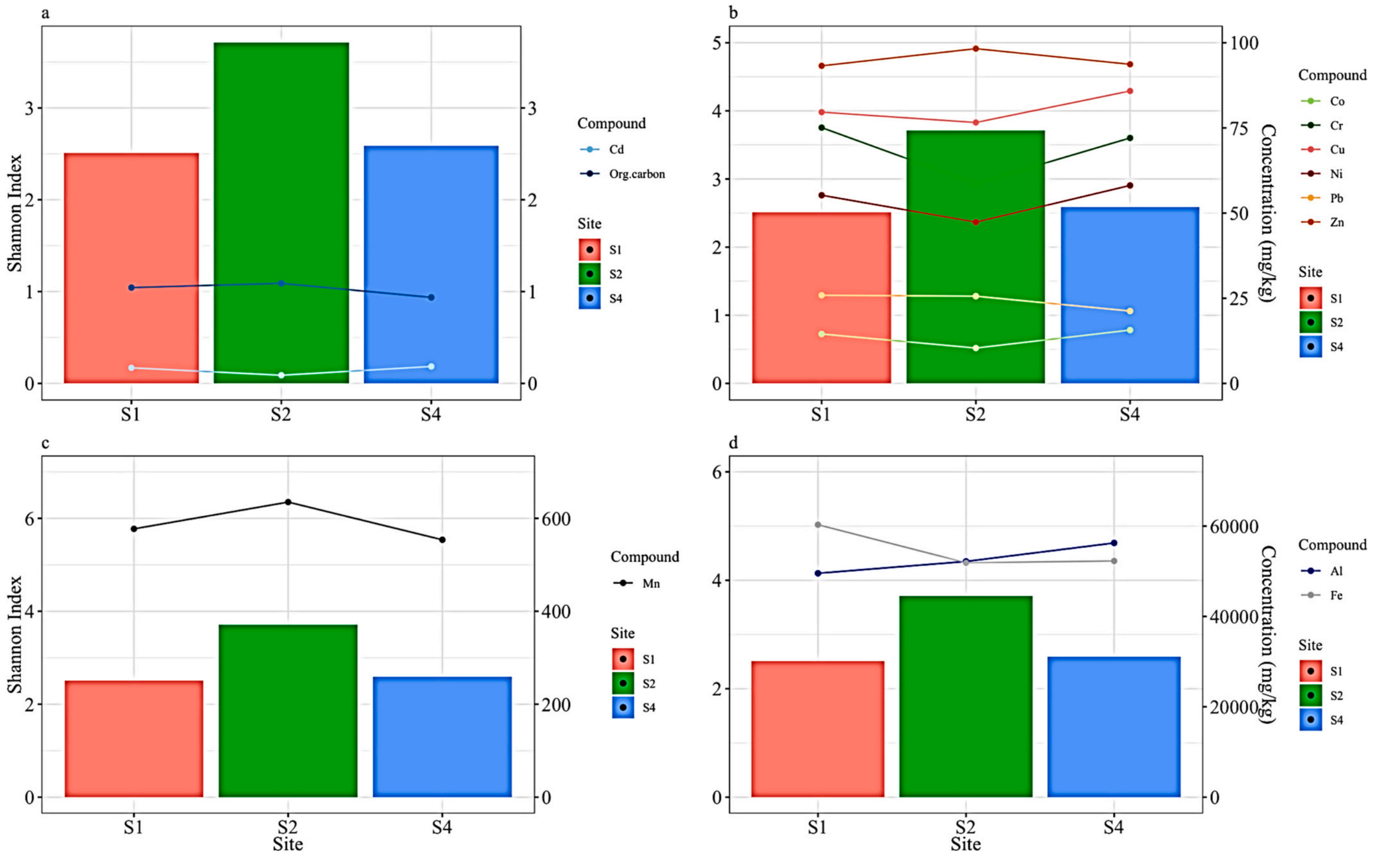

Fig. 6. Bar plot of alpha diversity calculated using Shannon Index for each site. Line chart corresponding to the secondary Y-axis depicts average of metal concentrations.

