Phytoaccumulation of Zinc and Iron by *jatropha curcas* Grown in Used Lubricating Oil-Contaminated Soil

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ABSTRACT Contamination of soil by heavy metals from used lubricating oil constitutes major environmental problems due to its negative impacts on humans and environment. Plants that possessed bioaccumulation potential offered green remediation approach for the removal of such heavy metal contaminants. In this study, heavy metal accumulation potential of *Jatropha curcas* was studied in soil contaminated with 2.5% and 1% (w/w) used lubricating oil and enhanced with organic wastes [banana skin (BS), brewery spent grain (BSG) and spent mushroom compost (SMC)] for 180 days under room condition. At the end of 180 days, accumulation of Fe and Zn were recorded in *J. curcas* tissues (roots, stems and leaves) in all the treatments. 9.94 mg/kg to 26.34 mg/kg of Fe and 4.14 mg/kg to 8.45 mg/kg of Zn were recorded in the roots of *J. curcas* in soil contaminated with 2.5% oil. Organic wastes amendments enhanced the bioaccumulation of Fe and Zn with about 25% compared with control treatments without organic wastes amendment. The result of this study suggests that *J. curcas* has a good potential for bioaccumulation of Fe and Zn in contaminated soil.

Keywords: *Jatropha curcas,* bioaccumulation, organic waste, used lubricating oil

INTRODUCTION

Lubricating oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents [1]. It is used to lubricate the parts of an automobile engine, in order to keep everything running smoothly [2]. The waste-lubricating oil, otherwise called spent oil or used-lubricant, obtained after servicing and subsequent draining from automobile, generators and industrial machines is disposed off indiscriminately in many countries. For example, in United States about 500 million gallons of used oil are being disposed indiscriminately every year [3, 4]. This waste-oil usually contains appreciable amount of toxic hydrocarbons and heavy metals such as Va, Pb, Al, Ni, Fe, Cr and Zn [5].

Soil contamination by heavy metals (e.g. Fe, Pb and Zn) from anthropogenic source such as pollution from used lubricant is consequently the most critical environmental problems as it poses significant impacts to the human health as well as the ecosystems. The contaminants are able to infiltrate deep into the layer of underground waters and pollute the groundwater as well as the surface water. Heavy metals in the soil will subsequently enter the human food web through plants and they are a risk to the ecosystem as they tend to bioaccumulate and can be transferred from one food chain to another. Heavy metals are identified in various food chains where the results are usually detrimental to microorganisms, plants, animals and humans alike.

Remediation of petroleum-contaminated system could be achieved by either physicochemical or biological methods. However, the attendant negative consequences of the physicochemical approach are currently directing greater attention to the exploitation of the biological alternatives [6]. The remediation of metal contaminated sites often involves expensive and environmentally invasive and civil engineering based practices [7]. A range of technologies such as fixation, leaching, soil excavation, and landfill of the top contaminated soil *ex situ* have been used for the removal of metals. Many of these methods have high maintenance costs and may cause secondary pollution [8] or adverse effect on biological activities, soil structure, and fertility [9]. A promising approach is the phytoremediation technology, where living plants are used to remove trace metals from impacted sites [8]. Variety of pollutant attenuation mechanisms possessed by plants makes their use in remediating contaminated land and water more feasible than physical and chemical remediation [10, 11].

The objectives of this study are to explore the potential of *J. curcas* to accumulate Fe and Zn present in used lubricating oil-contaminated soil and to investigate the effects of different organic wastes amendments on the ability of *J. curcas* in removing Fe and Zn from the contaminated soil.

MATERIALS AND METHODS

Sample collection

The soil samples used for the study were collected from a nursery section in Sungai Buloh, Selangor, Malaysia. Used lubricating oil was collected from Perodua car service centre, Petaling Jaya, while the organic wastes were collected from different locations; banana skin (BS) was collected from IPS canteen, University of Malaya, brewery spent grains (BSG) was collected from Carlsberg brewery, Shah Alam, Selangor and spent mushroom compost (SMC) was collected from Ganofarm Sdn. Bhd, Tanjung Sepat, Selangor.

Physicochemical properties of soil, organic wastes and used lubricating oil

Nitrogen content of soil used for phytoremediation and organic wastes was determined using the Kjeldahl method; while phosphorus, iron and zinc contents were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES optima 4100 DV, Perkin Elmer, USA) after acid digestion in a microwave oven. The pH was determined with pH meter (HANNA HI 8424) on 1:2.5 (w/v) soil/distilled water after 30 min equilibration. Triplicate determinations were made.

