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Research Article
Bioavailability of Pb and Cu in Sediments of Vegetated Seagrass, *Enhalus acoroides*, from Spermonde Islands, Makassar, South Sulawesi, Indonesia

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Abstract

Seagrass might impact metal bioavailability in sediments with physiological processes that occur at the root and rhizome. Seagrass growth may increase bioavailability due to oxygen transport from leaves to the root systems. This study aims to analyze the concentrations of bioavailable Pb and Cu in sediments with and without seagrass. This study was done at two sites in Spermonde Islands, South Sulawesi, Indonesia. All metals analysis and sediment parameters were conducted on dry, <63 μm grain size sediment samples. Metal speciation in sediment was determined using the Community Bureau of Reference (BCR) three steps sequential that extract exchangeable and acid soluble fraction, reducible and oxidisable fraction. The average concentrations of bioavailable fraction of Pb and Cu (fraction 1) was higher in vegetated sediments associated with *Enhalus acoroides* than in unvegetated sediments. Higher concentration of Cu in fraction 1 was also associated with higher concentration in Enhalus roots. This indicates that the presence of seagrass may increase the bioavailability of metals in sediments. Increased metal bioavailability in vegetated sediment will imply increase toxicity.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anthropogenic pollutants, such as metals can reduce the distribution and biomass of seagrass (Macinnis-Ng and Ralph, 2002). Bioavailability of metals in sediments is strongly influenced by processes that occur in the marine environment. This could affect the availability of metals to organisms that would affect further toxicity (Bernhard and Neff, 2001).

Metal bioavailability is influenced by sediment characteristics such as pH, redox oxidation potential, dissolved organic carbon, inorganic complexes, organic complexes and sediment particle size. Changes in redox potential in sediment will affect metal mobility. Study shows that an increase of oxygen content in anoxic sediments will increase metal released to surrounding environments and thus available to organisms (Clark *et al.*, 1998; Eggleton and Thomas, 2004; Kelderman and Osman, 2007). Dredging activities in sediment, bioturbation and seagrass physiological activites in root systems can influence oxidation state in sediments. Bioturbation by bottom organisms, oligochaetes and benthic bivalves, increases metal bioavailability by changing the redox potential through sediment resuspension (Peterson *et al.*, 1996; Ciutat and Boudou, 2003; Atkinson *et al.*, 2007).

Seagrass can absorb metals from the water column and sediment through the leaf and root-rhizome and then distributed to various comparments in plants (Prange and Dennison, 2000; Macinnis-Ng and Ralph, 2002; Sanz-Lazaro *et al.*, 2012). In coastal waters where the dissolved oxygen is low (suboxic to anoxic), heavy metals will generally be strongly bound to sulphites in sediments and reduce its bioavailability for the organism. However, marine plants such as seagrass can oxidize sediments in the root zone, through the diffusion of oxygen from the leaves to the roots, causing the oxidation of sulphite-metal compounds that can release metals into the sediment which then increases the potential bioavailability to biota (Pulich, 1987; Weis and Weis, 2004).

Lead (Pb) is a toxic metal, found naturally in the waters and has no biological function for the organism (non-essential). Human activities on land (anthropogenic), especially the use of additives in gasoline, paint industries, battery, increasing the concentration of Pb in the waters. According to Neff (2002), the majority of Pb in waters originate from the atmosphere. Copper (Cu) is an essential metal for the metabolism of biota, but increasing concentrations above the standard minimum requirement will have deleterious effects on biotas. The use of Cu a raw material in antifouling paints (TBT) is one of the main sources of these metals entering the sea (Srinivasan and Swain, 2007; Bao *et al.*, 2008). With the increasing development of coastal areas of Makassar, anthropogenic inputs of this metal will also affect sediment and biota living on it.

This study describes differences in Pb and Cu bioavailability in sediment of seagrass and non seagrass areas off the coast of Makassar, Spermonde Islands, South Sulawesi. The result aims to increase understanding of seagrass oxidation impact on metal bioavailability in sediment. It also improves knowledge of metal contamination and toxicity in different sediment types. Information from this study will improve risk assessment method for metal toxicity in sediments.

MATERIALS AND METHODS

Study sites: This study was conducted in Bonetambung Island and Gusung Tallang (Lae Lae Caddi Island), which are part of The Spermonde Islands, near Makassar, South Sulawesi, from March-April, 2013. Gusung Tallang and Bonetambung island are located approximately 1 and 17 km from coast, respectively (Fig. 1). Seagrass beds in Gusung Tallang has monospecies of *Enhalus acoroides*; whereas, in Bonetambung Island has multi-species and dominated by *Enhalus acoroides*. Sediment and seagrass samples were taken from seagrass and non-seagrass areas at each island.