(Sharma et al., 2020). Heavy metals, whether originating from natural geological processes or anthropogenic activities, can significantly influence fungal communities (Jiao et al., 2023). Some fungi exhibit resilience or adaptability to heavy metal stress, while others may be inhibited or outcompeted (Kamal et al., 2010). The concentrations of heavy metals in the sediment are intricately linked with sediment physiochemical parameters (Abdu et al., 2016). For instance, pH levels play a pivotal role as they affect metal solubility, thus influencing the bioavailability of metals to fungi. Additionally, sediment texture and composition influence metal retention and mobility, indirectly affecting fungal communities (Philippot et al., 2023). The organic matter content of the sediment serves as a crucial factor, influencing both heavy metal interactions and providing a substrate base for fungi. High organic matter content may mitigate the toxic effects of certain heavy metals on fungi (Stefanowicz et al., 2020). Conversely, heavy metals can alter sediment physiochemical characteristics, impacting nutrient availability and sediment structure, which, in turn, influence fungal diversity (Kamal et al., 2010). Therefore, the combined effects of heavy metals and sediment physiochemical parameters on fungal diversity highlight the need for a holistic understanding of these interactions to assess and manage sediment health in diverse ecosystems.

The escalating levels of heavy metal ions might exert a profound impact on both fungal diversity and its metabolic activity. These effects precipitate a selection process within the fungal population, leading to the dominance of species that can tolerate the challenging environmental conditions. Fungi, is among the diverse microbial organisms thriving in polluted environments, exhibit distinctive cellular adaptations that enable them to counteract the adverse effects of metallic pollution in their ecosystems. The overarching mechanism of metal tolerance in fungal cells, encompasses both intracellular and extracellular mobilization of metal ions (Harms et al., 2011; Mahanty et al., 2021; Valls and Victor De Lorenzo, 2002). Intracellular metal mobilization entails the sequestration of metal ions within the vacuole compartment or their binding to metal-binding proteins such as phytochelatin, metallothionein, and glomalin (Harms et al., 2011; Mahanty et al., 2021; Valls and Victor De Lorenzo, 2002). This process also involves the conversion of metal ions into organometallic complexes, which are subsequently volatilized or excreted from the cell using siderophores (Harms et al., 2011). On the other hand, extracellular metal mobilization encompasses biosorption, enzymatic transformation, and the excretion of organic acids (such as citrate and oxalate). These processes increase the solubility of metal ions by releasing H^+ ions, leading to the extracellular dissolution of heavy metal (Mahanty et al., 2021, 2023). In polluted environments, species that lack biochemical defences against pollutants cease to develop and perish. Conversely, tolerant species exhibit stress-induced enzymatic responses that disrupt normal cellular processes, hindering the natural growth of the organism. In contrast, non-polluted environments devoid of stress-induced factors allow normal/sensitive fungal cells to exhibit regular metabolic activity, fostering adequate fungal growth in harmony with the surrounding environment.

3.5. Exploring heavy metal and environmental factors' impact on sediment fungal load

The conducted principal component analysis (PCA) provided significant insights into the dataset's structure. PC1 and PC2 emerged as pivotal components, collectively elucidating 68.4 % of the total variance, with PC1 contributing 38.5 % and PC2 contributing 29.9 %. This highlights the substantial explanatory power of these two components in capturing the dataset's variability. Fig. 8 visually depicts the distribution of samples in the PCA space. Remarkably, distinct clusters are evident

