HEAVY METAL CONTAMINATION OF SEDIMENTS AND WATER OF INDIAN SUNDARBAN: IMPLICATIONS ON BIOFILM GROWTH

A Thesis
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In
Environmental Biotechnology
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by

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains a literature survey and original research work by the undersigned candidate, as part of her "***Master of Technology in Environmental Biotechnology***" studies. All information in this document has been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to the research work.

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CERTIFICATION

This is to certify that **Susmita Maity,** a final year student in Master of Technology in the department of Environmental Biotechnology Of School of Environmental Studies, Jadavpur University, bearing Registration No:- **160396 of 2021-2022,** Examination roll no**:- M4EBT23004,** has successfully completed the thesis work entitled "**Heavy Metal Contamination Of Sediments And Water Of Indian Sundarban: Implications On Biofilm Growth**" under the guidance of **Dr. Reshmi Das, UGC Assistance Professor** during her master degree curriculum. This work has not been reported earlier anywhere and can be approved for submission in partial fulfilment of the requirements for the Master of Technology in Environmental Biotechnology.

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DECLARATION

The thesis titled "**Heavy Metal Contamination Of Sediments And Water Of Indian Sundarban: Implications On Biofilm Growth**" is prepared and submitted for the partial fulfillment of the continuous assessment of "Masters of Technology in Environmental Biotechnology course of Jadavpur University of the said course for the session of 2021-2023. This thesis is a presentation of my original research work. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature, and acknowledgment of collaborative research and discussions.

The work was done under the guidance of Dr. Reshmi Das at the Jadavpur University at the School of environmental studies.

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This is to certify that this thesis is hereby approved as an original work, conducted and presented in a manner satisfactory to warrant its acceptance as a pre-requisite to the degree for which it has been submitted. It is implied that by this approval, the undersigned do not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn therein, but approved for the purpose for which it is submitted.

Final Examination for valuation of Thesis

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LIST OF ABBREVIATIONS

- EPS -- Exopolysaccharides
- HNO_3 -- Nitric Acid
- NaAc -- Sodium acetate
- HCl -- Hydrochloric acid
- EF -- Enrichment Factor
- Igeo -- Geo accumulation Index
- CF-- Contamination factor
- RI -- Potential ecological risk index
- FA -- Factor Analysis
- ICP-OES -- Inductively Coupled plasma optical emission spectrometer
- ICP-MS -- Inductively coupled plasma mass spectrometer

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ABSTRACT

Multi-species intertidal biofilms have a high level of stress tolerance due to their extended exposure to the dynamic environmental conditions facilitating in-situ sequestration of contaminants. Heavy metals tend to accumulate in these intertidal sediments and eventually get desorbed with changing physicochemical conditions. Once released to the overlying water they become bioavailable to living organisms, making the sediments a potential secondary source of metal pollutants. The focus of our study is to identify the partition of heavy metals (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, V, Zn) in biofilms (absorbed and adsorbed), water and sediments across salinity gradient in intertidal sediment of Sundarbans during the winter season (1^{st} week of January, 2023). In this study, we also measured the different physico-chemical parameters of water and we find out the correlation between the environmental factors and metals concentration. We also measured the CF, EF, Igeo, and RI of heavy metals according to the location to find out the source of those metals. The contamination factor (CF), geo-accumulation index (Igeo), and ecological risk index (ERI) highlighted that the sampling areas were mostly exposed to Ni, Cd contamination. The multivariate statistical techniques such as Factor Analysis (FA) have been widely used for identifying sources of pollution and their spatial distribution in the river network. To maintain the sound environment and healthy ecosystem of the river and the surrounding areas, need proper management and monitoring of water quality of the river.

Keywords: Intertidal, Biofilm, Heavy metal, Bioavailable, Environmental factors, Salinity gradient.

CHAPTER 1

INTRODUCTION

Heavy metal pollution in aquatic systems is becoming a continuous issue. Heavy metals are common pollutants that cause significant environmental concern since they are non-degradable and consequently persistent. Each metal has a tolerable limit past which they are generally poisonous, and some are even hazardous. Intertidal sediments are sinks for heavy metals because of their anoxic environment, tiny particle size, high sulfide, and organic matter content. Heavy metals pose a continuous hazard to human lives and the environment due to their considerable toxicity even at trace quantities, non-biodegradability (unlike organic contaminants), bioaccumulation, and biomagnification (Dutta et al., 2022). However, as water pH changes, particularly in view of acidifying ocean water, metals are released into the overlying water, making them bioavailable (Chowdhury and Maiti, 2016a). The anoxic condition, fine grain size, high sulfide content, and high organic matter of the sediments provide a suitable environment for the formation of metal sulfides, promoting heavy metal retention (Álvarez-Iglesias and Rubio, 2009); (Ranjan et al., 2012). However, it is generally considered that mangroves show the ability to accumulate metals and possess a certain tolerance to relatively high levels of heavy metal pollution (Kamaruzzaman et al., n.d.).

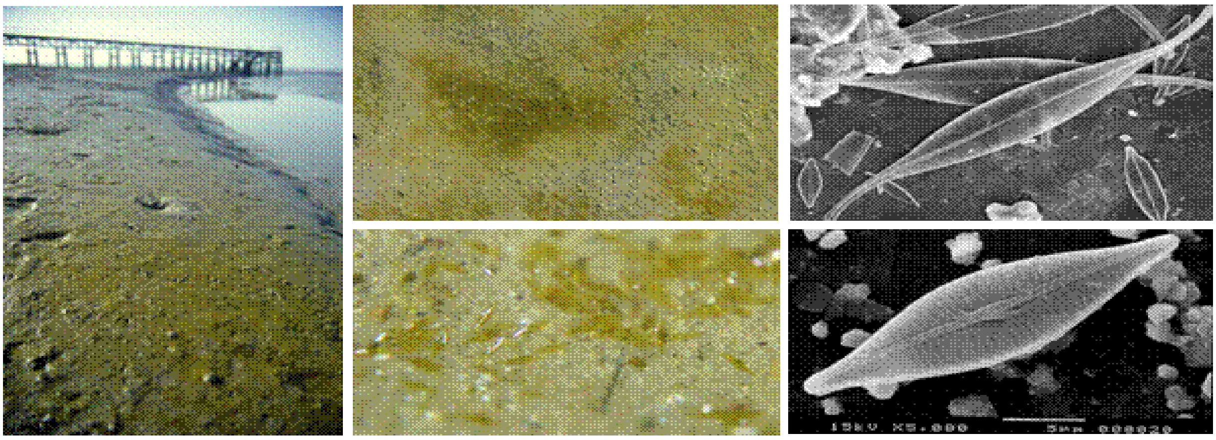


Figure 1: Biofilms over the river bank and its structure.

(Source: http://la.cesam.ua.pt/highlights/2007/EMM_v1_JoaoSerdio.htm)

However, metal sequestration in the sulfide phase is not a permanent phenomenon. With variation in the physical and chemical characteristics of pore water and tidal effects, part of these fixed metals gets desorbed into the intertidal environment and become bioavailable to living organisms, making the sediments a potential secondary source of metal pollutants (Peng et al., 2009).

As a result of rapid industrialization and large-scale human activities, pollution in water and soil is developing rapidly, which is a major global concern. Several traditional ways of remediation have been used, and these treatment technologies can be classified as physical, chemical, or biological treatment processes (Singh et al., 2021). Bioremediation is classified into two forms based on the site of the pollution treatment. In the case of in situ bioremediation, polluted water samples are treated in their original location, whereas ex situ remediation often occurs off-site (Ateia Ibrahim et al., 2016). In situ bioremediation is a wise solution as it minimizes the need for contamination removal from the site, reducing transportation costs and site disruption. Microbial biofilm mediated remediation is the most environmentally friendly and cost-effective biological technique for cleaning up water pollutants in all the biological methods of remediation (Manobala et al., 2019). Biofilms are effective for bioremediation because they absorb, immobilize, and decompose a variety of water contaminants (Igiri et al., 2018). Though many remediation techniques are currently available, the use of microbes has many advantages including cost effectiveness, few or no by-products, reusability, and many more.

The motivation of this research is to identify a natural phenomenon to immobilize the released metals in the sediments and thus prevent bioaccumulation. We found intertidal multispecies biofilms are capable of sequestering heavy metals from the water. With time the mats get buried in the sediments thus sequestering the metals (Dutta et al., 2022).

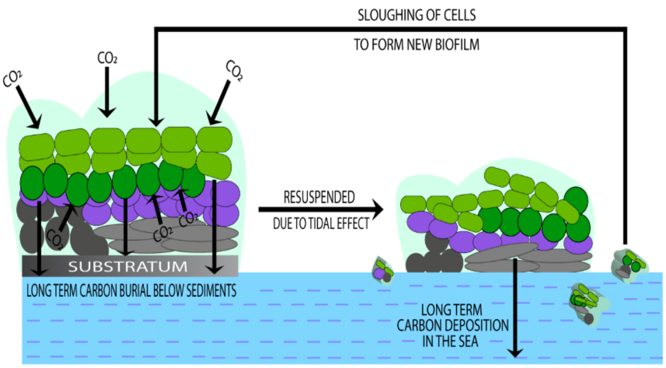


Figure 2: Longterm carbon fixation by intertidal biofilms.

