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Assessment of *Melaleuca cajuputi* as Heavy Metals Phytoremediator for Sewage Sludge Contaminated Soil

[1,3]Shibli Nik Mohd, [2]Nik Muhamad Majid,
[3]Noor Azhar Mohamed Shazili and [1,2]Arifin Abdu

[1]Department of Forest Management, Faculty of Forestry,
University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Institute of Tropical Forestry and Forest Products (INTROP),
University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Faculty of Science and Technology, University Malaysia Terengganu,
21200, Kuala Terengganu, Malaysia

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ABSTRACT

In 2011, total marine capture landings in Peninsular Malaysia decreased by 3.9% which amounted to 1,373,105 tonnes as compared to 1,428,881 tonnes in 2010. The decline is connected to marine pollution which mainly comes from land based sources. Coastal forests play an important role affecting the marine ecosystem health and need to be conserved as a buffer to minimize pollution input into marine ecosystem. Heath forest, a type of coastal forest that receives less attention compared to mangroves, also has similar role. *Melaleuca cajuputi* that dominates heath forest has high potential as a phytoremediator of heavy metals and also has high economic values. A greenhouse experiment was conducted to evaluate the potential of this species to extract heavy metals namely Zn and Cd from soil. Four levels of treatment were prepared where the test media was mixed with different amounts of sewage sludge (v/v) namely; T1 (control-soil only), T2 (30% sludge +70% soil), T3 (50% sludge + 50% soil) and T4 (70% sludge + 30% soil). After 90 days of experimental period, the accumulation of Zn was found to be high in the roots (137.19 mg kg^{-1}), followed by the leaves (135.0 mg kg^{-1}) and stems (102.24 mg kg^{-1}). As for Cd the highest concentration was in the roots (2.05 mg kg^{-1}), stems (1.78 mg kg^{-1}) and leaves (1.66 mg kg^{-1}). The species Transfer Factor (TF) values in the greenhouse experiment were > 1 but the Bio-Concentration Factor (BCF) values are≤ 1. This result shows that *M. cajuputi* is tolerant to Zn and Cd toxicity (low BCF) but able to transfer these elements (high TF) to the shoots for removal by leaf fall or by harvesting the shoots. In addition, the accumulation of Zn and Cd were high in the roots which suggests that Zn and Cd were efficiently immobilized (in the roots) from entering the marine ecosystem.

Keywords: Phytoremediation, *Melaleuca cajuputi*, Heavy Metals, Heathand Coastal Forests

1. INTRODUCTION

In 2011, the total marine capture landings in Peninsula Malaysia decreased by 3.9% which amounted to 1,373,105 tonnes as compared with 1,428,881 tonnes in 2010 (DOF, 2011). One of the reasons of declining marine capture landing might be due to pollution of the marine ecosystem. Biological effects from marine pollution result in economic effects (Ofiara and Seneca, 2006). There are different pathways by which contaminants can be introduced into the marine environment: direct input, riverine contribution and drawdown from the atmosphere (Kroger *et al*., 2002). Most sources of marine pollution are land based such as

Corresponding Author: Arifin Abdu, Department of Forest Management, Faculty of Forestry, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia Tel: +603-89467177 Fax: +603-89432514

agricultural run-off, industrial discharges and substances released from sewage treatment plants.

Coastal forests play an important role in marine ecosystem health and need to be conserved with a new perspective not only as mitigation from natural hazards such as tsunami and hurricane but as a buffer to minimize pollution input into the marine ecosystem. Zhang *et al*. (2010) stated that mangroves wetland especially with *Sonneratiaapelata* species has great potential in removing heavy metals and nutrients in coastal areas. Mangrove tree is a biochemical reactor that plays an important role in the physiological and biochemical processes including organic matter decomposition (Ramos-e-Silva *et al*., 2006).