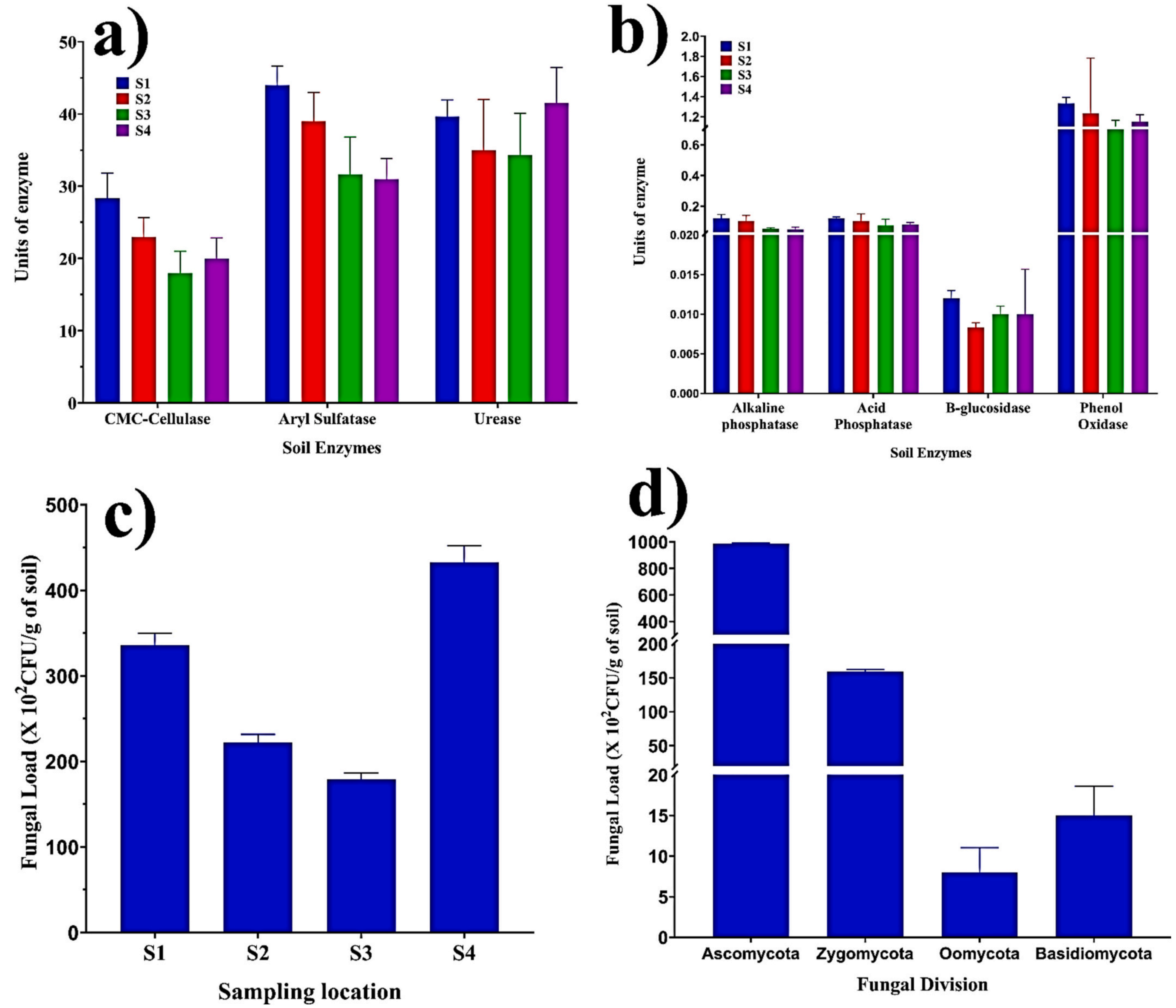

Fig. 7. a–b) Activity of different soil enzyme of sediment samples of Indian Sundarbans c–d) Fungal load at different sampling points.

within each sample site, indicating similar patterns in metal load values among sites. This clustering suggests a localized impact of PTMs contamination on fungal load within sediment across diverse sites. The biplot visualization further clarifies the relationships between variables and sample sites. The direction and length of arrows represent each variable's contribution to the principal components. Predominantly, arrows point towards higher values along PC1, indicating a strong positive association between PC1 and the examined variables (PTMs, OC, pH, EC, salinity, and total fungal load). Particularly, arrows corresponding to metal load variables extend notably towards higher values along PC1, implying a substantial influence of PTMs on fungal populations in sediment samples. Copper (Cu), categorized as a Class III metal, displayed a pronounced effect on fungal load, especially in sites like Gangasagar (S4), which showed high metal contamination. The PCA analysis suggested that Cu had the potential to significantly impact the enzymatic processes in fungi, leading to biodiversity loss. Copper can interfere with the catalytic functions of key fungal enzymes such as cellulase, phenol oxidase, and phosphatase, inhibiting their ability to process organic matter. This enzymatic disruption not only affects nutrient cycling but also creates an environment unfavourable for sensitive fungal species. As a result, only metal-tolerant fungi, such as those from the Chytridiomycota division, thrive under such stress, as observed in S4. Additionally, the pH values in sediment samples correlated with Cu and PTMs, with lower pH levels exacerbating the toxic effects of Cu by increasing its solubility, making it more bioavailable and harmful to the fungal community (Mahanty et al., 2021; Mishra et al., 2019, 2023). This underscores the significant contribution of metal load variations across sample sites to observed fungal load patterns. Additionally, the biplot highlights site S4 (Gangasagar) as having the highest metal load values, aligning with its position on the PCA plot. This suggests that heavy metals at this site exert a particularly strong influence on the observed dataset patterns, likely due to its elevated metal load levels. Interestingly, despite the deteriorating sediment quality in Gangasagar (S4), it exhibits the highest fungal load compared to other regions. This phenomenon can be attributed to the presence of metal-tolerant fungal species that have adapted to the high PTM concentrations. Analysis at the genus level and metagenomic analysis reveal a profound presence of fungi belonging to the Chytridiomycota division in this region, possibly indicating the adaptability of fungal species to PTMs. The resilience of these fungi, particularly those with a high

