

## ECOLOGICAL INDICATOR AGENTS FOR INORGANIC CONTAMINANTS STATE MONITORING THROUGH SONNERATIA ALBA, AVICENNIA ALBA AND RHIZOPHORA APICULATA

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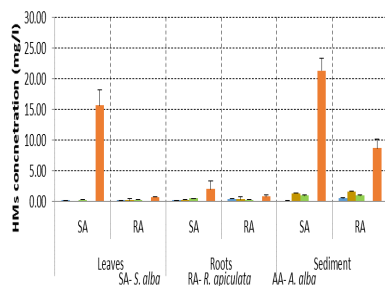
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### Graphical abstract



### Abstract

Mangrove forest ecosystems are threatened by direct impacts such as cutting and pollution due to agricultural, industrialization and urbanization activities. Mangrove forests are often regarded as unpleasant environments with little intrinsic value. Mangrove forests perform valued regional and site-specific functions. Mangrove species can take up excessive nutrients and also play a crucial role in creating a favourable environment for a variety of chemical, biological and physical processes that contribute to the inorganic removal and degradation of organic compounds. Among the many mangrove species, *Sonneratia alba*, *Avicennia alba* and *Rhizophora apiculata* have been studied for biomonitoring of toxic heavy metals elements (Fe, Cu, Zn, Pb and Mn) in a wide range of plant tissues (roots and leaves) and sediment composition at three different locations in Negeri Sembilan, west coast of Malaysia. The results established that there were significant differences between the three mangrove species, locations, plant tissues and sediment samples and their interaction for all the five heavy metals content. The findings revealed that leaf tissues for all species accumulated mostly Fe, Zn, Pb and Cu. Interestingly we noticed that different localities will accumulate different type of heavy metals, for instance *R. apiculata* leaf tissues were detected with higher concentration of Cu and Pb at Kampung Sungai Sekawang whereas in another two sites were detected with Cu, Fe, Zn and Pb. *S. alba* indicated that the most heavy metals highly accumulated was Zn followed by Pb and Cu at Pasir Panjang. In this study *A. alba* showed Zn was highly accumulated in leaf tissues at Pasir Panjang. Thus, those three mangrove species appear to have the greatest potential for use as an effective ecological indicator tools as green application for inorganic contaminants monitoring in mangrove ecosystems.

**Keywords:** *Sonneratia alba*, *Avicennia alba*, *Rhizophora apiculata*, phyto-indicator, heavy metals, ecological indicator, green technology

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## 1.0 INTRODUCTION

Since twenty years back, the Intergovernmental United Panel on Climate Changes (IPCC) has reported that the average global temperature has

increased between 0.15 and 0.3°C per decade due to loss of land and global warming [1-2]. In Malaysia context, mangroves are also facing a critical problem due to natural impacts. The report from IPCC and United Nations Environmental Programme

(UNEP) stated that, the current climate changes in Malaysia is 0.7°C with normal daily temperature at 40°C and have increased sea levels in Southeast Malaysia from one to two metres. As long as the temperature does not increase beyond the limitation between 0.7°C until 2°C, this phenomenon will only impact the coastline area in the form of soil erosion and will trigger mass coral bleaching in Malaysia, which will affect the aquatic ecosystem and habitat [3]. The increasing temperature affects mangrove species in changing a species' composition, phenological patterns which means flowering and fruiting, increase mangrove productivity and expanding mangrove ranges to higher latitudes where range is limited by temperature. Moreover, temperature is a major factor that greatly affects within the types of forest and geographically across the distribution of mangrove species that reduce seedling establishment [4-5].

Mangroves in Malaysia have been declined and loss about 1 % per year due to human activities in maximizing the use of land such as aquaculture (7%), agriculture (43%), urban development (20%) and other activities (30%). The population of mangrove was reduced from 26.8% in 1970 to 60% in 2000 meanwhile over exploitation of fishery resources causes environmental challenges through a rise of sea levels [6-9]. Due to that, mangrove forests continuously suffer by increasing demand in food supply and shrimp farming either for aquatic habitats or human consumption [10].