Soil preparation

Four kilogram (4 kg) of sieved (2 mm) soil each was contaminated with 2.5% and 1% (w/w) of used lubricating oil and thoroughly mixed, 5% (w/w) of different organic wastes (BS, BSG and SMC) were also mixed separately with the oil contaminated soil. Plastic bags were filled with the soil-oil-organic waste mixture and allowed to stabilize for four days before transplanting the seedlings into the contaminated soil. Control treatment consisting of bags of the plant without used lubricating oil or organic wastes was also set up. All the treatments were set up in triplicate at room temperature (28 ± 2 ^{0}C) with 24 hours fluorescent lamps. The plants were moderately watered every 2 days with tap water to prevent leaching from the plastic bags. The appearance of the plants in response to the oil in soil was monitored to determine if there is phytotoxicity of the oil to the plants. The design of the experiment is shown in Table 1.

At the completion of the experiment (180 days), the plants were uprooted. The soil samples were collected from the rhizosphere of each plant and analyzed for residual heavy metals (Fe, Zn) contents in the soil and the plant tissues (root, stem and leaves). The roots were rinsed thoroughly with tap water and the plant dry matter was determined after drying at 60 ^{0}C for 48 hours. The residual soil and the plant tissues were digested with mixture of concentrated acids in a microwave oven, followed by determination of Zn and Fe contents using inductively coupled plasma-optical emission spectroscopy (ICP-OES optima 4100 DV, Perkin Elmer, USA).

Rate of metal uptake by *J. curcas*

The uptake rate of Fe and Zn by *J. curcas* was determined using first order kinetic model of Kamath et al., [12]

$k = -1/t\ (\ln M/M_o)$

Where:

k = first order rate constant for metal uptake per month

t = time in month

M = mass of residual metal in the soil (mg/kg)

M_o = initial mass of metal in the soil (mg/kg)

Statistical analysis of the data was carried out using one – way ANOVA with SPSS Statistics version 17.0.

RESULTS AND DISCUSSION

Physicochemical properties of soil, organic wastes and lubricating oil used for phytoremediation

The physicochemical properties of soil, used lubricating oil and organic wastes used for phytoremediation are shown in Table 2. The soil had low nitrogen content (0.6%). Phosphorus content of the soil was 32.1 mg kg^{-1}. Of the organic wastes used, BSG had higher amount of nitrogen (1.02%) compared to BS (0.4%) and SMC (0.5%). Fe and Zn are the major metals detected in the lubricating oil used for the study; these metals have been reported by different authors in used lubricating oil [4, 5, 13, 14].

Concentration of Zn in the oil was more than that of Fe. However, the soil used for the phytoremediation

Table 1: Experimental Design

Treatment	Details of Treatment
A	4 kg soil + 2.5% oil + 5% BS + *Jatropha* plant
B	4 kg soil + 2.5% oil + 5% BSG + *Jatropha* plant
C	4 kg soil + 2.5% oil + 5% SMC + *Jatropha* plant
D	4 kg soil + 2.5% oil + *Jatropha*
E	4 kg soil + 2.5% oil only
F	4 kg soil + 1% oil + 5% BS + *Jatropha* plant
G	4 kg soil + 1% oil + 5% BSG + *Jatropha* plant
H	4 kg soil + 1% oil + 5% SMC + *Jatropha* plant
I	4 kg soil + 1% oil + *Jatropha* plant
J	4 kg soil + 1% oil only

Table 2: Physicochemical properties of soil, used lubricating oil and organic wastes used for phytoremediation

Substrate	(mg/Kg)			N (%)	pH
	Fe	Zn	P		
Used oil*	10.29	86.05	-	-	-
Soil (unpolluted)	76.34	0.02	32.1	0.6	6.8
BSG	-	-	20.6	1.0	6.7
BS	-	-	21.2	0.4	7.0
SMC	-	-	22.5	0.5	5.6
Soil+1% oil	77.02	32.15	33.6	0.4	6.4
Soil+2.5% oil	79.43	38.32	35.3	0.5	6.6

BSG: Brewery spent grain; BS: Banana skin; SMC: Spent mushroom compost
*: mg/L

contained 76.34 mg/kg of Fe compared to 10.3 mg/L present in the used lubricating oil. It also contained 0.02 mg/kg of Zn compared to 86.05 mg/L of Zn present in the used lubricating oil. This is an indication that accumulation of Fe in the plant parts might be from the Fe present in the soil used for the study while any accumulation of Zn may likely come from those present in the used lubricating oil utilized for the phytoremediation study.

