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Phytoremediation of Heavy Metals Contaminated Agricultural Soil Planted to *Jatropha curcas*

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ABSTRACT: *This study was conducted to assess the potential of Jatropha curcas in remediating soils contaminated with heavy metals from hydrocarbon fuel. Matured cuttings of Jatropha plant were planted in the plastic pots filled with soils contaminated with three different level of HMs concentration; 250mg/kg, 500mg/kg and 1000mg/kg respectively arranged in a Completely Randomized Design (CRD). The aim was to appraise if Jatropha curcas can be used to treat the heavy metals; Zn, Ni, Cd, Pb and Cu in the contaminated soils and determine whether the treated metals complied to soil permissible limit concentration of agricultural soils advocated by Nigerian and other related international standards. Findings indicated that all the analysed metals recorded a significant statistical difference with different level of soil contamination ($P<0.05$). However, despite their concentration achieved compliance at their initial soil contamination, Cu, Zn and Ni were highly removed by Jatropha plant though the latter metal failed compliance to Nigerian standard at both pre and post Jatropha planting period. Moreover, the metals showed highest removal efficiency in the range of 57%-95% at 500mg/kg hydrocarbon fuel soil contamination. In contrast, Cd metal concentration was low and above compliance limit at pre and post Jatropha planting period except at 1000mg/kg hydrocarbon fuel contamination that achieved compliance of United States (US) with removal efficiency in the range of 8%-42%. However, Pb metal concentration was high and variable at both initial contamination and concentration after Jatropha planting, with the metal achieving and failing compliance of some countries at certain level of concentration after the Jatropha phytoremediation. Overall, the outcome of this research indicates that the Jatropha plant has depurated the metals effectively from the contaminated soils particularly Cu, Zn and Ni at 500mg/kg level of contamination despite the fact that the phytoremediation process was within the period of two months. Hence, the continuation of the experiment is needed in order to fully determine the actual period and appropriate level of soil contamination required for the optimum metal phytoremediation and type of metals the Jatropha plant prefers most in terms of the remediation compared to others.*

KEYWORDS: hydrocarbon, jatropha, metals, irrigation, contamination

INTRODUCTION

Pollution of soil bodies with organic and inorganic compounds such as hydrocarbons and heavy metals (HMs) due to increase in industrialization, continuous carbon emission and other agents of environmental contamination is a major global pollution problem (Zubillaga *et al*., 2008; Ali et al., 2013; Srivastav et al., 2018; Sani et al., 2020). These compounds pollute the soil through anthropogenic activities and result in decreasing its quality in-terms of agricultural production and other related environmental potentials. The pollution could also be from point and non-point sources such as emission, industrial effluents, solid discharge from industries, vehicle exhausts and soluble salts, usage of insecticides or pesticides, dumping of industrial and metropolitan wastes in agriculture, and fertilizers application in excess respectively (Bhatia et al., 2015; Srivastav et al., 2018).

Soil contamination with HMs has become a worldwide problem and poses a serious threat to the environment including agricultural soils because it leads to soil fertility and quality deterioration at a higher concentration above permissible limit (Sani et al., 2020). Subsequently, leading to drop in agricultural yield and hazardous health effect to the crops grown on the affected soils and the receiving consumers as the HMs enter the food chain (Gupta et al., 2015; Srivastav et al., 2018; Sani et al., 2020; Singh et al., 2022).

HMs are trace elements, stable metalloids in nature that have density higher than 4.5 g/cm^3. They include lead (Pb), Copper (Cu), nickel (Ni), cadmium (Cd), zinc (Zn), mercury (Hg) and chromium (Cr) among others. Furthermore, they are non-biodegradable or non-destructible in nature making them easily to bio-accumulate in soils and sediments with subsequent potential adverse impacts to humans, soil and water bodies. These features, made them among the pollutants of concern globally by environmental agencies (Chopra et al., 2009; Ali et al., 2013; Sani et al., 2020).

Some HMs like Cu, Fe, Mn, and Zn, are essential for life processes, while others, like Cd, Ni, and Hg, have no physiological function but often result in detrimental disorders at a higher concentration above allowable limit (Yadav et al., 2017; Srivastav et al., 2018). For example, on health impact, these metals are carcinogenic, mutagenic, and teratogenic, and cause diseases like hair loss, brain and Kidney damage, liver cirrhosis, anxiety, depression, impaired development, reduced intelligence, loss of short-term memory, among others (Ali et al., 2013; Yadav et al., 2017; Sani et al., 2020). On the other hand, literature has documented that HMs in soils at a higher concentration above permissible limits affects the concentration of soil physical parameters such as aggregate stability (AS), porosity (P), hydraulic conductivity (HC) and bulk density (BD) (Almuktar et al., 2018; Sani et al., 2020) leading to poor texture and structure. Comparably, Khaskhoussy et al. (2015) and Sani et al. (2020) reported that accumulation of metals affected soil chemical properties by increasing soil salinity, electrical conductivity (EC), organic matter (OM), exchangeable cations, phosphorus (P) and micronutrients concentrations, and decreasing soil pH in their experiment. Though some studies reported no apparent changes in these parameters (Almuktar et al., 2018, Sani et al., 2020).

Considering the aforementioned negative impacts of these metals to human health, crop and soil quality, and overall environmental quality improvement, treating them is imperative but strenuous with common elimination methods. These methods include chemical, physical and biological but are not applicable everywhere due to high cost, irreversible changes in soil properties, destruction of soil microbes and creation of unwanted by-products during the process of the metals abatement (Ali et al., 2013; Srivastav et al., 2018). Hence, searching and applying a suitable metals removal technique that is environmentally friendly, ecologically balance, cost effective, less energy intensive, easy to operate with public acceptance (Ali et al., 2013; Subhashini and Swamy, 2013; Yadav et al., 2018) such as phytoremediation especially in the agriculture sector to improve soil quality remains necessary.

