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Heavy Metal Contents Of *Manihot Esculenta* Crantz Seedlings Grown on Crude Oil Contaminated Soil Amended With Bacteria Consortium Of *Pseudomonas* Sp And *Bacillus* Sp

S. Habila[1] and L. A. Akonye[2]
[1]*Department of Plant Science and Technology, Faculty of Natural Sciences, university of Jos Nigeria, P.M.B 2084, Jos, Plateau State Nigeria.*
[2]*Department of Plant Science and Biotechnology, Faculty of Biological Science, College of Natural and Applied Sciences, University of Port Harcourt, P.M.B.5323, Choba Port Harcourt, Rivers State, Nigeria.*

Abstract: *The heavy metal contents of Manihot esculenta seedlings grown in crude oil contaminated soil were investigated at the Botanical garden of the Department Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The treatments comprised of 0.00 % crude oil +cassava; 0.00 % crude oil + cassava + consortium; 10.00 % crude oil (w/w) + cassava, and 10.00 %crude oil (w/w) + Cassava + consortium. The crude oil was applied to the soil 12 days before the stems of Manihot esculenta were planted. The cassava stems in the treatments with the bacterial consortium was inoculated by dipping into the bacterial solution for one minute before they were planted. The experiment was arranged in a completely randomized design with five replicates. The results showed that crude oil contamination of soil had significant (P<0.05) effects of increasing the contents of heavy metal (including Aluminium, Iron, Copper, and Zinc) of the test plant when compared with values obtained for plants from the uncontaminated soil. It was also observed that the bacterial consortium significantly (P<0.05) enhanced the uptake of the heavy metals when compared with values obtained for seedlings from the non-inoculated stems. This study has shown that crude oil contamination of soil can lead to a gradual build up of heavy metals which when absorbed are capable of making the cassava shoots and roots potentially toxic and harmful if consumed as food by man and his animals.*
Keywords: *Crude oil-contaminated soil–Heavy metals, bacterial consortium, Manihot esculenta*

I. Introduction

Crude oil pollution is an inevitable consequence of oil exploration and exploitation activities both in oil producing and consuming areas due mainly to accidental discharge, human error, destruction of pipelines, transportation, natural causes among others (Akonye and Onwudiwe, 2004; Agbogidi *et al*., 2007). Crude oil has a complex mixture of thousands of hydrocarbons and related compounds that are toxic to various life forms (Agbogidi *et al*., 2007). Some of these substances are heavy metals which are stable in the environment. Heavy metals pollution of soil has been shown to be deleterious to plant germination, growth and yield (Agbogidi and Ilondu, 2012).

Phytoremediation is the technical term used to describe the treatment of environmental problems through the use of plants. Certain plants are able to extract hazardous substances such as arsenic, lead and uranium from soil and water (Cunningham and Lee, 1995). One example is Alpine pennycress, a plant which naturally accumulates high levels of cadmium and zinc from the environment (Srujana and Anisa, 2011). In Nigeria, the use of cassava plant in phytoremediation have been reported (Akonye and Onwudiwe, 2004; Habila *et al*., 2012). Microorganisms are added to the polluted environment in order to reinforce natural biological processes. If a treatability study shows no degradation in contaminated groundwater or soils, then inoculation with strains known to be capable of degrading the contaminants may be helpful. This process increases the reactive enzyme concentration within the bioremediation system and subsequently may increase contaminant degradation rates over the non-augmented rates, at least initially after inoculation ((Hayman and DuPont, 2001; Xin *et al*., 2009). Many microbes are described to have the genetic tools to mineralize recalcitrant pollutants such as poly aromatic hydrocarbons, chlorinated aliphatics and aromatics, nitroaromatics, and long-chain alkanes (Xin *et al*., 2009).). Although, studies exist on the heavy metal concentration of plants as affected by petroleum products and the use of microorganisms in bioremediation, there is scarcity of documented materials on *Manihot esculenta* grown on crude oil contaminated soil amended with a bacterial consortium of *Pseudomonas* sp and *Bacillus* sp. It is on this premise that this study was embarked upon in order to investigate the effects of crude oil contamination on heavy metal contents of cassava seedlings and the role of the bacteria consortium in the uptake of the metals by the plant.

