

Phytotreatment of soil contaminated with used lubricating oil using *Hibiscus cannabinus*

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Received: 1 October 2010 / Accepted: 9 August 2011 / Published online: 26 August 2011
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Abstract Soil contamination by hydrocarbons, especially by used lubricating oil, is a growing problem in developing countries, which poses a serious threat to the environment. Phytoremediation of these contaminated soils offers environmental friendly and a cost effective method for their remediation. *Hibiscus cannabinus* was studied for the remediation of soil contaminated with 2.5 and 1% used lubricating oil and treated with organic wastes [banana skin (BS), brewery spent grain (BSG) and spent mushroom compost (SMC)] for a period of 90 days under natural conditions. Loss of 86.4 and 91.8% used lubricating oil was recorded in soil contaminated with 2.5 and 1% oil and treated with organic wastes respectively at the end of 90 days. However, 52.5 and 58.9% oil loss was recorded in unamended soil contaminated with 2.5 and 1% oil, respectively. The plant did not accumulate hydrocarbon from the soil but shows appreciable accumulation

of Fe and Zn in the root and stem of *H. cannabinus* at the end of the experiment. The first order kinetic rate of uptake of Fe and Zn in *H. cannabinus* was higher in organic wastes amendment treatments compared to the unamended treatments, which are extremely low. The results of this study suggest that *H. cannabinus* has a high potential for remediation of hydrocarbon and heavy metal contaminated soil.

Keywords *Hibiscus cannabinus* · Used lubricating oil · Organic wastes · Hydrocarbons · Bioaccumulation

Introduction

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 (Anoliefo and Vwioko 1995; Adesodun and Mbagwu 2008). This waste-oil usually contains appreciable amount of toxic hydrocarbons and heavy metals such as Va, Pb, Al, Ni, Fe, Cr and Zn (Whisman et al. 1974). Environmental pollution with petroleum and petrochemical products has attracted much attention in recent decades. The presence of various kinds of automobiles and machinery vehicles has caused an increase in the use of motor oil. Spillage of used motor oils such as diesel or jet fuel contaminate our natural environment with hydrocarbon

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(Husaini et al. 2008). The hydrocarbons spread horizontally on the ground-water surface and partition into groundwater, soil pore, space air and to the surfaces of soil particles (Plohl et al. 2002).

Remediation of petroleum-contaminated system could be achieved by physical, chemical or biological methods. However, the attendant negative consequences of the physical and chemical approaches are currently directing greater attention to the exploitation of the biological alternatives (Okoh and Trejo-Hernandez 2006). Variety of pollutant attenuation mechanisms possessed by plants makes their use in remediating contaminated land and water more feasible than physical and chemical remediation (Greenberg 2006; Gerhardt et al. 2009). An estimated 350 species of plants naturally takes up toxic materials from the environment (Thieman and Palladino 2009). Phytoremediation of contaminated soils offers an environmentally friendly, cost effective, and carbon neutral approach for the cleanup of toxic pollutants in the environment (Dowling and Doty 2009). Phytoremediation appears attractive because in contrast to most other remediation technologies, it is not invasive and, in principle, delivers intact, biologically active soil (Wenzel 2009). The most common plant species used in phytoremediation of organic compounds includes willows, poplar and different types of grasses. Study by Mun et al. (2008) shows *H. cannabinus* as a potential plant for remediation of heavy metals-contaminated soil.

The choice of *H. cannabinus* for this study was because it is a non-edible plant and due to its commercial viability as a raw material for paper production. The objectives of the study is to assess the potential of *H. cannabinus* in removing hydrocarbons and heavy metals in soil contaminated with used lubricating oil and to investigate the effects of different organic wastes amendments on the ability of *H. cannabinus* in removing hydrocarbons from the contaminated soil.

Materials and methods

Sample collection

The soil with no history of hydrocarbon contamination used for the phytoremediation studies was collected from the Nursery section of Asian-

European Institute, University of Malaya. The *H. cannabinus* seeds were purchased from National Tobacco Board of Malaysia, Kelantan, Malaysia. Used lubricating oil was collected from Perodua car service centre, Petaling Jaya, while 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 Gano mushroom farm, 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 kilograms (4 kg) of sieved (2 mm) soil each, 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 (12 cm × 12 cm) were filled with the soil-oil-organic waste mixture and allowed to stabilize for 4 days before planting the seeds of *H. cannabinus* into the contaminated soil. Control treatment consisting of bags of the plant without used lubricating oil or organic wastes was also set up. Additional control treatment comprising of autoclaved soil containing 0.5% (w/w) NaN_3 was also set up to determine non-biological loss of waste lubricating oil from the soil. All the treatments were set up in triplicate at the experimental site exposed to sunlight and rainfall for a period of 90 days. 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 as shown in Table 1.

