

HEAVY METALS DISTRIBUTION AND FRACTIONATION IN MANGROVE SEDIMENTS LINKED TO ORGANIC DEPOSITS VIS-À-VIS ACCUMULATION IN *RHIZOPHORA* SPP. AT TANJUNG PIAI, JOHOR, MALAYSIA

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Abstract. Mangrove ecosystems are crucial for ecological processes. However, mangrove forests are currently under peril due to urbanization, aquaculture expansion, and growing pollutant burdens. Marine debris including organic deposits (ODs) is intentionally or unintentionally deposited into the marine environment, which could alter the natural ecology of the ecosystem. ODs from Tanjung Piai, Johor, Malaysia have high concentration of heavy metals (HMs), specially Cd and Pb, with respective concentration of 2.45 mg kg⁻¹ and 82.41 mg kg⁻¹, which surpassed the compost guidelines of the European and United Kingdom. Four sampling sites were established in Tanjung Piai based on ODs levels: T1: without ODs, T2: new ODs, T3 and T4: decomposed ODs 90 m and 150 m from shoreline, respectively. T3 and T4 had the highest Cu and Pb. Modified sequential extraction European Community Bureau of Reference (BCR) reveals that Fe and Mn are highly mobile for mangrove species uptake, with concentrations of these HMs in *Rhizophora* spp. leaves higher than Cd, Pb, and Zn, implying that these species did not meet the criteria for hyperaccumulators due to low metals accumulation, necessitating further research to identify species suited for phytoextraction of HMs.

Keywords: *mangroves, Tanjung Piai, heavy metals, fractionation, Rhizophora spp., phytoextraction*

Introduction

Mangrove ecosystems are among the most significant intertidal wetlands in tropical or subtropical coastal regions. In coastal environments, mangrove sediments can act as sinks for anthropogenic contaminants (especially heavy metal contaminants) due to the anaerobic properties, reductive and rich in sulfides, organic matter and high productivity (Nath et al., 2014). As urbanization and industrialization grow in coastal regions, mangrove ecosystems will play an increasingly important role in controlling heavy metal pollution (Sarath et al., 2021). In order to estimate the toxicity of metallic trace elements in soil, it is necessary to determine not only the total concentration, but also the forms in which heavy metals occur – as the implications for the prediction of changes in their solubility that may occur in soil as a result of changes in environmental conditions (Zhang et al., 2010). Several physical properties of particular elements determine their mobility in soil, as well as the properties of the soil.

Generally, heavy metal pollution is usually resistant to environmental factors. Heavy metals have a significant impact on environmental nutrient cycles, as well as the quality of food (Strayer, 2014). Heavy metals may enter the food chain in various ways and accumulate in living organisms (Kumar et al., 2019). Plants typically absorb and accumulate toxic metals from soils, water, or the atmosphere. Among the components of ecosystems, soils are the largest receivers of toxic metals (Kormoker et al., 2021). Anthropogenic sources and soil parent material can affect the total metal concentration in soils. It is important to understand the levels of heavy metals in sediments, in terms of their mobility, their ecological hazards, as well as their availability to plants (Charzyński et al., 2017). Consequently, heavy metals mobility, bioavailability and ecological toxicity to plants are primarily governed by their fractions. The exchangeable forms in these fractions are considered bioavailable, while the bound forms of carbonates, iron, and manganese oxides can also be considered bioavailable (Zhang et al., 2014). However, most residues are not available to plants or microorganisms.

Sequential extraction procedures (SEPs) have been widely used to assess heavy metal speciation in soils (Choleva et al., 2020). By using selective reagents, the heavy metals will be dissolved in different components to provide a more realistic estimation of their environmental impact (Du et al., 2020). The SEPs proposed by the European Community Bureau of Reference (BCR) are widely used for analyzing heavy metals fractions. The BCR sequential extraction method consists of three steps: exchangeable and bound to carbonates; reducible (bound to Fe – Mn oxides); and oxidizable (bound to organic matter and sulfides). In the last step, metals bond with minerals that are soluble in strong acids. With sequential extraction technique, comprehensive information on trace metal origin, availability, mobilization and transportation can be obtained.

Geographically, Tanjung Piai mangrove forest occupies a very unique geographical location. Located at 1°16.00' North, is the southernmost point of mainland Asia, or more precisely, continental Eurasia (Hui et al., 2019). It has been an ongoing battle since Tanjung Piai National Park was established in 1997 to protect its mangrove forest, which has faced serious erosion issues due to intensity of waves generated by thousands of oil tankers that sail by every year, as well as pollution events from oil spills and illegal ballast water dumping (Kunasekaran et al., 2018). The Tanjung Piai mangrove forest is also threatened with marine debris that contains organic deposits which result in the decaying mangrove roots and slowly kills the mangrove trees, due to its acidic composition and high levels of heavy metals (Wan Rasidah et al., 2015). Although quite a number of studies have examined the heavy metal concentration in this area, none have assessed the mobility of heavy metals in Tanjung Piai mangrove forest. Therefore, it is necessary to determine the concentration and distribution of heavy metals in this area to assess the current condition of its ecosystem and to compile baseline data for monitoring in the future. Phytoremediation is a green approach that use several types of plants, known as phytoremediators, to remove heavy metals (HMs) from soil. *Rhizophora apiculata* (*R. apiculata*) is an important mangrove species in Peninsular Malaysia in this regard. Because of its deep root structure, this species is well recognized worldwide for its capacity to enhance water quality by trapping sediments and extracting minerals contained in saltwater (Sarath et al., 2021). However, little study is being done in Malaysia on the phytoextraction potential of several *Rhizophora* spp.

The study aims to investigate the concentration and deposition of selected heavy metals, i.e. Cd, Cu, Fe, Mn, Pb and Zn, in the mangrove sediment around the Tanjung

Piai mangrove forest. The concentration of heavy metals in mangrove sediments and growth of mangrove can be affected by anthropogenic activities such as sewage discharge, waste dumping or shipping activities. Nevertheless, studies on the effects of organic deposits on sediments heavy metals are still lacking. Due to the presents and composition of organic deposits at the study sites were in different composition, therefore heavy metals contents were compared in the sediments not the organic deposit present at the study sites in Tanjung Piai, Johor, Malaysia. This study can enhance the understanding of heavy metal contamination in the Tanjung Piai mangrove forest. The information will contribute to effective monitoring of both environmental quality and sustainable development for decision makers involved in coastal ecosystem management in Malaysia.

Materials and methods

Site description

Tanjung Piai mangrove forest

Tanjung Piai mangrove forest located in Pontian is one of the Ramsar sites in Johor. Ramsar sites are wetlands that have been identified according to the Ramsar Convention on Wetlands based on the representative, rare and unique or the importance of the wetlands to the conservation of biological diversity. Tanjung Piai National Park located 1° 16' 04.2" north latitude and 103° 30' 30.2" east latitude in Johor is the most Southern tip of Peninsular Malaysia which made up mostly of mangrove and mudflats. Located strategically between Malaysia, Singapore and Indonesia, this is the most significant place geographically as the southernmost point of Mainland Asia, which attracted both local and foreign tourists to explore the tip.

Design of the study

The sampling sites were selected at different localities based on the presence and extent of organic deposit (Figs. 1 and 2). Three dominant species of *Rhizophora* spp. in Tanjung Piai mangrove forest have been selected for the leaves sampling. Table 1 describes the sampling sites for sediments and leaves of *Rhizophora* spp: *Rhizophora stylosa*, *Rhizophora apiculata*, and *Rhizophora mucronata* that had been established in Tanjung Piai mangrove forest.



Figure 1. Map of the study area at Tanjung Piai mangrove forest, Johor



Figure 2. Sampling area at Tanjung Piai mangrove forest, Johor, Malaysia

Table 1. The description of each plot in Tanjung Piai, Johor

Plot	Description
T1	Site without organic deposit material <ul style="list-style-type: none"> • There is no organic material present at this plot • 25 m from shoreline
T2	Site with new organic deposit material <ul style="list-style-type: none"> • The presence of organic deposit on surface of the sediment • 40 m from shoreline • 1°16'00.2"N 103°30'27.9"E
T3	Site with decomposed organic deposit material <ul style="list-style-type: none"> • The organic deposit material mixed in the sediment • 90 m from shoreline • 1°16'03.3"N 103°30'25.4"E
T4	Site with decomposed organic deposit material <ul style="list-style-type: none"> • The organic deposit material mixed in the sediment • 150 m from shoreline • 1°16'03.7"N 103°30'25.0"E

Sediment sampling at T1 were divided into two groups which are:

1. T1F: Sampling site facing sea
2. T1B: Sampling site facing mangrove forest

Organic deposit sampling

Fresh organic deposit was collected around the T2 study sites. Samples were collected in June 2016. Organic deposit samples were collected on top of the soil using stainless steel shovel. Samples were kept in a zip lock plastic bag and transferred directly to the laboratory for analysis. Samples were dried in the oven at temperature to constant weight (60 °C for 48 to 72 h), and ground in an agate mortar.