(Source: Mandal et al., 2021. Marine Pollution Bulletin)

During winter especially on the 7th and 8th of January, 2023 biofilms, water, and sediments were collected from Patharpratima, Namkhana, Kakdwip & Canning across salinity gradient in a proper way for the further analysis of that samples. On that day water salinity and temperature were measured but other parameters of water like pH were measured at the laboratory. And heavy metal analysis of water and sediment was also done. But due to less amount of biofilms, we were unable to analysis the concentration of the metal from biofilms. We also collected the water samples at 20-minute intervals along the entire route to the sampling site and we have drawn a contour map of pH and salinity using ArcGIS 10.4.1 version that is shown in figure 3.

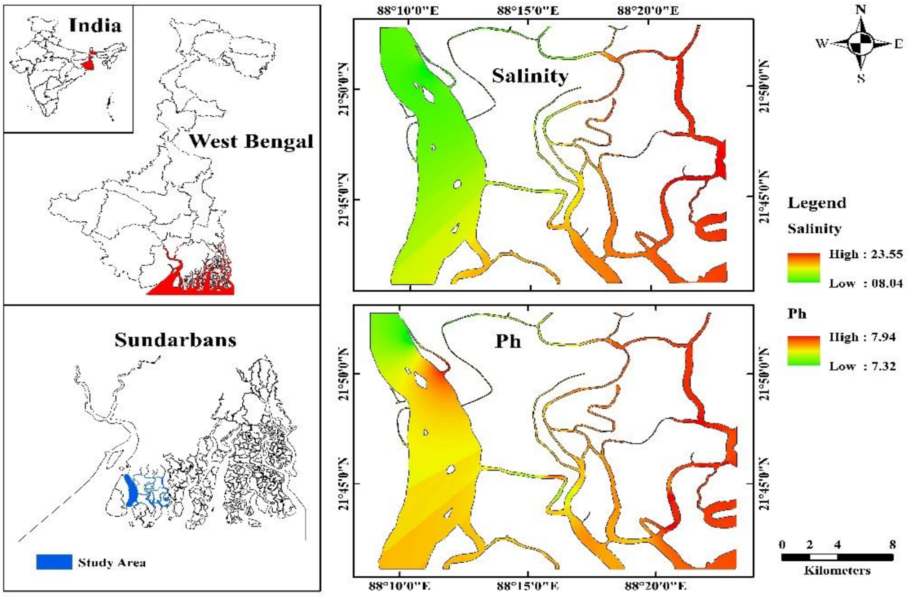


Figure 3: Contour map of water pH and salinity.

With decreasing water pH, part of the metals that are currently fixed in the sediments will be mobilized and solubilized in the above water. Hence in the future ocean water-soluble metal concentrations will increase thus facilitating biological uptake and trophic transfer.

Previously the role of intertidal biofilms of the Sundarbans in the sequestration of polyaromatic hydrocarbons was evaluated by Balu et al. (2020). To the best of our knowledge, majority of the previous work looked into metal uptake was performed by cultured biofilm. But for the first time, we will investigate metal uptake in natural environment without culturing the biofilms in the laboratory.

Therefore, the present study was performed primarily to evaluate the potential of naturally occurring intertidal phototropic biofilms to absorb and adsorb heavy metals along with the estimation of the biological parameters, such as chlorophyll, exopolysaccharides (EPS), metallothionein. In this study, we also evaluate the geochemical factors such as contamination factor, enrichment factor, geo-accumulation index of the heavy metals, and potential ecological risk index including possible anthropogenic influences in the Sundarbans mangrove ecosystem. These measurements provided us with an understanding of the biochemical responses of the biofilms to heavy metal exposure and their potential for future bioremediation studies.

The combined use of FA , EF, Igeo, and RI can successfully detect the comprehensive and single pollution levels of elements in sediment, which is significant for determining the degree of heavy metals pollution in sediment. The Origin 2023b used for multivariate statistical analysis, which is factor analysis.

CHAPTER 2

AIMS

&

OBJECTIVES

The prime aim of this present dissertation work is the natural fixation of increased water-soluble metals from the future acidifying ocean water. Majority of the previous work investigated metal uptake by cultured biofilm. This work for the first time will investigate metal uptake in the natural environment.

The study was carried out with the following objectives:

- To measure the different parameters of water and observe how it affects the growth of biofilms.
- To measure the partition of heavy metals (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, V, Zn) in water and sediments across salinity gradient.
- To calculate heavy metal pollution potential in the study area, various sediment quality indices have been used that is contamination factor (CF), enrichment factor (EF), geo-accumulation index (Igeo), and potential ecological risk index (RI).
- To identify the correlation between environmental factors and heavy metals concentration, factor analysis is to be done. And the possible source of contamination is also found out by this statistical analysis.

CHAPTER 3

LITERATURE REVIEW

A literature survey on the topic showed that there is no review study concerning trace metals pollution in different compartments (like biofilms, river water, and biofilm-mediated sediment) of the Sundarbans mangroves in India. Although there are review studies on trace metals in mangroves where the Sundarbans have been reported at a global scale (Kumar et al., 2019), they are insufficient to present a detailed scenario of trace metals contamination in different compartments of the Sundarbans. A survey of the literature was conducted using different websites such as Google Scholar, and Science Direct. Different keywords were used to search including “Metals in Sundarbans”, “Uptake of heavy metals by biofilms in Sundarbans”, “Toxic effects of heavy metals in Sundarbans", and "Different sources of heavy metals in Sundarbans ".

Krupadam et al. used sequential extraction to study the operationally determined chemical forms of Co, Cr, Cu, Ni, Pb, and Zn and their spatial distribution in the sediments of the Tapi estuary. It was found that oxidizable binding fraction was the most crucial phase for binding of all metals followed by Fe-Mn oxide and carbonate fractions. This study investigated the influence of anthropogenic activities on metal binding and their chemical control in estuarine sediments (Krupadam et al., 2006).

Chatterjee et al. first documented regarding concentration, distribution, and possible sources of selected trace elements (Cu, Fe, Mn, Zn, Cr, Co, Ni, Pb, Al, B, and Ba) in core sediments (< 63 μ particle size) from the lower stretch of Hugli (Ganges) estuary, northeast coast of Bay of Bengal by ICP–AES and EDXRF to evaluate geochemical processes influencing their distribution and possible environmental consequences. However, the Hugli estuarine environment was getting polluted by Pb, Zn, and Cu and the affected sites might be classified as “moderately contaminated” by these heavy metals (Chatterjee et al., 2007).

Peng et al. provided a review of the geochemical forms, affecting factors, and remediation technologies of heavy metals in sediment. The in-situ remediation of sediment aimed at increasing the stabilization of some metals such as the mobile and the exchangeable fractions; whereas, the ex-situ remediation mainly aimed at removing those potentially mobile metals, such as the Mn-oxides and the organic matter (OM) fraction. For different sediment contaminated by heavy metal, the adoption of remediation technology usually depended on some unique characteristics of sediment, such as metal loads, size distributions of particles, and metal species distribution (Peng et al., 2009).

Wang et al. investigated the distribution and speciation of Cd, Cu, Pb, Fe, and Mn in the shallow sediments of Jinzhou Bay, Northeast China, which nonferrous smelting activities had heavily contaminated (Wang et al., 2010).

Chowdhuri and Maiti. identified the different sources, the extent of metal pollution (Cd, Cr, Mn, Ni, Pb, and Zn), and their potential ecological risk in Sundarban Biosphere Reserve. Pollution indexes like contamination factors (CF), geo-accumulation index (Igeo), ecological risk index (ERI), and multivariate statistics were widely used to evaluate the pollution in soil and water bodies (Chowdhury and Maiti, 2016a).

Watt et al. examined the distribution, possible sources, and ecotoxicological assessment of 51 trace elements covering 13 sampling stations in surface sediments of coastal regions of the Sundarban mangrove wetland and adjacent Hugli River estuary. This present study suggested that an application of the geochemical indices along with multivariate statistical methods was an effective tool to provide complementary information for identifying pollution status (Watts et al., 2017).

Igiri et al. offered a critical evaluation of the bioremediation capacity of microorganisms, especially in the context of environmental protection. Furthermore, from this study, the biosorption capacity concerning the use of bacteria, fungi, biofilm, algae, genetically engineered microbes, and immobilized microbial cells for the removal of heavy metals was discussed. The use of biofilm had shown synergetic effects with many-fold increases in the removal of heavy metals as sustainable environmental technology shortly. This technique was cost-effective and a green technology that had advantages such as faster kinetics, and high metal binding over a broad range of pH, and temperature. This review provided an opportunity to reveal the role of microbial cell, biofilm, and their metabolites in the remediation of heavy metals and environmental research (Igiri et al., 2018).

Kader and Narayan Sinha identified that the Sundarbans soil was contaminated with sludge and moderately contaminated with Cd and Co according to the contamination factor (CF), enrichment factor (EF), geo-accumulation index (Igeo), and ecological risk factor (ERF). Based on this study it was found that the *H. fomes* and *P. paludosa* were very much effective for the removal of Cd and Cu respectively (Kader and Narayan Sinha, 2018a).

Roy et al. unfolded the extent of metal pollution in the sediment of the Indian Sundarbans estuarine mangrove ecosystem and also investigated the source and distribution of the metals. Statistical analyses such as principal component analysis and correlation matrix identified different sources of metal contamination. Almost 700 tannery industries were located in the upstream region of the rivers, and several small and large-scale battery industries seem to be the main possible source of Cd, Cr, and Pb pollution. Analysis of the results indicated the alarming condition of this heritage site (Roy et al., 2018).

