Heavy Metal Uptake Responses in Plants Grown on Crude Oil-Polluted Soils as Prospects for Phytoremediation

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ABSTRACT: The demand and utilization of petroleum products have re-energized its exploration and exploitation globally and this upsurge in world production, refining and distribution of petroleum products have brought with it various problems of environmental pollution, which have effects on the ecosystems. Twenty (24) polyethylene pots each containing 7 kg of sandy loam soil mixed with 50 ml of crude oil, were arranged in the Botanical garden of the University of Ilorin, Nigeria, to assess their ability to phytoextract heavy metals in Crude oil-polluted soil. Seeds of Amaranthus hybridus L., Tithonia diversifolia, Abelmoschus esculentus L. and Zea mays were sown in polyethylene containers containing 7 kg of contaminated or Control soil. The containers were arranged in a complete randomized design. Plants were left to grow for two months with regular watering. Plants were harvested, separated into roots and shoots and oven-dried to constant weight. The experimental plants have been able to reduce the concentration of Cu in both soils by about 45% to 85%, Cr in the soil by 92.08% to 96.72%, as the residual concentration varied between 66.00 mg/kg and 99.00 mg/kg, Cd in the soil was reduced to 4.00 mg/kg and 17 mg/kg which represented 96.8% and 86.4% reduction. Tithonia had the highest Pb reduction in crude oil-polluted soil. Ni concentration was reduced by 85.84% by Tithonia planted in crude oil-polluted soil. 94.59% by Amaranthus hybridus planted in Control soil. These show that all the test plants were good phytoextractors of the metals.

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Phytoremediation is a novel plant-based remediation technology applied worldwide to soil, water and sediments polluted by inorganic or organic materials. It makes use of naturally occurring processes by which plants and microbial rhizosphere flora degrade and/or sequester pollutants. It is more economical than alternative mechanical and chemical methods of eliminating hazardous pollutants from soils (Bollag et al., 1994). Inorganic pollutants occur as natural elements in the earth crust or atmosphere, and anthropogenic activities such as mining, agriculture, traffic and industrial activities encourage their release into the environment, thereby causing toxicity (Nriagu, 1979). Inorganic pollutant cannot be degraded but can be phytoremediated through volatilization, phyto-stabilization or sequestration in harvestable plant part. Inorganic pollutants that can be phytoremediated include macronutrients like nitrates and phosphates (Nwoko et al., 2004) and trace elements such as Cr, Fe, Zn, Ni, Mn, Mo, and Cu (Lytle et al., 1998). Plant roots extract metal contaminants from soil, polluted water and waste water, and accumulate them in their root tissue. Plants’ roots uptake both organic and inorganic pollutants (Sinha et al., 2004). The bioavailability of a given compound depends upon the lipophilicity and the soil or water conditions e.g. pH and clay content. Considerable amount of the contaminants may be translocated above ground through the xylem and accumulated in the shoots. The roots and shoots are collected and incinerated to decompose the contaminants (Sinha et al., 2004). The objective of this study was to determine and report the uptake of Cu, Cr, Cd, Pb and Ni by Amaranthus hybridus L., Tithonia diversifolia, Abelmoschus esculentus L. and Zea mays grown on crude oil-polluted soils.

MATERIALS AND METHODS
The method of Ogunkunle et al. (2013) was adopted. The experiment was carried out in the Botanical garden of the University of Ilorin, Nigeria, with 24 polyethylene pots, each containing Seven (7) kg of
sandy loam soil mixed with 50 ml of crude oil. Twelve containers contained the crude oil-polluted soil while the remaining twelve containers contained the Control soil. Seeds of *Amaranthus hybridus* L., *Tithonia diversifolia*, *Abelmoschus esculentus* L. and *Zea mays* were sown in polyethylene containers containing 7 kg of contaminated or Control soil.

The seeds were sown after two weeks of spiking the soil with crude oil. Six containers per treatment were used for each plant species. The containers were arranged in a complete randomized design (CRD). Two weeks after planting, plants were thinned to four seedlings per container. Plants were left to grow for two months with regular watering with borehole water under natural photoperiod and ambient conditions. After two months, plants were harvested, separated into roots and shoots (stem and leaves together), and washed with tap water to remove soil particles. Plant samples were properly labelled and oven-dried to constant weight at 80 °C.