Mangrove ecosystems play a role as a transitional area between freshwater and marine ecosystems and an important 'sink' to trap pollutants either from inland freshwater or marine habitats. However, few studies have addressed the function of the mangrove ecosystem as an ecological indicator for heavy metal pollutants and nutrients excess in water or sediment. The ecosystem of mangrove species located in silt-rich and brackish water environment along tropical and near river estuaries, particularly along the coast of Malaysian Peninsular, are a good indicator of coast line wellbeing [11]. In addition, the most common metals that enter the estuarine ecosystem through industrial sources are Cu, Pb and Zn [12]. Heavy metals toxicity give impact towards landscape pattern and fragment of mangrove ecosystem because mangrove vegetation are carrying different capability to adsorb and accumulate heavy metals contaminants in different ways.

Changes in landscape conditions in the riparian zone may have a greater influence on water quality in broader scale and watershed conditions [13-14] although the importance of near-site, landscape conditions may vary, depending on the biophysical setting [15]. Scientists and environmental managers alike are concerned with the broad-scale changes in land use and landscape patterns and their cumulative impact on hydrological and ecological processes that affect stream, wetland, and estuary conditions [16-17]. Particular concern is the degree to

which landscape conditions at watershed scales influence nitrogen, phosphorus, and sediment loadings to surface waters [16, 18-19]. High levels of nutrients and sediments in water can pose an impact to human health and ecological risks [18]. Although there have been numerous attempts to monitor and model nutrient and sediment loadings to streams and estuaries [18, 20-21], no comprehensive approach exists to evaluate potential loadings to streams based on landscape composition and pattern across regional scales. Moreover, there is a need to identify those surface waters at greatest risk to high levels of nutrient and sediment loads so that actions can be taken to reduce the risk [22].

Therefore, the study aimed to evaluate the potential of *Sonneratia alba*, *Avicennia alba* and *Rhizophora apiculata* as landscape ecological indicator or state monitoring for inorganic contaminants of mangrove ecosystem for iron (Fe), copper (Cu), zinc (Zn), lead (Pb) and manganese (Mn). Several major parameters were identified before further research which are methods or tool of study, types of mangrove species as plant material, plant tissues and sediment, types of heavy metals as well as selection of locations. The assessment of mangrove species as phytoindicator, particularly of *Sonneratia alba*, *Avicennia alba* and *Rhizophora apiculata* are well documented in previous studies however research has thus far been restricted to single species, certain heavy metals and toxicities, types of plant tissues and sediment as well as localities. So, in this study the researcher compares the different capability of mangrove fragment as ecological indicator tools for inorganic contaminants toxicity. The hypothesis on mangrove species such as of *Sonneratia alba*, *Avicennia alba* and *Rhizophora apiculata* can be manipulated as ecological indicator tool for landscape monitoring to accumulate inorganic contaminants in mangrove ecosystem would significantly derive through leaf and root tissues also sediment.

## 2.0 MATERIALS AND METHODS

### 2.1 Site Selection

Three sites were selected along Port Dickson's coastline mangrove forests located at Tanjung Tuan (2°24'36"N 101°51'14"E), Kampung Sungai Sekawang (2°25'30"N 101°55'16"E) and Pasir Panjang (2°25'33"N 101°55'53"E) in the state of Negeri Sembilan. All the selected sites comprise of at least three major families of mangrove species (Avicenniaceae, Rhizophoraceae and Sonneratiaceae) occupied and dominated by *Avicennia alba* (Api-api putih), *Sonneratia alba* (Perepat) and *Rhizophora apiculata* (Bakau minyak).

## 2.2 Sample Collection and Preparation

Analysis of iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and lead (Pb) in mangrove sediments and plants were carried out by adopting the standard US EPA 3051 method [23] using Hach Spectrophotometer (Model DR5000).

Mangrove sediments, roots and leaves were sampled during low tides in open mudflat areas. Surface sediment samples of three sites were collected randomly at the depth of 0-30 cm with triplicates samples of each species in each site using hand auger (Eijkelkamp Agrisearch) and each species will have three biological samples at each site as further detailed [24]. The roots were carefully handpicked from the sediments at three different spots in triplicate and immediately wrapped with aluminium foil and labelled, whereas the leaves were collected in three samples of approximately 20 leaves from 3 - 5 mangrove trees as well as at three different spots and in triplicates. For each individual tree, 20 leaves were collected from each tree at 1.5 m height with girth at breast height of >20 cm. The samples were put inside a polyethylene bag and labelled for further analysis.