Organic deposit heavy metal analysis

The heavy metals content of the organic deposit was measured as follows: A crucible holding 0.5 g of organic deposit sample was weighed and heated for 5–6 h in a furnace

at 500 °C. Three drops of pure water were poured to each crucible, followed by 2 mL of strong hydrochloric acid (HCl). The crucible was then placed in the sand bath to dry off the HCl solution. The Agilent 725 ICP-OES was used to determine the elements. 10 ml of 20% nitric acid was pipetted into the crucible and heated on the sand bath for 30 min to 1 h, or until only 3 to 4 mL of nitric acid remained. The materials were filtered into a 25 ml volumetric flask, the crucible was washed, and the solution was brought up to volume. The heavy metal content was determined using an Agilent 725 ICP-OES.

Sediment and leaves sampling

A total of 10 sediments samples (0 – 10 cm and 10 – 30 cm, triplicates for each sample) from sampling plots were collected using sediment Augers. Samples were kept in a zip lock plastic bag and transferred directly to the laboratory for analysis. All sediments samples were dried in the oven at temperature not exceeding 40 °C. The samples were then ground and sieved after completely dried. Random 10 *Rhizophora* spp. trees were selected for leaves analysis. Leaves sampling were collected and placed in separate container according to the three species of *Rhizophora* spp. within the same study plot for heavy metals content analysis. Random leaf position were collected (upper, middle and basal leaves). Samples were dried to constant weight (60 °C for 48 to 72 h), and ground in an agate mortar. Portions of the sample were kept in a zip lock plastic prior to chemical analysis.

Heavy metal content in sediment at Tanjung Piai mangrove forest

The concentrations of heavy metals Cd, Cu, Fe, Mn, Pb, and Zn were measured using the Agilent 725 ICP – OES and the Aqua Regia extraction technique. 0.5 g of sediment samples were digested with 2 ml hydrochloric acid and 1 ml nitric acid in the digestion tube. The final digested mixture was filtered and heavy metals concentration was determined using an Agilent 725 ICP-OES.

Fractionation of heavy metals in sediment at Tanjung Piai mangrove forest

A four-stage BCR (European Community Bureau of Reference) sequential extraction was followed according to the procedure described fully in Rauret et al. (2000). The extraction process was divided into four stages was performed in triplicate.

Acid soluble and exchangeable portion (F1)

In a 100 mL centrifuge tube with 40 mL of 0.11 M acetic acid, one gramme of material was sonicated for 7 min at 22 ± 5 °C. After that, the mixture was centrifuged at 3000 g for 20 min using Eppendorf Centrifuge 5810. The extract was separated for analysis. After sonicating the residue for 5 min with 20 mL of deionized water, it was centrifuged for 20 min at 3000 g. The water was discarded.

Reducible fraction, bound to Fe/Mn oxides (F2)

The residue from the first step was added with 40 mL of fresh 0.5 M hydroxylamine hydrochloride solution, pH 1.5, and sonicated for 7 min at temperature 22 ± 5 °C. The mixture was then centrifuged at 3000 g for 20 min. For the reducible fraction analysis, the extract was separated. Similarly, to the first stage, the residue was washed with deionized water.

Oxidizable fraction, bound to organic matter (F3)

Residue from the second step was added with 20 mL of 30% hydrogen peroxide and sonicated for 2 min at temperature 22 ± 5 °C. Then, reduced the volume of H₂O₂ around 1 mL using water bath. The moist residue was added with 50 mL of 1 M ammonium acetate and sonicated for 6 min at 22 ± 5 °C. The mixture was then centrifuged at 3000 g for 20 min. For analysis, the extract was separated. Similarly, to the previous processes, the residue was washed with deionized water.

Residual fraction (F4)

The third-step residue was extracted with concentrated HNO₃ with addition of 30% H₂O₂. It was heated for 30 min at 80 °C before being left overnight. 20 mL distilled water was added to the mixture, which was then mixed using vortex mixture. It was then filtered and made up to volume in a 100 mL volumetric flask. The fractionation of heavy metals at each step content was determined by Agilent 725 ICP-OES.

Heavy metals content in Rhizophora spp. leaves

0.5 g of each dried sample was weighed into a porcelain crucible and dry-ashed in a muffle furnace (Thermolyne Type 30400 furnace) at 500 °C for five to 6 h. The residue was dissolved with 2 mL HCl on the sand bath until the HCl solution was completely dry. 10 mL of 20% nitric acid was added and heated for 30 min to 1 h on the sand bath, until roughly three to four mL of nitric acid remained inside the crucible. The samples were filtered into 25 mL volumetric flask, the crucible was rinsed, and the solution was prepared to the required volume. Agilent 725 ICP-OES was used to determine the heavy metals content.

Statistical analysis

IBM SPSS version 24 was used to analyze the statistical data (IBM Inc., Armonk, NY, USA). The data was given as mean \pm standard deviation of the mean. One-way analysis of variance (ANOVA) and Tukey's Test were used to find the mean difference of data between the variables. When $P \leq 0.05$ was used, the differences were considered statistically significant, and different letters in the same column or row were used to indicate them.

Results

Heavy metals content of organic deposits

The heavy metal content in the organic deposit samples is shown in *Table 2*. The heavy metals value of this study was compared with European countries (EU) and the United Kingdom (UK) for the limit established for compost, as well as a prior study done by Wan Rasidah et al. (2015), for the organic deposit discovered in Tanjung Piai, Johor where the sample of organic deposit were collected in 2013. The sampling site for organic deposit both of this study were at same location which is at T2 (1°16'00.2"N 103°30'27.9"E). The concentrations of Pb and Zn in the organic deposit were greater in the current research in Tanjung Piai than in prior studies and other sample locations. The Zn concentration was seven times higher than in the prior study, but it was still below the

EU and UK maximum ranges. Pb content was greater at bit than in earlier studies, with 82.41 mg kg⁻¹, but it above the EU and UK regulatory range. Despite the fact that the Cd value was lower than in the prior study, it surpassed the EU and UK compost limit. Tanjung Piai Cu content was 21.50 mg kg⁻¹, which was lower than the previous study's result of 27.6 mg kg⁻¹. The concentration of Cd and Cu in the current research is lower than the prior study. However, concentration of Cd were exceed the EU and UK limit range. Cu content were still below the EU and UK maximum range for compost.

Table 2. Comparison of heavy metal contained in organic deposit with previous study in Tanjung Piai, EU, UK compost limits

Heavy metal	EU limit range ^a	UK limit range ^b	Previous study ^c	Tanjung Piai ^d
			Tanjung Piai (2015)	(current study)
Cd	0.7	1.5	5.02	2.45 ± 0.30b
Cu	70	200	27.6	21.50 ± 0.10a
Fe	-	-	-	2.61 ± 0.30b
Mn	-	-	-	0.053 ± 0.01b
Pb	45	50	67.2	82.41 ± 0.52a
Zn	200	200	17.5	133.12 ± 1.53a

Means ± standard error of mean value followed by different letters in row are significantly different using repeated measures ANOVA

^aLimits set for compost applied in European countries and United Kingdom (Saveyn and Eder, 2014)

^cResults of heavy metal content in organic deposit from previous study in Tanjung Piai

^dMean of heavy metal content in organic deposit from the study

Heavy metals content in sediment

Tables 3–8 demonstrate the concentrations of heavy metals in sediment at all research plots in Tanjung Piai mangrove forest over a one-year period for both depths of 0 to 10 cm and 10 to 30 cm. Zn > Pb > Cu > Fe > Cd > Mn was the sequence in which the mean heavy metal content in the sediment declined.