**Elemental analyses of soil samples:** Analyses of the elemental contents of the soil, shoots and roots samples were determined with the adopted method of Abdulkadir et al. (2012). The air dried soil samples from each of the treatment were crushed, ground and powdered with a mortar and pestle. Each powdered soil sample (0.1 g) was carefully weighed into a test tube and a mixture of 0.5 ml Trioxo-nitrate V acid (HNO₃), 1.5 ml Perchloric acid (HClO₄) and 0.5 ml hydrofluoric acid (HF) were added to each sample. The sample was heated on a hot plate in a fume cupboard till colourless solution was formed. After cooling, the residue was transferred into 50 ml beaker and made to volume up to 10 ml with deionized distilled water. The digested samples were then analyzed for their various heavy metals (Cu, Cr, Cd, Pb and Ni) by using Atomic Absorption Spectrophotometry (AAS).

**Elemental analyses of plant (root and shoot):** Plant samples i.e., roots and shoots (stem and leaves) were crushed, ground and powdered separately with the help of a mortar and pestle. An amount of 0.1 g of each powdered plant sample was carefully weighed into a test tube and a mixture of 0.5 ml 70% Perchloric acid (HClO₄), 2.5 ml Trioxo-nitrate (V) acid (HNO₃) and 0.5 ml Tetraoxo-sulphate (VI) acid (H₂SO₄) were added to each sample. The sample was heated on a hot plate in a fume cupboard till the appearance of a clear solution. It was then set aside to cool. The residue was transferred into 50 ml beaker and made to the volume, up to 10 ml with deionized distilled water. The digested samples were then analyzed for their various heavy metals (Cu, Cr, Cd, Pb and Ni) by using Atomic Absorption Spectrophotometry (AAS).

**Data Analyses:** The data collected from the study were statistically analyzed with the use of SPSS. The means of the treatments were compared statistically with ANOVA, and the means separated using Duncan’s Multiple Range Test (DMRT). Graphs were plotted using Origin 7.0 software.

**RESULTS AND DISCUSSIONS**

The tables below show the concentrations of Cu, Cr, Cd, Pb and Ni detected in the shoots, roots and soils used for the experiment. In shoots of plants, the mean Cu concentration was highest (18.00±0.00 mg/kg) in *Amaranthus hybridus* planted in crude oil-polluted soil and lowest (4.00±1.00 mg/kg) in *Abelmoschus esculentus* planted in crude oil-polluted soil. However, when the means were compared statistically, Cu concentrations in shoots of *Tithonia diversifolia*, *Zea mays*, *Abelmoschus esculentus* and *Amaranthus hybridus* planted in crude oil-polluted and Control soils did not differ at p≤0.05. On the other hand, the mean concentration of Cu in the roots was maximum (21.50±5.50 mg/kg) in roots of *Tithonia diversifolia* planted in crude oil-polluted soil and minimum (10.00±1.00 mg/kg) in the root of *Abelmoschus esculentus* planted in crude oil-polluted soil.

When the means were compared, there existed no significant difference among the Cu concentrations in the roots of the four plants planted in both soil types at p≤0.05. Furthermore, the initial concentration of Cu in the soil was 20 mg/kg while the residual Cu concentrations in the two soils varied between 3.00±1.00 mg/kg and 11.00±0.00 mg/kg which were recorded in *Tithonia diversifolia* and *Zea mays* planted in crude oil-polluted soil and *Zea mays* planted in Control soil respectively. In general, the experimental plants have been able to reduce the concentration of Cu in both soils by about 45% (11.00 mg/kg) to 85% (3.00 mg/kg).