Triplicate samples of sediment, leaves and roots were then combined in aluminium foil and oven-dried at  $\pm 70^{\circ}\text{C}$  in three days before being digested for constant dry weight in a microwave digestion system (Milestone Start D), as detailed in the Method US EPA 3051 and Method of Dry Ashing [25]. After three days, the dried samples were homogenized in an agate mortar and blender and were then sieved using a 2 mm stainless steel sieve in order to remove debris and other coarse structures. The samples were then stored at  $4^{\circ}\text{C}$  for further analysis. Microwave-assisted acid digestion was performed on the samples according to US EPA Method 3051 [19] for sediment whereas leaves and roots were subjected to the Dry Ashing Method [25]. The method provides the acid digestion of the sample in a closed vessel device using temperature control microwave heating for metals (Cu, Pb, Zn, Fe and Mn) determination by spectroscopic method (HACH Spectrophotometer). All the samples were handled in fume hood.

## 2.3 Statistical Analysis

Analysis of variance (ANOVA) was calculated to test the validity of the data and the significance of the variation in the data of five heavy metals studied Fe, Zn, Cu, Pb and Mn toxicities for plant tissues and sediment of *Sonneratia alba*, *Avicennia alba* and *Rhizophora apiculata* at three different locations at Negeri Sembilan.

## 3.0 RESULTS AND DISCUSSION

Analysis of variance showed significant difference ( $p > 0.001$ ) between the three mangrove species, the three locations, the leaves, roots and sediment samples and their interaction for all the five heavy metals content. Those mangrove species, *Sonneratia alba*, *Avicennia alba* and *Rhizophora apiculata* accumulated all five heavy metals tested (Fe, Zn, Cu, Pb and Mn) at Kampung Sungai Sekawang, Pasir Panjang and Tanjung Tuan in present investigation are ranging from deficient, normal and toxic levels (Figure 1, Table 1). Little is known about the accumulation of three mangrove species which are *S. alba* (Sonneratiaceae), *R. apiculata* (Rhizophoraceae) and *A. alba* (Avicenniaceae) as phyto-indicator species for heavy metals contaminants specifically Cu, Fe, Pb, Mn and Zn in mangrove ecosystem. So far, only *R. apiculata* [26-27] been reported on heavy metals accumulation in leaves, roots and sediments, however there is no report on *S. alba* and *A. alba*. So far only five species were studied (*R. apiculata*, *R. mucronata*, *A. officinalis*, *S. caseolaris*) and mostly indicated Cu and Pb accumulation or contaminants followed by Zn, Fe and Mn [26-31]. In our study, we observed that *S. alba*, *R. apiculata* and *A. alba* also accumulated predominantly Cu, Pb, Fe and Zn at toxic levels.

Based on previous studies, leaf tissues accumulated substantially high Fe, Zn and Pb at toxic leaves followed by Cu. This study showed that leaf tissues were detected at toxic levels of Fe, Zn, Pb and Cu. Our study on leaf tissues of *S. alba* indicated that the most heavy metals highly accumulated was Zn followed by Pb and Cu which is contrary to [28] who reported that leaf tissues of *S. caseolaris* can detect high concentration of Pb followed by Cu and Zn. In this study, *A. alba* showed Zn was highly accumulated in leaf tissues at Pasir Panjang and a similar result was found on *A. marina* [29-33]. In contrast, Cu and Pb were highly accumulated in leaf tissues of *A. marina* compared to *R. apiculata* [27].