Table 1 Experimental design

Treatment	Details of treatment
A	4 kg Soil + 2.5% oil + 5% BS + Hibiscus plant
B	4 kg Soil + 2.5% oil + 5% BSG + Hibiscus plant
C	4 kg Soil + 2.5% oil + 5% SMC + Hibiscus plant
D	4 kg Soil + 2.5% oil + Hibiscus
E	4 kg Soil + 2.5% oil only
F	4 kg Autoclaved soil + 2.5% oil + 0.5% NaN ₃
G	4 kg Soil + 1% oil + 5% BS + Hibiscus plant
H	4 kg Soil + 1% oil + 5% BSG + Hibiscus plant
I	4 kg Soil + 1% oil + 5% SMC + Hibiscus plant
J	4 kg Soil + 1% oil + Hibiscus plant
K	4 kg Soil + 1% oil only
L	4 kg Autoclaved soil + 1% oil + 0.5% NaN ₃

Sampling and analysis

Soil samples (triplicate) were taken within the rhizosphere zone of the plant from each plastic bag every 30 days for analysis of total petroleum hydrocarbon (TPH) and hydrocarbon utilizing bacterial (HUB) counts. At the completion of the experiment (90 days), the plants were uprooted. The soil samples (triplicate) were collected from the rhizosphere of each plant and analyzed for residual TPH, heavy metals (Fe, Zn) contents and hydrocarbon utilizing bacterial counts. The root was rinsed thoroughly with tap water and the plant dry matter was determined after drying at 60°C for 48 h. The root tissue was extracted with mixture of hexane and dichloromethane in a Soxhlet extractor for 12 h to determine if the roots absorb the hydrocarbon from soil. The extracts were analyzed for hydrocarbons using gas chromatography with a mass-selective detector (GC/MSD) HP-6890 in scan mode. The GC was equipped with cross-linked 5% phenyl methyl siloxane capillary column; HP-5MS. Helium was used as carrier gas. The temperature program was started at 40°C and raised by 10°C/min until 300°C, which was maintained for 8 min (Palmroth et al. 2002). Another portion of the Hibiscus roots, stems and leaves from different soil treatments were dried at 80°C for 2 days, grind and digested with mixture of acids. They were analyzed with ICP-OES to determine if there was accumulation of metals from the oil and soil.

HUB counts in the soil was determined by plating a serially diluted 1 g of the soil on oil agar (OA) [1.8 g K₂HPO₄, 4.0 g NH₄Cl, 0.2 g MgSO₄·7H₂O, 1.2 g KH₂PO₄, 0.01 g FeSO₄·7H₂O, 0.1 g NaCl, 20 g agar, 1% (v/v) used lubricating oil in 1000 mL distilled water, pH 7.4 (Zajic and Supplission 1972)], and incubated at 30°C for 72 h. The colonies on each plate were counted and recorded as colony forming unit per gram of soil (CFU/g). The pure culture of the bacterial isolates were identified by Gram staining technique and API 20NE for Gram negative bacteria and BBL Crystal rapid identification kit for Gram positive bacteria. The total extent of used lubricating oil biodegradation in soil were determined by suspending 10 g of soil (dried with 10 g anhydrous sodium sulphate) in 20 ml of dichloromethane in a 250 ml capacity Erlenmeyer flask. After shaking for 1 h on an orbital shaker (Model N-Biotek-101), the solvent-oil mixture was filtered using Whatman number 4 filter paper into a beaker of known weight and the solvent was completely evaporated in a rotary evaporator. The new weight of the beaker (now containing residual oil) was recorded. Percentage biodegradation of used oil was calculated using the formula of Ijah and Ukpe (1992).