Several studies have documented that phytoremediation technology is used worldwide as a result of the aforementioned qualities (Yadav et al., 2012; Yadav et al., 2017; Srivastav *et al*., 2018) and most preferred efficient tool for soil contaminants removal including HMs pollution control. The technology is applied to treat variety of soil contaminations from different sources including agricultural and industrial, animal waste waters, sludge and mine drainage, petroleum wastewaters and recently applied successfully to treat soils irrigated with domestic waste water contaminated with hydrocarbons (Almukhtar et al 2018; Sani et al., 2020)**.** The technique is now gaining reputation and acceptance globally (Almuktar *et al*., 2018; Yadav et al., 2017; Srivastav et al., 2018; Sani et al., 2020; Singh et al., 2022) as a tool for environmental pollution control. The technique's function principle is akin to the purification process involving the combination of plants, soils, and microbial organisms to aid in the treatment of pollutants in the contaminated environment (Sani et al., 2020; Singh et al., 2022). Generally, the major removal pathways for these pollutants are phytoextraction, phytovolatilization, phytodegradation, phytostabilization, and phytofiltration (Ali et al., 2013; Pandey et al., 2012; Srivastav et al., 2018; Martin et al., 2020; Abdullahi and Abdurrahman, 2021; Singh et al., 2022) though depending on the type of pollutants; organic or inorganic.

In this study, the choice of phytoremediation to restore soils contaminated with HMs using *Jatropha curcas* (henceforth referred as Jatropha) was due to plant's versatility as perennial shrub, draught tolerant that grows in tropical and subtropical regions like Nigeria and associated characteristics of robustness, commercial feasibility for biodiesel production and non-edibility with high capacity of bioaccumulation and phyto-translocation (Agamuthu et al., 2010). Furthermore, it is fast growing and exhibits high biomass production ability (Abhilash et al., 2012; Ali et al., 2013), carbon sequestration capability, erosion control and can increase soil fertility (Mohammed et al., 2021). In addition, the plant has the ability to rehabilitate and improve soil quality degradation (Ogunwole et al., 2008; Pandey et al., 2012; Ali et al., 2013) as well as soil and water conservation improvement (Chen, 2006; Pandey et al., 2012).

Many studies revealed that HMs compounds in contaminated soils treated with Jatropha are attenuated predominantly via phytoextraction mechanism (Cluis, 2004; Cherian and Oliveira, 2005; Malik et al., 2012; Ali et al., 2013; Singh et al., 2022). However, some metals like Hg and Se are depurated via phytovolatalization (Ali et al., 2013), CrIII and CrVI via Phytostabilization, Zn, Cu, Cd, Cr, Pb and Ni via both phytoextraction and phytostabilization mechanisms (Abdullahi et al., 2017; Abdullahi and Abdulrahman, 2021). The abatement of these metals is carried out efficiently by the Jatropha fibrous roots that accumulate the metals and subsequently transfer them to above ground biomass (Ali et al., 2013; Leapheng et al., 2019; Martin et al., 2020; Singh et al., 2022) with high removal efficiencies (>80%) for some metals (Leapheng et al., 2019).

This study may provide useful information to petroleum, agricultural and related environmental industries to incorporate phytoremediation techniques in their pollution treatment technologies particularly for abatement of HMs compounds in petroleum hydrocarbon spills or industrial sources that may otherwise pollute the soil and water environment.

This study is focused on assessing the efficacy of phytoremediation using Jatropha plant in removing HMs compounds of sandy loam soils in Kano, Sudan Savannah Zone of Nigeria. Many works on HMs treatment via phytoremediation using Jatropha have been conducted in Nigeria (Agbogidi et al., 2013; Abdullahi et al., 2017; Awotedu and Ogunbamawo, 2019; Abdullahi and Abdulrahman, 2021; Mohammed et al., 2021; Singh et al., 2022). However, in Sudan Savannah zone, none or only few if any evaluated the phytoremediation efficiency of Jatropha on these metal compounds from hydrocarbon spill contaminated soils and concomitantly assessing whether the phytoremediated metals concentration are within the threshold limit of agricultural soils using national and international standards. Therefore, the aim of this study was to evaluate the performance efficiency of Jatropha plant in phytoremediation of different HMs compounds with the following objectives;

i. Determine the concentration HMs in the experimental soil at pre and post hydrocarbon spill pollution period;
ii. Determine the efficiency of Jatropha plant in removing the metals concentration in the soil at post hydrocarbon spill pollution period;
iii. Determine whether the concentration of the removed metals by Jatropha plant is within the acceptable limit of agricultural soil quality advocated by national and international standard organizations

MATERIALS AND METHODS

Research Area Description

The experiment was conducted in the Nursery unit of the Department of Forestry, Fisheries and Wildlife, Kano University of Science and Technology (KUST), Wudil, Kano State, in Sudan Savanna Zone of Nigeria. The geographical location of the site is between 11.5^0 latitude and 8.8˚ longitude, and about 37.5 m above sea level. The annual rainfall ranges from 850 to 870 mm, while minimum and maximum temperatures range between 26 and 33ºC with low relative humidity that ranges between, 40 to 51% (Dambazau and Olofin, 2008).