Kumar et al. reviewed to decipher the distribution of trace metals (Al, As, Cd, Cr, Cu, Hg, Pb, Ni, Mn, V, and Zn) in different compartments and human health risks in the Sundarbans mangrove ecosystems. The literature suggested relatively higher contamination of soils and sediments than fish, crustaceans, and water. Cd, Fe, Ni, and Pb are most likely to accumulate in the roots of mangrove trees, while Al, As, Co, Cr, Cu, Mn, and Zn tend to accumulate in the leaves (Kumar et al., 2019).

Kuerban et al. evaluated the overall pollution situation and source apportionment of heavy metals in soil (Hg, Cd, As, Pb, Ni, Zn, Cu, and Cr) using various methods including geo-accumulation index (Igeo), potential ecological risk index (RI) and positive matrix factorization combined with Geographical Information System (GIS) to quantify and identify the possible sources to these heavy metals in soils (Kuerban et al., 2020).

Singh et al. introduced the use of microbial biofilms for the remediation of contaminated water. A biofilm system was a well-developed, cost-effective, and eco-friendly technology in which different microbes such as bacteria, fungi, and microalgae form a colony. Microbial biofilm-based systems for wastewater treatment were a rapidly expanding research area. Overall, biofilms might be used for the bioremediation of wastewater pollutants (Singh et al., 2021).

Dutta et al. evaluated the potential of naturally occurring intertidal phototropic biofilms cultured in flasks to absorb and adsorb Cd and Co along with the estimation of the biological parameters, such as chlorophyll a, biomass, exopolysaccharides (EPS), and cell vitality (Dutta et al., 2022).

Kumar et al. also undertook a comprehensive assessment of sediment contamination and ecological risks posed by metal(loid)s in the Sundarbans using previously published data. There was a distinct difference in metal(loid) content, pollution level, and ecological risk in Bangladeshi and Indian parts of the Sundarbans, with the Indian counterpart experiencing relatively higher metal(loid) pollution (Kumar et al., 2023).

CHAPTER 4

EXPERIMENTAL METHODOLOGY

4. Materials & methods:

Different materials used for this project work and different methods are discussed below.

4.1. Study area and sampling:

Study area comprises of the major urban/rural and forested centers of Indian Sundarbans surrounding the reserve forest as a ring of settlements. The locations and description of the four sampling sites are shown in Figure 4 and Table 1 respectively.

Table 1: Description of sampling sites, coordinates along with respective anthropogenic stresses.

Location	**Coordinates**	**Nature of anthropogenic stresses**
Patharpratima	21°45′50.3″N ,88°19′21.1″E	Plastic pollution, boating activity, discharge of untreated waste from the adjoining market.
Namkhana	21°46′12″ N, 88°13′53″ E	Ferry services, fishing activities, Tourism.
Kakdwip	21°52′45″N, 88°11′29″ E	Ferry services, fishing activities, agricultural, domestic and aquacultural practices.
Canning	22°18′08.76″N,88°40′ 20.90″E	Huge traffic via rail route, land transport, as well as ferry services, tourism activities.

Intertidal biofilms, sediments, and river water were collected from Patharpratima, Namkhana, Kakdwip and Canning in South 24 parganas (Indian Sundarbans), West Bengal, India during the Winter season 7th & 8th January, 2023. The sampling sites Patharpratima is located on the adjacent of river Thakurani and Saptamukhi and Namkhana and Kakdwip are located on the river Muriganga (Balu et al., 2020).Canning is located on the western banks of the Matla river. Water was collected in 50ml falcon tubes in triplicate for the measurement of different parameters like pH, salinity, temperature, metals. Thin layer of biofilms were collected in 15 cm X 10 cm plastic boxes using plastic scoopers with proper labelling. All the falcon tubes, boxes used in this study were sterilized with UV light in Laminar Air Flow Cabinet for 1hrs. All the collected water and sediment samples were carried out to the laboratory safely and keep it under the rack one day for settle down. Geographical coordinates of the sampling site were acquired using GPS Map Camera.

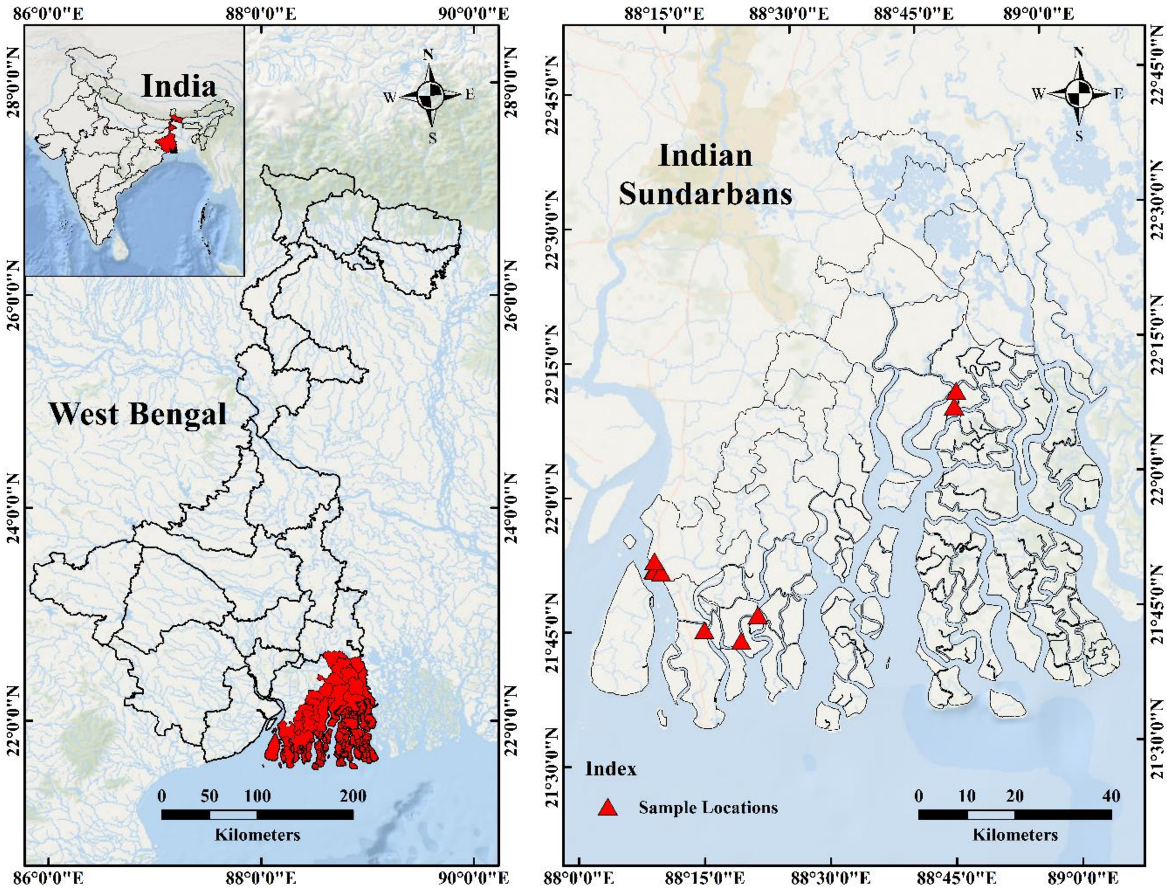


Figure 4: Study area and sampling locations.

4.2. Sample analysis: The collected water and sediment samples were analysed in the following ways.

4.2.1. Water analysis: New eppendorf falcon tubes were washed with 2% HNO_3 at 70°C at hot air oven overnight so that no contamination has occur during the metal analysis of that water. After that the falcon tubes were washed with Milli- Q water and dried out at the Laminar air flow cabinet. This acid washed falcon tubes were used for the further analysis of metal composition of the water. Water samples that were taken from Sundarban then filtered with Whatman paper and then with 0.2µm non pyrogenic single use syringe filter and transferred it to the previously washed falcons. Then 2 drops of extrapure HNO_3 were added to the filtered water and send it to the laboratory for metal analysis.

4.2.2. Sediment analysis: Small section of sediments were cut from the boxes and placed in petri plates. These petri plates were washed with 2% HNO_3 at 70ºC on the hot plate in Fume hood cabinet for 6 hrs. After that these petri plates were washed with the Milli-Q water and dried in the Laminar air flow cabinet. The sediments were then dried at 70º C at hot plates for two days to achieve constant weight. Finally, they were homogenised and grounded to fine powder using the Mortar pestle (Kader and Narayan Sinha, 2018a).

1 gm of sediment samples were taken at several teflon beakers for the quantification of metal. These teflon beakers were washed with Aqua regia (HCl: HNO_3 = 3:1) at 120º C on the hot plate for 24 hrs. Then these were cleaned with Milli-Q water. Then 3 ml of sodium acetate (pH 7) were added to each of the beaker. Then these beakers were shaken for 1 hrs. Wait for sometimes to settled own and the supernatant was taken to another beaker. Then the excess NaAc was evaporated at 140º C on the hot plate. 1 ml 70% trace metal grade HNO_3 (Sigma-Aldrich) was added to the beakers for the digestion at 100º C for 24 hrs to extract the absorbed metal from the sediment. Then the digested solution was evaporated and 2% HNO_3 was added. Then the supernatant was transferred to the centrifuge tube and centrifuge at 1000 rpm for 5 min. Finally, the supernatant was again transferred to the new centrifuge tube and send it to the laboratory for the bio-available fraction analysis of different metals by ICP-OES (Inductively coupled plasma - optical emission spectrometer) and ICP-MS (Inductively coupled plasma mass spectrometer) at IISC Bangalore.