II. Materials And Methods

The study was carried out at the Botanical Garden at the University Park of the University of Port Harcourt, Abuja Campus, Choba Port Harcourt, Rivers State. The cassava stem cuttings of the sweet variety 30572 were procured at the Rivers State Agricultural Development Program (ADP) Head Quarters, Rumuokoro, Obia/Akpo Local Government Council, Rivers state. The crude oil and the pure cultures of the bacteria grown on Nutrient Agar in Petri dishes were collected from Nigerian National Petroleum Corporation (NNPC) Zonal Office, Moscow Road, Port Harcourt Rivers state. Polyethylene bags used in the study were procured at the Mile 3 market in Port Harcourt, Rivers state.

The experiment was laid in a completely randomized design with five replications. The treatment combinations comprised of two levels of crude oil pollution (0% and 10% pollution) as follows:

Unpolluted soil + cassava
10% crude oil polluted soil + cassava
Unpolluted soil + cassava + inoculum
10% crude oil polluted soil + cassava + inoculum

10 kg of the unpolluted soil sample was weighed and properly mixed with 1 liter of crude oil (representing 10% pollution concentration). 2 kg of both the unpolluted and the polluted soil samples were measured and put in 20 polyethylene bags each. The experimental set up was allowed to stand for a period of twelve days to ensure homogeneity between the crude oil and soil sample used (Habila et al., 2012). A wire loop was sterilized by passing it through the flame from a Bunsen burner before collecting the microorganisms from the separate pure cultures of Pseudomonas sp and Bacillus sp. A loopful of each pure culture of the bacteria was placed in a cylinder containing 100 mls of sterile deionized water and the mixture was stirred gently but thoroughly to obtain the microbial suspension, hereafter referred to as the bacterial consortium of *Pseudomonas* sp and *Bacillus* sp.

Planting was carried out 12 days after crude application. The cassava stems used were on the average 14cm long, and were carefully selected to ensure uniformity. The edges (that is, the upper and lower edge) of the stem were not used. Incisions were made on both sides of the cassava stems and the microorganisms were applied literally by dipping the lower end of the stems into the bacterial consortium (inoculum). The incision was made on the lower end of the stem about 4cm. The inoculated cassava stems were planted in the respective polyethylene bags according to the treatment combinations.

At the twelfth week, the seedlings were harvested and separated into shoots and roots. These were oven-dried at 85^{o}C for 24 hours to get their dry weights following the procedure of Agbogidi and Eshegbeyi (2006). The plant tissues were ground to a powdered state. 1 g of each of the powder was weighted into a conical flask for wet-ashing before subjecting them to analysis for heavy metals by atomic absorption spectrophotometer at the Nigeria National Petroleum Corporation (NNPC), Research and Development analytical chemistry laboratory in Port Harcourt, Rivers state. Composite soil samples were collected from 0-2cm depths prior to treatment application and after harvest. The samples were used to determine soil heavy metal concentrations. Data obtained were subjected to analysis of variance and the significant means were separated with the Duncan's multiple range tests using SAS (2005).

III. Results And Discussion

There was an increase in the concentration of heavy metals in soils treated with crude oil when compared with soils without crude oil treatment (Figure 1). The level of heavy metals viz: aluminium, copper, iron and zinc in soils of oil impact were significantly ($P<0.05$) higher than the uncontaminated (Figure 2). Plant tissue analysis (Figures 3 and 4) also showed significantly higher amounts of heavy metals compared with tissues obtained from treatments not contaminated with crude oil. Heavy metal building in soils polluted with crude oil and its various products has been previously reported by Onyeike *et al*. (2000), Akubugwo et al. (2009), Agbogidi and Egbuchua (2010), Agbogidi and Ilondu (2012), who noted that trace elements abound in crude oil polluted soils and they have negative effects on the growth and nutritional value of fruit plants (ripe guava fruits). Heavy metals such as Cs, Cd, Pb, Al, Sn, Hg, Zn, Fe and Al are reported to be accumulated in plants by non-specific physico-chemical interactions as well as specific mechanisms of sequestration or transport (Misra, 1992; Gadd, *et al*., 1995).