Sample collection

Sediment: There were three replicates of vegetated (seagrass) and non-vegetated (non seagrass) sediment in each island. Sediment was sampled from the vegetated and non vegetated areas from oxic part of sediment surface (1-3 cm depth) using van Veen grab sampler. Samples were specifically taken from the center of the grab to avoid possible contamination from metal parts on the grab and placed into 1 kg plastic bags in a cool box during transport to the laboratory and then frozen in a freezer at 4°C until processing.

Seagrass: Three shoots of *Enhalus acoroides* were randomly collected from the vegetated area in each island. Below ground part (root and rhizome) of the plants were taken for metal content analysis. Prior to the metal analysis, the below ground part were stored overnight at -20°C.

Metal and bioavailability analysis: Sediment and below ground part of seagrass were analyzed for lead (Pb) and copper (Cu). Before analysis, sediments were dried in room temperatures for 4 days and oven dried for 16 h at 80°C. Sediment samples were sieved in dry conditions to attain sediment particles of <63 μm (Loring and Rantala, 1992; Yuan *et al.*, 2004; Hendozko *et al.*, 2010).
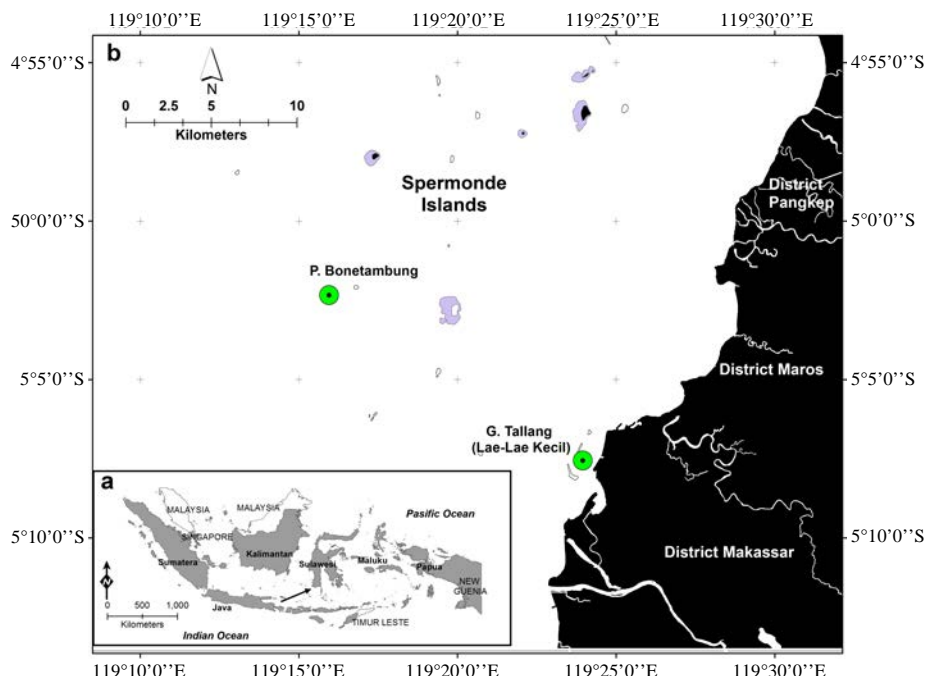
Fig. 1: Study sites in Spermonde Islands, South Sulawesi, Indonesia

Table 1: Three steps sequential procedures determining metals bioavailability in sediments

Steps	Fractions	Exracted components	Procedures
1	Acid soluble	Exchangeable ions and carbonates	40 mL CH_3COOH added on 1 g dry sediments (<63 μm), 16 h shaker
2	Reducible	Fe-Mn oxides	40 mL $NH_2OH.HCl$ (pH 2 with HNO_3), 16 h shaker
3	Oxidizible	Organic compounds and sulfites	10 mL H_2O_2, 1 h disgestion, 1 h water bath, added 10 mL H_2O_2, 1 h water bath, added 50 mL CH_3COONH_4 (pH 2 with HNO_3)

The bioavailable fractions of Pb and Cu in sediments was determined by the Community Bureau of Reference (BCR) Three-Steps Sequential Method (Ure *et al.*, 1993). The extractions procedures were summarized in Table 1. All reagents were of extra pure quality and all lab wares used were either new or thoroughly cleaned with 10% HNO_3 for 24 h before utilization.

Prior to metal extractions on seagrasses, below ground parts (roots and rhizomes) were cut in pieces and oven dry for 48 h in 60°C. Total concentration of Pb, Cu and Fe (was analysed as a sediment character) in sediments and total concentrations of Pb and Cu at the below ground part of the seagrasses were extracted by dry destruction method using HNO_3 (nitric acid) and $HClO_4$ (per chloric acid) (USEPA., 1994). Analysis of metals concentrations were performed on Atomic Absorption Spectrophotometer (AAS) Shimadzu AA-7000.