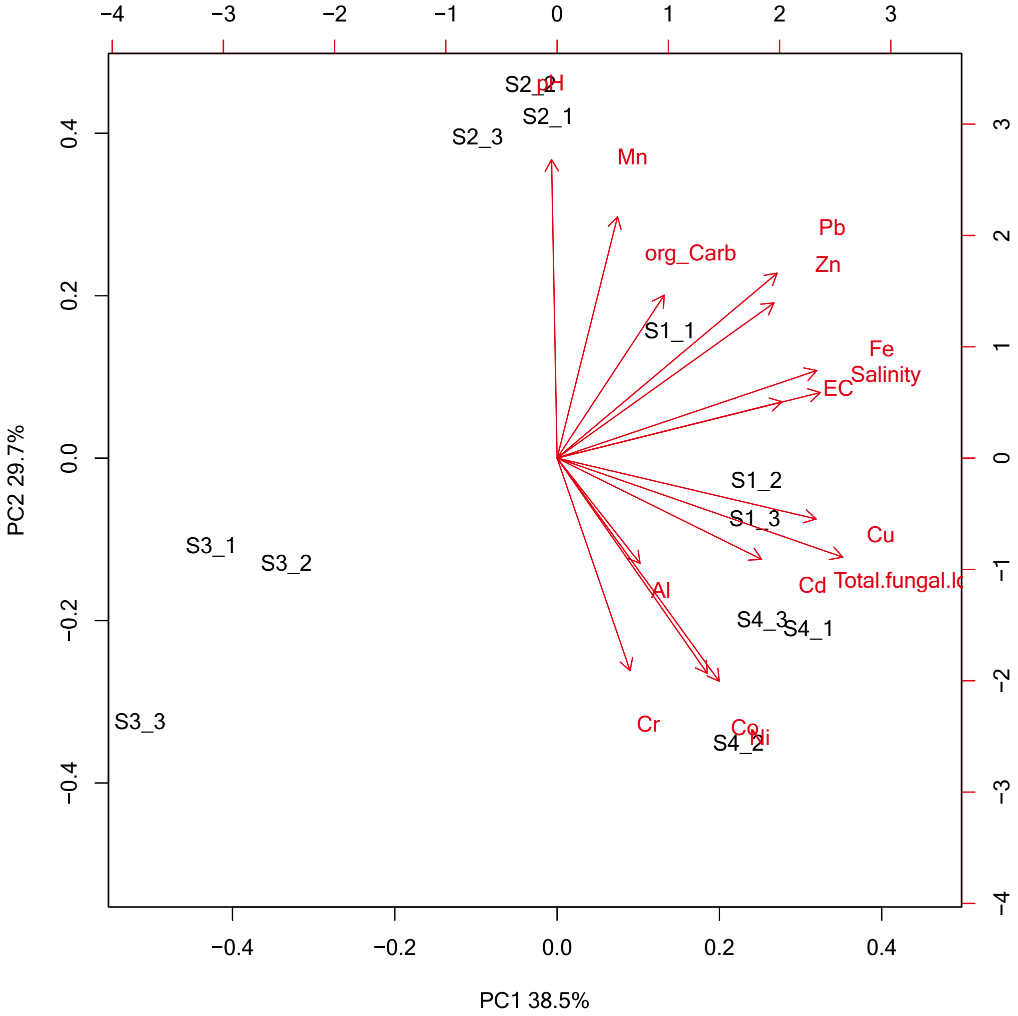

Fig. 8. Principal component analysis of metal loading across three sites.

tolerance to metals like Cu, highlights the selective pressure exerted by PTMs on fungal biodiversity. In summary, the PCA analysis uncovers complex relationships between environmental factors and sediment fungal load, with distinct patterns emerging across sample sites. These findings underscore the importance of considering site-specific environmental factors when assessing fungal communities in sediment ecosystems.