20 mg of sediment samples were also taken at different teflon beakers. Then the sediments were digested with HNO_3: HF in 3:1 ratio at 120º C on the hot plate for 24 hrs for the extraction of the metal. Then concentrated HNO_3 was added to evaporate the excess HF and refluxed for 1 hr. This process was repeated twice. Then 2% HNO_3 was added. After that sonication was carried out for 10 mins and the supernatant was transferred to the centrifuge tube and centrifuge at 1000 rpm for 5 mins. At last, the supernatant was taken to another centrifuge tube and send it to the laboratory for the total metal concentration of sediments by ICP-OES and ICP-MS (Ray et al., 2022) at IISC Bangalore.

4.3. Geochemical approach for assessment of the sediment contamination:

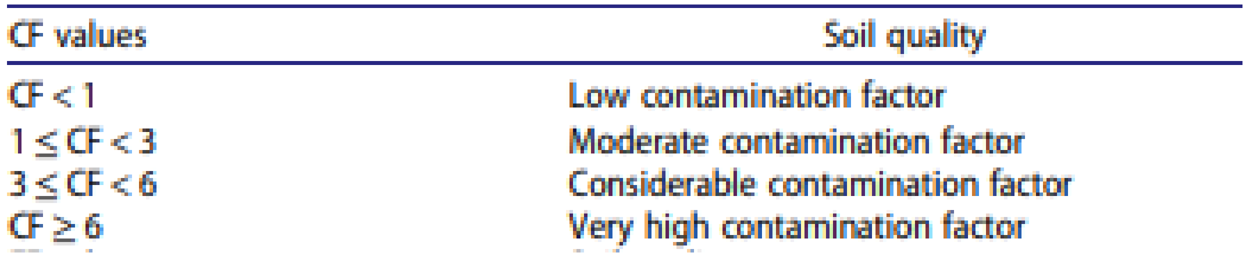
Various sediment quality indices have been used to infer heavy metal pollution potential in the study area. They were:

4.3.1. **Contamination factor**: A contamination factor (CF) describes the contamination of a given metal. The equation is:

$$\boxed{\mathbf{CF = Ci\ /Bi}}$$

where Ci is the content of metal i and Bi is the background concentration of the same metal (Kader and Narayan Sinha, 2018b).

Table 2: Classification of CF. [Source : (Kader and Narayan Sinha, 2018b)]



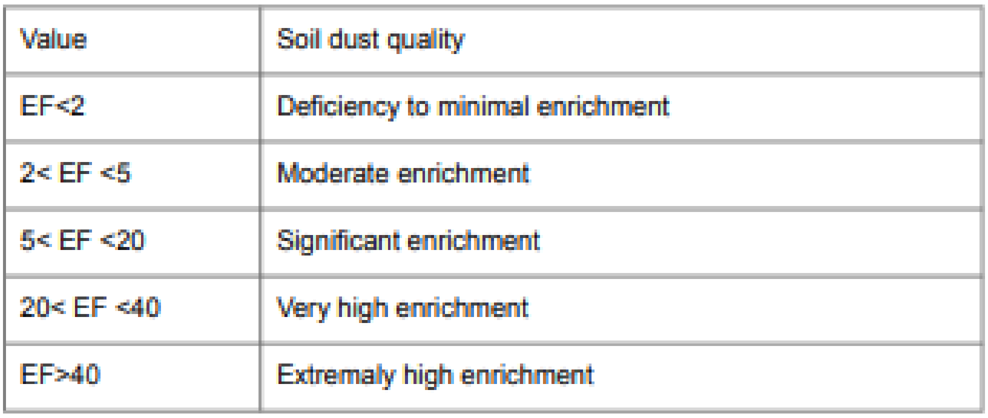
CF values	Soil quality
$CF < 1$	Low contamination factor
$1 \leq CF < 3$	Moderate contamination factor
$3 \leq CF < 6$	Considerable contamination factor
$CF \geq 6$	Very high contamination factor

4.3.2. Enrichment factor: To evaluate the anthropogenic sources of various non-crustal elements, the enrichment factor was determined. The EF calculations were mainly based on the average upper continental crustal composition described by Rudnick and Gao (2003) (Rudnick et al., 2003). An element's enrichment factor in a sediment sample is defined as:

$$\mathbf{EF = [\ (X/Ref)_{sample} \ / \ (X/Ref)_{Crust}\ ]}$$

where X and Ref are the element concentration in the sample and the reference element concentration in the continental crust, respectively (Kader and Narayan Sinha, 2018a) Aluminium, iron, and manganese were the most commonly used reference elements. Al was employed as a reference element in this experiment.

Table 3: Classification of EF**.** **[**Source: (Barbieri, 2016)]



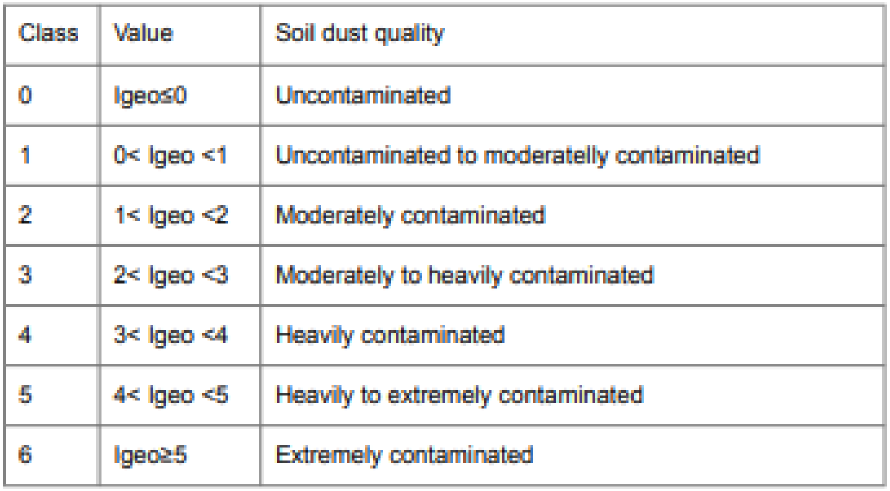
Value	Soil dust quality
EF<2	Deficiency to minimal enrichment
2< EF <5	Moderate enrichment
5< EF <20	Significant enrichment
20< EF <40	Very high enrichment
EF>40	Extremaly high enrichment

4.3.3. Geo-accumulation index: The degree of metal contamination in sediment was calculated by geo-accumulation index proposed by Muller. The Igeo is calculated using the following formula:

$$\mathbf{Igeo = \log_2[\frac{Ci}{1.5Bi}]}$$

Where Ci is the measured concentration of metal (i) in the sediment, Bi is the geo chemical background concentration of the metal (i), and 1.5 is the correction factor for the background matrix due to lithogenic effects (Kuerban et al., 2020).

Table 4: Classification of Igeo. **[**Source :(Barbieri, 2016)**]**



Class	Value	Soil dust quality
0	Igeo≤0	Uncontaminated
1	0< Igeo <1	Uncontaminated to moderatelly contaminated
2	1< Igeo <2	Moderately contaminated
3	2< Igeo <3	Moderately to heavily contaminated
4	3< Igeo <4	Heavily contaminated
5	4< Igeo <5	Heavily to extremely contaminated
6	Igeo≥5	Extremely contaminated

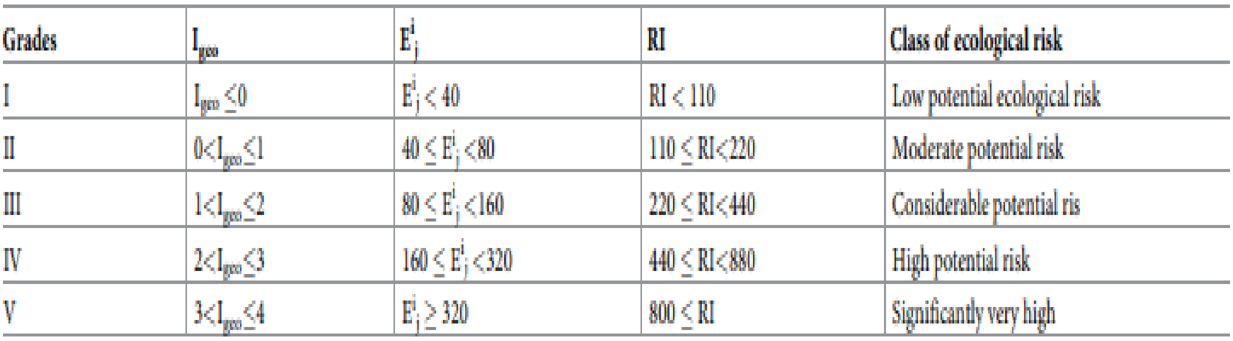
4.3.4. Potential ecological risk index: To assess the level of ecological risks, potential ecological risk index (PERI) methods were used, which were proposed by Hankinson, according to the characteristics of the heavy metals and their environmental behaviour. The PERI was highly associated with three coefficients, namely, the individual pollution coefficient (Cij), the response coefficient of heavy metal toxicity (Ti) and the potential ecological risk individual coefficient (Eij), and can be expressed as follows:

$$\mathbf{RI} = \sum_{i=1}^{n} \mathbf{Eij} = \sum_{\mathrm{i}}^{\mathrm{n}} (\mathbf{Ti} * \mathbf{Cij}) = \sum_{\mathrm{i}=1}^{\mathrm{n}} \left(\mathbf{Ti} * \frac{\mathbf{Ci}}{\mathbf{Bi}}\right)$$

where RI is the potential ecological risk index, E_{ij} is the potential ecological risk individual coefficient of heavy metal i at sample site j, and Ti is the toxicity response coefficient of heavy metal i.