Table 3. Cd content in sediments at each study plots

Site	Cd (mg kg ⁻¹)				
	Month 1	Month 3	Month 6	Month 9	Month 12
Sediment depth 0 to 10 cm					
T1F	1.96 ± 0.98b	3.19 ± 0.22ab	2.42 ± 0.14a	2.72 ± 0.31a	1.96 ± 0.14abc
T1B	3.12 ± 0.22a	2.44 ± 0.26c	2.31 ± 0.21a	2.36 ± 0.07a	1.84 ± 0.26bc
T2	1.67 ± 0.28b	1.13 ± 0.19d	1.26 ± 0.18b	1.45 ± 0.30b	1.36 ± 0.28c
T3	3.46 ± 0.28a	3.72 ± 0.24a	2.64 ± 0.13a	2.72 ± 0.21a	2.55 ± 0.05a
T4	3.38 ± 0.42a	2.66 ± 0.13bc	2.48 ± 0.42a	2.58 ± 0.63a	2.39 ± 0.19ab
Sediment depth 10 to 30 cm					
T1F	2.38 ± 0.36b	3.43 ± 0.32a	2.64 ± 0.11a	2.42 ± 0.24bc	1.63 ± 0.06c
T1B	3.43 ± 0.32a	3.59 ± 0.21a	2.72 ± 0.24a	2.65 ± 0.16b	1.34 ± 0.12c
T2	1.28 ± 0.23c	2.79 ± 0.18b	1.49 ± 0.17b	1.65 ± 0.42c	1.28 ± 0.22c
T3	3.12 ± 0.09a	3.22 ± a.0.12b	2.48 ± 0.37a	3.80 ± 0.20a	3.31 ± 0.20a
T4	2.14 ± 0.37a	2.67 ± 0.05c	2.16 ± a.0.40b	2.49 ± 0.37b	2.33 ± 0.36b

Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA

Table 4. Cu concentration in sediments at each study plots

Site	Cu (mg kg ⁻¹)				
	Month 1	Month 3	Month 6	Month 9	Month 12
Sediment depth 0 to 10 cm					
T1F	12.44 ± 0.49c	12.20 ± 0.52c	12.54 ± 0.28c	7.26 ± 0.13d	9.44 ± 0.36c
T1B	11.66 ± 0.62cd	10.29 ± 0.49d	12.32 ± 0.20c	8.66 ± 0.20c	9.54 ± 0.24c
T2	10.11 ± 0.72c	8.11 ± 0.80d	8.30 ± 0.81d	4.56 ± 0.42d	7.32 ± 0.38d
T3	24.43 ± 1.36a	26.01 ± 0.67a	21.75 ± 0.64a	14.80 ± 0.38a	20.31 ± 0.22a
T4	16.84 ± 0.16b	18.97 ± 0.56b	19.12 ± 0.53b	12.57 ± 0.06b	18.48 ± 0.22b
Sediment depth 10 to 30 cm					
T1F	12.37 ± 0.30c	12.54 ± 0.84c	7.35 ± 0.28c	10.45 ± 0.21c	8.44 ± 0.34c
T1B	9.53 ± 0.26d	12.32 ± 0.28c	7.55 ± 0.14c	11.04 ± 0.13c	8.49 ± 0.42c
T2	10.45 ± 0.26cd	8.29 ± 0.41d	4.10 ± 0.40d	7.45 ± 0.33d	7.69 ± 0.36c
T3	23.69 ± 0.67a	21.76 ± 0.62a	16.95 ± 0.17a	18.35 ± 0.30a	22.56 ± 0.34a
T4	18.61 ± 0.59b	19.12 ± 0.31b	12.97 ± 0.45b	16.40 ± 0.48b	18.85 ± 0.12b

Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA

Table 5. Fe content in sediments at each study plot

Site	Fe (mg kg ⁻¹)				
	Month 1	Month 3	Month 6	Month 9	Month 12
Sediment depth 0 to 10 cm					
T1F	3.42 ± 0.14c	2.91 ± 0.05ab	2.03 ± 0.10a	3.48 ± 0.10a	2.97 ± 0.02a
T1B	3.35 ± 0.20c	2.18 ± 0.24c	2.03 ± 0.04a	3.07 ± 0.05a	2.95 ± 0.17a
T2	2.19 ± 0.18d	1.39 ± 0.46d	1.23 ± 0.24b	1.39 ± 0.22b	1.58 ± 0.32b
T3	5.15 ± 0.27a	3.46 ± 0.09a	2.13 ± 0.08a	3.53 ± 0.29a	3.39 ± 0.37a
T4	4.45 ± 2.23b	2.36 ± b0.06c	2.01 ± 0.23a	3.57 ± 0.26a	3.06 ± 0.06a
Sediment depth 10 to 30 cm					
T1F	3.36 ± 0.28bc	2.98 ± 0.09a	2.13 ± 0.10a	3.17 ± 0.24b	2.72 ± 0.20a
T1B	2.54 ± 0.02cd	1.48 ± 0.45d	2.26 ± 0.02a	3.35 ± 0.06b	2.69 ± 0.19a
T2	2.38 ± 0.32d	2.33 ± 0.21b	1.30 ± 0.12b	2.25 ± 0.39c	1.44 ± 0.46b
T3	4.42 ± 0.35a	2.38 ± 0.17ab	2.07 ± 0.26a	4.71 ± 0.23a	3.16 ± 0.19a
T4	3.56 ± 0.49bc	2.49 ± 0.02bc	1.90 ± 0.26a	3.18 ± 0.36b	2.52 ± 0.12a

Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA

Table 3 shows the results of cadmium (Cd) content in sediments for both sediment depths. T3 > T4 > T1B > T1F > T4 > T2 was the decreasing order of Cd concentration in the sediments from 0 to 10 cm, and T3 > T1B > T1F > T4 > T2 was the decreasing order of Cd concentration in the sediments from 10 to 30 cm. T3 had the greatest Cd concentration in the sediments throughout the measurement periods at both depths, 0 to 10 cm and 10 to 30 cm, whereas T2 had the lowest Cd concentration. Cd concentrations vary from 1.28 mg kg⁻¹ to 3.80 mg kg⁻¹ across all plots.

The concentration of Cu in the sediments of Tanjung Piai mangrove forest is shown in Table 4. The maximum concentration was found at T3 for both the 0 to 10 cm and 10 to 30 cm sediment depths. The greatest concentration is 26.01 mg kg⁻¹ at 0 to 10 cm, and 23.69 mg kg⁻¹ at 10 to 30 cm. Cu concentrations are lowest in month 6 at T2, with 4.10 mg kg⁻¹ at 10 to 30 cm.

Table 6. Mn concentrations in sediments at each study plots

Site	Mn (mg kg ⁻¹)				
	Month 1	Month 3	Month 6	Month 9	Month 12
Sediment depth 0 to 10 cm					
T1F	0.08 ± 0.01a	0.07 ± 0.01a	0.04 ± 0.01a	0.07 ± 0.01a	0.06 ± 0.01a
T1B	0.07 ± 0.01a	0.07 ± 0.00a	0.04 ± 0.00a	0.05 ± 0.01b	0.06 ± 0.01b
T2	0.03 ± 0.00b	0.03 ± 0.01b	0.02 ± 0.01b	0.02 ± 0.00c	0.03 ± 0.01b
T3	0.01 ± 0.01c	0.01 ± 0.00c	0.02 ± 0.00b	0.01 ± 0.01c	0.02 ± 0.01b
T4	0.02 ± 0.01b	0.01 ± 0.1c	0.01 ± 0.01b	0.01 ± 0.01c	0.02 ± 0.01b
Sediment depth 10 to 30					
T1F	0.10 ± 0.00a	0.13 ± 0.01a	0.06 ± 0.00a	0.06 ± 0.01a	0.05 ± 0.01a
T1B	0.06 ± 0.01b	0.08 ± 0.01b	0.05 ± 0.01a	0.07 ± 0.00a	0.05 ± 0.01a
T2	0.03 ± 0.01c	0.06 ± 0.01b	0.02 ± 0.01b	0.03 ± 0.01b	0.03 ± 0.01b
T3	0.01 ± 0.01d	0.01 ± 0.00c	0.01 ± 0.00c	0.02 ± 0.01b	0.01 ± 0.01c
T4	0.01 ± 0.01d	0.01 ± 0.01b	0.01 ± 0.00c	0.01 ± 0.01b	0.02 ± 0.01bc

Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA

Table 7. Pb concentration in sediments at each study plots

Site	Pb (mg kg ⁻¹)				
	Month 1	Month 3	Month 6	Month 9	Month 12
Sediment depth 0 to 10 cm					
T1F	25.64 ± 2.61c	86.06 ± 2.54b	26.29 ± 1.70c	34.18 ± 2.73b	26.44 ± 1.62b
T1B	25.08 ± 0.64c	64.56 ± 3.97c	23.86 ± 2.51cd	26.13 ± 1.02c	26.52 ± 1.86b
T2	26.12 ± 1.08c	24.21 ± 2.71d	13.86 ± 3.52d	16.16 ± 3.97d	17.64 ± 3.27c
T3	69.23 ± 3.89a	117.31 ± 5.58a	51.24 ± 6.82a	56.67 ± 2.34a	21.23 ± 0.75bc
T4	48.19 ± 1.67b	78.69 ± 5.05b	28.63 ± 1.35b	34.8 ± 2.91b	42.97 ± 1.82a
Sediment depth 10 to 30 cm					
T1F	30.03 ± 1.19c	86.59 ± 1.80ab	24.74 ± 0.23b	26.25 ± 1.05c	26.031.25ab
T1B	27.39 ± 2.04c	44.18 ± 3.12d	27.40 ± 1.20b	26.83 ± 1.37c	25.27 ± 1.54b
T2	24.24 ± 1.59c	65.46 ± 3.56c	18.91 ± 1.29c	23.54 ± 1.77c	22.81 ± 2.85b
T3	67.07 ± 5.72a	88.54 ± 3.39a	34.26 ± 1.84a	59.36 ± 1.18a	27.86 ± 0.78a
T4	52.36 ± 2.57b	78.39 ± 3.23b	37.71 ± 3.55a	45.28 ± 1.64b	16.75 ± 0.83c

Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA

Table 5 shows the Fe content in the sediments of all plots in the Tanjung Piai mangrove forest. T3 has the greatest Fe concentrations at depths of 0 to 10 cm and 10 to 30 cm.

30 cm, respectively, with 5.15 mg kg⁻¹ and 4.17 mg kg⁻¹. At depths of 0 to 10 cm, there is no significant difference between plot T1F, T1B, T3 and T4 during month 6, month 9, and month 12 measurements. Among the other heavy metals tested in the sediment samples, Mn had the lowest concentration (Table 7). At T1F, the greatest Mn content is 0.13 mg kg⁻¹. Mn concentrations are lowest at T3 and T4, ranging from 0.01 to 0.02 at both depths. The second greatest concentration of Pb was found in the sediments, as reported in Table 7. Pb concentrations were greatest in all plots during M3, compared to other months in this research. From month 1 to month 3, the concentration increased, then decreased during month 6. T3 has the greatest concentration, 117.31 mg kg⁻¹. At T2, the lowest value of Pb was 16.16 mg kg⁻¹. Table 8 shows the Zn concentrations at all research plots. The highest concentrations of Zn were identified in T1F and T1B, whereas the lowest concentrations were observed in T2. Save for T2, which had the greatest concentration of Zn in month 3 for both depths, 0 to 10 cm and 10 to 30 cm, the maximum concentration of Zn was detected in month 6 in all plots except T2.

Table 8. Zn concentration in sediments at each study plots

Site	Zn (mg kg ⁻¹)				
	Month 1	Month 3	Month 6	Month 9	Month 12
Sediment depth 0 to 10 cm					
T1F	82.54 ± 2.35a	87.45 ± 0.44b	110.17 ± 2.52a	94.88 ± 3.67a	93.73 ± 2.81a
T1B	86.24 ± 1.18a	89.37 ± 2.35b	112.35 ± 0.52a	81.60 ± 3.69b	96.18 ± 2.68a
T2	44.01 ± 2.52c	126.35 ± 3.62a	82.24 ± 1.88b	34.55 ± 2.02d	53.02 ± 0.74c
T3	43.63 ± 1.65c	50.22 ± 5.44d	97.63 ± 5.34ab	67.07 ± 2.90c	72.35 ± 1.67b
T4	64.87 ± 2.88b	64.88 ± 2.49c	119.02 ± 4.35a	72.42 ± 4.24c	69.40 ± 3.26b
Sediment depth 10 to 30 cm					
T1F	88.43 ± 1.97a	90.01 ± 3.51a	115.86 ± 3.23a	95.23 ± 2.53a	94.98 ± 0.83a
T1B	65.59 ± 3.20b	88.84 ± 5.01a	116.86 ± 2.49a	88.29 ± 0.78b	83.87 ± 3.47b
T2	35.58 ± 3.14d	73.31 ± 1.07b	68.53 ± 3.95c	47.00 ± 0.21d	65.67 ± 3.92c
T3	57.61 ± 1.18bc	67.39 ± 3.70b	124.65 ± 0.69a	69.03 ± 0.13c	76.36 ± 3.47b
T4	52.93 ± 4.90c	82.62 ± 1.18a	91.26 ± 3.83b	73.16 ± 4.04c	56.72 ± 1.74c

Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA

Heavy metals content in *Rhizophora* spp. leaves

For this study, heavy metals were analyzed in the leaves of three different mangrove species. Heavy metal concentrations in plant leaves of *R. stylosa*, *R. apiculata*, and *R. mucronata* at four research plots in Tanjung Piai mangrove forest are shown in Table 9. Mn > Fe > Pb > Cu > Zn > Cd is the average concentration of heavy metals in the leaves, from highest to lowest. The greatest concentration detected in *Rhizophora* spp. leaves is Mn, which has a concentration of 657.60 mg kg⁻¹ in *R. apiculata* leaves at T1. *R. stylosa* and *R. mucronata* had the highest Mn concentrations at T1 in the four-study plot, with 489.87 mg kg⁻¹ and 374.12 mg kg⁻¹, respectively. Others with reading of Mn concentration more than 200 mg kg⁻¹ are *R. stylosa* at T2, *R. apiculata* at T4 and *R. mucronata* at T3.

Second highest concentration of heavy metals in *Rhizophora* spp. leaves is Fe with 469.99 mg kg⁻¹ in *R. mucronata* at T3, 303.05 at T4 and 226.84 at T1. *R. stylosa* in T3

and T4 also have high concentration of Fe with 346.59 mg kg⁻¹ and 350.00 mg kg⁻¹ followed by T1 with 283.57 mg kg⁻¹. *R. apiculata* leaves samples at T1, T3 and T4 also recorded Fe concentration with more than 200 mg kg⁻¹. All *Rhizophora* spp. leaves at T2 have the Fe concentration below than 200 mg kg⁻¹ but more than 100 mg kg⁻¹.

Table 9. Heavy metal content in *Rhizophora* spp. leaves

Site	mg kg ⁻¹					
	Cd	Cu	Fe	Mn	Pb	Zn
<i>Rhizophora stylosa</i>						
T1	nd	nd	283.57 ± 2.79b	489.87 ± 2.28a	0.73 ± 0.09c	nd
T2	nd	0.48 ± 0.05b	164.79 ± 2.43c	207.19 ± 1.06b	2.22 ± 0.04a	nd
T3	0.05 ± 0.01b	0.43 ± 0.02b	346.59 ± 5.48a	196.17 ± 0.94b	1.63 ± 0.07b	nd
T4	0.53 ± 0.03a	2.64 ± 0.13a	350.00 ± 1.50a	162.43 ± 6.18c	1.56 ± 0.04b	nd
<i>Rhizophora apiculata</i>						
T1	nd	nd	216.08 ± 2.17c	657.60 ± 2.98a	1.30 ± 0.04d	0.62 ± 0.04a
T2	nd	0.39 ± 0.33b	176.25 ± 1.04d	186.66 ± 2.33c	2.83 ± 0.07a	nd
T3	nd	1.63 ± 0.14a	276.71 ± 1.68a	189.53 ± 1.514c	2.29 ± 0.04b	nd
T4	nd	0.75 ± 0.07b	248.19 ± 0.93b	248.28 ± 2.09b	1.73 ± 0.04c	nd
<i>Rhizophora mucronata</i>						
T1	nd	0.06 ± 0.01b	226.84 ± 2.56c	374.13 ± 0.85a	1.57 ± 0.29b	0.05 ± 0.35a
T2	nd	nd	135.36 ± 1.59d	167.91 ± 1.74c	2.92 ± 0.10a	nd
T3	nd	1.51 ± 0.06a	469.99 ± 3.99a	224.93 ± 1.97b	1.17 ± 1.14b	nd
T4	nd	1.49 ± 0.08a	303.05 ± 1.23b	163.10 ± 2.05d	1.20 ± 0.05b	nd

Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA. Note: nd = not detectable

