# Accumulation of cadmium and lead in *Enhalus acoroides* (tape seagrass) species in Brgy. Alegria, Semirara Island, Caluya, Antique, Philippines

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**Abstract**

Seagrass meadows are a critical component of the marine ecosystems worldwide and are sensitive to changes in the environment. Semirara, Antique operates the biggest open pit coal mines in the Philippines since 1980. Heavy metals are one of the waste products from mining which can accumulate in living organisms, posing a threat to their health. In this study, seawater, sediment, and *Enhalus acoroides* (tape seagrass) samples were collected from Brgy. Alegria, Semirara and analyzed using Microwave Plasma - Atomic Emission Spectroscopy (MP-AES) to determine the accumulation of cadmium and lead. Results showed that concentrations for Cd and Pb in seagrass roots were as follows: Cd (<0.009) and Pb (0.061 ± 0.008) while concentrations in seagrass leave were: Cd (<0.009) and Pb (0.067 ± 0.005). Concentration of Cd decrease in the following order: seawater > seagrass leaves > seagrass roots > sediments while concentrations of Pb decrease in the order: seagrass roots > seagrass leaves > seawater > sediments.

***Keywords:*** *seagrass, cadmium, lead, Enhalus acoroides, mp-aes*

**Introduction.** Due to increasing demand for coastal resources as well as the growth of the human population to over 7 billion, coastal ecosystems are exposed to many pollutants including trace metals from anthropogenic activities through rivers or atmospheric deposition [1]. The heavy metal distribution in aquatic system require serious attention due to rise of global anthropogenic factors. Acid mine drainage (AMD) from mining sites has become one of the main causes of aquatic pollution. Trace elements naturally occur in the environment but it can have toxic effects at high concentrations even if they are considered micronutrients for living organisms like plants [2].

Seagrasses are specialized marine flowering plants which are ecologically and economically important by providing nutrition, physical habitat, and spawning ground for marine organisms such as marine turtles, sea cows, and fish [3]. Eighteen species of seagrass have been observed in the Philippines and among the dominant species in the Western Pacific region include *Cymodocea rotundata,, Enhalus acoroide, Syringodium iso etifolium, and Thalassia hemprichii* [4]. Although several studies have been established that seagrass accumulate trace metals, there is a need to conduct more studies because of the persistence of trace metals' difficult biodegradability and their tendency to concentrate on aquatic organisms [1,2,5,6,7,8].

Seagrass beds surveyed around Caluya group of islands found in Antique, Iloilo Province are the most extensive in Region VI [9]. It is also where Semirara Coal Mining Corporation (SMC) is located. In such, waste products from mining contains acid, heavy metals, and PAHs which can contaminate and accumulate in living organisms which poses a threat to their health and safety [10].

Very few studies have been conducted which talks about the environmental impacts of mining in SMC to its biota. Furthermore, there exists no reliable information available on the levels of trace metals in Semirara Island, specifically Brgy. Alegria thus, this study will contribute more knowledge about the effects of mine waste from SMC.

This study aimed to assess the concentrations of cadmium and lead in the dominant seagrass species in Brgy. Alegria, Semirara Island, Caluya, Antique, Philippines. It specifically aims:

(i) To measure the concentration of cadmium, and lead in the intertidal waters and sediment fractions of Brgy. Alegria, Caluya, Antique, Philippines.

(ii) To measure the concentration of cadmium, and lead from the a) roots and b) leaves of the dominant seagrass species in Brgy. Alegria, Caluya, Antique, Philippines

(iii) To compare the concentrations of cadmium, and lead obtained from the sediment samples, as the seagrass samples in Brgy. Alegria, Caluya, Antique, Philippines.