Sediment character analysis: Sediment samples from the oxic layer of fine grain sediment (<63 μm) were also analysed for: Total Organic Carbon (TOC) content by Walkley-Black titration method (ASTM., 2000); sulfur content spectrophotometrically using HNO_3 dan $HClO_4$ (Tabatabai and Bremner, 1970); $CaCO_3$ by titrimetric method (Allison and Moodi, 1965); sediment texture by hydrometer method, type and sediment grain size based on Wentworth Scale using dry sieving method (Boggs, 2006). Sediment redox potential was measured using Hanna Instrument (HI 8314) with ionode probe, Australia (IJ64).

Statistical analysis: Independent sample T-student test was performed to determine differences in the average concentration of metals in sediments and seagrasses from both study areas. Correlation analysis amongst metal concentrations in each fraction and sediment geochemical parameters was performed using Pearson Correlation. All statistical analysis were computed using SPSS version 16 and the graphs were performed with Microsoft Excel for Windows.

RESULTS

Sediment characteristics: Characteristics and geochemical properties of the sediments in the two sites, Gusung Tallang (Lae Lae Kecil) and Bonetambung Island, are presented in
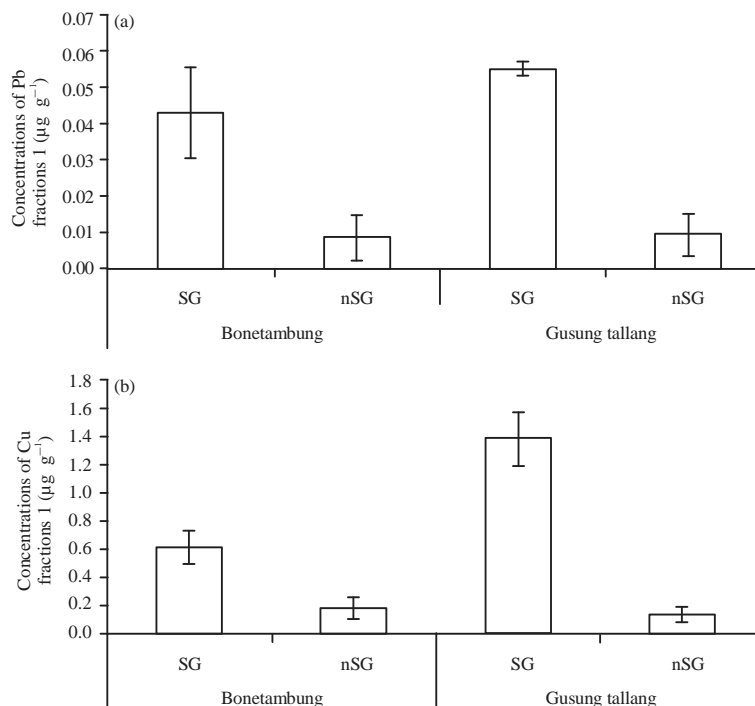
Fig. 2(a-b): Avarage concentrations of Pb and Cu in bioavailable fractions (fraction 1) in seagrass (SG) and non-seagrass (nSG) sediments from Bonetambung Island and Gusung Tallang (Mean±SE, n = 3)

Table 2: Sediment characteristics in Bonetambung Island dan Gusung Tallang

	Bonetambung		Gusung Tallang	
	SG	nSG	SG	nSG
Sand (%)	92.85±0.17	92.95±0.47	92.62±0.28	92.63±0.49
Mud (%)	7.15±0.17	7.05±0.47	7.38±0.28	7.37±0.49
$CaCO_3$ (%)	3.61±0.87	1.20±0.25	6.52±2.93	7.54±1.22
TOC (%)	1.53±00.7	1.55±0.16	1.72±0.11	1.88±0.20
Sulfur (%)	1.82±0.26	1.96±0.06	1.13±0.26	2.01±0.50
Total Fe ($\mu g\ g^{-1}$)	6.95±1.47	6.66±1.09	70.07±9.63	51.34±1.89
Redox Potential (mV)	51.33±19.29	91.90±24.61	-115.33±44.20	-52.37±22.81
GS<0.63 mm	0.89±0.15	0.67±0.11	0.22±0.03	2.66±0.76

*SG: seagrass and nSG: non-seagrass (mean±SE, n = 3)

Table 2. As can be seen, non-seagrass sediment of Gusung Tallang has slightly higher fine grain particles, but in general, sediment texture conditions on both locations consisted of >90% sand and only about 7% which is mud (silt+clay). Total organic carbon and sulfur content in both sites has also similar values.