However, the maximum Pb content was detected in *Rhizophora* spp. leaves at T2. 2.92 mg kg⁻¹ for *R. mucronata*, 2.83 mg kg⁻¹ for *R. apiculata*, and 2.22 mg kg⁻¹ for *R. stylosa*. Except for *R. stylosa* at T1, which had 0.73 mg kg⁻¹, all Pb values were over 1 mg kg⁻¹. Cu concentration was found to be greatest in *R. stylosa* at T4 with 2.64 mg kg⁻¹, followed by *R. mucronata* at T3 and T4 as well as *R. apiculata* at T3 with 1.51, 1.49, and 1.63 mg kg⁻¹, respectively. *R. mucronata* had the lowest Cu concentration of 0.06 mg kg⁻¹ at T1 and is not detectable at T1 for both *R. stylosa* and *R. mucronata* and T2 for *R. mucronata*. The Zn concentrations were only measurable at T1 for *R. apiculata* and *R. mucronata* and others were not detectable. The concentration for *R. apiculata* is 0.62 mg kg⁻¹ and only 0.05 mg kg⁻¹ detected for *R. mucronata*. Same as Cd concentration, most of the *Rhizophora* spp. leaves were not detectable for this study but only found in *R. stylosa* at T3 and T4 with concentration less than 1 mg kg⁻¹. Heavy metal concentration of Cu, Fe, Mn, Pb and Zn were found to be significantly different ($p < 0.05$) between *Rhizophora stylosa* species over the study plots, while the concentration of Cd was not significantly different. In *Rhizophora apiculata*, the concentration of Cu, Fe, Mn, Pb and Zn were found to be significantly different ($p < 0.05$) between each treatment plots. Next for *Rhizophora mucronata*, the heavy metals except for Cd were found to be significantly different over treatment plots in Tanjung Piai mangrove forest.

Fractionation of heavy metals content of mangrove sediment at different localities in Tanjung Piai mangrove forest

Fractionation of Cd

Figures 3 and 4 visually illustrate the average proportion of metals in fractionation at each research location at both depths; 0 to 10 cm and 10 to 30 cm respectively. The fractionation of heavy metals (Cd, Cu, Fe, Mn, Pb, and Zn) in the extracted solutions was conducted by Agilent 725 ICP-OES. The availability of heavy metals is the sum of residual and oxidizable fraction (F1 + F2). The mobility of heavy metals may be calculated by adding the residual fraction, oxidizable fraction, and reducible fraction (F1 + F2 + F3).

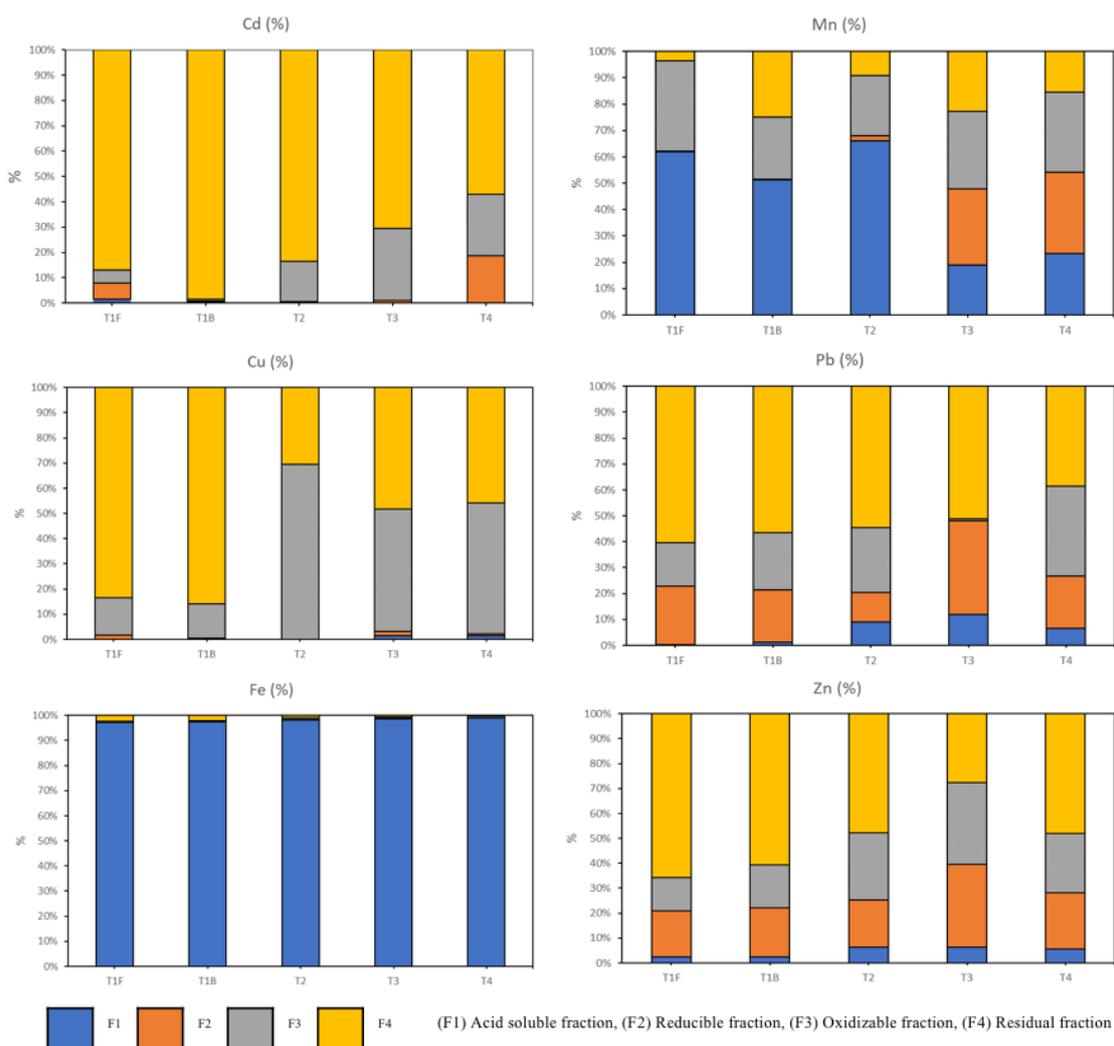


Figure 3. Fractionation of heavy metals at 10 cm at all study plots in Tanjung Piai, Johor

Cd fractionation was in the following sequence at both depths, 0 to 10 cm and 10 to 30 cm: Acid soluble fraction > Residual fraction > Oxidizable fraction > Reducible fraction. T1B has the largest percentage of residual fraction (F4) in the 0 to 10 cm depth range, with 98.56%, followed by T1F with 86.94%. T4 had the lowest residual fraction percentage, at only 57.05%. At a depth of 0 to 10 cm, the mobility and availability of

Cd decreases in the following order: T4 (42.95%) > T3 (29.59%) > T2 (16.46%) > T1F (13.55%) > T1B (1.44%) Cd mobility and availability were as follows between 10 and 30 cm: T2 (39.65%) > T3 (39.38%) > T4 (12.21%) > T1B (11.62%) > T1F (11.62%) (11.54%). At both sediment depths, Cd mobility and availability were not the same. When compared to 0 to 10 cm, Cd was more mobile and available at T4, but less mobile and available from 10 to 30 cm. However, in comparison to others, the proportion of Cd mobility and availability was low at T1F and T1B, at both sediment depths.