% biodegradation

$$= \frac{\text{weight of oil (control)} - \text{weight of oil (degraded)}}{\text{weight of oil (control)}} \times \frac{100}{1}$$

Rate of metal uptake by *Hibiscus cannabinus*

The rate of uptake of heavy metals (Fe and Zn) by *H. cannabinus* was determined by using first order kinetic model to calculate the uptake rate of each metal per month by the plants, as follows:

$$k = -1/t (\ln M/M_0)$$

where k is the first order rate constant for metal uptake per month, t is the time in month, M is the mass of residual metal in the soil (mg/kg), M₀ is the initial mass of metal in the soil (mg/kg).

Bioconcentration factor (BCF) and Translocation factor (TF) of Zinc and Iron by the *H. cannabinus* was calculated using the formula of Yadav et al. (2009).

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Average metal concentration in the whole plant tissue (mg kg}^{-1}\text{)}}{\text{Metal in the soil (mg kg}^{-1}\text{)}}$$

$$\text{Translocation factor (TF)} = \frac{\text{Concentration}_{\text{aerial}}}{\times 1/\text{Concentration}_{\text{root}}}$$

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 as shown in Table 2 revealed that 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%).

Loss of used lubricating oil in soil

The percentage biodegradation of used lubricating oil in soil contaminated with 2.5 and 1% oil are shown in Figs. 1 and 2. Percentage biodegradation at the end of 90 days in soil contaminated with 2.5 and 1% oil ranged from 2.9 to 86.4% and 6.5 to 91.8% in all the

treatments, respectively. Contaminated soil treated with BSG as a source of nutrient for Hibiscus recorded the highest loss of oil (86.4 and 91.8%) in 2.5 and 1% contaminated soil respectively; while soil treated with SMC only shows 66.1 and 67.1% oil loss in 2.5 and 1% oil contaminated soil at the end of 90 days respectively. However, the contaminated soil containing only Hibiscus plant without organic waste amendment recorded 52 and 58% oil biodegradation, while control soil without Hibiscus plant recorded 39.8 and 41.3% oil loss in 2.5 and 1% pollution at the end of 90 days. 11.1 and 14.1% oil loss in soil contaminated with 2.5 and 1% oil may be due to non-biological factors like evaporation, photo degradation etc., which was recorded in autoclaved soil treated with sodium azide after 90 days. The percentage of oil biodegradation in all the treatments amended with organic wastes were not significantly different at $P < 0.05$ significant level, but significant difference was recorded between the treatment amended with organic wastes and those without organic wastes, thus establishing the fact that organic wastes positively contributed to the degradation of the oil from the soil. This was evident in our previous studies where 10% organic waste (BSG) alone positively enhanced the biodegradation of used lubricating oil (95%) in soil contaminated with 10% used lubricating oil (Abioye et al. 2010).

High percentage loss of oil in soil treated with organic wastes (BSG, BS, and SMC) and Hibiscus

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

Substrate	Fe (mg/kg)	Zn (mg/kg)	P (mg/kg)	N (%)	pH
Used oil (mg/l)	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

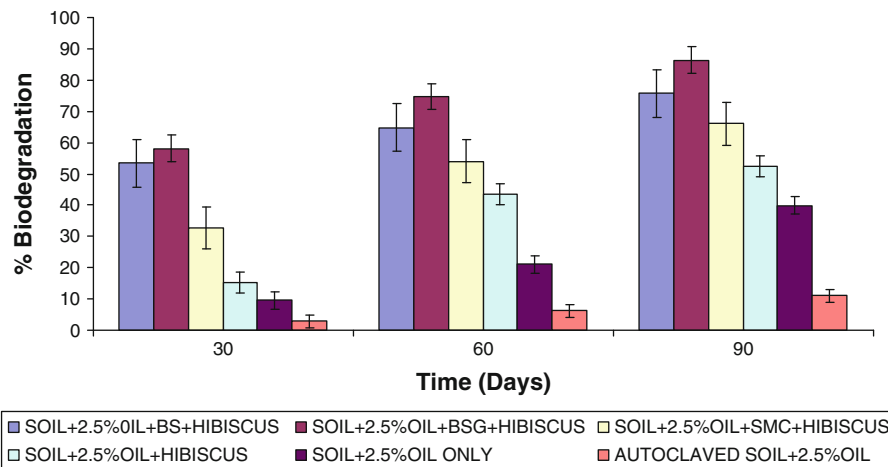


Fig. 1 Percentage biodegradation of used lubricating oil in soil contaminated with 2.5% oil. Vertical bars indicate standard deviation ($n = 3$)