Lack of studies on root tissues of mangrove species and very limited information regarding type of heavy metals contaminant make it difficult for us to make further comparison and discussion. Based on previous studies, only Cu, Pb and Zn were detected at toxic levels in root tissues [27,29,32,34] whereas in this study Cu, Pb, Zn and Fe were detected. Again we also found that the same species especially *A. marina* at different localities will accumulate different types of heavy metals. In India, *A. marina* root tissues only accumulated Zn and Pb; Cu and Pb in Malaysia whereas in Australia Cu, Pb, Zn were detected. Pb (364.2 mg/kg) was highly detected in root tissues of *A. marina* meanwhile Cu (96.2 mg/kg) was detected in similar range in *A. marina* and *R. apiculata* [27]. In this study, Cu, Fe, Zn and Pb were highly accumulated in root tissues of *S. alba*, *A. alba* and *R. apiculata* at all case studies respectively. High concentration of Zn was found in root tissues of *A.*

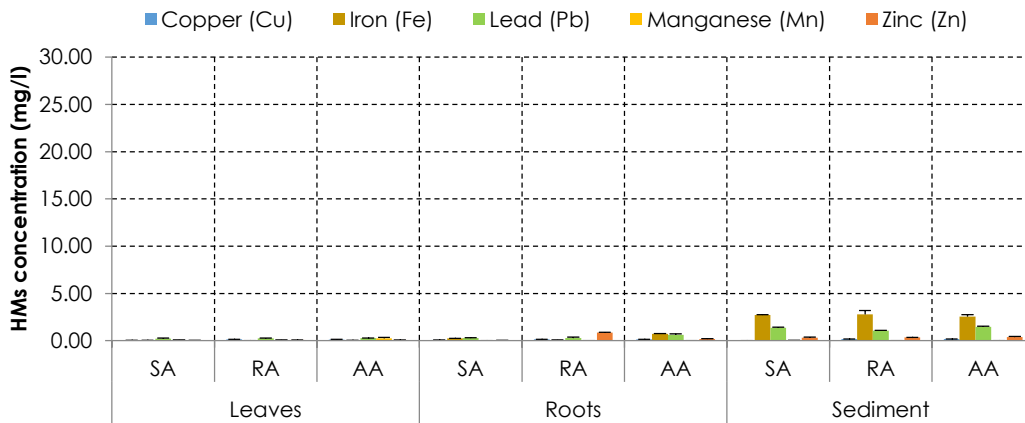
marina [29, 32] and in contrast Cu (25.51 mg/kg) was highly detected in root tissues of *A. marina* [34].

Mangrove sediments are considered a sink and source for trace metals as biogeochemical sink due to high concentration of organic matter and sulphates [35-36]. In this study, the accumulation of heavy metals contaminants by three mangrove species (*S. alba*, *R. apiculata* and *A. alba*) in sediment showed that Cu, Zn and Pb were detected in all case studies. Cu (300 mg/kg) was highly accumulated by *S. alba* and *R. apiculata* except at Kampung Sungai Sekawang. Fe (5120-5920 mg/kg) was highly accumulated by *A. Alba* in both sites except at Tanjung Tuan (species was not available) respectively. Meanwhile, previous studies showed similar elements were found including Cu, Pb and Zn but the composition differ with locality [27-30, 37-38]. For example in Saudi Arabia, *A. marina* sediment was found with Cu but in Iran Pb was detected meanwhile in India Zn and Pb were observed whereas in Malaysia Cu and Pb were mostly reported [34, 37]. The result showed that strong correlation between true mangrove species, localities, sources of contaminant, abiotic factors, mangrove plant tissues and heavy metals content and composition. The comparison studies range of toxic level essential for plant growth were reported by [39] for sediment and [40] for plant tissues as shown in Table 1. Generally, the range of single mangrove species accumulate of heavy metals contaminant in plant tissues and sediment depended on the environmental factors such as plant species, temperature, dissolved oxygen demand and secretion of roots [41].

Mangrove zonation is known to reflect the vegetation distribution from open zone (intertidal zone) to land mangrove (high intertidal zone) and several factors may influence the zonation pattern and frangment including environmental stress such as tidal inundation and salinity [42]. In this study, *S. alba*, *R. apiculata* and *A. alba* were found dominant as native mangrove species and occupying in intertidal zone depend on its inundation tolerance except at Tanjung Tuan (species was not available). Five heavy metals content were detected in different ranges of toxicity in leaf and root tissues as well as in sediment at open zone and only Cu, Zn and Pb were reported in previous studies. Fe is generally described as the principal metal that precipitates with sulphides compounds in anaerobic sediment [43]. However, this study indicated that Fe concentration was deficient in the selected sites. Mn. Heavy metals such as Cu, Pb and Zn are the greatest eco-toxicological concern that accumulate in mangrove ecosystems due to proximity to urban development [44] as well as from domestic garbage dumps to agricultural runoff [45].