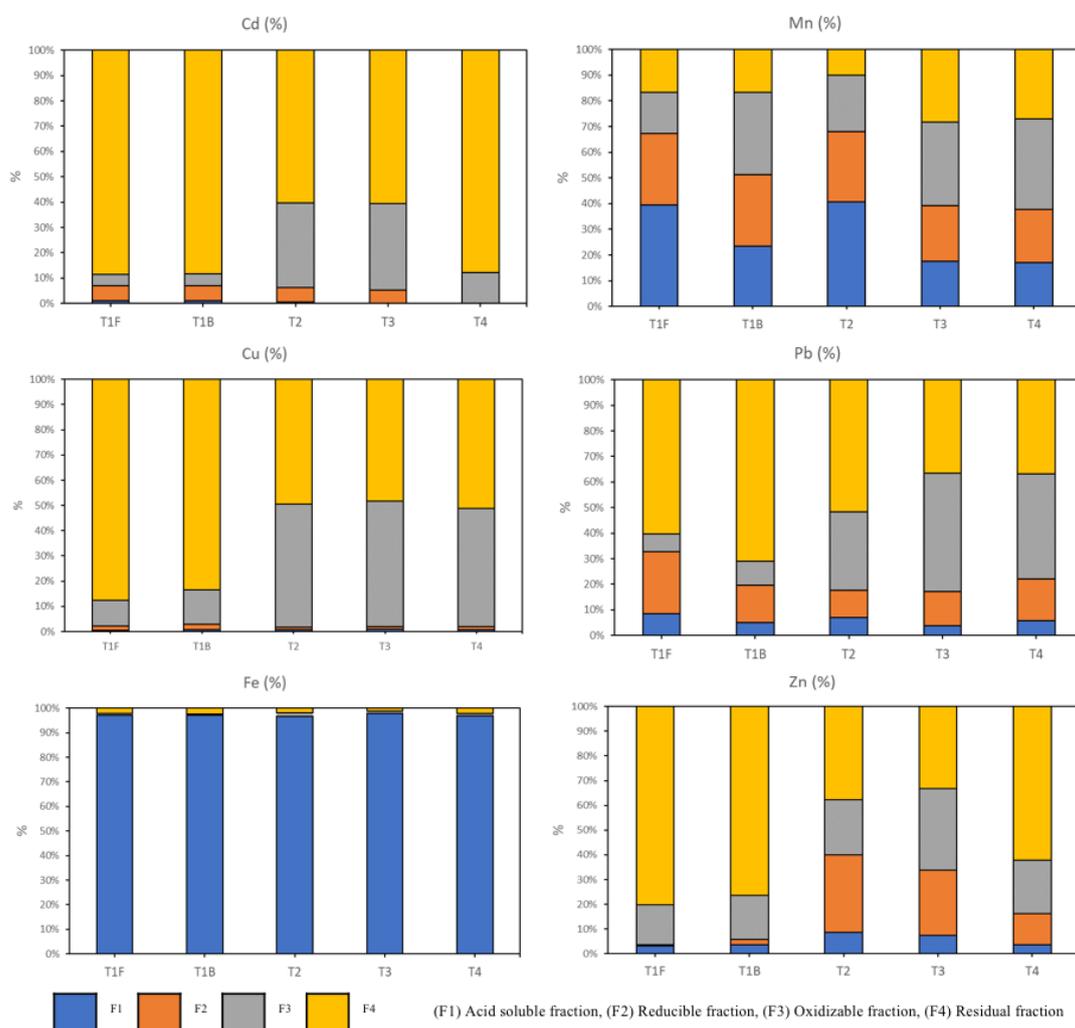


Figure 4. Fractionation of heavy metals at 30 cm at all study plots in Tanjung Piai, Johor

The largest percentages of oxidizable fraction (F3) are found at T3 (28.57%) and T4 (24.16%), whereas the percentage of oxidizable fraction is less than 20% at the other three plots. At T4, the reducible fraction (F2) accounted for 18.79%, while T1F accounted for 6.34%. Cd percentage for residual fraction (T4) was high at 10 to 30 cm depth at T1F, T1B, and T4, with 88.46%, 88.37%, and 87.79%, respectively. T3 and T2 contain substantial percentages of oxidizable fraction at this level, with 34.20% and 33.33%, respectively. Both the acid soluble fraction (F1) and the reducible fraction (F2) had percentages of less than 10%. The acid soluble fraction ranges from 0.00% to 1.16%, whereas the reducible fraction ranges from 0.00% to 5.18%.

Fractionation of copper (Cu)

Cu is divided into fractions. The following orders were followed at both depths: Acid soluble fraction > Residual fraction > Oxidizable fraction > Reducible fraction. Cd fractionation was also substantial from 0 to 10 cm, with 83.42% at T1F and 86.00% at T1B, similar to Cd. The residual proportion in the other three research plots was less than 50%. The oxidizable fraction was greatest at T2, with 69.52%, followed by T4 with 52.06%, and T3 with 48.62%. The oxidizable percentage was only 14.80% and 13.62% at T1F and T1B, respectively. At all research sites, the percentage of acid soluble fraction and reducible fraction was less than 3%. At each research plot, the mobility and availability of Cu at 0 to 10 cm deep were in the following order: T2 (69.52%) > T4 (54.27%) > T3 (51.80%) > T1F (16.59%) > T1B (16.59%) (14.00%). Cu mobility and availability were in the following order between 10 and 30 cm: T3 (51.62%) > T2 (50.54%) > T4 (48.76%) > T1B (16.5%) > T1F (12.4%). T1F and T1B had larger residual fractionation from 10 to 30 cm, with 87.5% and 83.50%, respectively.

T2, T3, and T4 had nearly identical percentages of residual and oxidizable fractions, with 48.93% (F3) and 49.46% (F4) at T2, 49.60% (F3) and 48.38% (T4) at T3, and 46.88% (F3) and 51.24% (F4) at T4. The range for acid soluble and reducible fractionation at all study site is in between 0.57% to 2.17%.

Fractionation of iron (Fe)

At both depths, the fractionation of Fe decreases in the following order: Residual fraction > Oxidizable fraction > Reducible fraction > Acid soluble fraction. Acid soluble and exchangeable fractionation (F1) was more than 95% at both depths, with values ranging from 97.24 to 98.74% from 0 to 10 cm and 96.54 to 97.77% at 10 to 30 cm. As a result, Fe mobility at all researched locations was extremely high at both depths. Other fractionations for Fe were less than 3%. Heavy metals' mobility and toxicity in sediment are primarily determined by their binding structures. Bioavailable substances are those that are exchangeable (F1) or bonded to carbonates (F2). Plants and microorganisms may be able to use the oxidizable fractions (F3), but the residual fraction is largely unavailable to them.

Fractionation of manganese (Mg)

The following was the order of Mn fractionation from 0 to 10 cm depth: Oxidizable fraction > Residual fraction > Reducible fraction > Acid soluble fraction. At 0 to 10 cm depth, T2 has the largest proportion of acid soluble (F1) with 66.03%, followed by T1F with 61.97%. The reducible fraction (F2) at T3 and T4 was high, at 28.96% and 30.94%, respectively, compared to the other three locations, where residual fraction ranged from 0.13% to 1.92%. Even though F2 fractionation was less than 2% at T1F, T1B, and T1, Mn availability was substantial due to the strong F1 fractionation at these three locations. Mn's movement was in the following order: T1F > T2 > T4 > T3 > T1B.

Mn fractionation was in the following sequence at 10 to 30 cm: Reducible fraction > Acid soluble fraction > Residual fraction > Oxidizable fraction. Reduced fraction (F2) at T1F, T1B, and T2 were high at 10 to 30 cm depth as compared to 0 to 10 cm depth. Mn was more accessible from 10 to 30 cm at T1F and T2, with a percentage of availability of more than 65%. With 89%, 82%, and 81%, Mn mobility is high at T2, T1B, and T1F, respectively.

Fractionation of lead (Pb)

Pb fractionation decreased from 0 to 10 cm depth in the following order: Acid soluble fraction > Residual fraction > Reducible fraction > Oxidizable fraction. Pb fractionation is done in the following order at 10 to 30 cm: residual fraction > oxidizable fraction > reducible fraction > acid soluble fraction. In comparison to other fractions, the residual fraction (F4) was large at both depths. T1F has the largest percentage of F4 from 0 to 10 cm, with 66.35%. Pb has a low acid soluble fraction (F1) when compared to Mn and Fe. At 0 to 10 cm, the F1 fraction ranges from 0.24% to 8.95%. Pb availability was poor at depths of 0 to 10 cm, with the total of acid soluble and reducible fractions at all four locations falling below 38%. The mobility of Pb is likewise poor, since the sum of three fractions (F1 + F2 + F3) is less than 50%, with the exception of T4, where the mobility of Pb is 61.43%. T1B has the largest proportion of residual fraction (70.96%) at 10 to 30 cm, followed by T1F with 60.31%. Pb availability was equally poor at this level, with mobility percentages ranging from 17.04% to 32.77%. At T3 and T4, Pb mobility was high, with percentages of mobility of 63.49% and 63.17%, respectively. The proportion of Pb mobility was less than 50% at the other three research sites.

Fractionation of zinc (Zn)

Zn fractionation decreased in the following sequence at both depths: Acid soluble fraction > Residual fraction > Oxidizable fraction > Reducible fraction > Study sites. T1F and T1B exhibit the largest residual fraction (F4) at both depths, with 65.81% and 60.62% respectively from 0 to 10 cm and 80.27% and 76.45% at 10 to 30 cm. This also suggests that Zn is not readily accessible or mobile in plants.