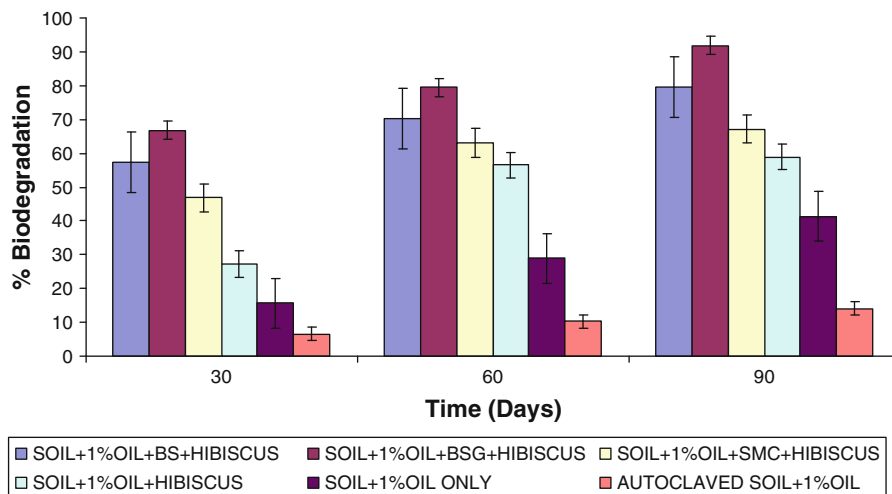


Fig. 2 Percentage biodegradation of used lubricating oil in soil contaminated with 1% oil. Vertical bars indicate standard deviation ($n = 3$)

might be due to the presence of appreciable quantities of nutrients (N & P) in the organic wastes, which possibly enhanced the growth of bacteria present at the rhizosphere of the plants. It may also be due to the fact that addition of organic wastes to the contaminated soil before planting of Hibiscus helps to loosen the compactness of the soil making sufficient aeration available for the indigenous bacteria present in the soil, thereby enhancing their biodegradative activities of the oil from the soil. The results are similar to the results obtained in our earlier studies with *Jatropha curcas* in which contaminated soil treated with BSG and *Jatropha* recorded 96.6% oil biodegradation after

180 days (Agamuthu et al. 2010). The result is also in agreement with the findings of Palmroth et al. (2002), who recorded 60% loss of diesel fuel in 30 days in diesel-contaminated soil planted with pine tree and amended with NPK fertilizer. The results revealed that addition of organic wastes into the contaminated soil planted with Hibiscus rapidly enhanced both the growth of *H. cannabinus* and biodegradation of oil in the soil. This is in agreement with the findings of Vouillamoz and Milke (2009) who observed that compost addition combined with phytoremediation increases the rate of removal of diesel fuel in soil.

Uptake of oil and metals by *H. cannabinus*

The GC/MS results of *H. cannabinus* root extract did not show any accumulation of hydrocarbon. This is in sharp contrast with the results of Palmroth et al. (2002), who observed uptake of diesel oil by grass roots, but agrees with the findings of Chaineau et al. (1997), who did not record uptake of hydrocarbons by maize root. The difference in the results of this study and that of Palmroth might be due to different types of oil used, lubricating oil is known to contain heavy range of hydrocarbons compared to diesel oil; this heavy ranged hydrocarbon in used lubricating oil might pose some challenges for uptake by the plant used. The result suggests that the mechanisms of hydrocarbon removal by *H. cannabinus* might be through rhizodegradation i.e. by the activities of microorganisms present at the rhizosphere of the plant whose activities might have been enhanced by the root exudates produced by *H. cannabinus*.

The ICP-OES results of digested plant tissues revealed appreciable accumulation of Fe and Zn in

the root and stem of *Hibiscus*, while no metal accumulation was detected in the leaves of all the treatments. Fe accumulation in the root of the plant in soil contaminated with 2.5 and 1% used lubricating oil ranged from 12.58 to 47.02 mg/kg and 10.58 to 38.37 mg/kg respectively; while Fe accumulation in the stem of *H. cannabinus* ranged from 1.26 to 2.37 mg/kg and 1.16 to 1.46 mg/kg in 2.5 and 1% oil pollution (Tables 3, 4). The results revealed the ability of *H. cannabinus* to accumulate Fe in the root and translocate this metal into the stem of the plant. Accumulation of Zn in the root of *H. cannabinus* ranges from 0.32 to 1.48 mg/kg in soil treated with 2.5% oil and from 0.35 to 0.91 mg/kg in soil treated with 1% oil. Zn concentration in the stem of *H. cannabinus* was higher than the accumulation in the root; it ranged from 0.32 to 1.64 mg/kg in soil contaminated with 2.5% oil and from 0.27 to 1.43 mg/kg in soil treated with 1% oil (Tables 3, 4).