It was also observed that plant tissues from the treatment amended with the bacterial consortium had significantly ($P<0.05$) higher heavy metal concentrations than those not amended (Figures 3 and 4). The inoculation of the cassava stem cuttings with the bacterial consortium seemed to have facilitated the oxidation and uptake of the heavy metals by the plant. Biological activity accounts for a large number of the environmental sinks for toxic metals whether derived from natural or anthropogenic sources (Habila *et al*., 2012). This is in harmony with the reports of Al-Merrill et al. (2009), Xin *et al*. (2009) and Habila *et al*. (2012) who reported that microorganisms has the ability to transform metals and metalloids species by oxidation or reduction into soluble forms that could be subsequently absorbed by plants.

The presence of metals in the uncontaminated soil indicates that heavy metals naturally occur in the environment and natural ecosystem (Salihu *et al*., 2012). This further shows that they are natural components of the ecological system associated with one or more functions which may not be harmful at reduced concentrations but could become toxic at increased concentrations. This finding is in harmony with the reports of Akubugwo *et al*. (2009). Heavy metals have also been shown to affect the physical, chemical, biological and microbial properties of soils (Akubugwo *et al*., 2009). Whenever heavy metals are absorbed by plants they are capable of making the plants potentially toxic and harmful to man as well as his animals if consumed as food (Agbogidi and Egbuchua, 2010; Habila *et al*., 2012). Like the trace elements, some of the heavy metals like zinc, copper selenium, are essential for the maintenance of body metabolism at moderate concentrations, but they are however, poisonous when in higher concentrations. Habila *et al*. (2012) noted that poor growth of crop plants in higher levels of oil treatment was primarily due to the toxic effect of heavy metals or mineral uptake. Agbogidi and Ilondu (2012) also maintained that poor performance of plants in crude oil impacted soil could be due to either synergistic or antagonistic relations.

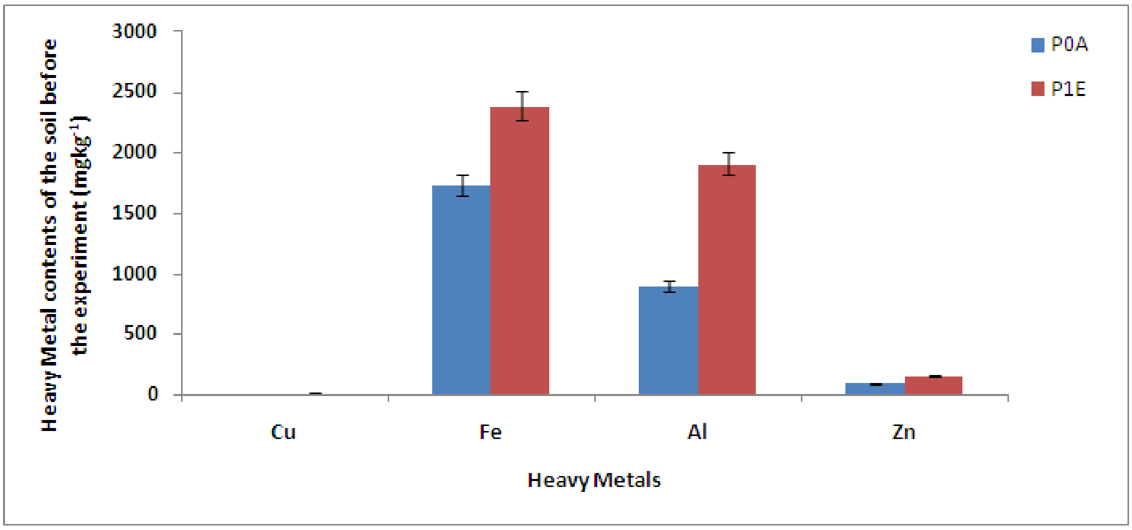

Figure 1: Heavy metal content of the soil before crude oil application (P_0A) and after crude application (P_1E)