Of all sediment parameters analysed, $CaCO_3$, sulfur content and redox condition are much different comparing both study areas. Calcium carbonate content is almost double and sulfur content is ten times higher in Gusung Tallang's than in Bonetambung Island. Sediment redox potential shows the more reduced condition in Gusung Tallang; whereas, the more oxidized condition in Bonetambung Island.

Metals in bioavailable fractions from seagrass and non-seagrass sediment: Only metals in fraction 1 are presented in this study, because fraction 1 (acid soluble fraction) has the most mobility and thus the most related to the bioavailable concentration. Average concentrations of Pb and Cu in fraction 1 are shown in Fig. 2.

Mean concentrations of Cu in fraction 1 in both study areas show significantly higher in seagrass than in non-seagrass sediment ($p < 0.05$). The Pb concentrations in fraction 1 is also significantly higher in seagrass than non-seagrass sediment ($p < 0.05$) in Gusung Tallang. Although, is not statictically significant, Concentrations of Pb in fraction 1 of seagrass sediment from Bonetambung Island is also higher than those of non-seagrass.
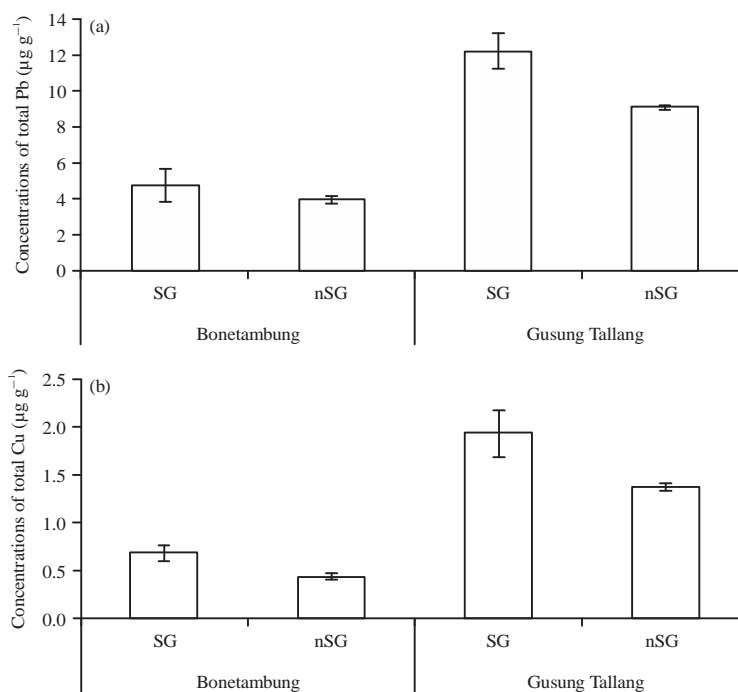
Fig. 3(a-b): Average concentrations of total Pb and Cu in seagrass (SG) and non-seagrass (nSG) sediments from Bonetambung Island and Gusung Tallang (Mean±SE, n = 3)

Only average concentration of Cu in fraction 1 from seagrass area in sediment from Gusung Tallang that is significantly higher comparing to those from Bonetambung Island (p<0.05).

Seagrass and total sediment metal concentrations in relation to bioavailable metal concentrations in sediments: Average concentrations of total Pb and Cu in sediment on both study areas are presented in Fig. 3. Concentrations of total Cu in sediment from Bonetambung Island and total Pb from Gusung Tallang are significantly higher in seagrass area than non-seagrass area (p<0.05). Total concentrations of Pb and Cu in each sediment area are almost three times higher in Gusung Tallang than in Bonetambung Island (p<0.01).

Average Pb and Cu concentrations in are shown in Fig. 4. Concentrations (µg g^{-1}) of Pb in Enhalus roots from Bonetambung Island and Gusung Tallang ranged from 1.90-3.68 (avg 2.52±0.58) and 2.78-5.66 (avg 4.15±0.83), respectively. Whereas, Cu concentrations (µg g^{-1}) 0.40-0.86 (avg 0.56±0.15) and 2.78-5.67 (avg 1.45±0.32), respectively. Although, there are no significant different between mean concentrations of Pb and Cu in seagrass roots from both Bonetambung Island and Gusung Tallang (p>0.05), but from the graph in Fig. 4, average concentrations of both metals are higher in seagrass roots from Gusung Tallang than from Bonetambung Island.
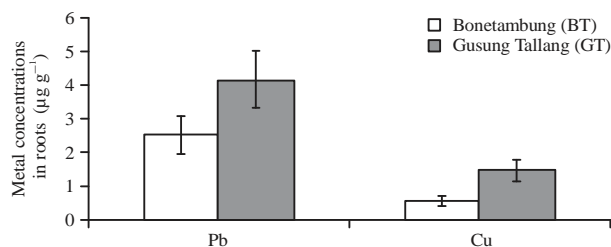