Copper (Cu) is an important element for plants and animals. Excessive concentrations of this metal are considered to be highly toxic. Roots and shoot of the plants contained comparable Cu concentrations. Cu concentrations in plants above 10-30 µg/g are regarded as poisonous (Macnicol and Beckett, 1985). Within roots, Cu is associated mainly with cell walls and is largely immobile. However, higher concentrations of Cu in shoots are always in phases of intensive growth and at the luxury Cu supply level (Tiffin, 1977). Relatively high concentrations of Cu in the *Tithonia diversifolia* and *Zea mays* in relatively high pH soil may be attributed to their biomass. This is in
agreement with the findings of Weis and Weis (2004) who reported that at higher pH conditions greater than 7.0 enhanced Cu uptake. The permissible limit of copper for plants is 10 mg/kg recommended by WHO (Zigham et al., 2012). The maximum permissible limit of Cu according to SEPA of China is 100 mg/kg. In some of the plant samples, concentration of copper was recorded above the permissible limit. In the soil samples, concentration of Cu was recorded above the recommended maximum level (100 μg/g) Chiroma et al. (2012). The present study classifies Tithonia diversifolia, Abelmoschus esculentus, Amaranthus hybridus and Zea mays as Cu accumulators having met the criteria for appraising the potential and efficiency of plants, which are their Remediation Factors and Phytoextraction Potentials. The mean concentration of Cu in the roots was highest (21.50±1.50 mg/kg) in roots of Tithonia diversifolia planted in crude oil polluted soil and lowest (10.00±1.00 mg/kg) in the root of Abelmoschus esculentus planted in crude oil polluted soil. Wagh et al. (2013) pointed out that Cu content of most plant is generally between 2 and 20 mg/kg in the plants as Cu strongly binds to soils it is very immobile and hence the plant roots are frequently higher in Cu concentration than other plant tissues. This could be why more Cu concentrations were found in the roots of the plants used for this study.

The heavy metals’ uptake responses of experimental plants grown in crude oil polluted and Control soils are shown in Table 2. The concentration of Cr in shoot was highest in the shoot of Abelmoschus esculentus (97.00±48.00 mg/kg) and lowest in shoot of Zea mays (15.00±0.00 mg/kg) planted in Control soil. When the mean values were compared, the Cr concentrations were found to be statistically the same for all experimental plants at p≤0.05.

The concentration of Cr in root was highest in Amaranthus hybridus (117.00±18.00 mg/kg) followed by Tithonia diversifolia (88.00±11.00 mg/kg) and lowest (12±0.00 mg/kg) in Zea mays. When the means were compared, Amaranthus hybridus planted in crude oil-polluted soil had the highest concentration of Cr, but not significantly greater than Zea mays and Tithonia diversifolia planted in crude oil-polluted soil at p≤0.05. The least Cr concentration was observed in Zea mays (12.00 mg/kg) planted in Control soil.

The experimental plants were able to reduce the initial concentration of Cr in the soil (1250 mg/kg) by 92.08% to 96.72%, as the residual concentration varied between 66.00 mg/kg (crude oil-polluted soil of Abelmoschus esculentus) and 99.00 mg/kg (crude oil-polluted soil of Zea mays). This shows that all the test plants are good. Chromium (Cr) is a non-essential metal to plant growth, and may be possible that plants do not have any specific mechanism for transport of Cr (Shanker et al., 2005). The soil used for this study had high concentrations of Cr. Results from the present study showed that all plant parts contained statistically the same Cr concentration. This is in contrast to assertion by Khairia (2012) who stated that Cr is immobilized in the vacuoles of the root cells and showed less translocation, thus rendering it less toxic. This may be a neutral toxicity response of the plants (Macnicol and Bekett, 1985). According to Macnicol and Bekett (1985), the toxic levels of Cr in plants range from 1 to 10 μg/g dry weight. The permissible limit of Chromium for plants is 1.30 mg/kg recommended by WHO. The maximum permissible limit of Cr according to SEPA of China is 250 mg/kg. In plant, all the parts contained chromium concentrations that were above the permissible limit. This could be because of the availability of Cr in large concentrations in the soil and the pH of the soil. The concentration of lead in the pretreated soil was above the recommended maximum level (100 μg/g) according to WHO (Chiroma et al., 2012). The present study classifies Tithonia diversifolia, Abelmoschus esculentus, Amaranthus hybridus and Zea mays as Cr accumulators having met the criteria for appraising the potential and efficiency of plants.