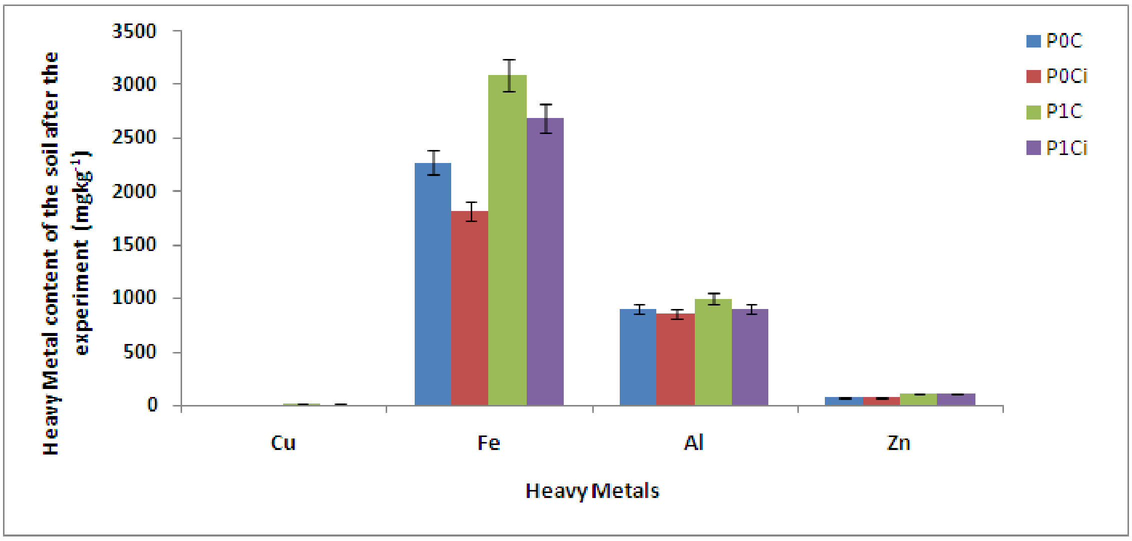

Figure 2: Effect of crude oil contamination on heavy metal contents of the soil after the experiment. P_0C (0% pollution + cassava), P_0C_i (0% pollution + cassava + inoculum), P_1C (10% pollution + cassava) and P_1C_i (10% pollution + cassava + inoculum)