Fig. 4: Average concentrations of Pb and Cu in *Enhalus acoroides* roots (Mean±SE, n = 3)

Relation between bioavailable fraction with total metal concentrations in sediment and seagrass roots: Bioavailable fractions of metals consist of all three fractions (fraction 1, 2 and 3) from speciation study. The most bioavailable and the most toxic form is fraction 1 because of high solubility and thus easily enter the organisms body. The other fraction (2 and 3) which are also the non-resistant, can be easily removed from sediment matrix and solubilized, thus become potentially toxic to organisms depending of physical and chemical parameters of such as redox potential changes, oxygen content and pH changes (Ramirez *et al.*, 2005).

In this study, the pearson correlation analysis (Table 3) were performed between all metal fractions (1, 2 and 3) and total concentration in sediment and in *Enhalus acoroides* roots. There is a strong positive correlation between Cu

concentrations in Enhalus roots and its total concentration in sediment (r = 0.868, p<0.05), fractions 1 and 3 (r = 0.848 and r = 0.876, respectively, p<0.05). There are no correlation between Pb in seagrass root with all metals analysed.

DISCUSSION

Sediment characteristics: While the sampling sites were selected to contrast terrigenic and biogenic sediment, it is not clear from the sediment analysis if the sediments should be classified as terrigenic or biogenic. Based on Al-Rousan *et al.* (2006), sediment type at the two sites is terrigenic because both have $CaCO_3$ content of <10% (Table 2). However, based on Fe content at both locations, sediment types in Gusung Tallang is more of terrigenic types, whereas Bonetambung Island is more biogenic sediment types. Badr *et al.* (2009) stated that iron enrichment in coastal areas constituted a supply of ferromagnesium from terrigenic material. Also, according to Chen *et al.* (1996), the iron content in biogenic sediments are generally very low due to the concentration of Fe on the surface of the sea water is very small. Gusung Tallang has higher content of iron (Fe) due to close proximity to the mainland and therefore sources of pollution from Makassar City.

These results contradicted to Erftemeijer and Middelburg (1993) who found a high content of $CaCO_3$ (>90%) in the sediment of Barranglompo Island, the island adjacent to Bonetambung Island; while in Gusung Tallang, a location close to the mainland (Makassar), contains about 10% of $CaCO_3$.

The $CaCO_3$ and Fe content of sediments may influence bioavailability of metals in sediment. According to John and Leventhal (1995), the most bioavailable fractions (dissolved fractions) consists of carbonate complexes which tend to increase with increasing pH and metals will be released with decreasing pH. The medium mobility is metals bound to Fe and Mn oxides in surface particulates matters and or sediments. Metals released is governed by redox potential in sediment, i.e. with higher redox potential (high oxidation states) tend to increase metal solubility and in reduced conditions, sulfate is reduced and metal will bound tightly to sulfide.

The higher average redox potential was measured in Bonetambung Island (71.63 mV) and according to Colman and Holland (2000), this was a suboxic condition of sediments; whereas, Gusung Tallang's sediment has a transitional redox potential from suboxic to anoxic condition (-83.85 mV). Redox potential condition may influence metals mobility in sediments. In oxic condition metals bound in sediment materials may be released to the water coloumn and become available to organisms.

Metal in bioavailable fractions from seagrass and non-seagrass sediments: It is clear from the speciation study that most bioavailable fractions (fraction 1) of Pb and Cu are higher in sediments with seagrass. This indicated that seagrass plant may influence metal bioavailability in sediments. According to Weis and Weis (2004), aquatic plants can oxidize sediments around the roots through oxygen which is transported from leaves to the roots. Mangrove *Avicennia* was found to oxidize below ground part of the plants which can lead to sulphites reduction and increasing metal concentrations in the exchangeable fraction (fractions 1).

Study conducted by Doyle and Otte (1997) also found higher metal concentrations in vegetated areas than non-vegetated areas, especially in the area around root plants. In the seagrass plants, transport oxygen from the leaves to the roots is used for respiration and nutrient uptake, due to slightly stretched membrane on the roots some oxygen will leach to sediment and will lead to occurrence of oxidation processes near the seagrass roots (Schwarz *et al.*, 2004). The oxidation processes can further lead to the release of sulphites-bound metals from sediments.

Higher Pb and Cu concentrations in the most bioavailable fraction 1 in Gusung Tallang compare to Bonetambung Island is most probably due to the close proximity to the mainland where anthropogenic wastes maybe transported from. According to Yap *et al.* (2002) and Yap and Wong (2011), metals in the non-resistant fractions (fraction 1, 2 and 3) are an indication that the source of metals are most likely due to anthropogenic input rather than natural input.