**Table 1**: Concentration of Cu in the soils and plants used for the experiment

| Plant Species         | Soil Concentration | Shoot Concentration | Root Concentration |
|-----------------------|--------------------|---------------------|--------------------|
| Amaranthus hybridus   | 15.00±2.82         | 21.50±7.77          | 20.00±5.65         |
| Tithonia diversifolia | 4.00±1.41          | 17.00±2.82          | 10.00±1.41         |
| Abelmoschus esculentus| 12.00±5.65         | 21.50±7.77          | 15.00±2.82         |
| Zea mays              | 4.00±0.00          | 10.00±1.41          | 14.00±4.24         |

Values represent mean ± standard deviation. Values with the same letter along the columns are not significantly different at p≤0.05.

Key: A = Initial concentration of metal in the soil; B = Residual concentration of metal in the Control soil; C = Residual concentration of metal in the Crude oil-polluted soil; D = Metal concentration in the shoots of plants in Crude oil-polluted soil; E = Metal concentration in the roots of plants in Crude oil-polluted soil; F = Metal concentration in the shoot of plants in Control soil; G = Metal concentration in the root of plants in Control soil.

OLAWEPO, GK; OGUNKUNLE, CO; FATOBA, PO; ANIMASAUN, DA; BEN-UWABOR, PO; DANZAKI, MM
Concentrations of Cd (mg/kg) in crude oil-polluted and Control soils and plants are shown in Table 3. The least Cd concentration (0.5±0.50 mg/kg) was found in the shoot of Tithonia diversifolia planted in crude oil-polluted soil while the highest concentration (13.50±0.50 mg/kg) was found in the shoot of Tithonia diversifolia planted in Control soil. However, there was no statistical difference in the concentrations of Cd in the shoots of other test plants when compared with that found in the shoot of Tithonia diversifolia planted in natural soil except for Tithonia diversifolia planted in crude oil-polluted soil. Conversely, the concentration of Cd in the roots showed that Abelmoschus esculentus (23.50±1.50 mg/kg) and Zea mays (20.00±2.00 mg/kg) planted in Control soil had the highest Cd concentrations. The lowest concentrations of Cd were found in the roots of Tithonia diversifolia and Abelmoschus esculentus planted in crude oil-polluted soil but their means were statistically the same with the root of Zea mays planted in crude oil-polluted soil and Amaranthus hybridus planted in both soils. At the same time, initial concentration of Cd in the soil was 125 mg/kg and this was reduced to residual concentrations that varied between 4.00 mg/kg and 17 mg/kg which represented 96.8% and 86.4% reduction. The test plants were able to reduce Cd both in crude oil polluted and Control soils hence are good phytoextractors of Cd.

The present study classifies Tithonia diversifolia, Abelmoschus esculentus, Amaranthus hybridus and Zea mays as cadmium accumulators. These species were able to phytoextract Cd above the permissible level. This could be because of the organic matter content of the soil and acidic nature of the soil. The permissible limit of Cadmium in plants, recommended by WHO is 0.02 mg/kg. The maximum permissible limit of Cd in the soil according to SEPA of China is 0.6 mg/kg. The concentration of cadmium in the soil sample used was above maximum permissible limit (MPL) (0.6 mg/Kg) (SEPA, 1995). Voogt et. al., (1980) upheld that Cd can be taken up by plant such as maize, spinach, wheat and rice. It is capable of accumulating in food chains and its uptake is irrevocable and its excretion is very slow, it is therefore very toxic in nature. Uba et al. (2008) in their assessment of heavy metals bioavailability discovered that extractable Cadmium was found to be above the critical permissible concentration of 3.0 mg/kg. The findings in this study corroborated the work of Egberongbe (2010) who reported that Tithonia diversifolia seedlings absorbed Cd and Pb in polluted soils, and the contents in the root were more than the contents in the shoot.