Jatropha Planting

Cuttings of *Jatropha curcas* were raised and planted in polythene pots in the University Nursery. A matured cutting of *Jatropha curcas* plant was transferred and planted in the plastic pots (4kg of soil mixed with 1kg of organic manure) contaminated with heavy metals from hydrocarbon fuel to simulate natural metals spill scenario that contaminate agricultural soils. The fuel was bought at Alhaji Baballe Jaji Company LTD filling station along Gaya road, Wudil Local Government. The plastic pots have a dimension of 32cm height, 106cm upper diameter and 69cm lower diameter. The experimental design applied for the experiment was Completely Randomized Design (CRD) with three (3) treatments replicated three (3) times making a total of nine (9) and additional one (1) control for comparison. The experimental pots were labeled according to their fuel composition; replications 1, 2 and 3 contain 250ml, 500ml and 1000ml of hydrocarbon fuel respectively, and each replication was thoroughly mixed together with 250ml of water.

Data Collection

Initially before the onset of the experiment, control and the experimental soils were subjected to laboratory analysis before being contaminated with hydrocarbon fuel and planted to Jatropha plant. The experiment was conducted for eight (8) weeks. During the experiment, the plants were watered after five days to avoid lack of water availability when there was no rainfall, and that was done only once during the period of the experiment as the experiment was conducted during rainy season. HMs in the contaminated soils were sampled and analysed at the end of the experiment.

Laboratory Analyses

Soil pH and EC were determined using pH and conductivity meters respectively, Organic Carbon was assessed by the dichromate oxidation method as detailed by Nelson and Sommers (1982) while Mechanical Analysis was by standard hydrometer method as outlined by Gee and Bauder (1986). Soil Bulk Density and Phosphorus were determined by Core method as outlined by Blake and Hartge (1986) and acid digestion as described by Murphy and Relay (1962) respectively whereas Total Nitrogen content was evaluated using the micro-Kjeldhal technique as expressed by Bremner (1982).

Heavy metals Evaluation

Heavy metals were analysed using Atomic Absorption Spectrophotometer (AAS) after acid digestion with diacid mixture of HCl and HNO3 (aqua regia) at 100 ^{o}C for 3h (Majid et al., 2012)

Statistical Analysis

SPSS statistical package was used to determine the mean concentration of heavy metals after the data was subjected to Analysis of Variance (ANOVA) technique, and the means were separated using Lease significant difference (LSD) at 5% level of significance. HMs removal efficiency in percentage (%) was computed using the method of Awotedu and Ogunbamawo (2019) where % metal (removal) efficiency= Cm-Cr/Cm*100. Cm: concentration of metals in the contaminated soil (mg/kg) and Cr: concentration of metals in remediated soil (mg/kg)

RESULT AND DISCUSSION

Experimental Soil Quality

The analyses of soil parameters in control soil before the start of the experiment and other soils after being contaminated with HMs from hydrocarbon fuel was depicted in Table 1. Findings indicated that the pH recorded in both control and the contaminated soils (250mg/kg, 500mg/kg and 1000mg/kg hydrocarbon contamination respectively) was within the range of 6.35-6.53 which is compliant to 6.5-8.5 permissible limit of agricultural soils (WHO, 2008) (Table 1).

The soil textural class was sandy loam in nature and the BD values were lower in the control soils compared with the contaminated ones and also within the range that does not restrict crop root growth and movement in soil (Yiferu et al., 2018). OC and N were low in all the contaminated soils but higher than control while P was also very high in all the contaminated soils and control according to Esu (1991) and Adamu et al.(2021). The EC concentration indicated that the soil was not saline (FAO, 1993) in both control and contaminated soils. However, all the analysed HMs in contaminated soils particularly Zn, Ni and Cu recorded relatively higher concentrations than control and were within the compliance limit of good agricultural soils. Though, Cd and Pb recorded relatively low and high concentrations respectively in the contaminated soil and higher than control, both the metals were variable in achieving compliance to some agricultural soil standards advocated by some regulatory agencies (CCME, 2001; FAO/WHO, 2001; FEPA, 2019) (Table 3).

Considering the aforementioned contaminated soil characteristics, the soil quality was marginal because most of the analysed parameters were not within the compliance limit of agricultural soils standard.

Note, all the aforementioned soil parameters and HMs in the contaminated soils were analysed before planting Jatropha in the contaminated soils.

Table 1: Overall mean values of control soils before contamination and contaminated experimental soils with HMs from hydrocarbon fuel in different level of contamination

Soil Parameters	Uncontaminated soil	Contaminated soil		
		A	**B**	**C**
pH	6.35	6.44	6.64	6.53
Particle size				
Sand (%)	77.78	80.45	73.12	82.45
Silt (%)	16.55	16.56	15.22	11.22
Clay (%)	5.67	2.99	11.66	6.33
BD (g/cm^3)	1.02	1.68	1.63	1.7
OC (%)	1.48	2.76	2.87	3.10
N (%)	0.21	0.16	0.53	0.89
P (mg/kg)	89.57	90.01	87.49	74.83
EC (dS/m)	0.12	0.92	0.99	1.27
Zn (mg/kg)	26.9	38.47	43.40	48.1
Ni (mg/kg)	1.87	18.24	27.37	29.77
Cd (mg/kg)	0.51	6.88	8.98	11.23
Pb (mg/kg)	6.01	143.51	157.97	167.22
Cu (mg/kg)	7.24	7.78	9.73	11.33

A, contaminated soil with HMs at 250mg/kg hydrocarbon fuel, B, contaminated soil with HMs at 500mg/kg hydrocarbon fuel, C, contaminated soil with HMs at 1000mg/kg hydrocarbon fuel