Although, sediment Pb and Cu concentration found in this study were below Sediment Quality Guidelines (SQGs) generated by NOAA's (Long *et al.*, 1995), i.e., Effects Range-Low (ERL) and Effects Range-Medium (ERM) concentration for Pb (46.7 and 218 µg g^{-1}, respectively) and Cu (34 and 270 µg g^{-1}, respectively). Similarly, Ambo-Rappe (2014) also found Pb and Cu concentration in sediment from seagrass area in Gusung Tallang and Bonetambung Island were still below the guideline for protection of aquatic biota. However, the bioavailability results in this study indicated possible risks of metal toxicity on organisms associated with seagrass.

Seagrass and total sediment metal concentrations in relation to bioavailable metal concentrations in sediments: Concentrations of Pb and Cu in Enhalus roots reflect the total metals concentrations in sediments, i.e., both total metals concentrations in sediment are higher in Gusung Tallang than Bonetambung Island (Fig. 3). Higher metals in Gusung Tallang is predicted because it is located near Makassar city, which is may contribute more metals to the areas.

Table 3: Pearson Correlation among metals in *Enhalus acoroides* and sediments

Concentrations	Seagrass root	
	Pb	Cu
Sediment (total)	0.658	0.868*
Fraction 1	-0.047	0.848*
Fraction 2	0.221	-0.051
Fraction 3	0.771	0.876*

*Correlation is significant at the 0.05 level (2-tailed), n = 3

Seagrass can absorb metals from the water column and sediment (Ambo-Rappe *et al.*, 2007). Absorption of metals from the water column can be performed through the plant leaves and from sediments through the roots and rhizomes. Once the metal is absorbed by seagrass, translocation may occur from the top to the bottom of the seagrass or otherwise. Essential metals such as Cu and Mn, will generally accumulate on the leaves because of metabolic needs; whereas the non-essential metals such as Pb and Cd will be accumulated more in the below ground of seagrasses, i.e. the roots and rhizomes (Prange and Dennison, 2000; Wasserman and Wasserman, 2002). In line with these study, the average total Pb concentration in the rooting section was higher than Cu in both study sites.

The positive significant Pearson Correlation analysis in Table 3 may indicates accumulation of Cu in seagrass sourced from metals in sediments and also closely associated with Cu in fraction 1 and fraction 3. Higher proportions of Cu in fraction 1 may increase its bioavailability and therefore could increase the concentration of Cu in the seagrasses, thus its toxicity to the plant. However, low average concentrations of Cu in seagrass roots compared to Pb further indicates the possibility of translocation of Cu from the below ground to the above ground of seagrasses, i.e., leaf, where necessary for metabolic processes of plants.

A significant positive correlation between the concentration of Cu in seagrass root with Cu in fractions 3 showed that the higher Cu bound to organic materials, sulfite and carbonate, the higher metal accumulate in the roots of seagrasses. With the changes in pH and redox potential from anoxic to oxic conditions, may release metals that bind strongly to the fraction 3 to become more easily absorbed by seagrasses. Changes in the oxidation condition may be due to the transfer of oxygen from the leaves to the roots. The possible higher metabolic rate (photosynthetic rate) of seagrass plants in Gusung Tallang than in Bonetambung Islands was indicated by higher Cu in fraction 1 in Gusung Tallang when compared to Bonetambung Island (Fig. 4).

Arifin *et al.* (2012) studied metal contamination in sediment of Indonesian waters from Jakarta Bay and Berau Delta, East Kalimantan. They found higher concentration of Pb in reducible fraction (fraction 2) in contaminated sediment from Jakarta Bay; whereas, in Berau Delta, concentration of Pb mostly in residual fraction that is strongly bound to sediment matrix, thus not available for take up by organisms.

The apparent absence of a significant correlation between the average concentration of Pb in seagrasses with metal in sediments and fractions may indicate that there were other factors affected the absorption of Pb to the seagrasses. Nevertheless, there was a fairly high correlation between Pb concentrations in seagrasses with the metal in sediments, this could indicate the source of Pb in seagrass roots systems derived from sediments.

CONCLUSION

This is the first study on bioavailability of metals on seagrass sediment. This study found the higher average concentrations of bioavailable fraction of Pb and Cu (fraction 1) are related to the vegetated sediments associated with *Enhalus acoroides*. Higher concentration of Cu in fraction 1 was also associated with higher concentration of Cu in Enhalus roots. This indicates that the presence of seagrass may increase the bioavailability of metals in sediments. Increased metal bioavailability in vegetated sediment will imply increase toxicity. Higher concentration of Pb and Cu at the root of seagrasses was related to the higher total metal concentration in sediments; suggested source of metals comes from sediments. Higher metals concentration in bioavailable fractions, in sediment bulk and also in Enhalus root from Gusung Tallang, especially Cu, further indicated that the source of Pb and Cu are most probably from anthrophogenic input rather than natural input.

