

Using *Orthosiphon stamineus* B. for Phytoremediation of Heavy Metals in Soils Amended with Sewage Sludge

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Abstract: Problem statement: Phytoremediation is a biological treatment where plants are used to remove pollutants from the environment to make them harmless. A study was conducted in a greenhouse at the Faculty of Forestry, Universiti Putra Malaysia on the potential of *Orthosiphon stamineus* B. planted in sewage sludge for heavy metals uptake. The objective of this study was to evaluate the ability of *O. stamineus* to remove toxic heavy metals from sewage sludge. **Approach:** A total of 36 of *O. stamineus* were treated at six different levels of soil mixture and sludge treatment including one control. The treatments were replicated six times. Growth parameters mainly height and basal diameter were measured for each plant within 10 weeks. Soil texture (pipette method), soil pH (glass-electrode method) and total carbon (C) (Los on Ignition) were determined. The concentration of heavy metals that include cadmium (Cd), chromium (Cr), zinc (Zn), copper (Cu) and lead (Pb) in the planting media and plant parts were analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). **Results:** There was a significant difference ($P \leq 0.05$) in height, basal diameter and number of leaves among the six treatments. The height increment values for all treatments in descending order are: T3 (10.22 cm), T2 (12.42 cm), T1 (13.92 cm), T5 (14.65 cm), T4 (16.20 cm) and C (16.97 cm). In terms of basal diameter, the highest incremental values in ascending order are T5 (0.99 mm), T1 (1.20 mm), T3 (1.31 mm), T2 (1.38 mm), T4 (1.53 mm) and C (1.75 mm). Soil texture for the control is categorized as clay and for T5 is silt loam. Soil reaction is acidic while total carbon ranges from 16.7% to 61.3 %. The concentrations of heavy metals in the planting medium were higher before planting as compared to after planting. For examples, the values for Cd, Cr, Zn, Cu and Pb in T5 (100% sewage sludge) before planting are 0.18 ppm, 23.45 ppm, 290.05 ppm, 17.07 ppm and 5.33 ppm, respectively. The values decreased after planting where 0.13 ppm for Cd, 24.30 ppm for Cr, 252.04 ppm for Zn, 18.56 ppm for Cu and 5.96 ppm for Pb were observed. *O. stamineus* absorbed heavy metals in the range of 0.01 ppm to 0.57 ppm for Cd, 0.08 ppm to 8.48 ppm for Cr, 0 ppm to 43.93 ppm for Zn, 0.11 ppm to 16.73 ppm for Cu and 0.01 ppm to 9.05 ppm for Pb in various plant parts of leaves, stems and roots. **Conclusion:** Most of the heavy metals taken up by the plants were stored in the leaves, whereas Zn seemed to be stored in all plant parts. These results indicate that *O. stamineus* has the potential to accumulate heavy metals particularly Zn, Pb and hence could be considered as a good phytoremediator species.

Key words: Heavy metals, *Orthosiphon stamineus*, phytoremediation, sewage sludge, phytoremediator species, chemical remediation methods, organic materials, microbial synthesized products, Completely Randomized Design (CRD), physico-chemical analysis

INTRODUCTION

Phytoremediation is termed as an in-situ or ex-situ engineered use of plants to remove or control

contaminant, or to foster contaminants breakdown by microorganisms in plant and used the term to evaluate the plants use to accumulate metals from the groundwater and soil (Schnoor *et al.*, 1995). Jamil *et*

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al. (2009) explained that phytoremediation techniques required very low costs to carry out. Likewise, (Meagher, 2000; Ahmadpour *et al.*, 2010) studied that this method is widely recognized and accepted as an ecologically responsible alternative to the environmental destructive chemical remediation methods. In addition, this particular technique is considered as an environment-friendly as it utilizes natural resources such as microorganisms (Jamil *et al.*, 2009). The process of phytoremediation comprises two distinctive physiological processes which are the uptake of heavy metals by the plant or by the root colonizing microbes.