Heath forest or *Melaleuca* forest, a type of coastal forest that receives less attention compared to mangroves, also has an important role in regulating pollution input into the marine ecosystem. According to Okubo *et al.* (2003) *Melaleuca* forest thrive son BRIS soils that are rich with pyrite and contain peat layer underneath the sandy soil. The decline of *Melaleuca* forest could pose pollution risk to the marine environment as the pyrites and peat layer would cause acid drainage with high heavy metal loads that is toxic to marine life. Cabon *et al.* (2010) found out that most of the metallic elements were released from the sediments into the seawater during the first fifteen minutes of acid exposure after which a high degree of pollution was induced if acids leached into seawater were not rapidly diluted.

The importance of conserving coastal forests has been recognized in numerous scientific literatures but knowledge on how wetland plants interact with pollution was incomplete (Stottmeister *et al*., 2003). Phytoremediation is a soil remediation technique using plants and also provide an effective way to control pollution from spreading (Majid *et al*., 2011; 2012a; 2012b). This technique utilizes plant's ability to extract pollutants, transform pollutants into non-toxic compounds or degrade pollutants utilizing only solar energy (Alkorta *et al*., 2004). Thus, the objective of this study was to assess the tolerance and accumulation of heavy metals namely Zn and Cd by *M. cajuputi* and to evaluate the potential of the species in extracting heavy metals from the soil.

2. MATERIALS AND METHODS

2.1. Experimental Design and Data Collection

The study was conducted in a complete random design experiment in the greenhouse. The contaminated test media were prepared by mixing (v/v) dewatered sewage sludge with normal garden soil. Each treatment pot was filled up to about 16 kg of test media. Four levels of treatment were prepared namely; T1 (control-soil only), T2 (30% sludge +70% soil), T3 (50% sludge + 50% soil) and T4 (70% sludge + 30% soil). The study was conducted for 90 days before the plants were harvested for laboratory analysis. No fertilizer was applied throughout the experiment as it was assumed that the treatment media would adequately supply the required nutrients for optimum growth. Growth of the *M. cajuputi* saplings was measured by the height and stem basal diameter taken every two weeks.

Plant samples were washed thoroughly with distilled water and dried in an oven at 60°C for two days whereas soil samples of the treatment media were air dried for about three days prior to laboratory analysis. Samples of treatment media were analyzed for pH, Cation Exchange Capacity (CEC), total carbon, total nitrogen and heavy metals namely Zn, Cu and Cd. Determination of media pH were measured with a pH-meter in water supernatant obtained from mixing the soil with distilled water in 1:5 ratios (w/v) for 15 min. Total carbon and nitrogen were determined by automated CHNS analyzer. CEC was determined by neutral ammonium acetate leaching as used by the United States Department of Agriculture-National Resources and Conservation Services (USDA-NRCS) Soil Survey Staff standard methods for soil analysis (Burt, 2004). Soil and plant heavy metal concentrations were extracted by wet digestion method with aqua-regia solution (Allen *et al*., 1974).

Heavy metals in the extraction solution were then determined with Atomic Absorption Spectrophotometer (AAS). Recovery tests for heavy metals extraction reliability was conducted using Standard reference material (SRM) certified materials for plant sample (material code 1573A; tomato leaves) and soil (material code HISS-1; marine sediment) obtained from United States National Institute of Standard and Technology (NIST) and over 90% recovery was obtained and maintained.

2.2. Statistical Analysis and Phytoremediation Potential Assessment

All data were subjected to Duncan's Multiple Range Test (DMRT) to detect and rank significant differences between treatments and groups. Assessing the potential of a plant species in phytoremediation was achieved by applying the Bio-Concentration Factor (BCF) and Translocation Factor (TF). Bio-Concentration Factor (BCF) is a concentration ratio of heavy metal concentration in plant tissues over concentration in soil (Liang *et al*., 2009). Values > 1 indicate heavy metals are accumulated in the biological tissues. On the other hand, Translocation factor (TF) is the ratio of concentration in plant shoots over the concentration in the roots (Colle *et al*., 2009). Values > 1 indicate that the species transfer or translocate heavy metals from roots to shoots or heavy metals are highly mobile in the species (Justin *et al*., 2011; Majid *et al*., 2012c; Sundarajoo *et al*., 2013).