**Methods.** Cadmium and lead concentrations were assessed in seagrass, sediment and seawater samples collected at Brgy. Alegria (12.00°N 121.38°E) in Caluya, Antique, Philippines. Samples were then transported via ferry to the Chemistry Laboratory of Philippine Science High School - Western Visayas Campus. Samples were then cleaned, dissected, dried, powdered, and subjected to wet digestion ($HNO_3$: $H_2O_2$) in preparation for analysis. Microwave Plasma - Atomic Emission Spectrophotometer (MP-AES) was used to assess the concentration of cadmium and lead in the digested samples.

*Sampling Site.* The sampling site is located near the mouth of Bigo Creek, a former coal washing facility and siltation pond of SMC, which is approximately 8 km away from the Himalian coal mine located in Brgy, Alegria [11]. Seagrass samples were identified using a dichotomous key and collected at Brgy. Alegria, Semirara Island, Caluya, Antique, Philippines (12.00°N 121.38°E).

*Sampling and Field Collection.* Clean Hands/Dirty Hands (CH/DH) Technique was employed during water sample collection. One hundred mL of seawater was collected in high density polyethylene (HDPE) bottles. Seagrass samples were excavated using a trowel. The samples were rinsed with seawater to remove sediment attached to the roots which was then stored in plastic bags acidified using 0.1% hydrochloric acid (HCl). The sediment samples were taken in the vegetated area. The depth of the collection was approximately 12 inches below the surface. Sediment samples were collected using a trowel and placed in clean and acidified plastic bags.

*Post Collection Preparation.* Collected water samples were filtered using Whatman Grade 595 filter paper. The pH of filtered water was adjusted to 4 ± 0.1 by addition of nitric acid. Seagrass samples were dissected into two parts: roots, and leaves using a scalpel and oven-dried at 60°C overnight. Dried samples were grounded using a mortar and pestle and sieved using 250 μm mesh. Upon arriving at the laboratory, sediment samples were oven-dried at 60°C. Dried samples were sieved, and finer grain size fraction of sediments were used for digestion.

*Sample Digestion.* 0.3 g of seagrass and sediment samples were digested to 5 mL of 65% $HNO_3$ and 2 mL of 20% $H_2O_2$ for 1 hour in hot bath. Digested samples were left to cool, filtered, and stored in a refrigerator until analysis. Seawater samples, on the other hand were digested using Ammonium Pyrrolidine Dithiocarbamate – Methyl Isobutyl Ketone (APDC-MIBK) complex. APDC (~99%, Sigma Aldrich) and MIBK (Ajax) was obtained from D'Malt Industrial Sales Corporation, Iloilo City and Yana Chemodities, Cebu, respectively. 35 mL of MIBK was added, then, followed by 7 mL of 1% APDC solution. The solution was equilibrated for 30 min on a mechanical shaker. The organic layer was separated and stored in HDPE bottles while the aqueous layer was stored for the preparation of standard solutions.

*Trace Metal Quantification.* Total concentration of cadmium and lead in seagrass, seawater, and sediments were analyzed using the Agilent 4200 Microwave Plasma-Atomic Emission Spectrometer (MP-AES) at DOST Region 6 OneLab in LaPaz, Iloilo City. with a method detection limit (MDL) of 0.03 mg/L and 0.006 mg/L for cadmium and lead, respectively. The method quantitation limit (MQL), on the other hand, is 0.009 mg/L and 0.021 for cadmium and lead, respectively.

*Safety Procedure.* Collection of data was done during low tide to ensure the safety of the sampling method. Personal protective equipment was also observed to prevent any possible harm from different organisms present in the area.

Safe handling and proper disposal were properly observed to minimize adverse impact to the marine ecosystem. Proper waste segregation was observed to avoid unwanted reactions in the laboratory. The waste containers were labeled properly to prevent possible biological hazards after disposal.

Wearing of personal protective equipment (PPE) was always observed inside the laboratory especially in dealing with toxic fumes from chemical reagents used in this study (nitric acid and hydrogen peroxide). All reagents and chemicals in the acid digestion process were handled inside a fume hood.

