

Accumulation and Phytoextraction Potential of Heavy Metals of *Enhalus acoroides* in The Coastal Waters of Lamongan, Java, Indonesia

(Penumpukan dan Potensi Fotopengekstrakan Logam Berat terhadap *Enhalus acoroides* di Perairan Pinggir Laut Lamongan, Java, Indonesia)

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ABSTRACT

This study quantified the concentration of heavy metals Cd and Cu in Enhalus acoroides from shallow seagrass habitat in the northern coastal waters of Lamongan, Indonesia. The objective of this study was to determine the concentration of these metals in different parts of seagrass E. acoroides and to assess their biomonitoring potential of heavy metals pollution in coastal areas. Heavy metal contents in the sediments, roots and leaves of E. acoroides were determined by Flame Atomic Absorption Spectrometry from four sampling sites. The results showed that the concentrations of Cu were significantly higher than the concentrations of Cd in both sediments and seagrass tissues in all stations. The concentrations of Cu were found in slightly similar values in the sediments, roots and leaves. On the other hand, the concentrations of Cd were higher in the sediments than in the roots and leaves of E. acoroides. In addition, Bioconcentration Factor (BCF) of Cu in E. acoroides was two times higher than BCF of Cd ($BCF_{Cu} = 1.09$; $BCF_{Cd} = 0.49$). In contrast, Translocation Factor (TF) of Cu was lower than TF of Cd that was 0.65 and 1.05, respectively. According to BCF and TF values, E. acoroides can be considered to have the ability to perform phytoextraction process especially Cd because E. acoroides was able to restrict its absorption of Cd from the environment ($BCF < 1$) and once it is absorbed, the plant has the ability to transfer it to the other body parts ($TF > 1$).

Keywords: Coastal waters; heavy metal pollution; Indonesia; Lamongan; phytoextraction; seagrass

ABSTRAK

Kajian ini mengukur kepekatan logam berat Cd dan Cu dalam Enhalus acoroides dari habitat lamun yang cetek di perairan utara pinggir laut Lamongan, Indonesia. Objektif kajian ini adalah untuk menentukan kepekatan logam ini pada bahagian lamun E. acoroides yang berbeza dan untuk menilai potensi pembioawasannya terhadap pencemaran logam berat di perairan pinggir laut. Kandungan logam berat dalam sedimen, akar dan daun E. acoroides ditentukan melalui Spektrometri Nyala Penyerapan Atom daripada keempat-empat sampel. Hasil kajian menunjukkan bahawa kepekatan Cu adalah lebih tinggi daripada kepekatan Cd dalam kedua-dua sedimen dan tisu lamun dalam semua stesen. Kepekatan Cu adalah lebih kurang sama dalam sedimen, akar dan daun. Sebaliknya, kepekatan Cd adalah lebih tinggi pada sedimen berbanding akar dan daun E. acoroides. Tambahan lagi, Faktor Pembiopekatan (BCF) Cu dalam E. acoroides adalah dua kali ganda lebih tinggi daripada BCF Cd ($BCF_{Cu} = 1.09$; $BCF_{Cd} = 0.49$). Faktor Translokasi (TF) Cu pula adalah lebih rendah berbanding TF Cd, iaitu 0.65 dan 1.05. Menurut nilai BCF dan TF, E. acoroides boleh dianggap mempunyai kemampuan untuk melakukan proses fotopengekstrakan, terutamanya Cd kerana E. acoroides boleh menyekat penyerapan Cd daripada persekitaran ($BCF < 1$) dan setelah ia terserap, tumbuhan mempunyai keupayaan untuk memindahkannya ke bahagian yang lain ($TF > 1$).

Kata kunci: Fotopengekstrakan; Indonesia; Lamongan; lamun; pencemaran logam berat; perairan pinggir laut

INTRODUCTION

Seagrasses play significant roles in coastal ecosystems and are exposed to a wide variety of pollutants including heavy metals (Brown et al. 2016; Govers et al. 2014; Thangaradjou et al. 2010). There have been many studies on the accumulation of heavy metals in the seagrass ecosystems (Riosmena-Rodríguez et al. 2010; Schlacher-Hoenlinger & Schlacher 1998a; Suwandana et al. 2011; Thangaradjou et al. 2013). Depending on the

species, seagrass can accumulate heavy metals differently (Thangaradjou et al. 2010) due to the differences in the metabolic condition and life stage of the individual species (Riosmena-Rodríguez et al. 2010).