The results of metal accumulation is similar to the study conducted by Hassinen et al. (2009), who reported accumulation of Zn and Fe in the root and

Table 3 Heavy metal concentrations in root of *Hibiscus cannabinus* in soil contaminated with 2.5 and 1% oil

Treatment	Heavy metals (mg/kg)			
	2.5% oil		1% oil	
	Fe	Zn	Fe	Zn
Soil + 2.5% oil + BS + Hibiscus	47.02	1.00	22.67	0.35
Soil + 2.5% oil + BSG + Hibiscus	12.58	1.48	10.58	0.91
Soil + 2.5% oil + SMC + Hibiscus	13.20	0.97	15.17	0.89
Soil + 2.5% oil + Hibiscus	16.01	0.32	38.37	0.37
Soil without oil + Hibiscus	29.87	ND	29.87	ND

ND not detected

Table 4 Heavy metal concentrations in stem of *Hibiscus cannabinus* in soil contaminated with 2.5 and 1% oil

Treatment	Heavy metals (mg/kg)			
	2.5% oil		1% oil	
	Fe	Zn	Fe	Zn
Soil + 1% oil + BS + Hibiscus	1.33	0.47	1.45	0.47
Soil + 2.5% oil + BSG + Hibiscus	1.63	1.64	1.16	1.43
Soil + 2.5% oil + SMC + Hibiscus	2.37	0.32	1.32	0.27
Soil + 2.5% oil + Hibiscus	1.45	0.53	1.46	0.37
Soil without oil + Hibiscus	1.26	ND	1.26	ND

ND not detected

shoot of hybrid aspen in the first year of planting on a metal contaminated site. Addition of organic wastes to the contaminated soil in addition to planting of *H. cannabinus* also promoted better biomass yield as well as better accumulation of Zn and Fe, this might be due to nutrients in the organic wastes, which enhanced the growth of *H. cannabinus* with lots of fibrous roots. The results is in line with the findings of Mun et al. (2008), who reported higher bioaccumulation of Pb in the root and stem of *H. cannabinus*, in their studies, the authors discovered higher accumulation of Pb in the root and stem when fertilizer was added to one of the treatment. However, the results was in contrast to that of Yadav et al. (2009), who reported that application of dairy sludge to soil contaminated with metal and metalloid significantly reduced the uptake of As, Cr and Zn by *Jatropha curcas*. The differences in the two results might be due to different plant and organic wastes used for the studies or it may be due to different environmental factors.

Rate of metal uptake by *H. cannabinus*

Table 5 shows the rate of uptake of Fe and Zn by *H. cannabinus* within the period of study (3 months). The rate of uptake of Fe and Zn in all the treatments ranged between 0.018 to 0.108 and 0.039 to 0.109 month⁻¹ respectively. The rate of uptake of Fe and Zn within 3 months of study was relatively high. The reason for this higher rate of metal uptake might be due to the plant physiological systems

which encourages high rate of uptake of metals and also may be because *H. cannabinus* grows faster and taller, attaining the height of 140 cm within a 3 month period. Higher rate of metal uptake was also recorded in all the treatments amended with organic wastes. This may be due to the potential of the organic wastes in enhancing the growth of *H. cannabinus*, which also directly promotes the rate of uptake of Fe and Zn in the amended treatments compared to the unamended treatments.