In this study, we adopted reference toxicity values for each heavy metal in the order of $T_{Zn} = 1$, $T_{Cr} = 2$, $T_{Cu} = T_{Ni} = T_{Pb} = 5$, $T_{Cd} = 30$ and $T_V = 2$. Where Ci is the measured concentration of metal (i) in the sediment, Bi is the geo chemical background concentration of the metal (i) (Kuerban et al., 2020). The classification conditions of potential ecological risks are shown in Table 5.

Table 5: Classification of E^i_j & RI. **[**Source: (Kuerban et al., 2020)**]**



Grades	I_{geo}	E^i_j	RI	Class of ecological risk
I	$I_{geo} \leq 0$	$E^i_j < 40$	$RI < 110$	Low potential ecological risk
II	$0 < I_{geo} \leq 1$	$40 \leq E^i_j < 80$	$110 \leq RI < 220$	Moderate potential risk
III	$1 < I_{geo} \leq 2$	$80 \leq E^i_j < 160$	$220 \leq RI < 440$	Considerable potential ris
IV	$2 < I_{geo} \leq 3$	$160 \leq E^i_j < 320$	$440 \leq RI < 880$	High potential risk
V	$3 < I_{geo} \leq 4$	$E^i_j \geq 320$	$800 \leq RI$	Significantly very high

4.4. Statistical analysis:

pH and salinity contour map was prepared by Arc GIS 10.4.1. version. The map of study area along with sampling sites were also prepared by Arc GIS 10.4.1. All the data analysis was done in the Microsoft Office Excel 2016. Factor analysis was done in Origin 2023b.

CHAPTER 5

RESULTS & DISCUSSIONS

5.1. Water quality assessment:

Throughout the sampling site of Sundarbans especially from the river Thakurani, Saptamukhi and Muriganga of Patharpratima, and Namkhana, Kakdwip respectively we have collected several water samples. We have measured several water parameters like pH, temperature, salinity to assess the status of pollution that was shown in the Table 6.

Table 6: Different water parameters throughout the sampling site.

Sample No	**Latitude (⁰ N)**	**Longitude (⁰ E)**	**pH**	**Temperature (° C)**	**Salinity (‰)**
1	21.796111	88.361751	7.92	24	22.85
2	21.792699	88.371815	7.91	24	22.98
3	21.782456	88.379162	7.87	24	23.43
4	21.768777	88.378261	7.85	24	23.55
5	21.766112	88.378442	7.94	22	23.34
6	21.76655	88.363934	7.92	22	23.44
7	21.765489	88.351005	7.91	22	23.51
8	21.765214	88.350557	7.87	22	23.54
9	21.74944	88.341117	7.82	22	22.87
10	21.739768	88.341587	7.93	22	22.74
11	21.728243	88.345218	7.91	22	22.13
12	21.726983	88.345162	7.90	22	22.18
13	21.719904	88.342835	7.93	22	22.13
14	21.708779	88.31931	7.71	22	20.96
15	21.708772	88.31922	7.81	22	20.88
16	21.708437	88.289803	7.82	22	20.55
17	21.72977	88.266068	7.77	22	19.33
18	21.742869	88.270523	7.65	21	16.9
19	21.75692	88.267365	7.85	22	15.08
20	21.75734	88.251956	7.64	22	14.18
21	21.868719	88.189378	7.32	16	14.74
22	21.859756	88.175617	7.42	16	15.32
23	21.859359	88.176048	7.57	16	9.07
24	21.853117	88.182977	7.62	16	8.04
25	21.852735	88.183911	7.87	17	8.88
26	21.850487	88.187948	7.91	17	10.17
27	21.838792	88.197487	7.94	17	11.48
28	21.837501	88.195461	7.92	17	12.01

This route corresponded to the ferry and ecotourism activity and fishing harbour. In winter all the activities were high and as in this time no rain fall was there, dilution rate will less so the pollution will be high. From Table 6 we can see that the pH of water varied from 7.32 to 7.94 which indicated that the water was slightly alkaline in nature. And temperature varied from 16 ^{0}C to 24 ^{0}C. And the salinity range of water was 8.04 ‰ to 23.55 ‰.

5.2. Metals concentration:

From Sundarban we have collected biofilms along with their adjacent sediment and water samples. In the laboratory, the bio-available fraction of sediment and total sediment was analysed for the different metal quantification by ICP-OES and ICP-MS. In different types of samples, different measuring units were used. Therefore, to make an easy comparison, the unit of trace metals concentration was converted into ppm and ppb for water analysis, except for sediments, it was expressed as μg/gm and ng/gm and mg/kg (Kumar et al., 2019). Due to the proximity to the urban environment and industry, mangroves received a wide range of pollutants in the form of wastewater, solid waste, particulate matters, and so on. Among the major pollutants from anthropogenic inputs were trace metals (MacFarlane and Burchett, 2002). The first receiver of trace metals in mangroves is water and sediments.

In the following sub-sections, levels of trace metals in sediment and water have been detailed.

5.2.1. Trace metals in sediments of Indian Sundarban: The metal concentration of bio-available fraction of sediments are shown in the table 7 and 8 respectively. Sediments are the first receivers of the trace metals from the source. We can see that Al, Fe, K, Mg, Na, Sr, Ca were present in the sediment in a significant concentration. But Li, B, Cr, Co, Ni, Zn, Ba, Pb were present in less concentration.

The trace metals concentration in sediments have been compared with the world's shale value (Turekian and Wedepohl, 1961) and it has been found that trace metals such as Ni showed a higher concentration (79 - 154 mg/kg) than the shale values (Table 9).The metal concentration of total sediments are shown in table 9. Besides, the concentration of Mg, Al, Ca, V, Cr, Mn, Fe, Cu, Zn, Pb, Co, Cd were 6338 - 1071, 20345 - 40894, 105 - 1552, 53 -100,40 - 82, 163 - 756, 25394 - 48243, 15 - 31, 43 - 110, 15- 25, 7.67 - 15, 0.084 - 0.163 mg/kg in the sediment.

Early studies found that the concentration of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn were 5.73, 89.5–148.6, 172.35, 150.1–195.1, 71,829.9, 978.4, 167.29, 46.24, 144.15, and 207.97 mg/kg in the sediments of Sundarbans (Kumar et al., 2019), which indicated an decreasing pattern of trace metals concentrations. However, early 90's study indicated that Zn concentration was 4.6–15.4 mg/kg in the sediments of the Sundarbans mangrove (Das et al., 1994), which is much lower than the present situation.

This pollution by trace metals could be due to domestic effluent discharge, oil residue from boats, over-siltation, intensive use of mechanized boats for transport and fishing, use of anti-fouling paints or burnt oil in mechanized boats, and herbicides and pesticides from nearby agricultural fields (Watts et al., 2017).

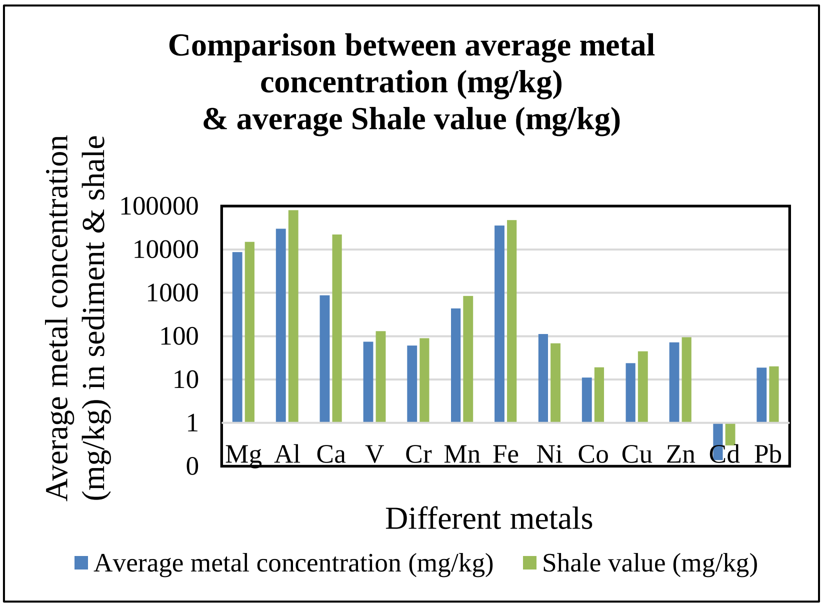


Figure 5: Average metals concentration in the total sediment & average shale value of those metals.

Table 7: Concentration of heavy metals in the bioavailable fraction of sediment (ICP-OES).