Comparison of Heavy Metals (HMs) Removal in post Jatropha Planting

Comparison of Zn and removal efficiency

The mean values of Zn in contaminated soils planted to Jatropha was depicted in Tables 2 and 3. The results indicated that all the metals were highly removed by Jatropha in different level of contaminated soils with hydrocarbon fuel concentrations (Table 2). The removal concentration was even below that of control soils. Furthermore, the Jatropha removal efficiency of the metal was relatively excellent (35-95%) with highest removal efficiency in 500mg/kg hydrocarbon fuel contaminated soil (Figure 1). The Anova result (Table 3) showed that there was a statistical significant difference ($P<0.05$) recorded between the metal removal by Jatropha and different hydrocarbon fuel contaminated soils (Table 3). Despite Zn recorded values in all the contaminated soils were compliant with standard of agricultural soils (CCME, 2001; FAO/WHO, 2001; FEPA, 2019), 500mg/kg hydrocarbon fuel contaminated soil recorded the highest removal concentration in comparison to other soils. The plausible reason for this could be attributed to level of the metals contamination from the applied hydrocarbon, period allowed for remediation and Jatropha species involved in the phytoremediation (Ahmadpour et al., 2010; Majid et al., 2012; Awotedu and Ogunbamawo, 2019). In this research, the phytoremediation efficiency of Jatropha on Zn metal indicated that it was at peak ($P<0.05$) at hydrocarbon concentration lower than 1000mg/kg, implying that Jatropha could positively promote the reduction of the metal in the contaminated soil at 500mg/kg contamination level compared to 250mg/kg and 1000mg/kg contaminated soils. This was probably possible due to the fact that Jatropha was suitable to restore the metal contamination at that level (500mg/kg) because the ability of rhizoremediation effect of the Jatropha plant and its roots ability was effective enough to stimulate the rhizosphere micro-organisms and excreting root exudates which facilitate the higher removal of the metal (Wang et al., 2011). However, the lower removal efficiency observed in 250mg/kg and 1000mg/kg of 35% and 53% respectively could be due to the fact that hydrocarbon fuel serves as microorganisms' source of energy (Wang et al., 2013) and higher concentration of it means higher microorganisms' activity and vice-versa. Hence, in 250mg/kg hydrocarbon contaminated soil, the activity is expected to be low and subsequent lower Zn removal compared to the 1000mg/kg contaminated soil. Nevertheless, the 1000 mg/kg fuel in the contaminated growth media could be toxic to the microorganisms and the phytoremediating plant roots, and this toxicity effect could have affected the capacity of the roots to reduce the metal efficiently thus leading to the lower observed removal efficiency of 53% (Figure 1) unlike in 500mg/kg fuel concentration that was not affected by the toxicity impact. The result of Zn removal of this research was comparable to findings reported elsewhere (Zakari et al., 2017; Singh et al., 2022) who reported high reduction of Zn metal by Jatropha curcus L as a result of its roots' effectiveness and microbial influence in the metal depuration process.

Soils containing high concentration of Zn metal is qualitatively poor and affect crops grown on the affected soils with apparent browning of collaroid roots and chlorosis particularly in vegetables (Islam et al., 2007). Moreover, when such affected crops are consumed by animals and humans, they can be infected by health associated problems such as dizziness and fatigue (Hess and Schmid, 2002).

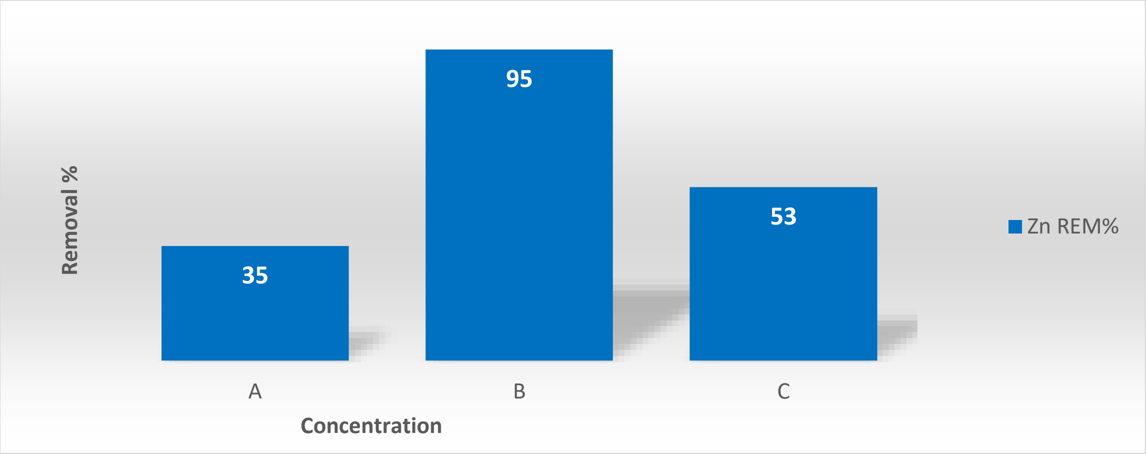
Comparison of Nickel (Ni) and removal efficiency