Seagrass absorbs heavy metals and store it in the above ground parts (shoots and leaves) and below ground parts (roots). Above ground and below ground parts could have significant differences in accumulating heavy metals. Lin et al. (2016) in their study of *Zostera*

japonica found a higher accumulation of Cu and Cd in the above ground parts, while the accumulations of Hg and As were higher in the below ground parts. This could be the result of differences in the metals uptake routes into seagrass from sediment to root/rhizome and from water to leaf/epiphyte pathways (Schlacher-Hoenlinger & Schlacher 1998b).

The ability of seagrass to accumulate heavy metals can be used to reduce metal pollution in the environment through phytoremediation process. There have been many studies conducted to evaluate the ability of plants in reducing metal pollution through phytoremediation process (Bonanno et al. 2017; Hidayati et al. 2009; Sari et al. 2018; Sidi et al. 2018). Yoon et al. (2006) identify two types of applications of phytoremediation in plants: phytoextraction, i.e. metal uptake and accumulation into plant shoots which can be harvested and removed from the ecosystems; and phytostabilization, i.e. the use of plants to minimize metal mobility in the contaminated areas. Bio-concentration factor (BCF) and translocation factor (TF) can be used as an indicator to estimate plant's potential for phytoremediation purpose. BCF is the ratio of metal concentration in the roots to that in the soil, while TF is the ratio of metal concentration in the shoots/leaves to the roots.

Indonesia as part of Southeast Asia is famous for the greatest diversity of seagrasses (Ooi et al. 2011). This ecosystem can act as a sink for pollutants in the coastal environment (Schlacher-Hoenlinger & Schlacher 1998b; Sidi et al. 2018). Lamongan is known as one of the busiest harbors in East Java, with high activities in fisheries and industries. These activities could have a significant impact on the ecosystems by the contribution of pollutant such as heavy metals. *E. acoroides* can be found along the northern coastal waters of Lamongan and the ecosystem is vulnerable to pollutants. Therefore, this study aimed to estimate metal concentrations (Cu and Cd) in the sediment, roots and leaves of *Enhalus acoroides* and to determine its ability in conducting phytoremediation process of heavy metals from the seagrass ecosystem in the northern coastal waters of Lamongan, Indonesia.

MATERIALS AND METHODS

STUDY AREA AND SAMPLING METHOD

Sampling was conducted in the northern coastal waters of Lamongan, East Java Province. There were four sampling stations chosen to collect sediment and seagrass samples. All sampling stations were characterized by the presence of pollutants from fisheries and domestic activities. Station 1 was located near Brondong Harbor which is busy with its fishery activity and Stations 2, 3, and 4 were located near residential areas with high contribution of the pollutant from domestic activities (Figure 1).

Sample collection was conducted in March 2016. Sediment samples were collected at 30-35 cm from the surface using 50 cm long and 5 cm in diameter of PVC core sampler. About 1 kg of sediment was taken and placed in a sealed plastic bag. Seagrass samples were taken by collecting the roots and leaves and rinsed with seawater to remove residue and epiphytes that attached to it. The roots and leaves were then placed in plastic bags. All the samples (seagrass and sediments) were collected in duplicate. Sediment and seagrass samples were put in an icebox to be transported to the laboratory for further analysis.

SAMPLE ANALYSIS

Sediment and seagrass samples analysis were conducted according to Lin et al. (2016) and Nguyen et al. (2017). Sediment samples were dried to constant weight at room temperature and once dried the samples were passed through a 1 mm diameter sieve. A 1 g quantity of dry sediment samples were oven digested at 90 °C overnight in an acid solution (H₂O₂/HNO₃, 2:3 v/v ratio). After digestion, the samples were diluted with Milli-Q water to final volume of 25 mL and analyzed for the Cu and Cd contents using a Shimadzu Flame Atomic Absorption Spectrophotometer Model AA-6800.

Seagrass samples were cut off using non-metal or ceramics scissors and dried at room temperature. Once dried, the samples were ground and homogenized in an agate mortar. Sample digestions were conducted by adding acid solution similar to sediments sample digestion process and the heavy metal concentrations were measured using FAAS. Quality assurance procedures for all samples were conducted using blank and standard solutions by developing calibration curves.