Sewage sludge is a liquid waste containing some solids, undecomposed organic materials and microbial synthesized products. The sludge includes washing water, urine, laundry waste and some other domestic wastes. Bahmanyar and Piradshiti (2008) believed that sewage sludge is suitable as fertilizer material because it contains constituents needed by plants for their growth. In addition, Korentajer (1991) found that sewage sludge exhibited high content of nutrients and organic matter which are favourable to be used as soil amendment medium. Untreated sludge and hydrated treated sludge release nitrogen slowly and this benefits the crops. Research done by Singh and Argawal (2007) recorded that application of sewage sludge to soil stimulates the nutrient cycling and reduces the demand on usage of commercial fertilizers. However, establishment of tree on highly contaminated mining waste or soil usually unsuccessful due to the high content of heavy metals that possess characteristics of pollutants and acts as inhibitor to the plant growth performance. Thus, when the metals are released or channeled into the soil, it may cause hazardous effects to the crops and also the consumers who utilize the products produced from the crops. Jones and Johnson (1989) also observed that the levels of heavy metals, such as Cd, Mn and Zn, in the soil and plant tissues increased after sewage sludge was applied and contributes to waterway and soil pollution. Kabata-Pendias and Pendias (2001) determined that Pb, Cd, Cr, Hg, Zn and Cu are the most common heavy metal contaminants. Therefore, the mobility of these metals in the soil itself creates interest among researchers in the field of ecotoxicology especially on the total capacity uptake of those metals by type of plants examined (Canet *et al.*, 1997; Sánchez-Martin *et al.*, 2004).

Orthosiphon stamineus B., family Lamiaceae, is widely distributed throughout South East Asia and locally known as 'Misai kucing' in Malaysia and Singapore. This medicinal herb can grow up to 1.5 meter height, exhibiting opposite pairs of leaf

arrangement, simple, glabrous, lanceolate leaf blade with serrate margin and green in colour. Leng and Lai-Keng (2003) described that *O. stamineus* vegetative propagation is via the cutting of the mature stem. The flower of *O. stamineus* is usually white-bluish in colour, campanulate in colour which gives it the cat's whisker structure. Besides that, Chin *et al.* (2007) and Affendy *et al.* (2010) found that *O. stamineus* is used vastly as traditional medicine to treat gallstones, fever and renal stones. To our knowledge, the information available on the potential of *O. stamineus* for accumulating heavy metals is limited or even lacking. Hence, this study was carried out to elucidate the potential of *O. stamineus* for the uptake of heavy metals on soil treated with sewage sludge.

MATERIALS AND METHODS

The study was conducted at a greenhouse of Faculty of Forestry, University Putra Malaysia (2° 59' 18.24" N latitude and 101° 42' 45.45" E longitude) from April 2009 until July 2009. The seedling of *O. stamineus* mother tree collected from Malaysian Agricultural Research and Development (MARDI) were germinated via cuttings of the mature stem and planted in 16 × 16 cm polybags. The proportion of growing medium for *O. stamineus* seedling was soil (3): organic materials (2): river sand (1). The seedlings were transplanted into suitable plastic pot (32.0 cm height, 106.0 cm upper diameter, 69.0 lower diameters) which was filled up with the mixture of soil and sewage sludge right after one month.

The experimental design used in this study was a Completely Randomized Design (CRD) and the pots were labeled according to its composition; Control (100% soil), Treatment 1 (80% soil and 20% sewage sludge), Treatment 2 (60% soil and 40% sewage sludge), Treatment 3 (40% soil and 60% sewage sludge), Treatment 4 (20% soil and 80% sewage sludge) and Treatment 5 (100% sewage sludge).

The height and diameter of *O. stamineus* planted was measured every 2 weeks during the study period using diameter tape while the basal diameter was measured using vernier caliper every 2 weeks. All the dead leaves were collected and kept for analysis in the laboratory. Root rot, infestation of insects, pathogen attack, nutrient deficiency and any abnormalities were jotted down.