The overall mean concentrations of Ni metal in all contaminated soils after Jatropha planting and the removal efficiencies were relatively fair and good particularly at 500mg/kg hydrocarbon fuel contamination (Tables 2 and 3)(Figure 2). Moreover, all the remediated metals concentrations were within the threshold international and Nigerian standard limits of good agricultural soils (Table 3). In addition, findings indicated that the high removal concentration of Ni by the Jatropha plant resulted in higher statistically significant (P<0.05) difference in 500mg/kg hydrocarbon fuel contaminated soils (11.23mg/kg) compared to the other soils. This high removed Ni concentration observed could be ascribed probably due to the same reason stated previously with reference to Zn metal removal above (Wang et al., 2011, 2013). This indicates that 500mg/kg hydrocarbon fuel is the appropriate dose suitable for Ni removal without any toxicity challenge to the Jatropha plant roots and the rhizosphere microbes which together remove the metals effectively. The concentration of Ni metal removed in this research was in agreement with data reported recently elsewhere (Abdullahi and Abdulrahman, 2021). However, some studies reported lower removal efficiencies and concentrations of Ni metal (<30% and <10mg/kg) respectively by Jatropha plant in their researches (Agbogidi et al., 2013; Awotedu and Ogunbamawo, 2019; Martin etal., 2020). The plausible reason for the differences between the current research result and that of the reported literature could be due to differences in the sources, degree of concentration, composition and type of HMs involved in the contamination (Pandey et al., 2012; Ali et al., 2013; Srivastav et al., 2018; Martin et al., 2020), period of metals phytoremediation by the Jatropha plant, population and the activity of the rhizosphere microbes associated with depuration of the metal (Ali et al., 2013; Wang et al.,2013).

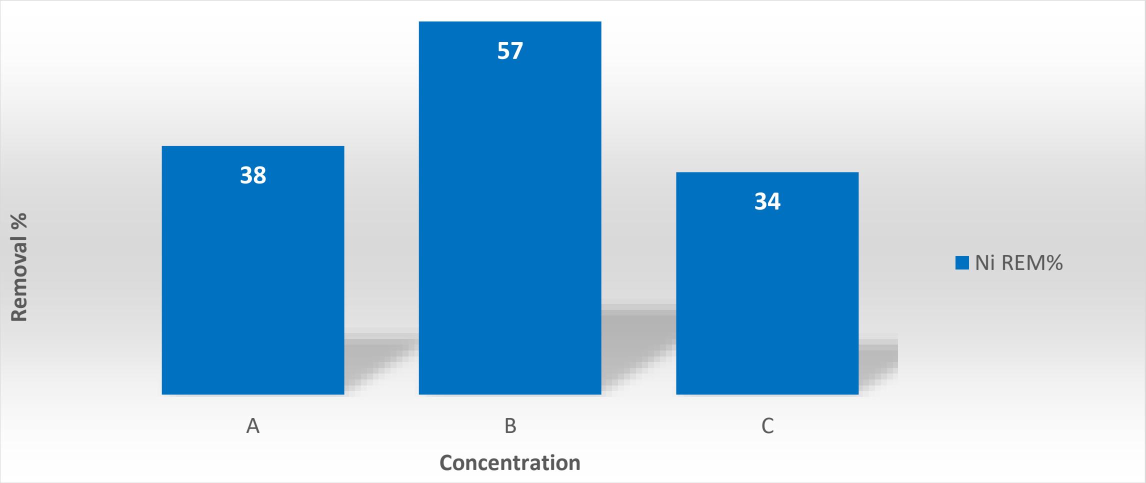
High Ni concentration in soils contaminates the soil growth media with negative impact to the crops grown on the media which can accumulate the metal, and subsequently cause serious health risks to consumers (Islam et al., 2007; Chopra et al., 2009; Ali et al., 2013) upon the consumption of the affected crops.

Comparison of Cadmium (Cd) and removal efficiency

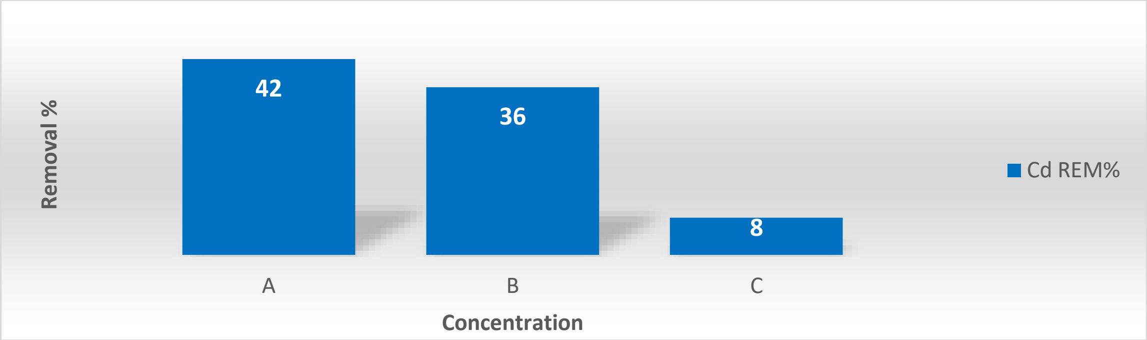
The mean average concentration of Cd and other HMs after the planting of Jatropha plant was depicted in Tables 2 and 3**.** The results indicated that the removed metal concentration was relatively high and above compliance to soil quality permissible standard advocated by both national and international regulatory agencies (FEPA, 2019) and (CCME, 2001; FAO/WHO, 2001) respectively in all contaminated soils with the exception of United States of American Standard (USA) (Table 3). This difference is attributable to differences in policy setting of standards pertaining soil quality of different countries' regulatory agencies. With regards to removal efficiency, though the removal % was relatively low (8%-42%), Figure 3 illustrated a decreasing trend with 250mg/kg hydrocarbon fuel contaminated soil recording highest removal efficiency (42%) followed by 500mg/kg (36%) and 1000mg/kg (8%) correspondingly. Moreover, this removed Cd metal recorded a significant difference statistically (P<0.05) across the contaminated soils (Table 3). This difference could be attributed to adequate amount of carbon and nitrogen as energy sources for the rhizosphere microbes from the applied hydrocarbon fuel in the 250mg/kg contaminated soil which was not too much to be toxic to them (Wang et al., 2013; Leapheng et al., 2019) in comparison to 500mg/kg and 1000mg/kg contaminated soils respectively. Hence, the activity of the microorganisms was stimulated which led to the increase of the Cd metal removal under the soil contaminated with the 250mg/kg hydrocarbon fuel (Figure 3). Many studies reported that Jatropha plants were very efficient in removing Cd in contaminated soils due to stimulation of microorganism's activity at the rhizosphere vicinity by the influence of added optimum carbon and nitrogen nutrients released from the applied organic and inorganic amendments in the soil including hydrocarbon (Ong et al., 2011; Wang et al., 2013; Leapheng et al., 2019; Mohamed et al., 2021). On the other hand, impediment in inorganic contaminants removal including metals as a result of high contamination has been reported previously confirming the data of the current study (Awotedu et al., 2019). However, a study (Leapheng et al., 2019) reported higher removal efficiency (64%) above that of the current study (8-42%). The plausible reason for this difference could be accredited to low level of initial soil contamination of the Cd metal (0.58mg/kg) in the reported literature compared to over 140mg/kg level of contamination assessed in the current study. Furthermore, the duration of the phytoremediation process was up to 90days in comparison to 60days evaluation period of the present research.