Sample name	Al (µg/gm)	Fe (µg/gm)	K (µg/gm)	Mg (µg/gm)	Na (µg/gm)	Sr (µg/gm)
P1	30	15	429	188	4919	3.94
P2	79	35	537	262	7302	5.19
P3	24	12	417	187	5304	3.65
N4	21	10.33	347	172	5143	2.41
K6	22	11	247	172	5103	3.02
K7	20	9.96	239	140	5444	2.52
K8	3.21	2.30	40	68	5742	3.30
C12	29	16	360	174	5213	3.25
C13	14	10.15	336	215	5216	4.21
C14	13	6.94	378	164	5704	2.38

Table 8: Concentration of heavy metals in the bioavailable fraction of sediment. (ICP-MS).

Sample name	Li (ng/gm)	B (ng/gm)	Ca (ng/gm)	Cr (ng/gm)	Co (ng/gm)	Ni (ng/gm)	Zn (ng/gm)	Ba (ng/gm)	Pb (ng/gm)
P1	24	286	70564	15	3.60	11	6.06	95	6.66
P2	31	536	61051	29	8.13	21	12	16	16
P3	19	373	45400	6.79	2.22	6.78	4.07	65	4.39
N4	14	226	46587	7.37	3.45	7.68	5.50	157	4.97
K6	11	190	46127	5.43	1.25	4.62	4.03	118	4.16
K7	16	265	48844	8.75	1.76	6.02	5.06	166	5.29
K8	25	326	48658	25	2.82	9.53	5.69	107	5.62
C12	25	326	48717	25	2.83	9.54	5.69	108	5.63
C13	35	611	46139	3.25	3.25	33.94	18	111	14
C14	19	274	43004	6.92	3.25	6.82	3.64	75	4.31

Table 9: Concentration of heavy metals in the total sediment.

Sample name	Mg (mg/kg)	Al (mg/kg)	Ca (mg/kg)	V (mg/kg)	Cr (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
P1	9041	28119	861	56	45	323	28178
P2	7371	2035	193	76	68	188	3358
P3	10365	39160	1476	100	82	756	48243
N4	8922	34647	1552	77	60	583	38642
K6	10377	40894	1340	81	67	629	40798
K8	7274	24819	844	66	53	446	32526
C12	10791	34120	617	86	71	452	39512
C13	7591	21735	105	76	63	163	32591
C14	6338	25765	913	53	40	370	25394

Sample name	Ni (mg/kg)	Co (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
P1	88	8.67	17	46	0.084	16
P2	107	9.70	25	108	0.095	18
P3	154	15	31	80	0.149	25
N4	124	13	24	110	0.163	22
K6	129	13	25	75	0.231	22
K8	104	11	23	58	0.154	17
C12	127	13	29	68	0.126	20
C13	103	9.71	27	61	0.139	15
C14	79	7.67	15	42	0.094	16

5.2.2. Trace metals in water of Indian Sundarban:

An overview of different metals present in the water samples of Indian Sundarban are shown in table 10. Most of the researches have been conducted using water from the inflowing rivers rather than groundwater in the Sundarbans. In most of the cases, the concentrations of trace metals in water were below the standard level set by the World Health Organization (WHO) (WHO, 2011), and India (BIS, 2012) except Cu.

The concentration of Al in the water bodies were not estimated in any research, and the other elements were estimated haphazardly (Table 10). Although this finding indicates a lack of sufficient research on water in the Sundarbans ecosystem, the concentrations of B, K, Sr, Cu were reported frequently in water bodies (Table 10). In water, the metals such as B, K, Sr and Cu are mainly originated from anthropogenic activities (Chowdhury and Maiti, 2016a). Nevertheless, trace metals can be originated from natural activities like weathering and erosion (Bakshi et al., 2018). No matter natural or anthropogenic origin, trace metals are deposited in the sediments of mangrove as oxides and/or oxy-hydroxides (Noël et al., 2015). Then, in the presence of organic matter, bacterial decomposition of trace metal oxides and/or oxy-hydroxides takes part to form soluble phases (For example, organic and sulfide form of trace metals), thus increasing their bioavailability and release in pore water (Kumar et al., 2023).

Nevertheless, based on the available data and in terms of trace metals pollution, water bodies in the Sundarbans area were relatively safe. This less pollution in water by trace metals may be due to the tidal forces that keep the water bodies in dynamic level make trace metals concentration dilute (Kumar et al., 2023).

Table 10: Concentration of heavy metals in the water. [*Source: (BIS 2012)]

Sample no	B (ppm)	K (ppm)	Sr (ppm)	Al (ppb)	Ca (ppb)	Ni (ppb)	Cu (ppb)	Ba (ppb)
P1	**3.05**	**297**	**5.34**	15	71287	2.91	1421	14
P2	**3.08**	**302**	**5.39**	15	72671	3.19	**3358**	13
P3	**2.98**	**282**	**5.29**	13	70659	2.93	**2619**	16
N4	**1.90**	**169**	3.39	10.02	51243	2.07	701	30
K6	**1.94**	**171**	3.66	12	60429	2.74	1230	43
K7	**2.04**	**181**	3.76	14	60006	2.91	1195	38
K8	**1.25**	**103**	2.35	7.70	43689	1.88	499	40
C12	**2.59**	**246**	**4.58**	18	62020	2.86	**1551**	21
C13	**1.47**	**146**	2.58	42	62854	2.99	**1651**	19
C14	**1.28**	**123**	2.21	22	61314	2.64	1488	20
Indian standard value of these trace metals in water * (in ppm)	1	12	4	0.2	200	0.02	1.5	0.7

5.3. Assessment of sediment contamination:

Sediment quality indices act as an essential indicator which helps in deriving the elemental contamination due to anthropogenic interference against the background sediment value. We have calculated the EF and Igeo using the formula described in the experimental methodology section. The value of EF and Igeo of different metals present in the different sampling location (Patharpratima, Namkhana, Kakdwip, Canning) are shown in the table 11 and table 12 respectively.

According to Sakan et al. (2009), EF values between 0 and 1 indicate that the elements are entirely from crustal materials or natural processes, whereas EF values higher than 1 suggest that the sources are more likely to be anthropogenic (Sakan et al., 2009). The average EF values ranged from 0.092 to 6.897 (Table 11) indicating that the enrichment of all elements (except Ni, Mn, Cd, Fe, V, Cu, Zn) involved greater crustal influence rather than anthropogenic influence (Watts et al., 2017). Here we can see Ni, Mn (in Namkhana), and Cd (in Kakdwip) have significant enrichment which depicting a strong source of anthropogenic activities, Fe, V (in Patharpratima and Canning), Cu (in Patharpratima, Kakdwip, and Canning), Zn, Cd (in Patharpratima, Namkhana, and Canning) have moderate enrichment. Mg, Al, Ca, Cr, Co, V (in Namkhana and Kakdwip), Mn (in Patharpratima, Kakdwip and Canning), Cu (in Namkhana) have deficiency to minimal enrichment (Ref. Table 3).

Table 11: Enrichment factor of heavy metals present in the sediment.

Location		Patharpratima	Namkhana	Kakdwip	Canning
EF	Mg	1.665	1.403	1.463	1.650
	Al	1	1	1	1
	Ca	0.092	0.142	0.106	0.064
	V	2.215	1.858	1.878	2.219
	Cr	1.974	1.543	1.625	1.890
	Mn	1.520	**6.869**	1.720	1.270
	Fe	2.255	2.004	2.005	2.147
	Ni	**6.897**	**6.198**	**6.157**	**6.568**
	Co	1.798	1.713	1.728	1.739
	Cu	2.425	1.998	2.113	2.534
	Zn	3.245	3.845	2.467	2.559
	Cd	3.392	4.269	**5.305**	3.982
	Pb	3.257	2.992	2.804	2.974

Igeo compares the contamination of a particular element with a standard background value to assess how much a particular site is polluted than the basic mineral composition. Igeo value of the sediment was asses for the quantification of the sediment's contaminant as per the seven enrichment classes.

And the sediments were uncontaminated from Mg, Al, Ca, V, Cr, Mn, Fe, Co, Cu, Pb as Igeo < 0. Igeo value was found to be positive only for Ni, Zn (in Namkhana) and Cd (in Namkhana and Kakdwip) which indicated it was uncontaminated to moderately contaminated status, means that these metals were coming from anthropogenic sources (Ref. Table 4).

The moderately contamination of the soil might have occurred due to uneven discharge of industrial and effluent waste water, uncontrolled utilization of different fertilizers and pesticides, chemogenic weathering of the earth crust and several other anthropogenic factors (Mahanty et al., 2021).

Table 12: Geo-accumulation index of heavy metals present in the sediment.

Location		Patharpratima	Namkhana	Kakdwip	Canning
Igeo	Mg	-1.330	-1.330	-1.346	-1.445
	Al	-2.065	-1.819	-1.896	-2.168
	Ca	-5.512	-4.632	-5.139	-6.142
	V	-0.918	-0.925	-0.986	-1.018
	Cr	-1.084	-1.193	-1.195	-1.250
	Mn	-1.461	-0.996	-1.113	-1.823
	Fe	-0.893	-0.816	-0.892	-1.066
	Ni	**0.721**	**0.813**	**0.727**	**0.548**
	Co	-1.219	-1.042	-1.106	-1.369
	Cu	-0.787	-0.820	-0.816	-0.826
	Zn	-0.367	**0.124**	-0.593	-0.812
	Cd	-0.303	**0.275**	**0.512**	-0.174
	Pb	-0.362	-0.238	-0.408	-0.595

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The potential ecological risk individual coefficient and contamination factor (CF) of different heavy metals at different sampling site were used to evaluate the potential risk index of multiple metals (Table 14). In this study, the C_d was defined as the sum of all contamination factors (CF). The C_d ranged from 10. 214 to 13.067 (Table 13) with the elevated value at Namkhana implicating that this station has a considerable C_d from anthropogenic pollution (Watts et al., 2017).