At T2, both depths show modest acid soluble fractionation, with less than 10%. At T2, the residual proportion of 0 to 10 cm was greater than that of 10 to 30 cm. It has a limited availability but is simple to transport since the mobility percentage at both depths is more than 50%. Next, when compared to other research sites, the oxidizable percentage at T3 was high at both depths. With 32.66% in the 0-10 cm range and 32.98% in the 10-30 cm range. This also suggests that Zn was easily transportable from the sediment to the plant at T3. With 48.13% residual (F4) at 0 to 10 cm and 62.20% at 10 to 30 cm, T4 has the largest proportion of residual (F4). At T4, Zn mobility is low at 10 to 30 cm and high at 0 to 10 cm. The availability of Zn at T3 and T4 at both depths was poor since the total of F1 and F2 was less than 40% at this research location. Even though it is easily movable, there is not much of it in the sediment.

Discussion

The mobility and immobility of heavy metals along with their availability in sediment largely depend on their types of binding forms. The mobility and availability of the metals decrease in order of acid soluble fraction > reducible fraction > oxidizable fraction > residual forms (Zimmerman and Weindorf, 2010). The first two fractions, acid soluble and reducible fractions constitute a more available form of the metals. The last two fractions, oxidizable fraction and residual fraction form a less available pool (Alvarez et al., 2002). According to Rauret (1998), the concentration of the first three fractions (acid soluble + reducible + oxidizable) are mobile fractions.

Cd, Pb, and Zn are prevalent in the residual fractions of all samples. Cd, Pb, and Zn were abundant in the residual phase but not in the other geochemical phases, indicating that these metals were more stable in this environment than the other metals. In T2 and T3, the Cu fraction is found to be more bound to organic matter than the other elements. Because of the high formation of organic – Cu compounds, copper can easily complex with organic matters (Fagbote and Olanipekun, 2010). The presence of a high concentration of acid-soluble Fe and Mn indicated that the metal existed in the reduced form (Tessier et al., 1979). The fractional distribution of cadmium indicates that the majority is bound to the residual fraction at all of the Tanjung Piai mangrove forest sites. Only a negligible amount of Cd (1.44% to 42.95%) was released from the non-residual fraction and 57.05% to 98.56% was released from the residual fraction. The high concentration of metal present in the inert phase (residual) is of lattice and detrital origin and can be attributed to natural sources (Singh et al., 2003).

A higher proportion of heavy metals in the non-residual fraction indicates a greater proclivity to become bioavailable. The higher percentages of Fe and Mn in the bioavailable non-residual fraction indicated that their bioavailability and mobility in Tanjung Piai mangrove forest sediments were high. As a result, the potential hazards of Fe and Mn were greater than those of Cd, Pb, and Zn, which were mostly found in the residual fraction. Heavy metal contamination can result in increased heavy metal concentrations in the non-residual fraction, which reflects the degree of anthropogenic influence (McLaren et al., 2004). High mobility and greater availability of Fe and Mn in this study indicate environmental pollution and can pose a critical toxicity risk in plant production areas over time.

The results of heavy metal content in *Rhizophora* spp. leaves show high concentrations of Fe and Mn, which support the fractionation results of Fe and Mn at each plot where their mobility percentage is high, according to *Table 9*. Heavy metal mobility and plant uptake progress through the solution phase. Plant heavy metal uptake is determined not only by its activity in solution, but also by the relationship that exists between solid-phase ions and solution ions (Violante et al., 2010). Mn was found in significant amounts in the reducible fraction; this Mn exists as oxides and may be released if the sediment is subjected to more reducing conditions (Panda et al., 1995). According to Peng et al. (2004), a significant amount of Mn may be released into the environment (reducible fraction) if conditions become more acidic.

Heavy metals like iron, copper, vanadium, and manganese occur naturally in the environment and, depending on their concentration, might function as plant nutrients. Some, such as mercury, lead, cadmium, and chromium, may be dispersed indirectly as a result of human activities and may be harmful even at low concentrations (Khosropour et al., 2019). *Table 9* illustrates the heavy metal concentrations in *Rhizophora* spp. leaves, with Mn having the greatest concentration relative to the other heavy metals. Mn plays an important role in a variety of photosynthetic activities (Ariyanto et al., 2019). Plants with low Mn levels have fewer chloroplasts, reduced chlorophyll content, poorer net photosynthetic efficiency, and are more susceptible to pathogen infections (Alejandro et al., 2017). However, high Mn concentrations may be toxic to the plant. The toxic concentration of Mn is highly dependent on plant species and genotype (Fernando and Lynch, 2015). Excess Mn can inhibit the uptake and translocation of essential elements like Ca, Fe, P, and Mg (Yamaji et al., 2013), cause a decrease in photosynthetic rate (Lambers et al., 2015), and inhibit chlorophyll biosynthesis (Blamey et al., 2015).

Fe is the second most abundant metal found in *Rhizophora* spp. leaves after Mn. Fe, like Mn, is involved in chlorophyll synthesis, and the cause of chlorosis (yellowing) at the leaf surface is associated with Fe deficiency (Yoneyama, 2021). Competition from other cations in the soil, such as calcium and manganese, may cause a lack of Fe uptake (Kathpalia and Bhatla, 2018). Because of the low redox potential of waterlogged soils, the concentration of soluble iron can increase by several orders of magnitude. Excessive Fe uptake is potentially toxic and can promote the formation of reactive oxygen-based radicals, which can damage vital cellular constituents via lipid peroxidation (Mezzaroba et al., 2019). According to Karimian et al. (2018), an increase in the concentration of available Fe in acidic or flooded soils can result in excessive Fe adsorption, often reaching toxic levels. Aside from that, the presence of organic matter, such as organic deposits, can increase Fe availability, presumably by supplying soluble complexing agents that interfere with fixation (Vardhan et al., 2019).

Among the heavy metals found in the sediments, Zn had the highest concentration. Zn is essential for cellular metabolism and can be regulated by organisms in their bodies (Chaiyara et al., 2013). Because of its environmental persistence, toxicity, and ability to be incorporated into food chains, zinc is regarded as a serious pollutant in aquatic ecosystems (Kishe and Machiwa, 2003). Mangrove plants were also known to absorb and accumulate heavy metals in tissues, with Zn mostly accumulating in the roots and less so in the leaves and stems (Kumar et al., 2010). This explains why zinc was mostly undetectable in the leaves of *Rhizophora* spp. in Table 9.

Conclusion

The chemical composition of organic deposits in this study have high concentration of Pb and Zn compared to the pervious study. The content of Pb and Cd in Tanjung Piai's organic deposit are higher than the European and American bio-waste limits. Zn > Pb > Cu > Fe > Cd > Mn were the heavy metal concentrations in the sediments in decreasing order. Fe and Mn fractionation show that these two metals are the most mobile and have the greatest availability. Both metals had high concentrations in leaf samples, indicating that they are easily transportable from sediment to plant. As a result, more research into ecological risk and human health risk assessment is needed to measure human and terrestrial animal exposure in the proximity of the mangrove forest.

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REFERENCES

- [1] Alejandro, S., Cailliatte, R., Alcon, C., Dirick, L., Domergue, F., Correia, D., Curie, C. (2017): Intracellular distribution of manganese by the trans-Golgi network transporter NRAMP2 is critical for photosynthesis and cellular redox homeostasis. – *The Plant Cell* 29(12): 3068-3084.
- [2] Alvarez, E. A., Mochón, M. C., Sanchez, J., Rodríguez, M. T. (2002): Heavy metal extractable forms in sludge from wastewater treatment plants. – *Chemosphere* 47(7): 765-775.