**Results and Discussion.** There currently exists eight (8) seagrass species in the Caluya group of Islands [10]. Brgy. Alegria, Semirara Island is near the mouth of a creek approximately 8 km from the Himalian coal mine, located near the core of the mining activities [11]. Results show that one seagrass species, *E. acoroides*, is found to thrive in the seagrass beds of Brgy. Alegria, Semirara, Antique, covering an estimated area of 275 $m^2$. The *E. acoroides* seagrass bed thrives very well in the area, where it can grow up to one meter long.

Cadmium (Cd) concentrations (mg/L) of seagrass leaves, seagrass roots, sediments, and seawater are shown in Table 1. The highest concentrations were observed in seawater samples, followed by seagrass leaves, and seagrass roots while lowest concentrations were observed in sediments. Concentrations of seagrass leaves, seagrass roots, sediment, and seawater samples were all below MQL but above MDL. The means for all the samples were <0.009. With respect to MDL, the mean concentration of Cd in leaves, roots, sediment and seawater is 0.007 mg/L, 0.006 mg/L, 0.003 mg/L and 0.008 mg/L, respectively.

Also shown in Table 1 are lead (Pb) concentrations (mg/L) in seagrass leaves, seagrass roots, sediment, and seawater. The highest concentrations were observed in seagrass leaves followed by seagrass roots while sediments and seawater samples were observed to have the lowest concentrations with negative value. Seagrass leaves and roots were both above the MQL and MDL while sediment and seawater samples were both below MQL and MDL.

The coastal area of Brgy. Alegria can be classified as class SC waters. Generally, the water quality guidelines for lead in class SC waters should not be above 0.05 mg/L while cadmium concentrations should not exceed 0.005 mg/L. Specifically, the parameters for class SC waters near mining industries for cadmium and lead should be within 0.01 mg/L and 0.1 mg/ L, respectively.

The results have shown that the concentration of both cadmium and lead in seawater in the coastal area of Brgy. Alegria, did not exceed DENR parameters, for class SC waters near mining sites but exceeded

**Table 1.** Mean concentrations of cadmium and lead in seagrass leaves, seagrass roots, sediment, and seawater samples (MQL)

| | Cadmium concentration (mg/L) | Lead concentration (mg/L) |
|---|---|---|
| Seagrass leaves | <0.009 | 0.061 ± 0.008 |
| Seagrass roots | <0.009 | 0.067 ± 0.005 |
| Sediments | <0.009 | <dl |
| Seawater | <0.009 | <dl |

cadmium concentrations for secondary general water quality guidelines [13].

In *E. acoroides*, the concentration of Cd decreases in the following order: seagrass roots > seawater > seagrass leaves > sediments while concentrations of Pb decrease in the order: seagrass roots > seagrass leaves > seawater > sediments. These results confirm that *E. acoroides* tends to accumulate Cd and Pb in the plant tissues itself as opposed to other external compartments such as sediments [1]. Data from this study do not completely agree with those previous studies [14] wherein lead concentration in sediments is higher compared to those of seagrass roots and seawater.

Cd and Pb are known to have high toxic effects and have been the primary cause of polluting the environment. Elements such as Cd and Pb in the environment increase through combustion of coal, presence of mining site and sewage [15,16]. Lead naturally comes from the activity of burning fossil fuels, mining, and manufacturing industries while cadmium is widely distributed in nature as a result of erosion and sedimentation. Thus, cadmium concentrations greater than the average can be due to contamination due to anthropogenic activities.

The metals analyzed in this study were selected as they represent the most common trace metals that can affect coastal communities. Both lead and cadmium were present in the seagrass tissues whereas negative values were obtained in the sediment and seawater samples for lead analysis. seagrass roots have higher concentration for both cadmium and lead.