Zinc removal efficiency (%) in Hydrocarbon fuel contaminated soils at different concentrations



Nickel removal efficiency (%) in Hydrocarbon fuel contaminated soils at different concentratio



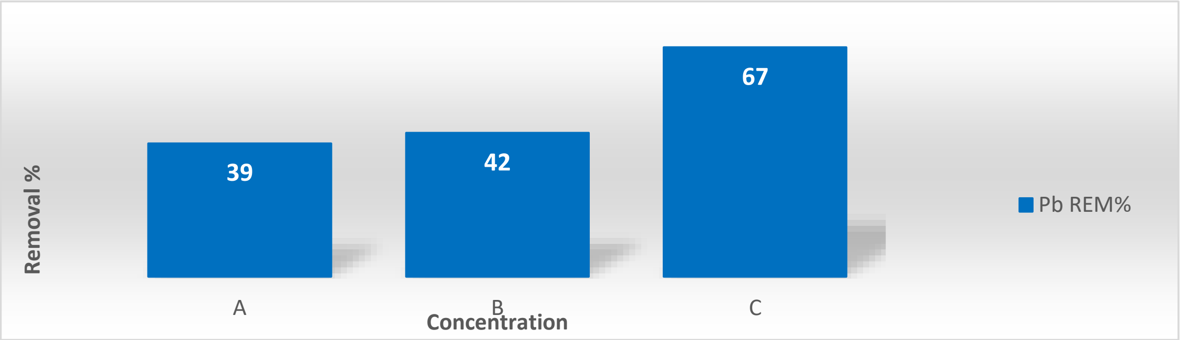
Cadmium removal efficiency (%) in Hydrocarbon fuel contaminated soils at different concentrations

Soils containing high amount of Cd above permissible standard leads to Cd soil toxicity and affect the roots and shoots growth, oxidative stress, genotoxicity in addition to impediment of the photothynthetic processes and root absorption mechanism of the crops grown on the affected soils. Furthermore, consuming the Cd metal via affected crops or vegetables by humans or animals has carcinogenic, mutagenic and teratogenic effect (Ali et al., 2013; Sani et al., 2020).

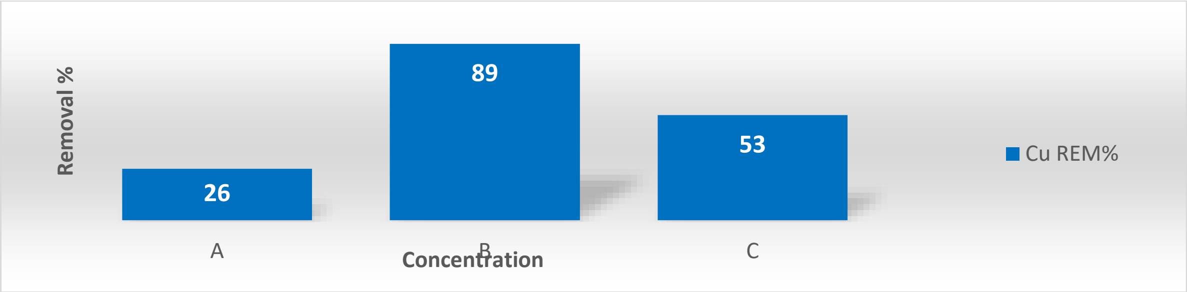
Comparison of Lead (Pb) and removal efficiency

Overall mean values and removal efficiencies with regards to Pb metal in pre and post Jatropha plant planting were shown in Table 2 and Figure 4. The result shows that, the metal in all the contaminated soils were relatively removed in high concentration (55.53mg/kg-91.77mg/kg) after Jatropha planting from the initial concentration in the range of 143.51mg/kg to 167.22mg/kg before the planting of Jatropha plant. The recorded Pb concentrations were compliant with some international agricultural soil quality standards particularly in the case of 250mg/kg and 500mg/kg hydrocarbon fuel contaminated soils except for Canada and Nigeria (Table 3). Moreover, compliance was not achieved with soil contaminated with 1000mg/kg hydrocarbon fuel to Nigerian standard (Table 3).