ACKNOLEDGEMENTS

REFERENCES

ASTM., 2000. Standard test methods for moisture, ash and organic matter of peat and other organic soils. Method D 2974-00, American Society for Testing and Materials, West Conshohocken, PA, pp: 43-49.

Al-Rousan, S., M. Rasheed, F. Al-Horani and R. Manasrah, 2006. Geochemical and textural properties of carbonate and terrigenous sediments along the Jordanian coast of the gulf of aqaba. J. Oceanogr., 62: 839-849.

Allison, L.E. and C.D. Moodi, 1965. Carbonate. In: Methods of Soil Analysis, Black, C.A. (Ed.). Part 2, American Society Agronomy, Madison, WI., USA., pp: 1379-1396.

Ambo-Rappe, R., 2014. Developing a methodology of bioindication of human-induced effects using seagrass morphological variation in Spermonde Archipelago, South Sulawesi, Indonesia. Mar. Pollut. Bull., 86: 298-303.

Ambo-Rappe, R., D.L. Lajus and M.J. Schreider, 2007. Translational fluctuating asymmetry and leaf dimension in seagrass, *Zostera capricorni* Aschers in a gradient of heavy metals. Environ. Bioindic., 2: 99-116.

Arifin, Z., R. Puspitasari and N. Miyazaki, 2012. Heavy metal contamination in Indonesian coastal marine ecosystems: A historical perspective. Coastal Marine Sci., 35: 227-233.

Atkinson, C.A., D.F. Jolley and S.L. Simpson, 2007. Effect of overlying water pH, dissolved oxygen, salinity and sediment disturbances on metal release and sequestration from metal contaminated marine sediments. Chemosphere, 69: 1428-1437.

Badr, N.B.E., A.A. El-Fiky, A.R. Mostafa and B.A. Al-Mur, 2009. Metal pollution records in core sediments of some Red Sea coastal areas, Kingdom of Saudi Arabia. Environ. Monit. Assess., 155: 509-526.

Bao, V.W.W., K.M.Y. Leung, K.W.H. Kwok, A.Q. Zhang and G.C.S. Lui, 2008. Synergistic toxic effects of zinc pyrithione and copper to three marine species: Implications on setting appropriate water quality criteria. Mar. Pollut. Bull., 57: 616-623.

Bernhard, T. and J. Neff, 2001. Metals bioavailability in the Navy's tiered ecological risk assessment process. Issued Papers Report, April 2001, pp: 1-15. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.202.7403&rep=rep1&type=pdf

Boggs, S., 2006. Principles of Sedimentology and Stratigraphy. 4th Edn., Pearson Prentice Hall, Inc., New Jersey, USA., ISBN-13: 9780131547285, Pages: 676.

Chen, S.Y., S. Ambe, N.Takematsu and F. Ambe, 1996. The chemical states of iron in marine sediments by means of mossbauer spectroscopy in combination with chemical leachings. J. Oceanogr., 52: 705-715.

Ciutat, A. and A. Boudou, 2003. Bioturbation effects on cadmium and zinc transfers from a contaminated sediment and on metal bioavailability to benthic bivalves. Environ. Toxicol. Chem., 22: 1574-1581.

Clark, M.W., D. McConchie, D.W. Lewis and P. Saenger, 1998. Redox stratification and heavy metal partitioning in *Avicennia*-dominated mangrove sediments: A geochemical model. Chem. Geol., 149: 147-171.

Colman, A.S. and H.D. Holland, 2000. The Global Diagenetic Flux of Phosphorus from Marine Sediments to the Oceans: Redox Sensitivity and the Control of Atmospheric Oxygen Levels. In: Marine Authigenesis: From Global to Microbial, Glenn, C.R., L. Prevot-Lucas and J. Lucas (Eds.). SEPM Publication, Tulsa, Oklahoma, pp: 53-75.

Doyle, M.O. and M.L. Otte, 1997. Organism-induced accumulation of iron, zinc and arsenic in wetland soils. Environ. Pollut., 96: 1-11.

Eggleton, J. and K.V. Thomas, 2004. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ. Int., 30: 973-980.

Erftemeijer, P.L.A. and J.J. Middelburg, 1993. Sediment-nutrient interactions in tropical seagrass beds: A comparison between a terrigenous and a carbonate sedimentary environment in South Sulawesi (Indonesia). Mar. Ecol. Prog. Ser., 102: 187-198.