3.6. *Negative impact of the PTMs on fungal population and consequent disturbance of the ecological balance*

The implications drawn from this study regarding the deleterious effects of heavy metals on fungal populations and their severe consequences for the ecological balance in the Indian Sundarbans are of paramount significance. The findings suggest that the presence of PTMs adversely influences the fungal community, essential contributors to nutrient cycling and ecological processes. The negative impact on sediment enzymes, including crucial ones involved in nutrient cycling and organic matter decomposition, indicates a disruption in the ecological functions performed by fungi. This disturbance in the fungal population, integral to maintaining the delicate balance of the ecosystem, can have severe consequences. Firstly, the compromised fungal diversity and altered metabolic activities suggest potential disruptions in nutrient cycling and organic matter decomposition processes (Kamal et al., 2010). These disturbances can lead to imbalances in nutrient availability, affecting the overall health of mangrove vegetation and potentially triggering a cascade of ecological consequences (Chowdhury et al., 2019; Karaca et al., 2010; Pérez-De-Mora et al., 2006). Secondly, the intricate relationships between fungi and plants in the Sundarbans, crucial for nutrient uptake and overall plant health, may be disrupted. The negative impact on fungal populations could compromise the symbiotic associations between fungi and mangrove plants, potentially leading to reduced resilience of the vegetation in the face of existing and emerging environmental stressors (Chot and Reddy, 2022). Thirdly, the disturbance in the ecological balance could have ripple effects throughout the entire Sundarbans ecosystem. Beyond the immediate effects on fungi and sediment enzymes, the repercussions may extend to other organisms within the food web, impacting biodiversity and ecosystem stability (Bjerregaard et al., 2022; Ranjan et al., 2018; Sharma et al., 2022; Zhang et al., 2023a, 2023b). Moreover, the Indian Sundarbans, already facing threats from climate change, rising sea levels, and anthropogenic activities, may be further challenged in its ability to adapt and recover due to the compromised resilience resulting from heavy metal-induced disturbances (Hassan et al., 2019; Subramanian et al., 2023). In practical terms, the study underscores the urgency of addressing heavy metal pollution in the Sundarbans. Conservation efforts and management strategies must be implemented to mitigate the negative impact on fungal populations, restore ecological balance, and safeguard the overall health of this critical mangrove ecosystem. Furthermore, the study emphasizes the need for a holistic approach to environmental management that considers the intricate interactions between different components of the ecosystem to ensure its long-term sustainability.

4. Conclusion

In this study, sediment samples collected from the mangrove habitat in the Indian Sundarbans exhibited alkaline and hypersaline characteristics. The elemental characterisation and sediment indices revealed a gradual decline in sediment quality. According to sediment quality guidelines, the elemental profile of the sediments indicated significant deterioration. Metal ions such as Pb, Cu, Ni, Cd, Zn, and Cr adversely impacted the quality of the sediments. The toxic nature of these metals is shown to inhibit the activity of crucial sediment enzymes, including CMC-cellulase, β-glucosidase, aryl sulfatase, urease, and phosphatases, which play fundamental roles in nutrient cycling and organic matter decomposition. This inhibition and alteration of enzyme activities suggest a potential disruption in essential processes, leading to imbalances in nutrient availability and organic matter decomposition rates within the Sundarbans ecosystem. Furthermore, the study implies that the PTMs adversely affects the fungal community. This negative influence on fungal populations could have cascading effects on the ecological balance of the mangrove ecosystem, potentially compromising the health and resilience of mangrove vegetation. The correlation of sediment PTM levels with fungal diversity and enzymatic activities represents a novel approach in environmental studies. Our findings highlight the interconnectedness of these factors and contribute to a more holistic perspective on the impact of PTMs on mangrove habitats. This pioneering study not only expands the current knowledge base but also establishes a foundation for using manglicolous fungi as a potential biological indicator of environmental health in vulnerable mangrove ecosystems. The dataset generated from this research serves as a crucial resource for future studies aiming to monitor and conserve mangrove habitats. By identifying key indicators and understanding the relationships between PTMs, fungal diversity, and enzymatic activities, our work paves the way for more targeted conservation efforts and sustainable management practices. Overall, this research represents a significant step forward in the field of environmental science and underscores the importance of interdisciplinary approaches to unravel the complexities of ecosystem health.

CRediT authorship contribution statement

Shouvik Mahanty: Writing – original draft, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kirthana Pillay:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Emilie A. Hardouin:** Writing – review & editing, Formal analysis. **Demetra Andreou:** Writing – review & editing. **Marin Cvitanović:** Writing – review & editing, Visualization, Validation. **Gopala Krishna Darbha:** Writing – review & editing. **Sukhendu Mandal:** Resources. **Punarbasu Chaudhuri:** Resources. **Santanu Majumder:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.117233.

Data availability

All the data present in the manuscript and its associated supplementary file. The raw sequences are publicly available on NCBI SRA database with project identification number PRJNA918540.

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