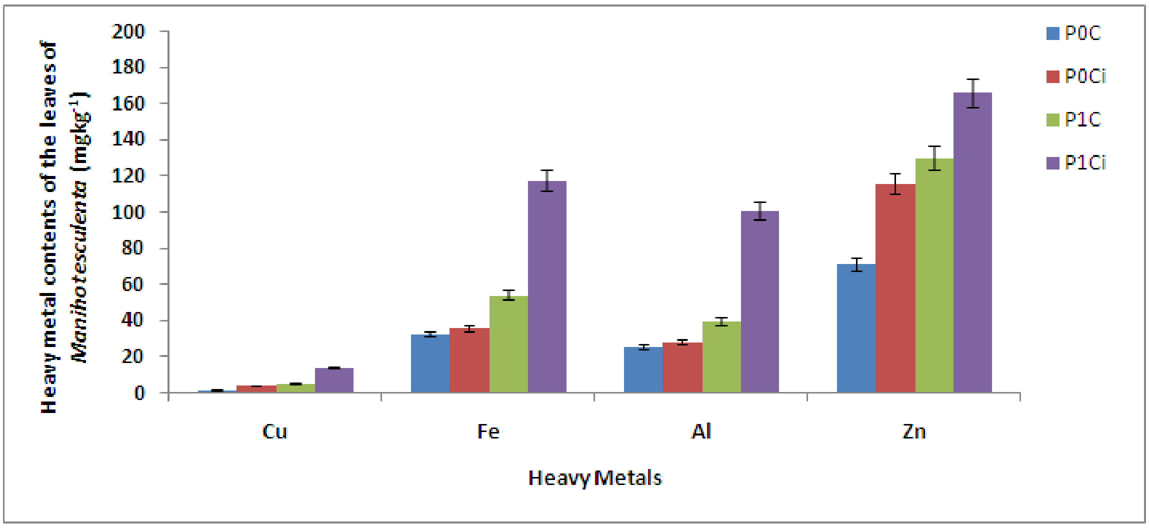

Figure 3: Effect of crude oil contamination on the heavy metal contents of the leaves of M. esculenta after the experiment. P_0C (0% pollution + cassava), P_0C_i (0% pollution + cassava + inoculum), P_1C (10% pollution + cassava) and P_1C_i (10% pollution + cassava + inoculum)

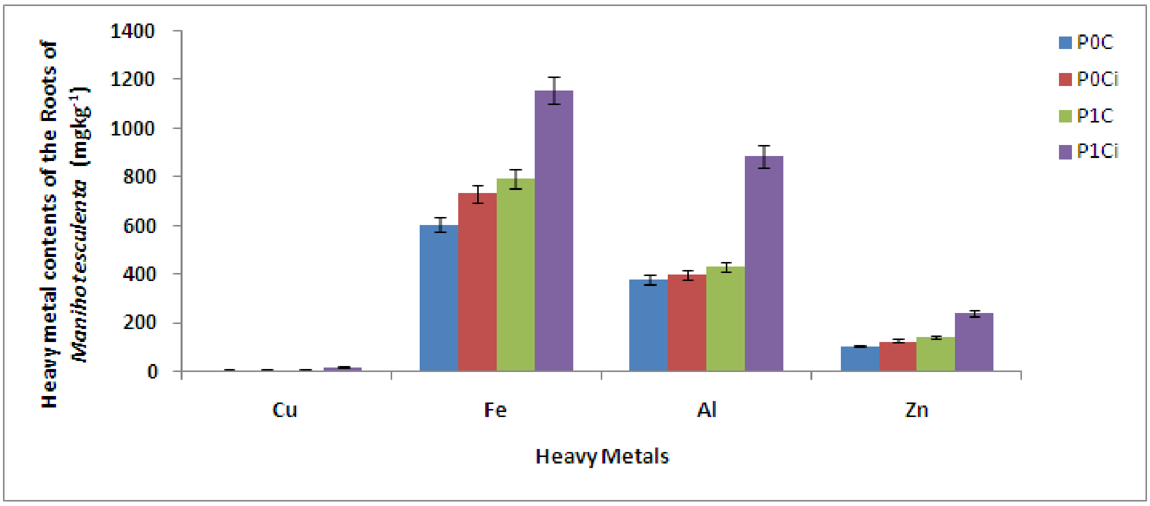

Figure 4: Effect of crude oil contamination on heavy metal contents of the roots of M. esculenta. P_0C (0% pollution + cassava), P_0C_i (0% pollution + cassava + inoculums), P_1C (10% pollution + cassava) and P_1C_i (10% pollution + cassava + inoculum)

IV. Conclusion

The present study investigated the heavy metal contents of Manihot esculenta seedlings grown in soil contaminated with crude oil in Port Harcourt, River State, Nigeria. The result indicated that crude oil contamination has a significant effect of increasing the concentrations of heavy metals including copper, aluminium, iron and zinc when compared with seedlings from the uncontaminated soils. This study has shown that plant species including Manihot esculenta can bio accumulate heavy metals which when ingested by man and his animal can constitute health risks. It was also observed that bacterial consortium of Pseudomonas sp and Bacillus sp enhanced the degradation and uptake of the heavy metals by Manihot esculenta.

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