DATA ANALYSIS

Bioconcentration factor (BCF) and translocation factor (TF) were used to analyze phytoremediation potential of *E. acoroides*. BCF was calculated from the ratio of metal concentration in the root and the concentration in the sediments, meanwhile, the ratio of metal concentration in the root and in the leaf was used to determine TF (Hu et al. 2019). Significant differences in metal concentration between sampling stations and concentrations of metal in sediment and seagrass tissues were determined using a two-way analysis of variance (ANOVA). Statistical tests were assessed at the significant values of 0.05 and carried out with SPSS 16.0 for Windows.

RESULTS AND DISCUSSION

METAL CONCENTRATIONS AND DISTRIBUTIONS IN SEDIMENTS AND SEAGRASS TISSUES

At all sampling stations, the mean concentrations of Cu were higher than the concentrations of Cd in

both the sediments and seagrass tissues (Figure 2). Cu concentrations in the sediments, roots and leaves were in the range of 0.20-0.31 $\mu\text{g g}^{-1}$, 0.01-0.45 $\mu\text{g g}^{-1}$ and 0.01-0.67 $\mu\text{g g}^{-1}$ in all sampling stations, respectively. On the other hand, the concentrations of Cd were in the range of 0.07-0.12 $\mu\text{g g}^{-1}$ in the sediments, 0.01-0.06 $\mu\text{g g}^{-1}$ in the roots, and 0.01-0.07 $\mu\text{g g}^{-1}$ in the leaves in all sampling stations. The results of this study were very much lower compared to the study of Ismarti et al. (2017), Nguyen et al. (2017) and Sidi et al. (2018). In addition, compared to the study conducted by Sugiyanto et al. (2016) in Pantai Paciran, Lamongan, Cd concentrations in the sediment found in this study were higher, but the concentrations in the seagrass roots and leaves were found rather low.

A two-way ANOVA test showed that the distributions of Cu has no significant difference among sampling stations ($p=0.37$) and also among sediment and seagrass tissues ($p=0.72$). Cu concentrations were found very much alike in all sampling stations for sediments, roots, and leaves of seagrass (Figure 2A). By using similar statistical analysis, no significant difference of Cd concentration between sampling stations ($p=0.07$). However, this study detected a significant difference in the mean of Cd concentrations among sediments, roots, and leaves of seagrass ($p<0.05$). Cd concentrations in the sediments were higher than in the roots and leaves (Figure 2B). Moreover, the concentrations of Cd in seagrass tissues were found mostly higher in the leaves than in the roots (Figure 2B). Above ground tissue of seagrass tend to accumulate a higher level of Cd compared to underground tissues. A study reported by Bonanno and Martino (2017) and Llagostera et al. (2011) also showed the same findings using seagrass *P. oceanica* and *C. nodosa*, respectively. Leaves of seagrass accumulated metals including Cd not only from roots translocation but also perform a higher absorption rate of the dissolved metals from surrounding waters (Rosalina et al. 2019; Zakhama-Sraieb et al. 2015). This result also indicated that there is a removal strategy conducted by seagrass species by storing the amount of metals in the leaves and then performed metal reduction because of a high rate of alteration in the leaves of seagrass (Bonanno & Martino 2017).

METAL ACCUMULATIONS IN *Enhalus acoroides*

The accumulations of Cu and Cd in the seagrass tissues were observed from BCF and TF values and it shows two categories (Table 1). According to Yoon et al. (2006), BCF and TF < 1 means that the plant is not optimal to do phytoextraction process, while BCF and TF > 1 shows the ability of the plant to conduct phytoextraction. Phytoextraction is the ability of plants to transfer pollutants to its other body parts such as shoots or leaves that can be harvested, thus reducing the level of pollutant in the environment.

BCF > 1 shows the concentration of metal is higher in the roots than the concentration in the sediments. *E.*

acoroides in this study shows BCF > 1 for Cu in almost all sampling stations, except at Station 3. On the other hand, the concentrations of Cd in the roots of *E. acoroides* were lower than the concentration of Cd in the sediments, resulted in the low value of BCF (< 1) in all sampling stations. Cu is one of the essential metals (Bastami et al. 2014; Saher & Siddiqui 2016; Sari et al. 2018) and that can be the reason of high absorption of the metal in *E. acoroides*, unlike Cd that is categorized as non-essential metal which the biological function is still unknown. A similar result has been found in the study of Ambo-Rappe et al. (2011) on the impact of metal exposure to the growth and leaf asymmetry of *Halophila ovalis*. Their study observed that the growth of *H. ovalis* is related to the ability of the plants to include or exclude the metals in relation to its function in the metabolic process. Plants tend to absorb more essential metals such as Cu and resulted in the toxicity even at a lower concentration, while Cd as a non-essential metal that is not required for growth is actively excluded in the absorption process by plants.