Uptake of Heavy metals by *Jatropha curcas*

Portion of the Jatropha roots, stems and leaves from different soil treatments were dried at 60 ^{0}C for 48 hours, ground and 0.5 g digested with mixture of acids were analyzed with ICP-OES to determine if there was accumulation of metals from the oil and soil. The results revealed appreciable accumulation of Fe and Zn in the roots, stems and leaves of Jatropha. Fe and Zn were detected to have accumulated in the root of Jatropha after 180 days of study. Fe accumulation in the root of the Jatropha plant in soil contaminated with 2.5% and 1% used lubricating oil ranged from 9.94 mg/kg to 26.34 mg/kg and 14.61 mg/kg to 23.40 mg/kg respectively in different treatment.

Zn accumulated in the root of the plant ranged from 4.14 mg/kg to 8.45 mg/kg and 6.24 mg/kg to 7.83 mg/kg in soil contaminated with 2.5% and 1% used lubricating oil, respectively as shown in Table 3. The result is in contrast with the findings of Palmroth et al., [15], who reported that there was no accumulation of heavy metals in the plant tissue in soil contaminated with weathered hydrocarbons and heavy metals amended with NPK and biowaste compost. The differences observed in the results might be due to different contaminated soil used. In this study; fresh hydrocarbon-contaminated soil was used while in the study of Palmroth et al., [15] weathered hydrocarbon-contaminated soil was used. The differences can also be attributed to differences in the plants used for the studies, while in this study *J. curcas* was used; they used pine and poplar for their studies.

Translocation of metals (Fe & Zn) from the root of Jatropha plant to the stem and leaves was recorded in all treatments with used lubricating oil. The quantity of Fe detected in the stem of Jatropha plant ranged between 2.46 mg/kg and 9.74 mg/kg in soil treated with 2.5% oil and between 2.46 mg/kg and 6.12 mg/kg in soil treated with 1% oil (Table 4). Accumulation of Fe in the leaves was very minimal with the highest quantity been 3.06 mg/kg in the soil treated with 1% used lubricating oil and amended with BSG (Table 5). Bioaccumulation of Zn in the stem and leaves of Jatropha plants varies greatly based on different organic wastes amendment. Soil contaminated with 1% used lubricating oil and amended with BSG recorded highest accumulation of Zn (3.10 mg/kg) in the stem of Jatropha (Table 4). Highest accumulation of Zn (1.34 mg/kg) in leaves of Jatropha was recorded in 2.5% oil contamination amended with BSG (Table 5). From the results of the bioaccumulation of Fe and Zn in Jatropha tissues describe above, it shows clearly the ability of *J. curcas* to accumulate heavy metals (Fe, Zn) in the different plant tissues. Accumulation of these metals was more pronounced in the Jatropha amended with organic wastes compared to the treatment with only Jatropha without organic wastes amendments. The organic wastes might have contributed positively to the ability of the plant and to the bioavailability of these metals in the polluted soil thereby enhancing the capacity of the plant to uptake the metals into the different plant parts.

Table 3: Heavy metal content in roots of *J. curcas* in soil contaminated with 2.5% and 1% used lubricating oil

	Heavy metals (mg/Kg)			
	2.5% oil		1% oil	
Treatment	Fe	Zn	Fe	Zn
Soil + 2.5% oil + BS + Jatropha	12.1	8.45	21.10	6.24
Soil + 2.5% oil + BSG + Jatropha	19.47	6.21	23.40	7.83
Soil + 2.5% oil + SMC + Jatropha	26.34	4.14	19.57	6.31
Soil + 2.5% oil + Jatropha	9.94	6.43	21.28	7.02
Soil without oil + Jatropha	14.61	ND	14.61	ND

ND: Not detected

Table 4: Heavy metal content in stems of *J. curcas* in soil contaminated with 2.5% and 1% used lubricating oil