The CF shows that Ni, Cd, Zn (in Patharpratima & Namkhana), Pb (in Patharpratima, Namkhana & Kakdwip) was "moderately polluted" ($1 \leq CF < 3$) and the remaining metals (V, Cr, Cu, Zn and Mn) were in "none to medium" pollution category (Table 13). It was seen that Ni had a higher pollution level in most of the sites followed by Cd. Intensive fishing and ferry services, sewage drainage from the mainland and other commercial activities were supposed to be potential sources of Ni in the sediment at all the sampling location. The highest cadmium pollution was recorded in Kakdwip (CF = 2.139) may be due to the nongreen disposal of rechargeable batteries. Moderate category of Pb pollution was observed in three locations (Patharpratima, Namkhana & Kakdwip) may be due to their dependence on water transport using fossil fuel, which seeps into the waters and hence comes into the sediment phase (Chowdhury and Maiti, 2016a).

Table 13: Contamination factor (CF) & degree of contamination (C_d) of heavy metals present in the sediment.

Location		Patharpratima	Namkhana	Kakdwip	Canning
CF	V	0.794	0.790	0.757	0.741
	Cr	0.707	0.656	0.655	0.631
	Ni	**2.472**	**2.635**	**2.482**	**2.193**
	Cu	0.869	0.850	0.852	0.846
	Zn	**1.163**	**1.635**	0.995	0.854
	Cd	**1.216**	**1.815**	**2.139**	**1.329**
	Pb	**1.167**	**1.272**	**1.131**	0.993
C_d		11.373	13.067	12.245	10.214

The technique for predicting the potential ecological risk of the heavy metal contamination was used for monitoring water pollution and understanding the result of the increasing concentration of heavy metals in sediments and their subsequent release into the water, which could threaten the ecological health (Rahman et al., 2014). From the table 14 it is shown that the potential ecological risk index (RI) value is < 110 which indicates that all the sediments have low potential ecological risk.

But from the potential ecological risk individual coefficient (Ei) value it is shown that all the heavy metals [V, Cr, Ni, Cu, Zn, Pb and Cd (in Patharpratima and Canning)] have low potential ecological risk at different sampling site. Only Cd at Namkhana and Kakdwip have moderate potential risk (Ref. Table 5) and the source could be the disposal of rechargeable batteries (Chowdhury and Maiti, 2016a).

Table 14: Potential ecological risk individual coefficient (Ei) & Potential ecological risk index (RI) of heavy metals present in the sediment.

Location		Patharpratima	Namkhana	Kakdwip	Canning
Ei	V	1.587	1.580	1.514	1.481
	Cr	1.415	1.312	1.310	1.262
	Ni	12.360	13.175	12.411	10.963
	Cu	4.34513	4.24799	4.25901	4.22931
	Zn	1.1623	1.635	0.995	0.854
	Cd	36.470	**54.444**	**64.163**	39.883
	Pb	5.837	6.361	5.653	4.965
RI		63.177	82.755	90.306	63.637

5.4. Pollution characteristics through multivariate statistical analyses:

Factor analysis (FA) was applied on the metal concentrations in the water bodies and sediment to obtain groups of similar metals (Chowdhury and Maiti, 2016a). Normalized variables (original variables) were transformed into the rotated components to extract significant principal components (PCs) by suppressing the contribution of variables with minor significance. Furthermore, these PCs were subjected to varimax rotation with loading coefficients (>0.1) to generate PCs factors/groups. Out of several methods, multivariate statistical analyses with FA were selected (Chowdhury et al., 2016).

5.4.1. Factor analysis for the minor elements:

- Pearson correlation matrix showed the linear relationship of the analyzed parameters with each other. The correlation between salinity and temperature was 0.752 (Table 15) and 0.730 (Table 16) for sediment and water analysis respectively which indicated that salinity and temperature had strong positive correlation with each other for both sediment and water as the value is > 0.5.

  The correlation between pH and temperature was -0.071 (Table 15) and -0.063 (Table 16) and the correlation between pH and salinity was in the range of -0.037 (Table 15) and -0.037 (Table 16) which indicates pH had no relationship with temperature and salinity for both sediment and water.

  Here, Cu, Pb, Cr were strongly positively correlated with Ni; Cr and Pb were also strongly positively correlated with Cu; Cr had strong positive correlation with Pb in the sediment analysis (Table 15), which indicated that these metals had similar source and that was anthropogenic.

  Cd showed a strong negative correlation with temperature and salinity.

  Fluctuation of salinity was a prime factor in the coastal areas, which influenced partitioning and bioavailability of metals (Himadri and Anilava, 2000). However, the relationship varied with the prevailing environmental conditions and types of metal. In this present study, Pb, Cu were shown strong positive correlation with salinity in water analysis.

Table 15: Correlation matrix of minor elements for sediment.

	pH	Temperature	Salinity	Ni	Cu	Cd	Pb	Cr
Ph	1							
Temperature	-0.071	1						
Salinity	-0.037	**0.752**	1					
Ni	0.316	-0.259	-0.071	1				
Cu	0.051	-0.103	0.031	**0.851**	1			
Cd	0.001	**-0.838**	**-0.632**	0.497	0.270	1		
Pb	0.423	-0.233	-0.038	**0.934**	**0.641**	0.472	1	
Cr	0.185	-0.101	0.160	**0.907**	**0.955**	0.298	**0.773**	1

Table 16: Correlation matrix of minor elements for water.

	pH	Temperature	Salinity	Cu	Pb	Cd
pH	1					
Temperature	-0.063	1				
Salinity	-0.037	**0.730**	1			
Cu	0.132	0.470	**0.793**	1		
Pb	-0.140	0.149	**0.533**	0.157	1	
Cd	-0.224	-0.034	0.228	0.371	0.383	1

- The result of FA revealed that eigen values that were > 1 accounted for 91.127 % (Table 17) and 81.950 % (Table 18) of the total variance indicating that different controlling factors or sources are responsible for the trace metal distributions in sediments and water. First factor (F1) accounted for 49.782 % and 43.968 % of the total variance of the 8 variables in the sediment analysis and water analysis respectively, while F2 and F3 accounted for 28.274 % and 22.234 % and 13.072 % and 15.748 % of the total variance in the sediment assessment (Table 17) and water analysis (Table 18) respectively (Watts et al., 2017).

Table 17: Eigenvalue and variance of minor elements for sediment.

Factor	Eigenvalue	Percentage of Variance (%)	Cumulative (%)
1	3.983	49.782	49.782
2	2.262	28.274	78.055
3	1.046	13.072	91.127

Table 18: Eigenvalue and variance of minor elements for water.

Factor	Eigenvalue	Percentage of Variance (%)	Cumulative (%)
1	2.638	43.968	43.968
2	1.334	22.234	66.202
3	0.945	15.748	81.950

- There are different methods for the rotation, but the most common method is the analytical varimax rotation. Hence, FA was performed with varimax orthogonal rotation to produce three varifactors with Cd, Cr, Cd, Ni, Pb, pH, T, Salinity (Table 19 & Table 20).

From the rotated loading table (Table 19) we can see that pH has the largest value in terms of amount for the 3rd factor and thus this wass assigned to the 3rd factor. As, pH had very weak negative correlation with temperature and salinity, so change in temperature and salinity slightly affect the change in pH.

Temperature and salinity had the greatest value in terms of amount in the 2nd factor and are therefore assigned to the 2nd factor in the sediment (Table 19). Climate change and rising temperatures might increase evapotranspiration, including the evaporation of water from sediments. As a result, water evaporated and the salt remained in the sediment, increasing the sediment salinity.

Rotated solution also showed that Ni, Cu, Pb and Cr were coming together indicating a similarity of source and the anthropogenic origin. Varimax orthogonal rotation optimized the loadings in each factor and it is evident that Cu, Cr, Pb and Ni had a loading value of > 0.5 in factor 1 of sediment dataset (Table 19), whereas Cd and Pb were seen to be in factor 2 in water assessment (Table 20). This indicated that these metals had a similar source of origin in the river sediment and water of Indian Sundarbans and the source was anthropogenic.

Cr was originated from the mixing of tannery effluents containing high toxic chromium compounds along with organic matter and salt content (Saha et al., 2006).

Intensive fishing and ferry services, sewage drainage from the mainland and other commercial activities were supposed to be potential sources of Ni in the sediment (Chatterjee et al., 2007).

The most toxic of the heavy metals was Pb, which found its way in coastal waters through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries etc. Antifouling paints used to prevent growth of marine organisms at the bottom of the boats and trawlers also contain lead as an important component. These paints were designed to constantly leach toxic metals into the water to kill organisms that may attach to bottom of the boats, which ultimately transported to the sediment and aquatic compartments (Mitra et al., 2012).