### Table 2: Concentration of Cr in the soils and plants used for the experiment

|          | Amaranthus hybridus | Tithonia diversifolia | Abelmoschus esculentus | Zea mays |
|----------|---------------------|-----------------------|------------------------|----------|
| A        | 125.00              | 125.00                | 125.00                 | 125.00   |
| B        | 8.00±0.50           | 18.00±0.50            | 20.00±0.00             | 30.00±0.00 |
| C        | 10.00±0.00          | 12.00±0.00            | 15.00±0.00             | 20.00±0.00 |
| D        | 5.00±0.00           | 10.00±0.00            | 10.00±0.00             | 15.00±0.00 |
| E        | 2.00±0.00           | 5.00±0.00             | 5.00±0.00              | 10.00±0.00 |
| F        | 1.00±0.00           | 2.00±0.00             | 2.00±0.00              | 4.00±0.00  |
| G        | 0.50±0.00           | 1.00±0.00             | 1.00±0.00              | 2.00±0.00  |

Values represent mean ± standard deviation. Values with the same letter along the column are not significantly different at p≤0.05.

Table 4 shows the uptake responses of Tithonia diversifolia, Zea mays, Abelmoschus esculentus and Amaranthus hybridus to Pb in crude oil-polluted and Control soils. Pb concentration was maximum at 109.00±3.00 mg/kg in the shoots of T. diversifolia planted in crude oil-polluted soil and minimum (25.50±5.50 mg/kg) in the shoot of Z. mays planted in Control soil. However, all the experimental plants planted in crude oil-polluted soil had statistically the same Pb concentration in their shoots.

The concentration of Pb in the roots of the test plants was statistically higher in the roots of Tithonia diversifolia, Zea mays, and Abelmoschus esculentus planted in Control soil.

OLAWEPO, GK; OGUNKUNLE, CO; FATOBAY, PO; ANIMASAUN, DA; BEN-UWABOR, PO; DANZAKI, MM
The range of Pb concentration in the roots of the plants was found to be from 116±5.00 mg/kg (in *Amaranthus hybridus* planted in Control soil) to 27.50±0.50 mg/kg (in *Zea mays* planted in Control soil). Furthermore, by the interaction of the roots of the plants with the polluted soils, the initial concentration of Pb in the soil was 625 mg/kg and this had been reduced by each plant to between 39.00±13.00 mg/kg to 69.00±8.00 mg/kg which represented 93.76% and 88.96% reduction respectively. *Tithonia diversifolia* had the highest Pb reduction in crude oil-polluted soil followed by *Abelmoschus esculentus* in crude oil-polluted soil which was the same statistically (p≤0.05) with that of *Zea mays* in Control soil. Generally, the experimental plants were able to reduce the concentration of Pb in the soil. Lead (Pb) is not essential and also toxic to plants. Pb is believed to be the metal of least bioavailability and the most highly accumulated metal in root tissue while Pb shoot accumulation is much lower in most plant species (Kabata-Pendias, 2001). This is not in agreement with the results obtained from the plants used in this study as there was no significant difference in the Pb concentration obtained in the root and shoot of the plant species. This may be as a result of the pH or the difference in the nature of the soils used. Pb translocation and uptake studies showed that Pb is mobile within the plant under certain conditions such as the nature of the plant (Meers et al., 2005). Moreover, Blaylock and Huang (2000) reported that shoot Pb concentrations reached a value similar to the concentration found in intact roots of the same species, when it is immersed in a nutrient solution containing Pb. John (2013) observed the Pb concentrations of the sunflower and mustard. The Pb and Cd concentrations in the shoots compared to the roots were about 54 % and 30 % respectively. He attributed this to the high insolubility of Pb and that it tends to form highly stable adsorption complexes. John (2013) reported that *Helianthus annuus* (Sunflowers) had shoot Pb concentrations to be 60 mg/kg while *Brassica juncea* (Indian mustard) plants had 35 mg/kg, and the average dry weight for sunflowers was 0.60 g, while mustard dry weight was 0.40 g and obtained no significant differences in dry weight between treatments used. The concentration of Pb obtained in his work is similar to those obtained in this study.