Higher levels of Cd were observed in the seagrass roots than other compartments. This can confirm that preferential uptake of Cd from water is different from the sediments. However, it has been reported that there exists a positive correlation between Cd levels in *E. acoroides* leaves as opposed to the sediments [15]. Majority of contaminants are absorbed through the roots and are then translocated to the leaves. Higher concentrations of Cd could be attributed to the increase in metabolic rate of these tissues during growth. This phenomenon could explain higher Cd levels that were observed in the leaves than to other compartments, since Cd is an essential metal for growth in *E. acoroides* species. Generally, higher Cd concentrations were observed in recent years. Furthermore, increasing trends in Cd concentrations have been reported as a worldwide phenomenon since 99% of Cd in the atmosphere is due to anthropogenic emissions [17].

Lead levels in seagrass roots are also comparatively lower in comparison to other previously reported studies. For seagrass tissues, lead concentrations from the analysis are generally lower compared to the study of Sidi et al. 2018. *E. acoroides* limits mobility of lead (Pb) to minimize its toxic impacts [9]. This could also be possibly due to its low bioavailability from soils and sediments, where Pb is strongly bound to the organic matter and other components. This feature has rendered it to have high phytoremediation potential and could cater the increasing amount of anthropogenic activities and contaminants in marine ecosystems.

Trace metal contamination in seagrass varies among plant compartments, showing different levels in roots and leaves. Furthermore, they are more susceptible to heavy metal accumulation because of the composition of their cell walls. The walls are composed of polysaccharide groups that act as binding site for metal and metalloid cations [13]. Seagrass trace metal values may also vary seasonally, with lower trace metal value in growing season and higher trace metal value in dormant season [20]. *E. acoroides* species are specialized marine plants that have adapted to nearshore environment.

This study presents the distribution of cadmium and lead in the coastal ecosystem of Brgy. Alegria, Semirara, Caluya, Antique, Philippines. The results have reported that seagrass tissues can accumulate the metals: lead and cadmium. Accumulation of said metals in *E. acoroides* shows the potential of seagrass as marine bioindicators for long term effects of anthropogenic activities, specifically coal mining, and is important for assessing marine ecosystem health. These seagrass beds are shelters and may serve as food for different marine organisms, in such, these organisms may take up toxic substances which in large amounts may affect their health.

*Error Analysis.* It took approximately 25 hours before the samples arrived in Iloilo. Processed samples were stored for approximately two (2) weeks before being subjected to MP-AES analysis.

**Conclusion.** The mean concentration of cadmium in sediments and in seawater is 0.003 mg/L and 0.008 mg/L, respectively. While the concentration of lead in sediments and in seawater is below detection limit. The mean concentration of cadmium in seagrass leaves and seagrass roots is 0.007 mg/L and 0.006 mg/L, respectively. While the mean concentration of lead in seagrass leaves is 0.061 mg/L and 0.067 for seagrass roots. Seagrass roots have the highest concentration for both cadmium (0.003mg/L) and lead (0.007 mg/L). On the other hand, sediments sample have the lowest concentration for both cadmium (0.003mg/L) and lead (below detection limit).

**Recommendations.** In trace metal quantification, Atomic Absorption Spectrophotometer (AAS) may be used as an alternative for MP-AES. In sediment collection, PVC corer can be used instead of a trowel. It is recommended that seasonality, and environmental factors such as ocean currents must be identified prior to the collection. It is further recommended that future studies collect samples all throughout Caluya while taking into consideration the ocean currents where mine effluents can wash up. Moreover, it is also recommended that samples will be collected in different times of the year to further determine the variations of heavy metal concentrations in different seasons.

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**References**

[1] Ahmad S, Azman S, Said MIM, Baloo L. 2015. Biomonitoring of metal contamination in an estuarine ecosystem using seagrass. Journal of Environmental Health Science and Engineering. 13:41-45.

[2] Bonanno G, Di Martino V. 2017. Trace element compartmentation in the seagrass Posidonia oceanica and biomonitoring applications. Marine Pollution Bulletin. 116:196-203.