Bioconcentration and translocation factors of metals in *H. cannabinus*

Table 6 shows the BCF and TF of Zn in the *H. cannabinus*. Highest BCF (0.0814) was recorded in soil polluted with 2.5% oil and amended with BSG, while highest TF in stem was recorded in soil treated with 2.5% oil without organic waste amendment, the result agrees with the findings of Yadav et al. (2009), who reported high TF in soil without organic wastes amendments. Table 7 shows the BCF and TF of Fe in the *H. cannabinus* plant. Highest BCF (0.6588) was recorded in soil polluted with 1% oil without organic waste amendment; while highest TF (0.1795) in stem was recorded in soil treated with 2.5% oil and *H. cannabinus* plant amended with SMC. The TF of Zn recorded in this study was lower than those reported by Adesodun et al. (2010), who recorded TF factor of Zn greater than 1 in soil contaminated with Zn and remediated with sunflower. The differences might be due to different plants used for the studies. There was no significant

Table 5 Rate of uptake of Fe and Zn by *Hibiscus cannabinus*

Treatment	Rate of uptake (month ⁻¹)	
	Fe	Zn
Soil + 1% oil + BS + Hibiscus	0.089	0.081
Soil + 1% oil + BSG + Hibiscus	0.072	0.109
Soil + 1% oil + SMC + Hibiscus	0.055	0.077
Soil + 1% oil + Hibiscus	0.018	0.050
Soil + 2.5% oil + BS + Hibiscus	0.108	0.092
Soil + 2.5% oil + BSG + Hibiscus	0.097	0.101
Soil + 2.5% oil + SMC + Hibiscus	0.084	0.096
Soil + 2.5% oil + Hibiscus	0.045	0.039
Soil without oil + Hibiscus	0.075	0.000

Table 6 Bioconcentration factor (BCF) and translocation factor (TF) of Zn in Hibiscus remediated soil

Treatment	Zinc (Zn)	
	BCF	TF (in stem)
Soil + 2.5% oil + BS + Hibiscus	0.0634	1.4300
Soil + 2.5% oil + BSG + Hibiscus	0.0814	1.1081
Soil + 2.5% oil + SMC + Hibiscus	0.0336	0.3278
Soil + 2.5% oil + Hibiscus	0.0222	1.6563
Soil + 1% oil + BS + Hibiscus	0.0256	1.3352
Soil + 1% oil + BSG + Hibiscus	0.0727	1.5766
Soil + 1% oil + SMC + Hibiscus	0.0361	0.3034
Soil + 1% oil + Hibiscus	0.0229	1.0109
Soil without oil + Hibiscus	0.0000	0.0000

Table 7 Bioconcentration factor (BCF) and translocation factor (TF) of Fe in Hibiscus remediated soil

Treatment	Iron (Fe)	
	BCF	TF (in stem)
Soil + 2.5% oil + BS + Hibiscus	0.6087	0.0283
Soil + 2.5% oil + BSG + Hibiscus	0.1789	0.1296
Soil + 2.5% oil + SMC + Hibiscus	0.1960	0.1795
Soil + 2.5% oil + Hibiscus	0.2198	0.0906
Soil + 1% oil + BS + Hibiscus	0.3994	0.0653
Soil + 1% oil + BSG + Hibiscus	0.1942	0.1096
Soil + 1% oil + SMC + Hibiscus	0.2727	0.0870
Soil + 1% oil + SMC + Hibiscus	0.6588	0.0381
Soil without oil + Hibiscus	0.3919	0.0422

difference between the BCF of Hibiscus remediated soil amended with organic wastes and those without organic wastes amendments. The reason for this might be that the added organic wastes enables the Hibiscus plants in soil amended with organic wastes to stabilize some of the metals through phytostabilization mechanism; hence the bioaccumulation of metals were minimal in the plant tissues.

Bacterial counts

Four different hydrocarbon-utilizing bacteria (HUB) were identified (*Pseudomonas aeruginosa*, *Bacillus*

sp., *Micrococcus* and *Acinetobacter* sp.) from the different treatment. These bacterial species might possibly contribute to the degradation of used lubricating oil at the rhizosphere region of the plant due to their increased number in this plant region. The counts of HUB in 2.5% and 1% contaminated soil are shown in Figs. 3 and 4. Soil treated with BSG and *H. cannabinus* recorded high counts of HUB (93 and 84×10^5 CFU/g) in both soil contaminated with 2.5 and 1% oil respectively at the end of 90 days, this is similar to our previous study where we reported that BSG enhanced the multiplication of HUB better than BS and SMC Abioye et al. (2010). However, treatment with *H. cannabinus* alone recorded low counts of HUB compared with those amended with organic wastes (43 and 38×10^5 CFU/g) in 2.5 and 1% oil pollution. This is similar to the results of our initial findings with *J. curcas* Agamuthu et al. (2010).