Soil samples from each pot were collected before and after planting and kept in standard plastic containers and were air-dried before their physico-chemical analysis. The texture of the soil in each pot was determined according to pipette method. Soil pH

and total carbon as well as heavy metal concentration (Cr, Cd, Zn, Cu and Pb) were determined. pH was determined via glass-electrode at 1:5 soil to solution ratio after reciprocal shaking for 1 hour (Arifin *et al.*, 2007). Total carbon analysis was determined by using conventional method, Los on Ignition (Heiri *et al.*, 2001). Five g of air-dried soil were kept in oven for 8 hours at 550°C and the calculation was carried using the following equation:

$$\text{Total C} = \frac{[\text{BO}-\text{AO}]}{\text{BO}} \times 100\% \quad (1)$$

Where:

BO = Soil before oven-dried

AO = Soil after oven-dried.

ICP-MS was used for digestion of element soluble in aqua regia. One hundred and fifty ml of concentrated hydrochloric acid were mixed together with 50 ml of concentrated nitric acid gradually in a polypropylene container. Each weighed and grinded samples was placed into the digestion tube and 20 ml of aqua regia was added. The tubes were swirled in a fume hood and left overnight before being heated. Then, the tubes were heated at 80°C for an hour before being increasing to 140°C for another hour until a clear solution was observed. The hot samples in tubes were left to cool to room temperature before 50 ml of distilled water was added. The samples were then filtered through ash-less medium grade filter study into the plastic bottle. Distilled water was added until 100 ml mark. A blank was prepared for each batch. The sample solution was analyzed by ICP-MS and blank was run accordingly for each batch.

Acid digestion (ICP-MS) was also used to determine the concentration of heavy metals in plant parts. Fresh samples of plant parts were weighed and dried in oven at 60°C for 24 hours and shredded into small pieces before digestion. Oven-dried weight was taken to obtain the biomass. Plant biomass was measured separately according to leaves, stems and roots and was calculated. The loss in weight upon drying is the weight originally present. Moisture content is expressed as percentage of the oven-dried weight. The symbol P_w is used to denote this percentage. The P_w is the ratio of weight of the samples water to the weight of oven-dried sample multiplied by 100:

$$P_w = \frac{[\text{WW}-\text{DW}]}{\text{WW}} \times 100\% \quad (2)$$

Where:

WW = Wet weight of soil sample

DW = Dry weight of soil sample

All data obtained in terms of growth, heavy metals in soil, sludge and plant parts were subjected to one way Analysis Of Variance (ANOVA). Duncan Multiple Range Test (DMRT) was employed to detect any significant differences among and between the treatments of planting medium and growth parameters such as height, basal diameter and number of leaves. Comparison using t-test also had been done to detect any significant differences between before and after planting.

RESULTS AND DISCUSSION

General properties of the soils: All treatments were categorized as clay in texture, except for T5 (100% sewage sludge) which is silt loam. The percentage of silt ranges from 8.04-56.70% while the percentage of clay and sand ranges from 20.09-85.15 and 5.84-25.65%, respectively. Clay and silt loam are termed in fine textured. The results revealed that soil pH after planting has decreased or became more acidic for all treatments except for 100% control of soil where the pH increasing (up to 6.24). The pH range before planting was from pH 5.39 to 5.98, while after planting the pH it was from 4.73 to 6.24.

This information was useful to identify the deficiency of important nutritional elements in soil samples. Plants need water and nutrients for their growth and the capacity of soils to store water and nutrient increases when their percentage of clay increases. Clay has higher capacity than silt loam to store water and nutrients due to its large surface area. Previous studies had shown that negative charge derived from clay minerals in the soils play important role in determining the soil nutrient or fertility status of acid tropical soils (Arifin *et al.*, 2007). Generally, soil with medium texture (loam) is the most desired for plant growth. After planting soil becomes more acidic because potassium, calcium and magnesium are reduced due to uptake by plants. As the pH goes above 7 the availability of boron, copper and zinc begins to decline. As such, soil pH should be kept in the range of 6.4 to 7.0 for good plant growth.