[3] Tupan C and Azrianingsih R. 2016. Accumulation and deposition of lead heavy metal in the tissues of roots, rhizomes, and leaves of seagrass Thalassia hemprichii (Monocotyledoneaa, Hydrocharitaceae). AACL Bioflux. 9(3):580-589.

[4] Arriesgado DM, Kurokochi H, Nakajima Y, Matsuki Y, Uy W, Fortes M, Campos WL, Nadaoka K, Lian C. 2016. Population genetic diversity and structure of a dominant tropical seagrass, Cymodocea rotundata, in the Western Pacific Region. Marine Ecology.

[5] Bonanno G, Di Martino V. 2016. Seagrass Cymodocea nodosa as a trace element biomonitor: Bioaccumulation patterns and biomonitoring uses. Journal of Geochemical Exploration. 169:43-49.

[6] Llagostera I, Cervantes D, Sanmarti N, Romero J, Perez M. 2016. Effects of copper exposure on photosynthesis and growth of the seagrass *Cymodocea nodosa*: An experimental assessment. Bulletin of Environmental Contamination and Toxicology. 97(3):374-379.

[7] Sidi N, Aris AZ, Yusuff FM, Looi LJ, Mokhtar NF. 2018. Tape seagrass (*Enhalus acoroides*) as a bioindicator of trace metal contamination in Merambong Shoal, Johor Strait, Malaysia. Marine Pollution Bulletin. 126:113-118. doi: 10.1016/j.marpolbul.2017.10.041

[8] Werorilangi S, Samawi F, Tahir A, Faizal A, Massinai A. 2016. Bioavailability of Cu and Pb sediments in vegetated seagrass, *Enhalus acoroides*, from Spermonde Islands, Makassar, South Sulawesi, Indonesia. Research Journal of Environmental Toxicology. 10: 126-134.

[9] Campos W, Acabado C, Guarte D, Paraboles L, Felix L, Burgos L, and Bagarinao A. 2013. Survey of Reef Areas, PCRA of Reef Flat and Seagrass Beds, and Fisheries Profile of Key Barangays. *Unpublished*

[10] Liu X, Bai Z, Yu Q, Cao Y, Zhou W. 2017. Polycyclic aromatic hydrocarbons in the soil profiles (0–100 cm) from the industrial district of a large open-pit coal mine, China. Royal Society of Chem. 7:28029-28037.

[11] Barnuevo A. 2011. Preliminary impact assessment of coal mine effluents on mangrove vegetation in Semirara Island, Antique, Philippines. Philippine Journal of Natural Sciences. 16:27-36.

[12] Fortes MD. 2013. A Review: Biodiversity, Distribution, and Conservation of Philippine Seagrasses. Philippine Journal of Science. 142(Special Issue):95-111.

[13] Water Quality Guidelines and and General Effluent Standards of 2016. Department of Environment and Natural Resources Administrative Order 2016-08. (May 24, 2016).

[14] Tupan CI, Herawati EY, Arfiati D, Aulanni'Am AA. Profile of lead (Pb) heavy metal in water, sediment and seagrass (Thalassia hemprichii) in Ambon Island, Maluku, Indonesia. 2014. Journal of Biodiversity and Environmental Science. 2014;5(4):65–73.

[15] Thangaradjou T, Nobi EP, Dilipan E, Sivakumar K, Susila S. 2009. Heavy metal enrichment in seagrasses of Andaman Islands and its implication to the health of the ecosystem. Indian Journal of Marine Sciences. 39(1): 85-91.

[16] Tiwari S, Singh S, and Garg S. 2013. Induced phytoremediation of metals from fly ash mediated by plant growth promoting rhizobacteria. Journal of Environmental Biology. Vol. 34, 717-727.

[17] Ancora S, Bianchi N, Butini A, Buia MC, Gambi MC, Leonzio C. Posidonia Oceanica As A Biomonitor Of Trace Elements In The Gulf Of Naples: Temporal Trends By Lepidochronology. Environmental Toxicology and Chemistry. 2004;23(5):1093. doi:10.1897/02-59