3. RESULTS AND DISCUSSION

3.1. Chemical Properties of the Treatment Media

Table 1 shows selected chemical properties of the treatment media. The control media without sewage sludge added was acidic with pH of 4.94 but when sewage sludge was added to the subsequent treatments the pH increased to 5.63 in treatment T4. Similarly, the CEC in T1 was very low (3.0 cmol kg^{-1}) but increased significantly (41.83 cmol kg^{-1}) in T4. Total carbon also increased from 0.5 to 3.1% whereas total nitrogen from 0.1% in T1 to 0.5% in T4. The significant difference among the treatment levels was due to high organic materials in the sludge (Amuda *et al*., 2008) but the differences depends on the age and the origin of the sewage sludge (Soler-Rovira *et al*., 1996) where in this experiment the differences of nitrogen and carbon were not very large.

The concentrations of Zn and Cd in the treatment media increased significantly with increased amount of sewage sludge. The control or T1 treatment has Zn at 26.67 mg kg^{-1} and Cd at 0.22 mg kg^{-1}. The concentrations then increased to 131.01 mg kg^{-1} and 10.36 mg kg^{-1} respectively in T4 treatment. According to Alidadi *et al*. (2007) organic materials in the sewage sludge contribute to the increased pH and CEC values. This is the reason why sewage sludge is often used as soil amendment to neutralize acid soils (Hani *et al*., 1996; Bramryd, 2002). However, sewage sludge is the contributor to the heavy metal contamination in agricultural soil (Merrington *et al*., 2003; Paramasivam *et al*., 2006) and this can be seen in the treatment media with 50 and 70% sewage sludge mix (T3 and T4) where Zn and Cd concentrations in these treatments exceeded the normal level of uncontaminated soil in Malaysia of 92.0 mg kg^{-1} of Zn and 0.032 mg kg^{-1} of Cd (Zarcinas *et al*., 2004).

3.2. Distribution of Zn and Cd in Different Plant Parts

Figure 1 show the concentration of Zn and Cd in the roots, stems and leaves of *M. cajuputi* after 90 days. The concentration of Zn ranged from 230-320 mg kg^{-1} but there were no significant difference ($p \leq 0.05$) among the treatments with sewage sludge except for the control (80 mg kg^{-1}). However, Cd concentration was not significantly different ($p \leq 0.05$), ranging from 2.05-5.29 mg kg^{-1} in all treatments except for treatment T3 which was the lowest (less than 2.9 mg kg^{-1}).

The non-significant different in the plant Zn and Cd concentration between the treatments indicate Zn and Cd were efficiently regulated by the species irrespective of the concentrations in the treatments as uptake of both element was actively transferred across the cell wall by specific carrier proteins (Kramer *et al*., 2007; Mejare and Bulow, 2001). This also shows that *M. cajuputi* is very tolerant to Zn and Cd contamination.

The distribution of Zn and Cd in the different plant parts were generally high in the roots ranging from 111.03-137.19 and 0.74-2.05 mg kg^{-1}, respectively. This shows that *M. cajuputi* is an efficient excluder species that manage to retain high levels of heavy metals in the roots. However, some Zn was transferred to the leaves (81.44-135 mg kg^{-1}) and a small amount accumulated in the stems (43.11-102.24 mg kg^{-1}). Cd has been found to have accumulated in the stems (0.23-1.78 mg kg^{-1}) more than the leaves (0.49-1.66 mg kg^{-1}). Accumulation of Zn in the leaves was due to its importance in biochemical reaction in photosynthesis, whereas Cd which is non-essential and toxic was transformed and stored in the stems and finally expelled as *M. cajuputi* frequently shed its papery bark.

3.3. Evaluation of *M. Cajuputi* as Phytoremediator Species

Table 2 shows the Bio-Concentration Factor (BCF) and Translocation Factor (TF) as an evaluation of phytoremediation potential of *M. cajuputi*. The value of BCF was high in the control treatment for Zn (1.3) and Cd (7.4). However, in other treatments, the BCF values were <1 indicating that *M. cajuputi* is not an accumulator species. On the other hand, TF values of *M. cajuputi* in every treatments (except T3 for Cd) show values >1, indicating that Zn and Cd were efficiently transferred to the shoots. This might be due to the high transpiration rate of the species which is common for salt tolerant plants (Nguyen *et al*., 2009; Cramer *et al*., 1999).