The highest content of total carbon was recorded in T5 which exhibited almost identical range from 0.17-0.21%. Besides, total carbon was high in all treatments that were treated with sewage sludge indicating that sewage sludge contains higher organic matter compared to the control treatment (mineral soil). Rice (2002) found that the soil fertility improved when organic matter increased. Soil organic matter also improves water holding capacity, thus increasing the plants' ability to withstand short droughts. Kerrigan and Nagel believed that fine textured soil increased the ability of plant to absorb heavy metals.

Height increment, basal diameter increment and number of leaves of *O. stamineus*: The results showed that there are significant differences ($P \leq 0.05$) among the treatments in terms of height increment, basal diameter increment and number of leaves (Table 1). Sewage sludge supplied nutrients such as potassium, magnesium and others to the growing *O. stamineus* which improved the growth. Plant height increased for the first six weeks, after which it leveled off (week 8 to week 10). On the other hand, plant in T5 showed a conspicuous decrease after week 8.

Basal diameter increment showed similar pattern with that of height increment where it increased significantly from week two to eight. After week 8, the diameter increment was constant for all treatments except for T5 that decreased slightly. It was observed that the control treatment had the highest diameter increment.

Another parameter observed was the increment of leave number where there were significantly different among the treatments. Plant in T2 (60% soil; 40% sewage sludge) showed a conspicuous decrease after week 8 compared to other treatments. The best treatment was T4 (20% soil; 80% sewage sludge) which showed higher number of leaves compared to other treatments. The lowest number of leaves was recorded by the control treatment (100% soil), producing only 153 leaves. This is consistent with the finding of Heppner (1993) who showed that environmental condition was the probable cause of leaf dropping.

Biomass of *O. stamineus* plant parts: The plant biomass shows significant difference ($P \leq 0.05$) among the treatments where after 10 weeks, treatment T5 and T3 (40% soil; 60% sewage sludge) shows the highest biomass for above ground plant parts; leaves (84.71 g) and stems (75.00 g). The lowest biomass was found for plants before planting with values of 35.0 g for leaves and 37.66 g for stems.

The average dry weight of root biomass ranges between 82.71 and 98.61 g, while the highest root biomass ranges between 82.71 and 98.61 g (Table 2). The highest root biomass for below ground was found in the control treatment. In less contaminated soils, factors limiting plant growth are macronutrient deficiencies and physical condition, leading to poor water holding, aeration and root penetration.

Heavy metals concentration in *O. stamineus* growth medium: Table 3 shows the concentration of Cd, Cr, Zn, Cu and Pb in soil before and after planting. T-test analysis gave significant difference ($P \leq 0.05$) of heavy metals in soil between before and after planting ($P \leq 0.05$). Plant in treatment T5 recorded the highest Cd concentration in the planting media with values of 0.18 mg kg⁻¹ and 0.13 mg kg⁻¹ for before and after planting period, respectively.

Table 1: Height increment, basal diameter increment and number of leaves of *O. stamineus* by 10 weeks

Treatment	Height (cm) and basal diameter (mm)		
	Height	Basal diameter	Leaves
Control	16.97a	1.75a	153d
T1	13.92b	1.20d	230c
T2	12.42c	1.38c	162d
T3	10.22d	1.31c	275b
T4	16.20a	1.53b	332a
T5	14.65b	0.99e	325a

Note: Different letter within a column indicate significant differences between means following Duncan Multiple Range Test ($P \leq 0.05$); ns, no significant differences ($P \leq 0.05$); nd, not determined. Control, (100% soil); T1, (80% soil and 20% sewage sludge); T2, (60% soil and 40% sewage sludge); T3, (40% soil and 60% sewage sludge); T4, (20% soil and 80% sewage sludge); T5, (100% sewage sludge)