Conclusion

The results of this study demonstrate the potential of *H. cannabinus* together with organic wastes amendments to remediate hydrocarbons contaminated soil. Though no accumulation of hydrocarbon was detected in the Hibiscus tissues, however there was bioaccumulation of heavy metals (Fe & Zn) in the root and stem of *H. cannabinus*. The use of *H.*

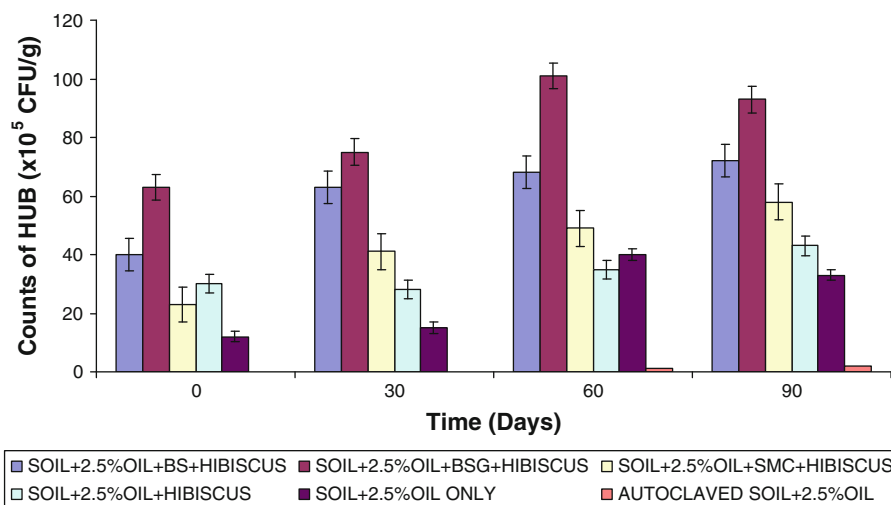


Fig. 3 Counts of hydrocarbon utilizing bacteria in soil contaminated with 2.5% used lubricating oil. Vertical bars indicate standard deviation ($n = 3$)

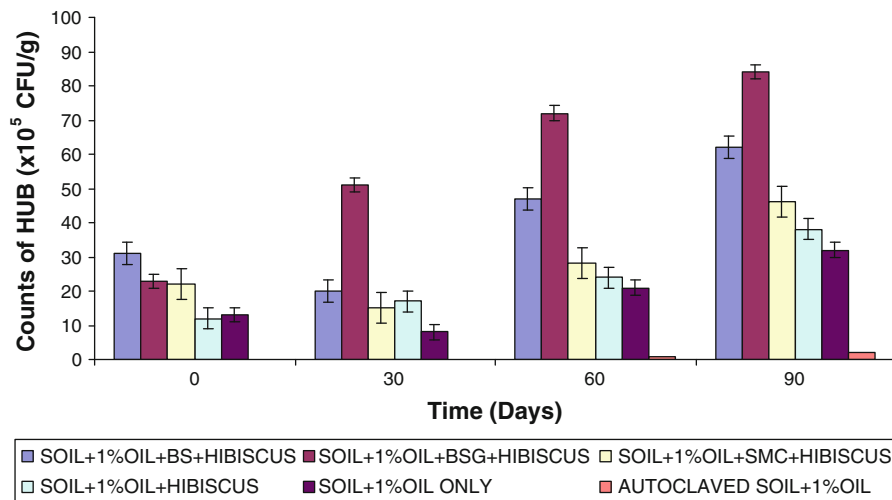


Fig. 4 Counts of hydrocarbon utilizing bacteria in soil contaminated with 1% used lubricating oil. Vertical bars indicate standard deviation ($n = 3$)

cannabinus (an economically viable plant) will therefore serve as an alternative method in removing oil contaminants and metals from soil while at the same time promoting the growth of *H. cannabinus*, which is highly useful to paper manufacturing industries.

Acknowledgments The authors would like to acknowledge the support of University of Malaya IPPP Grant PS 244/2008C and FRGS/1/10/SG/UM/01/6. Also, we would like to thank the managements of Carlsberg brewery for providing brewery spent grain and Gano farm for the provision of spent mushroom compost.

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