Pb and Cd were observed in areas affected by shortage of electricity, while Pb pollution attributed to the dependence on water transport (operated by fossil fuels), the anthropogenic origin of Cd source could be the disposal of rechargeable batteries (Chowdhury and Maiti, 2016b).

Cu is an essential trace metal for animal metabolism but at high levels is a very toxic substance to aquatic life (Bryan et al., 1983). The main sources of Cu in the coastal waters were antifouling paints (Goldberg, 1975) and this metal entered into the water body through industrial effluents containing $CuSO4$ used in metal plating and fishing operations (Goldberg, 1975); (Mitra and Choudhury, 1993). This was later transported to the sediment.

Table 19: Rotated loading (Method = Varimax) of minor elements for sediment.

Variable	Factor 1	Factor 2	Factor 3	Communality
pH	0.126	-0.004	**0.976**	0.968
Temperature	-0.095	**0.942**	-0.039	0.897
Salinity	0.135	**0.893**	-0.039	0.817
Ni	**0.945**	-0.198	0.218	0.980
Cu	**0.942**	-0.014	-0.117	0.902
Cd	0.346	**-0.875**	-0.033	0.886
Pb	**0.828**	-0.185	0.391	0.873
Cr	**0.983**	0.029	0.035	0.968

Table 20: Rotated loading (Method = Varimax) of minor elements for water.

Variable	Factor 1	Factor 2	Factor 3	Communality
pH	0.003	-0.171	**0.937**	0.907
Temperature	**0.908**	-0.150	-0.155	0.872
Salinity	**0.920**	0.335	0.010	0.958
Cu	**0.733**	0.376	0.350	0.801
Pb	0.262	**0.677**	-0.202	0.568
Cd	-0.004	**0.900**	-0.029	0.811

5.4.2. Factor analysis for the major elements:

- From the correlation matrix (Table 21 & Table 22) it is shown that Al, Mn, Fe were strongly positively correlated with Mg; Mn, Fe, Ca were strongly positively correlated with Al; Fe and Ca were strongly positively correlated with Mn; and Fe was also strongly positively correlated with Ca as the values were > 0.5 in sediment analysis which means that all of these metals have similar source of origin and that was crustal. Salinity had strong positive correlation with temperature in both sediment analysis (Table 21) and water analysis (Table 22).

  Al and Fe has strong negative correlation with pH as the value was < -0.5.

Al and Fe were strongly positively correlated with each other in water analysis that means they have similar source and that is crustal.

Ca showed strong positive correlation with temperature and salinity in water analysis.

Table 21: Correlation matrix of major elements for sediment.

	pH	Temperature	Salinity	Mg	Al	Mn	Fe	Ca
pH	1							
Temperature	-0.071	1						
Salinity	-0.037	**0.752**	1					
Mg	0.159	-0.194	0.088	1				
Al	0.211	-0.404	-0.172	**0.847**	1			
Mn	0.376	-0.437	-0.336	**0.650**	**0.913**	1		
Fe	0.316	-0.258	-0.052	**0.783**	**0.762**	**0.754**	1	
Ca	0.466	-0.347	-0.358	0.464	**0.821**	**0.915**	**0.502**	1

Table 22: Correlation matrix of major elements for water.

	pH	Temperature	Salinity	Al	Fe	Ca
pH	1					
Temperature	-0.063	1				
Salinity	-0.037	**0.730**	1			
Al	**-0.704**	0.404	0.392	1		
Fe	**-0.731**	-0.165	0.005	**0.603**	1	
Ca	0.063	**0.530**	**0.962**	0.254	-0.018	1

- The result of FA (Table 23 & Table 24) revealed that eigen values that were > 1 accounted for 87.970 % and 93.376 % of the total variance indicating that different controlling factors or sources are responsible for the trace metal distributions in sediments and water. First factor (F1) accounted for explains 54.865 % and 46.852 % of the total variance of the 8 variables in the sediment analysis and water analysis respectively, while F2 and F3 accounted for 20.567 % and 36.300% and 12.538 % and

10.224% of the total variance in the sediment assessment (Table 23) and water analysis (Table 24) respectively.

Table 23: Eigenvalue and variance of major elements for sediment.

Factor	Eigenvalue	Percentage of Variance (%)	Cumulative (%)
1	4.389	54.865	54.865
2	1.645	20.567	75.432
3	1.003	12.538	87.970

Table 24: Eigenvalue and variance of major elements for water.

Factor	Eigenvalue	Percentage of Variance (%)	Cumulative (%)
1	2.811	46.852	46.852
2	2.178	36.300	83.152
3	0.613	10.224	93.376

- FA was performed with varimax orthogonal rotation to produce three varifactors with Mg, Al, Fe, Mn, Ca, pH, T, Salinity (Table 25 & Table 26).

  From the rotated loading table (Table 25 & Table 26) we can see that pH had the largest value in terms of amount for the 3rd factor and 1st factor in sediment and water analysis respectively. As, pH has very weak negative correlation with temperature and salinity, pH does come together with temperature and salinity.

  Temperature and salinity has the greatest value in terms of amount in the 2nd factor and were therefore assigned to the 2nd factor in the sediment as with increasing temperature, salinity was also increased. But in case of water, temperature and salinity were assigned in two different factors, factor 3 and 2 respectively. As, with increasing water temperature water density decreased and as a result water salinity also decreased.

  Varimax orthogonal rotation optimized the loadings in each factor and it was evident that Mg, Al, Fe, Mn and Ca had a loading value of > 0.5 in factor 1 of sediment dataset (Table 25), whereas Al and Fe were seen to be in factor 1 and Ca is seen in factor 2 in water assessment (Table 26). This indicated that these metals had a similar origin in the river sediment and water of the Indian Sundarbans, and that these elements were deficient in certain of the phases in comparison to crustal abundance in the study area (Chatterjee et al., 2007).

Table 25: Rotated loading (Method = Varimax) of major elements for sediment.

Variable	Factor 1	Factor 2	Factor 3	Communality
pH	0.117	0.035	**0.942**	0.902
Temperature	-0.232	**0.884**	0.023	0.836
Salinity	0.034	**0.951**	-0.080	0.912
Mg	**0.936**	0.081	-0.033	0.885
Al	**0.940**	-0.230	0.152	0.959
Mn	**0.812**	-0.357	0.384	0.933
Fe	**0.868**	-0.027	0.161	0.780
Ca	**0.621**	-0.365	0.557	0.830

Table 26: Rotated loading (Method = Varimax) of major elements for water.

Variable	Factor 1	Factor 2	Factor 3	Communality
pH	**-0.923**	0.100	-0.098	0.871
Temperature	0.035	0.429	**0.876**	0.953
Salinity	0.085	**0.921**	0.377	0.998
Al	**0.828**	0.203	0.387	0.877
Fe	**0.899**	0.057	-0.318	0.912
Ca	-0.003	**0.986**	0.140	0.991

CHAPTER 6

CONCLUSION

In the last century, industrial processes have been creating waste all around the world, resulting in contaminated sediments and subsequent, far-reaching dispersal into water ecosystem including river, pond, groundwater, marine. The pollutants have accumulated and are still being created a major problem for us, Biofilm system is a well-developed, cost effective, and ecofriendly technology in which different microbes such as bacteria, fungi, and microalgae form a colony. Microbial biofilm-based systems for wastewater treatment are rapidly expanding research area.

In this present study we can see that the sediments were contaminated with heavy metals pollution from several nonpoint source, such as seepage of fossil fuel during water transportation activities, discharge of industrial waste in the upstream of river and non-green disposal of rechargeable batteries. The sediment analysis around the Sundarban mangrove forest indicated that ----

1) The sediments were contaminated with Ni. The average Ni concentration of these study area was 113 mg/kg which was higher than the average shale value 68 mg/kg. The main source of Ni pollution was intensive fishing and ferry services, sewage drainage from the mainland.
2) Ni, Cd, Zn, Mn, and Pb were the main anthropogenic pollutants. This finding was substantiated by EF, Igeo, CF, and further analysis by RI.
3) RI showed that only Cd at Namkhana and Kakdwip have moderate potential risk due to the fishing harbour and disposal of rechargeable batteries.
4) From the factor analysis result it was shown that the Ni, Cu, Cd, Pb, and Cr pollution occurred due to the anthropogenic activities but the source of Mg, Al, Mn, Fe, and Ca pollution was mainly crustal enrichment.

Among the four sampling locations (Patharpratima, Namkhana, Kakdwip, and Canning) it was observed that the pollution level was quite higher at Kakdwip. As a result of this, the growth of the biofilms were observed significantly at Kakdwip. The sample of biofilms collected from different sampling locations are shown in figure 6.

(a) (b)

(c) (d)

Figure 6: Biofilm samples from four sampling locations. (a) Patharpratima, (b) Namkhana, (c) Kakdwip, (d) Canning.

The rivers of the Sundarbans, India are contaminated with the B, K, and Cu and their average concentration in the water bodies are 2.16 ppm, 202 ppm and 1571 ppb respectively, and the probable sources could be the seepage of low-grade fossil fuel from ecotourism boat/launch/ferry transport and non-green disposal of rechargeable batteries.

The combined uses of different approaches indicate that the study area is slightly contaminated with different heavy metals due to anthropogenic activities which were confirmed by the correlation, Enrichment factor and FA. Continuous monitoring and assessment of these heavy metals should be carried out in future to check the pollution status to upgrade the brackish environment by ameliorating anthropogenic activities.

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