Table 2: Average dry weight biomass for leaves, stem and roots

Treatment	Weight (g)		
	Leaves	Stem	Roots
Before	35.00d	37.66d	45.83c
Control	71.23b	63.48b	98.61a
T1	69.83b	61.70b	87.95b
T2	44.27c	50.00c	82.71b
T3	82.65a	75.00a	89.10b
T4	46.56c	65.07b	89.32b
T5	84.71a	60.84b	87.65b

Note: Different letter within a column indicate significant differences between means following Duncan Multiple Range Test ($P \leq 0.05$); ns, no significant differences ($P \leq 0.05$); nd, not determined. Control, (100% soil); T1, (80% soil and 20% sewage sludge); T2, (60% soil and 40% sewage sludge); T3, (40% soil and 60% sewage sludge); T4, (20% soil and 80% sewage sludge); T5, (100% sewage sludge)

Table 3: Heavy metals concentration in growth medium of *O. stamineus*

Treatment		Concentration (ppm)				
		Zn	Pb	Cr	Cu	Cd
Control	B	10.50a	1.04c	1.15a	1.01c	0.02c
	A	2.76b	0.52d	1.12b	0.26d	0.00c
T1	B	27.26a	1.64c	12.9a	3.58c	0.07d
	A	23.27b	1.18d	3.12b	2.93d	0.06d
T2	B	36.80a	1.43c	2.69a	2.79c	0.09a
	A	31.73b	1.32d	2.94b	2.28d	0.04b
T3	B	46.50a	2.00c	2.45a	8.90c	0.07e
	A	40.59b	1.49d	1.86b	3.01d	0.05e
T4	B	69.21a	1.88c	4.96a	4.23c	0.08b
	A	65.88b	2.11d	7.29b	5.07d	0.08b
T5	B	290.05a	5.33c	23.45a	17.07c	0.18a
	A	252.04b	5.96d	24.40b	18.56d	0.13b

Note: Different letter between soil before (B) and after (A) planting in each treatment for each element were significantly different following Duncan Multiple Range Test ($P \leq 0.05$). Control, (100% soil); T1, (80% soil and 20% sewage sludge); T2, (60% soil and 40% sewage sludge); T3, (40% soil and 60% sewage sludge); T4, (20% soil and 80% sewage sludge); T5, (100% sewage sludge)

This shows that Cd concentration in the soil of treatment T5 had decreased by about 0.05 ppm. The control treatment gave the lowest Cd concentrations of 0.02 ppm for before planting and 0.01 ppm for after planting. This result also shows that the concentration of 5%. Consequently, the growth of plant was good in treatment T5.

The results of Cr determination in the soil and sewage sludge before and after planting showed there were significant differences ($P \leq 0.05$). The concentrations of Cr in soil and sewage sludge before and after planting ranges from 1.15 to 23.45 ppm and 1.12 to 24.30 ppm, respectively. Treatment T5 gave the highest Cd concentration with a value of 23.45 ppm for after planting and 24.30 ppm for before planting. The lowest concentration was given by the control treatment with respective values of 1.15 and 1.12 ppm for before and after planting. From the results, T1 gave a conspicuous decrease of about 24%. For Cr, the concentration is nearly same for before and after planting because Cr is not taken up by plants. Treatments T2, T4 and T5 showed an increased concentration after planting *O. stamineus*. It is shown that this species could not uptake this element in high concentration. On the other hand, the height, basal diameter and number of leaves increment were the lowest in treatment T5. Reduction of germination, roots, shoots and leaves growth for a particular species (Barton *et al.*, 2000).