	Heavy metals (mg/Kg)			
	2.5% oil		1% oil	
Treatment	Fe	Zn	Fe	Zn
Soil + 2.5% oil + BS + Jatropha	8.15	3.04	5.35	2.83
Soil + 2.5% oil + BSG + Jatropha	9.74	2.45	6.12	3.10
Soil + 2.5% oil + SMC + Jatropha	4.18	2.63	3.83	1.83
Soil + 2.5% oil + Jatropha	3.04	1.81	3.16	1.02
Soil without oil + Jatropha	2.46	ND	14.61	ND

ND: Not detected

Table 5: Heavy metal content in leaves of *J. curcas* in soil contaminated with 2.5% and 1% used lubricating oil

	Heavy metals (mg/Kg)			
	2.5% oil		1% oil	
Treatment	Fe	Zn	Fe	Zn
Soil + 2.5% oil + BS + Jatropha	1.13	0.18	1.91	0.36
Soil + 2.5% oil + BSG + Jatropha	2.92	1.34	3.06	0.74
Soil + 2.5% oil + SMC + Jatropha	1.43	0.24	2.10	0.31
Soil + 2.5% oil + Jatropha	1.75	0.45	1.93	0.28
Soil without oil + Jatropha	0.62	ND	0.62	ND

ND: Not detected

These results are similar to the results of Santosh et al., [16] who reported appreciable accumulation of Zn, Cr and As in the root stem and leaves of *J. curcas* in soil contaminated with different concentrations of Zn, Cr and As and amended with dairy sludge and bio-fertilizer. However, it contradicts the findings of Tordoff et al., [17] and Walker et al., [18] who reported that organic amendments of soil contaminated with metals always decreased the bioavailability of the metal in the soil. The differences in the results might be because the metals in the contaminated soil in this study are from used lubricating oil whereas the metals in the findings of Tordoff et al., [17] came from direct contamination of soil with the salts of these metals. Jatropha root accumulated higher percentage of Zn and Fe than other parts of the plant; this implies that Jatropha root can be an important sink for bioavailable Zn and Fe. A correlation between Fe and Zn concentrations in the roots, stems and leaves was found in this study. This suggests that possibly Fe and Zn were co-transported in Jatropha plant and thus share the same transport mechanisms.

Rate of metal uptake by *Jatropha curcas*

The uptake rate of each metal (Fe and Zn) per month by *J. curcas* was determined using first order kinetic model. The results in Table 6 revealed that rate of uptake of Fe and Zn by Jatropha within the period of six month study was higher in soil contaminated by 1% used lubricating oil than those of soil contaminated by 2.5% oil. However, soil amended with organic wastes recorded higher rate of Fe and Zn uptake in all the treatments compared to unamended soil. The reason for higher metal uptake in soil contaminated with 1% oil might be due to the fact that plants in the soil polluted with 1% oil did not experience much stress due to low level of oil contamination compared to those in 2.5% oil pollution, hence they are able to grow better and uptake the metal at higher rate than those in 2.5% oil pollution. The results in Table 6 also point to the fact that soil amended with BSG recorded higher rate of Fe and Zn uptakes in both level (2.5% and 1%) of oil pollution. The reason for this higher uptake rate shown by this treatment can be attributed to the rate of plant growth in this treatment which was much taller and better than plants in other treatments.

CONCLUSION

The results of this study demonstrated the potential of *J. curcas* together with organic wastes amendments to bioaccumulate heavy metals (Fe & Zn) present in the used lubricating oil and soil used for the phytoremediation studies. The root of *J. curcas* accumulated substantial (26.34 mg/kg and 8.4 mg/kg)

Table 6: Rate of uptake of Fe and Zn by *J. curcas* studied under laboratory condition

	Rate of uptake (month^{-1})	
Treatment	Fe	Zn
Soil + 1% oil + BS + Jatropha	0.046	0.031
Soil + 1% oil + BSG + Jatropha	0.058	0.034
Soil + 1% oil + SMC + Jatropha	0.048	0.026
Soil + 1% oil + Jatropha	0.039	0.022
Soil + 2.5% oil + BS + Jatropha	0.035	0.022
Soil + 2.5% oil + BSG + Jatropha	0.042	0.033
Soil + 2.5% oil + SMC + Jatropha	0.037	0.021
Soil + 2.5% oil + Jatropha	0.029	0.019
Soil without oil + Jatropha	0.034	0.000

quantities of Fe and Zn, respectively and these metals were also detected at the above ground portion of the Jatropha plant. The use of *J. curcas* (an economically viable plant) will therefore serve as an alternative method in removing heavy metal contaminants from soil.

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