The overall mean Pb concentration in 1000mg/kg hydrocarbon fuel contaminated soil was highest compared to 500mg/kg contaminated soil which recorded higher values than 250mg/kg contaminated soil. This difference was statistically significant ($P<0.05$) as shown in Table 3 which shows an assessment of the statistically significant differences between HMs and different hydrocarbon fuel contaminations in the analysed soils. Moreover, Figure 4 indicated that 1000mg/kg hydrocarbon contaminated soil recorded the highest removal efficiency (67%) compared to 500mg/kg and 250mg/kg contaminated soils that recorded 42% and 39% respectively. Despite complying with almost all permissible soil quality standards (Table 3), the high removed Pb values recorded in the 1000mg/kg contaminated soil compared to other contaminated soils could be ascribed probably to high amount of soil microorganisms and their activity in the rhizosphere vicinity of the Jatropha plant roots induced from the high applied hydrocarbon fuel. Subsequently, this might have led to the reduction of the Pb metal concentration greatly leading to the high observed removal efficiency in the 1000mg/kg hydrocarbon contaminated soil (Table 3 and Figure 4) compared to other contaminated soils since previous studies indicated that hydrocarbon fuel is composed of certain quantities of carbon and nitrogen and in soils, these nutrients induce increase and growth of microbial population in the soil rhizosphere. In addition, the nutrients serve as the source of energy to the living soil microorganisms and enhance their activity, which subsequently lead to greater removal of organic and inorganic contaminants including HMs (Wang et al., 2009, 2013). This has been supported by several studies who expounded that Jatropha curcus plant has removed huge amount of Cd, Cr and Pb in highly HMs contaminated soils (Devi Chinmayee et al., 2014; Vurayai et al., 2017) confirming the data of the current study.



Lead removal efficiency (%) in Hydrocarbon fuel contaminated soils at different concentrations



Copper removal efficiency (%) in Hydrocarbon fuel contaminated soils at different concentrations

The concentration and removal efficiency of Pb in all the hydrocarbon contaminated soils of this research were different with data reported in some studies elsewhere (Awotedu et al., 2019; Leapheng et al., 2019). The plausible reason for these differences could be attributed to the initial quantity and level of the HM contamination in the soil, duration of the phytoremediation experiment and the type of energy sources for the rhizosphere microbes involved in the remediation process (Ali et al., 2013).

High Pb concentration in soils above permissible standard leads to soil toxicity with subsequent soil quality and fertility reduction. Moreover, Pb accumulates in the leafy parts of vegetables and in the grain yield of some crops (Sidhu and Narwal, 2004; Chopra et al., 2009), and upon consumption of these crops leads to disease problems such as impaired development, reduced intelligence, loss of short-term memory, learning disabilities and coordination problems, among others (Ali et al., 2013; Sani et al., 2020) in the host consumer

Table 2:Overall mean concentration of HMs in uncontaminated and contaminated soils at pre and post Jatropha planting period under different level of hydrocarbon fuel contamination all in mg/kg

		A		**B**		**C**	
HMs	Uncontaminated soil	Jatropha **A**[a]	Jatropha **A**[b]	Jatropha **A**[c]	Jatropha **A**[d]	Jatropha **A**[e]	Jatropha **A**[f]
Zn	26.9	38.47	25.02	43.40	2.24	48.1	22.47
Ni	1.87	18.24	11.73	27.37	11.23	29.77	19.77
Cd	0.51	6.88	4.01	8.98	5.75	11.23	10.33
Pb	6.01	143.51	87.73	157.97	91.77	167.22	55.53
Cu	7.24	7.78	5.73	9.73	1.09	11.33	5.36

A[a] : HMs in contaminated soil before jatropha planting at 250mg/kg hydrocarbon fuel application; A[b], HMs in contaminated soil after jatropha planting at 250mg/kg hydrocarbon fuel application, A[c]; HMs in contaminated soil before jatropha planting at 500mg/kg hydrocarbon fuel application, A[d]; HMs in contaminated soil after jatropha planting at 500mg/kg hydrocarbon fuel application, A[e]; HMs in contaminated soil before jatropha planting at 1000mg/kg hydrocarbon fuel application; A[f]; HMs in contaminated soil after jatropha planting at 1000mg/kg hydrocarbon fuel application

Comparison of Copper (Cu) and removal efficiency

The mean values of Cu after Jatropha planting as depicted in Tables 1and 2 were relatively high and variable in all hydrocarbon contaminated soils and within agricultural soil quality threshold limit of national and international regulatory agencies (CCME, 2001; FAO/WHO, 2001; FEPA, 2019). In the statistical analysis table however, soil contaminated with 500mg/kg hydrocarbon fuel recorded highest removal concentration value of Cu fallowed by soils contaminated with 1000mg/kg and 250mg/kg respectively (Table 3). Comparably, the result of removal efficiencies of Cu metal (Figure 5) indicated that the highest removal efficiency of 89% was observed under 500mg/kg in comparison to 53% and 26% observed under 1000mg/kg and 250g/kg hydrocarbon contaminated soils in that order. This difference in concentration and removal efficiency was statistically significant ($P<0.05$). Despite Cu concentration removed in all contaminated soils were compliant with soil quality threshold standard, the plausible reason for the statistical difference observed in 500mg/kg hydrocarbon contaminated soils (Table 3) compared to other soils could be ascribed to high amount of C and N nutrients in the applied hydrocarbon fuel (Wang et al., 2009, 2013) that serve as source of energy for the rhizosphere microbes.