The t-test analysis showed significant difference in Zn concentration before and after planting ($P \leq 0.05$). The lowest value was detected in the control treatment with a value of 10.50 ppm for before planting and 2.76 ppm for after planting, while the highest concentration was recorded in T5 with 290.05 ppm and 252.04 ppm before and after planting, respectively. Moreover, the results showed that the plant was able to uptake Zn needed for its growth. The increasing acidity of soils may also liberate the bound pool of Zn, leading initially to the increased concentration of free Zn ions in the soil solution. Several plant species, grasses and herbs included, are capable of tolerating Zn toxicity symptoms even at a relatively low Zn level (Pahlsson, 1989).

The results for Cu analysis showed that there were significant differences between before and after planting ($p \leq 0.05$). The concentrations of Cu in the soil and sewage sludge before and after planting ranges from 1.01 to 17.07 ppm and 0.26 to 18.56 ppm, respectively. Treatment T5 has the highest Cu concentration in the planting media with a value of 17.07 ppm before and 18.56 ppm after planting; in the control it was 1.01 ppm before and 0.26 ppm after the

planting period. The control, T1, T2 and T3 treatments showed reduction in Cu concentration because of plant uptake. However, T4 and T5 showed an increased Cu concentration where it increased from 0.84 ppm to 1.49 ppm due to plant uptake. The excess of Cu may be stored in the planting medium.

The concentration of Pb differed significantly before and after planting ($P \leq 0.05$). The highest concentration was detected in T5 with a value of 5.33 ppm before and 5.96 ppm after planting. The lowest concentrations were recorded in the control treatment with values of 1.04 ppm and 0.52 ppm before and after planting, respectively. It was found that sewage sludge has higher Pb content compared to the control and the Pb concentration increased with increasing percentage of sludge. Hence, Pb can be found easily in soils treated with sewage sludge.

Heavy metals concentration in *O. stamineus* plant parts:

Table 4-6 showed the concentration of heavy metals in *O. stamineus* plant parts. These tables show the accumulation pattern of Cd concentration in plant parts. Cadmium concentration increased in the order of leaves < stems < roots. T1 recorded the highest Cd concentration in the leaves and the lowest Cd concentration was in T4. For stems, Cd concentration was the highest in the control treatment. The concentration of Cd in the roots was found to be lower compared to that in the leaves and stems, with a value of 0.01 ppm. Cadmium is not an element needed for plant growth. The movement of Cd in plant is limited and it is known that Cd is relatively more toxic to plants than Pb.

In the case of Cr concentration in plant parts, the highest concentration was recorded in the leaves, while stems and roots showed lower concentration. For the control treatment, the highest concentration was in the leaves with a value of 8.48 ppm, while the lowest concentration was found in T5 with value of 0.52 ppm. For T1, Cr concentration was the highest in stems (1.15 ppm), while T5 gave the lowest concentration with a value of 0.08 ppm. In contrast, Cr concentration in roots was in the range of 0.11 ppm to 0.47 ppm. There are many factors that affect these results. One of the factors was that the heavy metal was leached into the groundwater. It also depends on the local environment and soil factor. Toxic effects of Cr on plant include alteration in the germination process and reducing the growth of roots, stems and leaves. Hence, exposure of plant to high level of Cr decreases its dry matter production and yield. Chromium also causes deleterious

Table 4: Concentration of heavy metals in *O. stamineus* leaves

Treatment	Concentration (ppm)				
	Zn	Pb	Cr	Cu	Cd
Control	21.25a	9.05a	8.48a	16.73a	0.01a
T1	38.94b	3.77c	2.09b	0.34c	0.57b
T2	34.17c	0.59d	0.92c	0.57c	0.02a
T3	34.26c	0.77d	1.14c	1.33b	0.03a
T4	32.80d	8.00b	0.92d	0.50c	0.01a
T5	38.96b	0.88d	0.52d	1.09b	0.03a

Note: Different letter within a column indicate significant differences between means following Duncan Multiple Range Test ($P \leq 0.05$); ns, no significant differences ($P \leq 0.05$); nd, not determined. Control, (100% soil); T1, (80% soil and 20% sewage sludge); T2, (60% soil and 40% sewage sludge); T3, (40% soil and 60% sewage sludge); T4, (20% soil and 80% sewage sludge); T5, (100% sewage sludge)