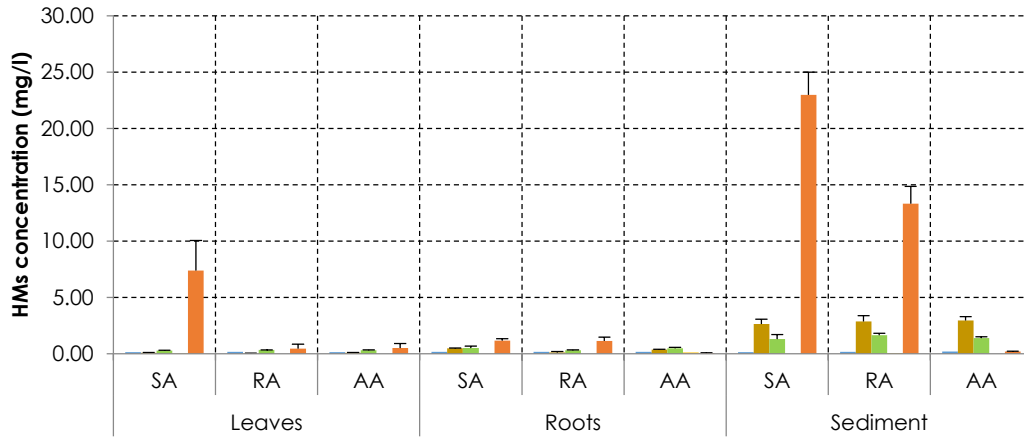
On the other hand, three types of mangrove species dominated the selected locations except for *A. alba* which was not available at Tanjung Tuan. Avicennaceae was an important species in open zones and the disappearing of this species might be due to the contamination of that area. The domestic sewage, tourism activities, land reclaiming and commercial activities were the main sources of heavy metals contaminant whereas 77% of pollutants in 2011 increase into 76% in 2012 at Port Dickson [46].

A.



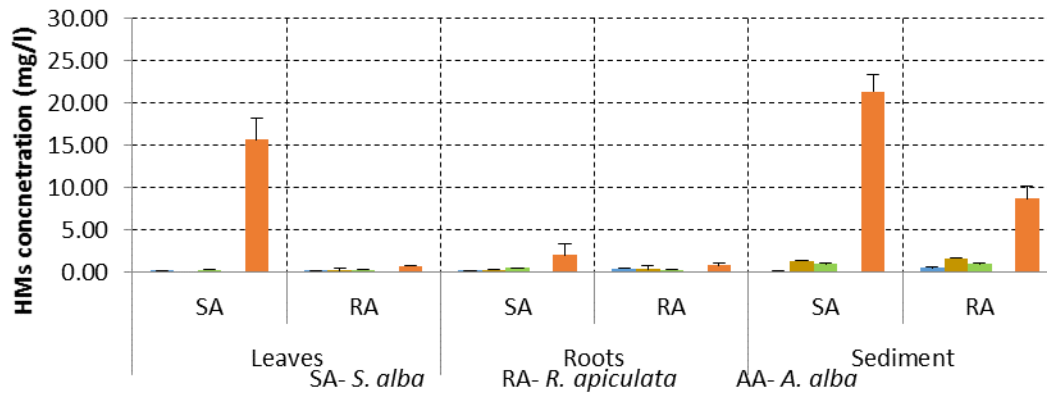
Heavy metals (HMS) content of mangrove tissues and sediment in 3 mangrove species at Kampung Sungai Sekawang

B.



Heavy metals (HMS) content of mangrove tissues and sediment in 3 mangrove species at Pasir Panjang

C.