Station 3 is the only station in the study areas with BCF < 1 for Cu and this is related to the low concentration of Cu in the sediment of Station 3. Sampling at Station 3 was conducted in the water that is connected to the open sea that influenced the accumulation of the metal in the sediment (Lin et al. 2013). This result is supported by the study of Ali et al. (2016) that found the increasing concentration of heavy metals in the sediment during low water movement. Low concentration of Cu in the sediments resulted in the low concentration of Cu in the roots and eventually lowering the value of BCF. Even though BCF at Station 3 was less than 1, but the TF value was the highest (1.99) among other sampling stations. TF value > 1 shows the ability of plants to transfer pollutant from the roots to the other body parts. All stations, except Station 2, were observed to have TF > 1 for Cu. These differences might be the result of the metal transfer mechanisms from the environment to the plants due to the difference in the seagrass morphology (Bonanno et al. 2017; Schlacher-Hoenlinger & Schlacher 1998b).

E. acoroides has the ability to do phytoextraction process of Cd, as can be seen from the BCF values that are less than one in all sampling stations and TF values that are more than 1 in most stations (Table 1). BCF less than one shows *E. acoroides* restricted the absorption of Cd from the environment. However, once the metal is absorbed, the plants will optimize its ability to transfer the metal to other body parts, in this case is leaves (TF > 1). This mechanism can be used to reduce Cd level in the water and plants because the leaves that have contained metals then can be harvested (Timofeeva et al. 2017; Van der Ent et al. 2020; Yoon et al. 2006). Moreover, plants have the ability to actively exclude metals that are not required for growth or development to minimize toxicity by isolating it in the storage tissues, where it does not interfere with the metabolic activity (Ambo-Rappe et al. 2011).

A comparison of the accumulation process of Cu and cadmium in different seagrass species between this study and other studies from different geographical areas is summarized in Table 2. Bioconcentration and translocation values in this study were very much lower compared to those studies especially for Cd. Similar

results were observed for the bioconcentration factor of Cu in *E. acoroides* (Nguyen et al. 2017) and *Z. japonica* (Lin et al. 2016). These differences might be the result of the variability in the physical and chemical factors, plant morphology and also metal contaminants between different study areas.

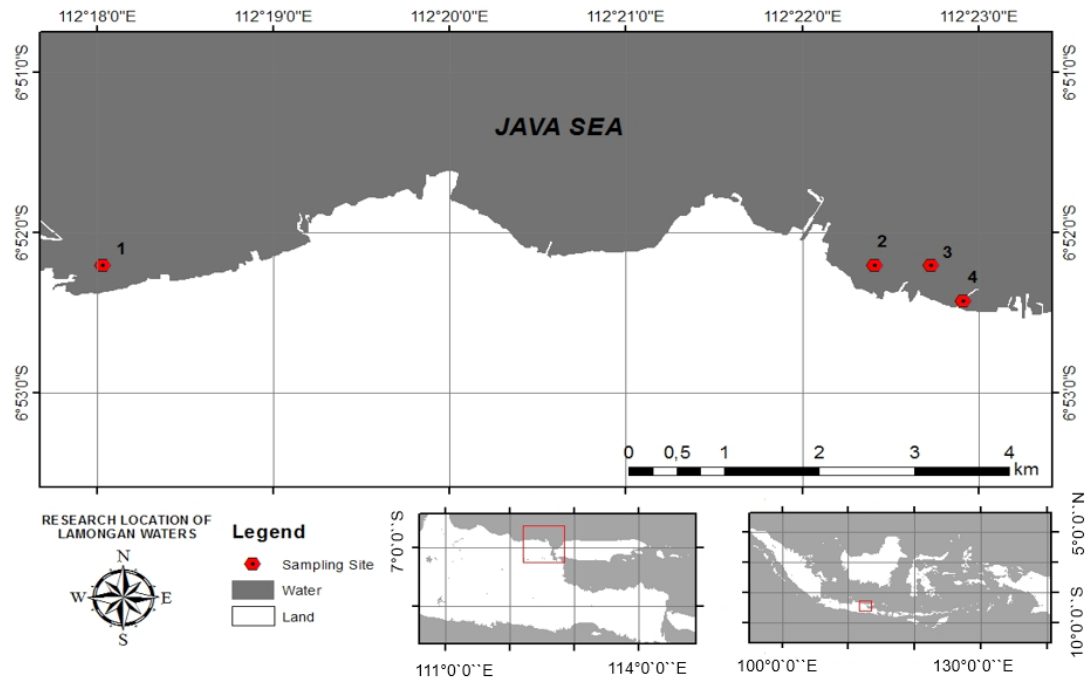


FIGURE 1. Map of the sampling sites in the northern coastal water of Lamongan, Indonesia