This implies that, 1000mg/kg hydrocarbon fuel is toxic despite containing high amount of C and N compounds in comparison to the 500mg/kg and could not provide a conducive environment for microorganisms to fully and greatly reduce the Cu metal in the rhizosphere of the phytoremediation plant (Wang et al., 2011). In contrast, 500mg/kg hydrocarbon fuel level of contamination is not toxic but suitable and provider of conducive rhizopheric environment for the microorganisms and Jatropha plant roots association. This enhances microbial activity in the Jatropha root vicinity resulting to high removal of the Cu metals observed in the 500mg/kg hydrocarbon contaminated soils and has been in agreement with some findings reported elsewhere (Majid et al., 2012; Awotedu et al., 2019). However, the Cu concentration removed in this research is much lower than the data reported in the literature (Abdullahi et al., 2017). The reason could be ascribed to initial level of contamination of the metal, duration of the phytoremedation process and the plant involved in the phytoremediation (Ali et al., 2013; Wang et al., 2013)

Table 3:Overall mean concentration and significant differences ($P<0.05$) of HMs in contaminated soils at post Jatropha planting period compared to international threshold values for HMs concentration in agricultural soils (mg/kg)

HMs	Post Jatropha planting HMs concentration			Regulatory Agencies					
	A	**B**	**C**	**USA**	**UK**	**EU**	**Canada**	**WHO/FAO**	**FEPA**
Zn	25.02^{c}	2.24^{a}	22.47^{b}	1400.0	200-300.0	300.0	200.0	300.0	300.0
Ni	11.73^{b}	11.23^{a}	19.77^{c}	210.0	50-110.0	75.0	50.0	50.0	10.0
Cd	4.01^{a}	5.75^{b}	10.33^{c}	19.5	3.0	3.0	1.4	3.0	3.0
Pb	87.73^{b}	91.77^{c}	55.53^{a}	150.0	300.0	300.0	70.0	50.0	1.75
Cu	5.73^{c}	1.09^{a}	5.36^{b}	170.0	80-200.0	140.0	63.0	100.0	70-80.0

Source: CCME (2001), WHO/FAO (2001) and FEPA (2019). Note, A, HMs concentration in contaminated soils planted to Jatropha at 250mg/kg hydrocarbon fuel application, B, HMs concentration in contaminated soils planted to Jatropha at 500mg/kg hydrocarbon fuel application, HMs concentration in contaminated soils planted to Jatropha at 1000mg/kg hydrocarbon fuel application respectively. Mean values on the rows with different super scripts are significantly different ($P\leq0.05$).

High Cu concentration in soils above permissible standard leads to soil toxicity with subsequent soil quality and food chain contamination. Moreover, its accumulation in food crops and subsequent consumption of the food leads to disease problems such as brain and kidney damage, liver cirrhosis and chronic anaemia, stomach and intestinal irritation (Ali et al., 2013)

CONCLUSION AND RECOMMENDATIONS FOR FURTHER RESEARCH

The main objective of this research was to evaluate the phytoremediation efficiency of Jatropha plant on different metal compounds from hydrocarbon spill contaminated soils and concomitantly assessing whether the phytoremediated metals concentration are within the threshold limit of national and international standards. Findings indicated that Cu, Zn and Ni out of the assessed metals were below the standard threshold limit for agricultural soil quality as recommended by regulatory agencies at both initial without Jatropha and final contamination after Jatropha planting except for the latter metal that was not for Nigerian standard. However, in terms of removal efficiency and concentration, Jatropha plant removed the metals relatively high in the range of 26%-89% for Cu, 35%-95% for Zn and 34%-57% for Ni respectively with high removal efficiency in the range of 57%-95% particularly at 500mg/kg hydrocarbon fuel soil contamination. In contrast, Cd metal concentration was low but above compliance limit of agricultural soil quality in both pre and post Jatropha planting in the contaminated soils except for 1000mg/kg contaminated soil after Jatropha planting that achieved compliance. Moreover, pertaining removal efficiencies, the metal recorded a decreasing trend with 42%, 36% and 8% efficiency for 250mg/kg, 500mg/kg and 1000mg/kg hydrocarbon contaminated soils respectively. Pb metal on the other hand, showed variability in compliance achievement in both pre and post Jatropha planting period in the contaminated soils, with some concentrations achieving compliance to some regulatory agencies' standard while some were not. Furthermore, the Pb removal efficiencies showed an increasing trend with 39%, 42% and 67% recorded under 250mg/kg, 500mg/kg and 1000mg/kg in that order. This indicates that level and degree of the metals contamination, type of the metal to be phytoremediated, time of the phytoremediation process involved and ability of the Jatropha plant roots in the remediation process influence the metals removal.

Overall, the study findings indicated that despite moderate to low concentration of the assessed metals and within the compliance limit of qualitative agricultural soils, Jatropha plant is a good remover of HMs from hydrocarbon fuel contaminated soils particularly at moderate level. Moreover, the plant phytoremediated the metals in just eight weeks, indicating that the plant could have removed the metals more had the experiment extended to greater than the research period. In addition, the result indicated that the Jatropha plant is selective in the metals depuration considering the variable metals removal concentration and removal efficiencies even in the lowest level of contamination.

Concerning recommendations, there is need for the experiment to continue for a longer period of time to fully assess the influence of time and response of microorganisms to effective metals phytoremediation by the Jatropha plant roots. Moreover, contribution of the Jatropha plant to soil fertility and quality should also be assessed.

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