Table 5: Concentration of heavy metals in *O. stamineus* stem

Treatment	Concentration (ppm)				
	Zn	Pb	Cr	Cu	Cd
Control	31.03b	15.90b	0.48b	0.75a	0.17a
T1	41.21a	16.00a	1.15a	0.78a	0.15a
T2	43.93a	6.84c	0.39b	0.33b	0.17a
T3	9.03c	0.04d	0.13b	0.17c	0.01a
T4	13.51c	0.02d	0.09b	0.29b	0.01a
T5	8.26c	0.01d	0.08b	0.13c	0.01a

Note: Different letter within a column indicate significant differences between means following Duncan Multiple Range Test ($P \leq 0.05$); ns, no significant differences ($P \leq 0.05$); nd, not determined. Control, (100% soil); T1, (80% soil and 20% sewage sludge); T2, (60% soil and 40% sewage sludge); T3, (40% soil and 60% sewage sludge); T4, (20% soil and 80% sewage sludge); T5, (100% sewage sludge)

Table 6: Concentration of heavy metals in *O. stamineus* roots

Treatment	Concentration (ppm)				
	Zn	Pb	Cr	Cu	Cd
Control	4.99d	nd	0.11b	nd	0.01a
T1	14.95b	nd	0.14b	0.17b	0.01a
T2	17.33a	nd	0.10b	0.19b	0.01a
T3	9.63c	nd	0.08b	0.19b	0.01a
T4	nd	nd	0.47a	0.49a	0.01a
T5	16.51b	nd	0.18b	0.27a	0.01a

Note: Different letter within a column indicate significant differences between means following Duncan Multiple Range Test ($P \leq 0.05$); ns, no significant differences ($P \leq 0.05$); nd, not determined. Control, (100% soil); T1, (80% soil and 20% sewage sludge); T2, (60% soil and 40% sewage sludge); T3, (40% soil and 60% sewage sludge); T4, (20% soil and 80% sewage sludge); T5, (100% sewage sludge)

effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition.

Zinc uptake by plant increased in the order of leaves < stems < roots. In this study it was shown that T5 had the highest concentration of Zn in the leaves, while the control treatment gave the lowest concentration of 21.25 ppm. For stems, the highest concentration was recorded in the T2 with a value of 41.21 ppm, while T5 showed the lowest concentration of 8.26 ppm. However, the concentration in the roots of

T5 was the highest with a value of 16.51 ppm, while T4 gave the lowest concentration of Zn (0 ppm). Zinc concentration was highly concentrated in the leaves, while stems and roots contained low amount. From previous study, it was found that Zn toxicity in plants limits the growth of both root and shoot (Fontes and Cox, 1998). Zinc toxicity also causes chlorosis in the younger leaves, which can extend to older leaves after exposure to high soil Zn level (Ebbs and Kochian, 1997). Excess Zn can also give rise to manganese (Mn) and copper (Cu) deficiencies in plant shoots. Such deficiencies have been ascribed to hinder transfer of these micronutrients from roots to shoots. This hindrance is based on the fact that the Fe and Mn concentration in plants grown in Zn-rich media are greater in the roots than the shoots (Ebbs and Kochian, 1997).

Low amount of Cu was taken up by *O. stamineus* because Cu is a micronutrient. The accumulation of Cu was in the order of leaves < stems < roots. The control treatment recorded the highest Cu concentration in the leaves. On the other hand, Cu concentration was high in the control medium with a value of 16.73 ppm, while T1 recorded the lowest concentration in the leaves (0.34 ppm). Copper concentration in the stems ranges from 0.13 ppm to 0.78 ppm. The highest concentration was recorded in T1 (80% soil: 20% sewage sludge) and the lowest concentration was recorded in T5. The concentration of Cu in the roots was found to be lower compared to that in the leaves and stems, ranging 0.11 ppm to 0.49 ppm. Usually, visible symptoms of heavy metal toxicity, like that of Cu, are small chlorotic leaves and early leaf fall. As a result the growth of plant is stunted and initiation of roots and development of root laterals are poor. The reduction in root development may result in a lower water and nutrient uptake, leading to disturbances in the metabolism and growth retardations. A study carried out by Sánchez-Martin *et al.* (2004) on total heavy metal content in a soil treated with sludge showed a slight increase in Pb, Cu and Ni concentration in plant parts.