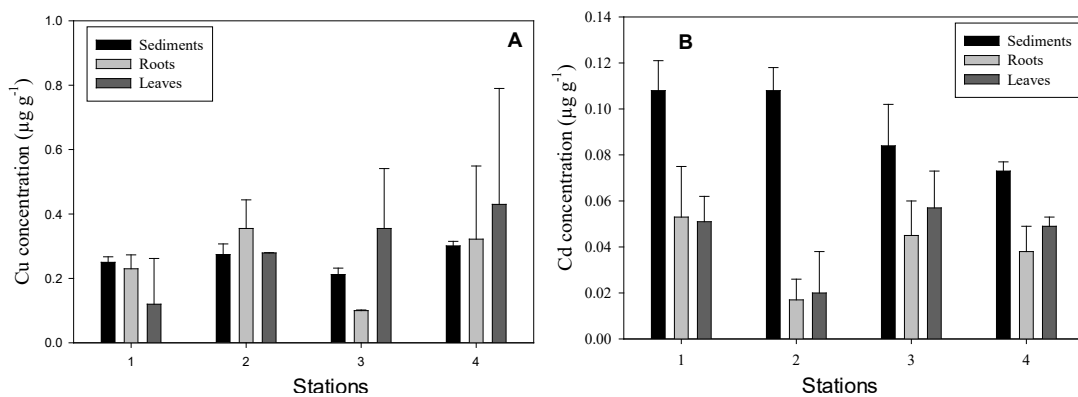


FIGURE 2. The metal concentrations of Cu (A) and Cd (B) in the sediments, roots and leaves of *E. acoroides* in the northern water of Lamongan, Indonesia

TABLE 1. BCF and TF of Cu and Cd in *E. Acoroides*

	Station			
	1	2	3	4
Cu				
BCF	1.08	1.18	0.40	1.17
TF	1.54	0.73	1.99	1.34
Cd				
BCF	0.49	0.16	0.53	0.52
TF	0.95	1.15	1.27	1.29

TABLE 2. Bioconcentration (root to sediment) and translocation factors (leaf to root) of different seagrass species reported from other studies

Species	Location	Cu		Cd		References
		BCF	TF	BCF	TF	
<i>Enhalus acoroides</i>	Northern water of Lamongan, Indonesia	0.4 – 1.18	0.73 – 1.99	0.16 – 0.53	0.95 – 1.29	This study
<i>Enhalus acoroides</i>	Merambong Shoal, Johor Strait	–	2.6	–	5.8	(Sidi et al. 2018)
<i>Enhalus acoroides</i>	Coast of Khanh Hoa, Vietnam	0.1 – 0.9	–	3.7 – 9.3	–	(Nguyen et al. 2017)
<i>Enhalus acoroides</i>	Paciran Beach, Lamongan, Indonesia	–	–	3.74	1.19	(Sugiyanto et al. 2016)
<i>Cymodocea nodosa</i>	Sicily, Italy	2.44	1.07	1.30	0.87	(Bonanno & Di Martino 2016)
<i>Posidonia oceanica</i>	Sicily, Italy	4.15	0.54	5.21	1.34	(Bonanno et al. 2017)
<i>Posidonia australis</i>		0.35	0.53	3.27	0.85	
<i>Cymodocea nodosa</i>		2.96	1.11	1.89	1.08	
<i>Zostera japonica</i>	Yellow River, China	0.69 – 1.06	–	11.91 – 30.69	–	(Lin et al. 2016)

CONCLUSION

This study showed the variability of the absorption process of heavy metals Cu and Cd in *E. acoroides*. Metal function in metabolic process influences the absorption process in which Cu that is known as essential metal

is highly absorbed by the plants as shown in the BCF values bigger than one. On the other hand, *E. acoroides* restricted the absorption of Cd from the sediments to the roots because this metal is known as non-essential metal. However, once the plants absorb it, they have the ability

to translocate the metal to other organs such as leaves that can be harvested, thus optimize the phytoextraction process to reduce heavy metal in the environment.

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