In general, plants that have BCF and TF values of >1 are sought for heavy metal extraction (Alkorta *et al*., 2004). Results from this experiment however, shows low BCF but high TF.

Table 1. Selected chemical properties of the treatment media before planting

Treatment	pH	CEC (cmol/kg)	TC (%)	TN (%)	Zn (mg/kg)	Cd (mg/kg)
T1 (Control)	4.94^{c}	3.00^{c}	0.5^{c}	0.1^{c}	26.67^{d}	0.22^{c}
T2(30% SS)	4.78^{c}	8.01^{bc}	2.6^{b}	0.4^{b}	79.74^{c}	2.60^{b}
T3 (50% SS)	5.12^{b}	12.07^{b}	3.0^{a}	0.4^{b}	102.96^{b}	10.07^{a}
T4 (70% SS)	5.63^{a}	41.83^{a}	3.1^{a}	0.5^{a}	131.01^{a}	10.36^{a}

Note: Averages within columns followed by different letters indicate statistically difference ($p \leq 0.05$) with Duncan's Test; SS = Sewage Sludge

Table 2. BCF and TF for *M. cajuputi* in the greenhouse experiment for each treatment

	Treatment	Zn	Cd
BCF	T1	1.3	07.4
	T2	0.8	00.4
	T3	0.3	00.1
	T4	0.4	00.2
TF	T1	4.6	02.7
	T2	3.1	01.1
	T3	2.0	00.2
	T4	2.9	11.1

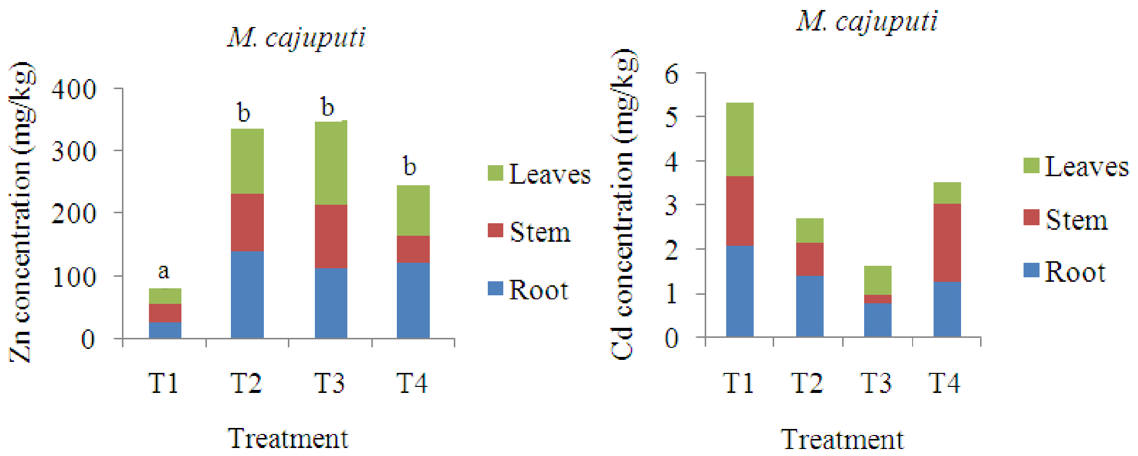

Fig. 1. Concentrations of Zn and Cd in different plant parts of *M. cajuputi*

This means that *M. cajuputi* is not Zn and Cd hyperaccumulator species but with high biomass of *M. cajuputi* that is commonly observed in the natural coastal forests, its ability to store Cd in the stems and high transpiration rate indicate that this species has high potential as a phytoremediator. This also shows how *M. cajuputi* coastal forests function as a buffer zone to control land based pollution especially heavy metals from entering the marine environment.

4. CONCLUSION

M. cajuputi has high potential as a phytoremediator and also has high economic value. *M. cajuputi* coastal forests provide a natural buffering ability to minimize heavy metal pollution from entering the marine environment. Conservation of this coastal forest which is dominant in the east coast of Peninsula Malaysia is highly desired to support the limited distribution of mangrove areas in similar location apart from the potential of the species for commercial use.

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