Hendozko, E., P. Szefer and J. Warzocha, 2010. Heavy metals in *Macoma balthica* and extractable metals in sediments from the southern Baltic Sea. Ecotoxicol. Environ. Safe., 73: 152-163.

John, D.A. and J.S. Leventhal, 1995. Bioavailability of Metal. In: Preliminary Compilation of Descriptive Geoenvironmental Mineral Deposit Models, Du Bay, E.A. (Ed.). U.S. Geological Survey, Colorado, USA.

Kelderman, P. and A.A. Osman, 2007. Effect of redox potential on heavy metal binding forms in polluted canal sediments in Delft (The Netherlands). Water Res., 41: 4251-4261.

Long, E.R., D.D. MacDonald, S.L. Smith and F.D. Calder, 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manage., 19: 81-97.

Loring, D.H. and R.T.T. Rantala, 1992. Manual for the geochemical analyses of marine sediments and suspended particulate matter. Earth Sci. Rev., 32: 235-283.

Macinnis-Ng, C.M.O. and P.J. Ralph, 2002. Towards a more ecologically relevant assessment of the impact of heavy metals on the photosynthesis of the seagrass, *Zostera capricorni*. Mar. Pollut. Bull., 45: 100-106.

Neff, J.M., 2002. Bioaccumulation in Marine Organisms: Effect of Contaminants from Oil Well Produced Water. Elsevier, Amsterdam, Netherlands, ISBN-13: 9780080527840, Pages: 468.

Peterson, G.S., G.T. Ankley and E.N. Leonard, 1996. Effect of bioturbation on metal-sulfide oxidation in surficial freshwater sediments. Environ. Toxicol. Chem., 15: 2147-2155.

Prange, J.A. and W.C. Dennison, 2000. Physiological Responses of Five Seagrass Species to Trace Metals. Mar. Pollut. Bull., 41: 327-336.

Pulich, W.M., 1987. Subtropical Seagrasses and Trace Metal Cycling. In: Proceedings of the symposium on Subtropical-Tropical Seagrasses of the Southeastern United States, 12 August 1985, Durako, M.J., R.C. Phillips and R.R. Lewis (Eds.). Vol. 42, Florida Department of Natural Resources, Gainesville, Florida, USA., pp: 39-52.

Ramirez, M., S. Massolo, R. Frache and J.A. Correa, 2005. Metal speciation and environmental impact on sandy beaches due to El Salvador copper mine, Chile. Mar. Pollut. Bull., 50: 62-72.

Sanz-Lazaro, C., P. Malea, E.T. Apostolaki, I. Kalantzi, A. Marin and I. Karakassis, 2012. The role of the seagrass *Posidonia oceanica* in the cycling of trace elements. Biogeosciences, 9: 2497-2507.

Schwarz, A.M., F. Matheson and T. Mathieson, 2004. The role of sediment in keeping seagrass beds healthy. Water Atmosphere, 12: 18-19.

Srinivasan, M. and G.W. Swain, 2007. Managing the use of copper-based antifouling paints. Environ. Manag., 39: 423-441.

Tabatabai, M.A. and J.M. Bremner, 1970. An alkaline oxidation method for determination of total sulfur in soils. Soil Sci. Soc. Am. Proc., 34: 62-65.

USEPA., 1994. Sediment sampling. SOP No. 1994, United States Environmental Protection Agency (USEPA), Washington, DC., USA.

Ure, A.M., P. Quevauviller, H. Muntau and B. Griepink, 1993. Speciation of heavy metals in soils and sediments. An Account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the commission of the European communities. Int. J. Environ. Anal. Chem., 51: 135-151.

Wasserman, J.C. and M.A.V. Wasserman, 2002. Cu, Fe, Mn and Zn cycling in seagrass (*Zostera noltii* Hornemann) stands from the arcachon bay (Atlantic French Coast). Mundo Vida, 3: 67-77.

Weis, J.S. and P. Weis, 2004. Metal uptake, transport and release by wetland plants: Implications for phytoremediation and restoration. Envir. Int., 30: 685-700.

Yap, C.K., A. Ismail, S.G. Tan and H. Omar, 2002. Concentrations of Cu and Pb in the offshore and intertidal sediments of the west coast of Peninsular Malaysia. Environ. Int., 28: 467-479.

Yap, C.K. and C.H. Wong, 2011. Assessment Cu, Ni and Zn pollution in the surface sediments in the southern Peninsular Malaysia using cluster analysis, ratios of geochemical nonresistant to resistant fractions and geochemical indices. Environ. Asia, 4: 53-61.

Yuan, C.G., J.B. Shi, B. He, J.F. Liu, L.N. Liang and G.B. Jiang, 2004. Speciation of heavy metals in marine sediments from the East China Sea by ICP-MS with sequential extraction. Environ. Int., 30: 769-783.