- [3] Ariyanto, D., Gunawan, H., Puspitasari, D., Ningsih, S. S., Jayanegara, A., Hamim, H. (2019): The differences of the elements content in *Rhizophora mucronata* leaves from asahan reGENCY, North Sumatra, Indonesia. – Polish J. Nat. Sci 34(4): 481-491.
- [4] Blamey, F. P. C., Hernandez-Soriano, M. C., Cheng, M., Tang, C., Paterson, D. J., Lombi, E., ... Kopittke, P. M. (2015): Synchrotron-based techniques shed light on mechanisms of plant sensitivity and tolerance to high manganese in the root environment. – Plant Physiology 169(3): 2006-2020.
- [5] Chaiyara, R., Ngoendee, M., Krautrachue, M. (2013): Accumulation of Cd, Cu, Pb and Zn in water, sediments, and mangrove crabs (*Sesarma mederi*) in the upper Gulf of Thailand. – Science Asia 39: 376-383.
- [6] Charzyński, P., Plak, A., Hanaka, A. (2017): Influence of the soil sealing on the geoaccumulation index of heavy metals and various pollution factors. – Environmental Science and Pollution Research 24(5): 4801-4811.
- [7] Choleva, T. G., Tsogas, G. Z., Vlessidis, A. G., Giokas, D. L. (2020): Development of a sequential extraction and speciation procedure for assessing the mobility and fractionation of metal nanoparticles in soils. – Environmental Pollution 263: 114407.
- [8] Du, X., Gao, L., Xun, Y., Feng, L. (2020): Comparison of different sequential extraction procedures to identify and estimate bioavailability of arsenic fractions in soil. – Journal of Soils and Sediments 20(10): 3656-3668.
- [9] Fagbote, E. O., Olanipekun, E. O. (2010): Speciation of heavy metals in soil of bitumen deposit impacted area of Western Nigeria. – Eur. J. Sci. Res. 47: 265-277.
- [10] Fernando, D. R., Lynch, J. P. (2015): Manganese phytotoxicity: new light on an old problem. – Annals of Botany 116(3): 313-319.
- [11] Hui, N. Y., Mohamed, M., Othman, M. N. A., Tokiman, L. (2019): Diversity and behaviour of mudskippers of Tanjung Piai, Pontian, Johor. – IOP Conference Series: Earth and Environmental Science 269(1): 012037.
- [12] Karimian, N., Johnston, S. G., Burton, E. D. (2018): Iron and sulfur cycling in acid sulfate soil wetlands under dynamic redox conditions: a review. – Chemosphere 197: 803-816.
- [13] Kathpalia, R., Bhatla, S. C. (2018): Plant Mineral Nutrition. – In: Bhatla, S. C., Lal, M. A. (eds.) Plant Physiology, Development and Metabolism. Springer, Singapore, pp. 37-81.
- [14] Khosropour, E., Attarod, P., Shirvany, A., Pypker, T. G., Bayramzadeh, V., Hakimi, L., Moeinaddini, M. (2019): Response of *Platanus orientalis* leaves to urban pollution by heavy metals. – Journal of Forestry Research 30(4): 1437-1445.
- [15] Kishe, M. A., Machiwa, J. F. (2003): Distribution of heavy metals in sediments of Mwanza Gulf of Lake Victoria, Tanzania. – Environment International 28: 619-625.
- [16] Kormoker, T., Proshad, R., Islam, S., Ahmed, S., Chandra, K., Uddin, M., Rahman, M. (2021): Toxic metals in agricultural soils near the industrial areas of Bangladesh: ecological and human health risk assessment. – Toxin Reviews 40(4): 1135-1154.
- [17] Kumar, J. I. N., Sajish, P. R., Kumar, R. N., George, B., Viyol, S. (2010): An assessment of the accumulation potential of lead (Pb), zinc (Zn) and cadmium (Cd) by *Avicennia marina* (Forssk.) Vierh. in Vamleshwar Mangroves near Narmada Estuary, West Coast of Gujarat, India. – World Journal of Fish and Marine Sciences 2(5): 450-454.
- [18] Kumar, S., Prasad, S., Yadav, K. K., Shrivastava, M., Gupta, N., Nagar, S., ... Malav, L. C. (2019): Hazardous heavy metals contamination of vegetables and food chain: role of sustainable remediation approaches. A review. – Environmental Research 179: 108792.
- [19] Kunasekaran, P., Rozak, N. I. N., Adam, S. M., Shuib, A. (2018): Perception of local communities on the indicators of governance in Tanjung Piai National Park. – International Journal of Business and Society 19(S1): 79-87.
- [20] Lambers, H., Hayes, P. E., Laliberte, E., Oliveira, R. S., Turner, B. L. (2015): Leaf manganese accumulation and phosphorus-acquisition efficiency. – Trends in Plant Science 20(2): 83-90.

- [21] McLaren. R. G., Kanjanapa. K., Navasumrit, P., Gooneratne. S. R. and Ruchirawat, M. (2004): Cadmium in the water and sediments of the Chao Phraya River and associated waterways, Bangkok, Thailand. – *Water, Air, Soil Pollut.* 154: 385-398.
- [22] Mezzaroba, L., Alfieri, D. F., Simão, A. N. C., Reiche, E. M. V. (2019): The role of zinc, copper, manganese and iron in neurodegenerative diseases. – *Neurotoxicology* 74: 230-241.
- [23] Namieśnik, J., Rabajczyk, A. (2010): The speciation and physico-chemical forms of metals in surface waters and sediments. – *Chemical Speciation & Bioavailability* 22(1): 1-24.
- [24] Nath, B., Chaudhuri, P., Birch, G. (2014): Assessment of biotic response to heavy metal contamination in *Avicennia marina* mangrove ecosystems in Sydney Estuary, Australia. – *Ecotoxicology and Environmental Safety* 107: 284-290.
- [25] Onrizal, O., Amelia, R., Amri, K., Sulistiyono, N., Mansor, M. (2019): Stand structure and diversity of restored mangroves at abandoned pond in eastern coast of North Sumatra. – *IOP Conference Series: Earth and Environmental Science* 305(1): 012050.
- [26] Panda, D., Subramanian, V., Panigrahy, R. C. (1995): Geochemical fractionation of heavy metals in Chilka Lake (east coast of India)—a tropical coastal lagoon. – *Environmental Geology* 26(4): 199-210.
- [27] Peng, S. H., Wang, W. X., Li, X., Yen, Y. F. (2004): Metal partitioning in river sediments measured by sequential extraction and biomimetic approaches. – *Chemosphere* 57(8): 839-851.
- [28] Rauret, G. (1998): Extraction procedures for the determination of heavy metals in contaminated soil and sediment. – *Talanta* 46(3): 449-455.
- [29] Sarath, N. G., Puthur, J. T. (2021): Heavy metal pollution assessment in a mangrove ecosystem scheduled as a community reserve. – *Wetlands Ecology and Management* 29(5): 719-730.
- [30] Saveyn, H., Eder, P. (2014): End-of-Waste Criteria for Biodegradable Waste Subjected to Biological Treatment (Compost & Digestate): Technical Proposals. – Publications Office of the European Union, Luxembourg.
- [31] Singh, M., Müller, G., Singh, I. B. (2003): Geogenic distribution and baseline concentration of heavy metals in sediments of the Ganges River, India. – *Journal of Geochemical Exploration* 80(1): 1-17.
- [32] Strayer, D. L. (2014): Understanding how nutrient cycles and freshwater mussels (Unionoida) affect one another. – *Hydrobiologia* 735(1): 277-292.
- [33] Tessier, A., Campbell, P. G., Bisson, M., (1979): Sequential extraction procedure for the speciation of particulate trace metals. – *Anal. Chem.* 51(7): 844-851.
- [34] Vardhan, K. H., Kumar, P. S., Panda, R. C. (2019): A review on heavy metal pollution, toxicity and remedial measures: current trends and future perspectives. – *Journal of Molecular Liquids* 290: 111197.
- [35] Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. G., Pigna, M. (2010): Mobility and bioavailability of heavy metals and metalloids in soil environments. – *Journal of Soil Science and Plant Nutrition* 10(3): 268-292.
- [36] Wan Rasidah, K., Mohd Zaki, M. I., Mohd Fakhri, I. (2015): Muddy Substrates of Malaysia Coast. – Institut Penyelidikan Perhutanan Malaysia, Kepong.
- [37] Yamaji, N., Sasaki, A., Xia, J. X., Yokosho, K., Ma, J. F. (2013): A node-based switch for preferential distribution of manganese in rice. – *Nature Communications* 4(1): 1-11.
- [38] Yoneyama, T. (2021): Iron delivery to the growing leaves associated with leaf chlorosis in mugineic acid family phytosiderophores-generating graminaceous crops. – *Soil Science and Plant Nutrition* 1-12.
- [39] Zhang, M. K., Liu, Z. Y., Wang, H. (2010): Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. – *Communications in Soil Science and Plant Analysis* 41(7): 820-831.

- [40] Zhang, C., Yu, Z. G., Zeng, G. M., Jiang, M., Yang, Z. Z., Cui, F., ... Hu, L. (2014): Effects of sediment geochemical properties on heavy metal bioavailability. – Environment International 73: 270-281.
- [41] Zimmerman, A. J., Weindorf, D. C. (2010): Heavy metal and trace metal analysis in soil by sequential extraction: a review of procedures. – Int. J. Analyt. Chem. 1-8. DOI: 10.1155/2010/387803.