### Table 4: Concentration of Pb in the soils and plants used for the experiment

|                | *Amaranthus hybridus* | *Tithonia diversifolia* | *Abelmoschus esculentus* | *Zea mays* |
|----------------|------------------------|-------------------------|--------------------------|-----------|
| A              | 625.00±0.00            | 625.00±0.00             | 625.00±0.00              |           |
| B              | 40.50±0.79             | 47.50±2.12              | 40.00±0.00               | 40.00±0.00 |
| C              | 69.00±11.31            | 39.00±18.38             | 67.50±6.36               | 62.00±5.65 |
| D              | 66.50±7.77             | 109.00±4.24             | 93.50±17.67              | 99.00±1.41 |
| E              | 38.00±9.69             | 104.50±4.94             | 99.50±12.82              | 102.50±6.36 |
| F              | 98.00±2.82             | 73.50±10.60             | 79.00±15.55              | 75.00±7.77 |
| G              | 116.00±7.07            | 78.50±9.19              | 86.00±36.76             | 27.50±0.70 |

Values represent mean ± standard deviation. Values with the same letter along the column are not significantly different at p≤0.05

The residual concentration of Ni in the shoots and roots of the experimental plants and crude oil-polluted and Control soil are shown in Table 5. The concentrations of Ni in the shoots varied from 30.00±30.50 mg/kg to 114.50±8.50 mg/kg. The highest concentration numerically, was found in *Tithonia diversifolia* planted in crude oil-polluted soil while the lowest was found in shoot of *Abelmoschus esculentus* planted in Control soil. Statistically, there was no significant difference in the concentrations of Ni in the selected plants at p≤0.05. Also in the roots of the selected plants, Ni was highest in *T. diversifolia* planted in crude oil-polluted soil followed by *Zea mays* planted in crude oil-polluted soil. The residual concentration of Ni was highest (150.50 mg/kg) in *Tithonia diversifolia* planted in crude oil-polluted soil and lowest in Control soil of *Amaranthus hybridus* (57.50 mg/kg). The initial soil Ni concentration of 1062.5 mg/kg was reduced by the plants by 85.84% (by *Tithonia diversifolia* planted in crude oil-polluted soil) to 94.59% (by *Amaranthus hybridus* planted in Control soil). In general, the selected plants were able to reduce the level of Ni significantly in the polluted soils. Nickel (Ni) has been considered to be an essential trace element for human and animal health (Khaoria, 2012). Prasad (2004) in his work observed that Nickel concentration in non-polluted soils to be between 5-50 mg/ kg, and the plants between 0.4-3 mg/ kg. The permissible limit of Nickel in plants, recommended by WHO, is 10 mg/kg. The maximum permissible limit of Ni according to SEPA of China is 60 mg/kg. The concentration of Nickel in pretreated soil was above maximum permissible limit by SEPA (1995) i.e. 60 mg/kg. Chen and Cutright (2001) reported that sunflower (Asteraceae) can also be utilized for the removal of Cd, Cr, and Ni in polluted soil. The present study classify *Tithonia diversifolia*, *Abelmoschus esculentus*, *Amaranthus hybridus* and *Zea mays* as Ni accumulators. The least Cd concentration (0.5±0.50 mg/kg) was found in the shoot of *Tithonia diversifolia* planted in crude oil-polluted soil while the highest concentration (13.50±0.50 mg/kg) was found in the shoot of *Tithonia diversifolia* planted in Control soil.

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Conclusion: In this study, four plant species namely *Tithonia diversifolia*, *Abelmoschus esculentus*, *Amaranthus hybridus* and *Zea mays* against five heavy metals, namely copper (Cu), lead (Pb), chromium (Cr), cadmium (Cd) and nickel (Ni) were evaluated based on the criteria stated. On the basis of the results gotten, the plants could be classified as accumulators of heavy metals.

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Table 5: Concentration of Ni in the soils and plants used for the experiment

| Soil Type          | Ni (mg/kg) | Soil Type          | Ni (mg/kg) |
|--------------------|------------|--------------------|------------|
| *Amaranthus hybridus* | 1062.50    | *Tithonia diversifolia* | 1062.50    |
| *Abelmoschus esculentus* | 1062.50    | *Zea mays* | 1062.50    |

Values represent mean ± standard deviation. Values with the same letter along the column are not significantly different at p<0.05.

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