Heavy metals (HMS) content of mangrove tissues and sediment in 3 mangrove species at Tanjung Tuan

Figure 1 Comparison of heavy metals content (mg/l) in mangrove plant tissues and sediment at three different locations

Table 1 Comparison of heavy metals content in mangrove plant tissues and sediment among Kampung Sungai Sekawang, Pasir Panjang and Tanjung Tuan

A. Heavy metals content (mg/l) at Kampung Sungai Sekawang

Mangrove species	HMs	Leaf	Toxicity status	Root	Toxicity status	Sediment	Toxicity status
<i>S. alba</i>	Cu	0.05	T	0.06	T	0.01	D
	Fe	0.03	T	0.23	T	2.71	D
	Pb	0.22	T	0.28	T	1.35	T
	Mn	0.03	N	0.01	D	0.02	D
	Zn	0.04	N	0.05	T	0.32	T
<i>R. apiculata</i>	Cu	0.14	T	0.14	T	0.17	T
	Fe	0.01	N	0.05	T	2.79	D
	Pb	0.24	T	0.29	T	1.07	T
	Mn	0.03	N	ND	D	ND	D
	Zn	0.09	N	0.85	T	0.34	T

Mangrove species	HMs	Leaf	Toxicity status	Root	Toxicity status	Sediment	Toxicity status
<i>A. alba</i>	Cu	0.12	T	0.15	T	0.18	T
	Fe	0.04	T	0.72	T	2.56	D
	Pb	0.27	T	0.62	T	1.45	T
	Mn	0.23	T	0.01	D	0.01	D
	Zn	0.08	T	0.18	T	0.39	T

## B. Heavy metals content (mg/l) at Pasir Panjang

Mangrove species	HMs	Leaf	Toxicity status	Root	Toxicity status	Sediment	Toxicity status
<i>S. alba</i>	Cu	0.13	T	0.16	T	0.15	T
	Fe	0.09	T	0.49	T	2.63	D
	Pb	0.29	T	0.52	T	1.31	T
	Mn	0.03	N	ND	D	ND	D
	Zn	7.40	T	1.17	T	23.00	T
<i>R. apiculata</i>	Cu	0.17	T	0.17	T	0.16	T
	Fe	0.04	T	0.12	T	2.86	D
	Pb	0.32	T	0.30	T	1.65	T
	Mn	ND	D	ND	D	ND	D
	Zn	0.44	T	1.13	T	13.33	T
<i>A. alba</i>	Cu	0.15	T	0.17	T	0.19	T
	Fe	0.07	T	0.38	T	2.96	D
	Pb	0.32	T	0.47	T	1.39	T
	Mn	ND	D	0.10	N	ND	D
	Zn	0.52	T	0.06	T	0.21	T

## C. Heavy metals content (mg/l) at Tanjung Tuan

Mangrove species	HMs	Leaf	Toxicity status	Root	Toxicity status	Sediment	Toxicity status
<i>S. alba</i>	Cu	0.17	T	0.19	T	0.15	T
	Fe	0.05	T	0.25	T	1.35	D
	Pb	0.28	T	0.48	T	0.96	T
	Mn	0.03	N	ND	D	ND	D
	Zn	15.67	T	2.00	T	14.67	T
<i>R. apiculata</i>	Cu	0.18	T	0.41	T	0.50	T
	Fe	0.18	T	0.34	T	1.67	D
	Pb	0.29	T	0.30	T	0.94	T
	Mn	ND	D	0.03	N	0.10	N
	Zn	0.70	T	0.80	T	8.67	T
<i>A. alba</i>	Species not available						

\*Toxicity status: D= Deficient N= Normal T= Toxic

\* ND: Not Detected

#### 4.0 CONCLUSION

In conclusion, the differences in indicating and accumulating of heavy metals contaminant by true

mangrove plant species shows a relative difference due to several factors. Strong correlation between mangrove sediment and mangrove plant tissues where high heavy metals content were



accumulated in leaf and root tissues explained the greatest variance increased of heavy metals accumulation in mangrove sediment. The three mangrove species studied namely *S. alba*, *R. apiculata* and *A. alba* were found to have great potential for phyto-indicator species to accumulate and remove heavy metal specifically Zn, Pb followed by Cu, Fe and Mn from mangrove plant tissues and sediment in intertidal zone. Those mangrove species also can act as ecological tool for green application for changes of landscape pattern and fragment.

In order to determine which key factors influence the plant mechanism to accumulate or to indicate the type of heavy metals in mangrove ecosystem and accumulation rate, a greater understanding of how mangrove fragment species influence heavy metals toxicity and composition in response to interaction with environment factors will emerge.

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