For the control treatment, the highest Pb concentration was in the leaves. We found that only a small amount of Pb was taken up by plant. Plant accumulated Pb in the order of leaves < stems < roots. Lead concentration was high in the control medium. For T1, the lowest concentration of Pb was in the leaves. The Pb concentration in the stems ranges from 0.01 ppm to 16.00 ppm. The highest concentration was recorded in T1, while the lowest concentration was recorded in T5.

Relationship between heavy metals concentration in the soil and in plant parts:

The concentration of Cd and Pb in the stem was correlated with those in the soil. Their concentration in the soil increased in the order of order of Cd < Pb < Cu < Cr < Zn. Their concentration was higher in the leaves than either stems or roots. The accumulation pattern in the leaves was in the order of Cd < Cr < Cu < Pb < Zn. Studies carried out by Kim *et al.* (2003) showed almost similar findings. They also confirmed that the phytoextraction coefficients increased in the order Pb < Cu < Zn < Cd. Therefore, more Pb and Zn are taken up by plant than that of Cd. These heavy metals are transported from roots to the different parts of the plant. Greger (2004) found that Cd and Zn were more mobile than Cu and Pb.

This study found that the concentration of the heavy metals in the soil was correlated with those in the stem. This is in contrast with the study of Baye *et al.* (2008) who found that there was no correlation between bioaccumulation Cd by medicinal plants and its concentration in soil. The presence of Cd in plant at high level may cause a decrease in photosynthesis and the uptake of water and nutrients (Mohanpuria *et al.*, 2007). However, the concentration of Cd normally encountered in the environment does not cause acute toxicity. Waldron (1980) showed that major hazard to human health from Cd is its chronic accumulation in the kidney cortex.

The Cr concentration was nearly same before and after planting and this could be due to non-essentiality of Cr by plant (Sánchez-Martin *et al.*, 2004; Shanker *et al.*, 2005). High level of Cr may cause serious environmental contamination in soils, sediments and groundwater (Shanker *et al.*, 2005). In this study no correlation was found between Cr in the soil and plant parts. This is agreement with the finding of Bowen (1979) who found that the concentration in leaves parts of plants showed little relationship with the overall the content of Cr in the soils.

Plant can uptake Cu which is a micronutrient (Thomas *et al.*, 1998). Our study found that Cu concentration in the soil was highest in T5. Excessive uptake of Cu may cause plant injury (Lewis *et al.*, 2001). No correlation was found between Cu in plant parts and that in soil.

Our results showed that soil Pb was highest in T4, while that for plant part it was T5. Sánchez-Martin *et al.* (2004) believed that Pb present in sludge did not give rise to its increase in plant unless the concentration was very high.

CONCLUSION

The *O. stamineus* was found to have the ability or potential to vanish the toxicity of heavy metals especially Zn in leaves (38.96 ppm). Besides that, *O. stamineus* absorbed heavy metals and stored it at various plant parts (leaves, stems and roots) in the range of 0.01 ppm to 0.57 ppm for Cd, 0.08 to 8.48 ppm for Cr, 0 ppm to 43.93 ppm for Zn, 0.11 to 16.73 ppm for Cu and 0.01 to 9.05 ppm for Pb. This plant species can be considered as a good phytoremediator to absorb heavy metals of contaminated soils, especially zinc. Further studies on the possibility of *O. stamineus* planted in soil containing with sewage sludge to be commercialized as food product needs to be done to evaluate the safety and hazardous to human health.

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