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Evaluation of okro (*Abelmoschus esculentus* L. Moench.) exposed to paint waste contaminated soil for growth, ascorbic acid and metal concentration

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ABSTRACT: Six (6) accessions of okro (*Abelmoschus esculentus* L. Moench.) were evaluated for growth in paint waste contaminated soil. Estimates of number of flower buds, flowers formed, fruits and days to 50% flowering were recorded. Others were fresh and dry weights, Fe and Pb concentrations in plant parts (root, stem and fruits), and ascorbic acid content of dry fruits. Soil chemical analysis revealed increased levels of pH, organic C, K, Ca, Na and Al. Generally, the contaminant decreased fresh weight of fruits, whole plants, average number of flower buds and flowers formed. In two of the six accessions, the flowering occurred earlier when compared with control and others experienced delay in contaminated soil. Also, the levels of ascorbic acid content of dry fruits were higher in three accessions. *A. esculentus* demonstrated the ability to take up Fe and Pb from soil and their translocation up to the fruits from roots. Fe concentration in plant tissues were higher in the plant grown in contaminated soil. The least concentrations of Fe in tissues were observed in the fruits. The Pb concentration in plant tissues of plants grown in both control and contaminated soil were far less than the soil concentration observed. Pb was detected in all tissues analysed (root, stem and fruit) indicating its translocation from the root through stem to fruits.

Key Words: Okro; *Abelmoschus esculentus*; Contaminated soil; Paint waste; Ascorbic acid; Environmental pollution.

Introduction

Paints are inorganic substances produced for coating construction materials, buildings, automobiles and electrical appliances, etc. The processes of utilizing and factory disposal processes allow paints and its derivatives as a solid, liquid and gaseous wastes escape into the surrounding environment especially into soil and water bodies. Paint wastes serve a veritable source of metal contamination to soil and water bodies (EPA/ROC, 1994). So its production and uses are sources of producing hazardous and non hazardous solid, liquid and gaseous particulate waste to soil (EPA/ROC, 1994). Particulate wastes commonly identified with paint wastes (water and solvent base) contain heavy metals such as Pb, Cr, Hg, and Zn (Anonymous 2002).

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These contaminants from paints enter into the environment especially natural aquatic system basically due to improper disposal processes. In addition, water base paint contains biodegradable substances such as surfactants and cellulose thickeners. These substances when broken down cause reduction of oxygen level in the aquatic environment. This is deleterious to aquatic lives. Sebastiaini *et al* (2004) recognized various means by which land is contaminated with heavy metals toxicants. These include urban activities, agricultural operations and industrial processing. The industrial processing adds its quota from metalliferous mining, tannery, smelting and paint factories. Resulting wastes from these processes are usually disposed in landfills. This solution negatively affects human health, flora and fauna around the landfills (Lasat, 2002). Consequently, the productivity of crops plants and livestock is affected. The management of heavy metal contaminated water or bio-solids is becoming challenging as stricter regulations are imposed to improve water and soil fertility (Sebastiani *et al*, 2004). In Nigeria, many of the paint factories need a sustainable alternative to disposal of paint waste in open, vacant and fallow plots and abandoned farmlands.

In this study, the effect of paint waste (water based) contaminated soil on biomass (dry and fresh weights), yield (flower buds, flowers, fruit and number of days to flowering), ascorbic acid content of fruit and concentration of iron (Fe) and lead (Pb) in plant parts of six local accessions of *Abelmoschus esculentus* (L) Moench. (Okro; dwarf type) were determined. Okro is a vegetable commonly cultivated in various agricultural production systems especially in home gardens in Nigeria. It is grown for its fruits, seeds, floral parts, stem and leaves. In these forms, they are consumed when immature and beaten into a gelatinous soup. The soup is used for consuming coarse textured starchy food commonly the diet of the people. It contributes to protein, mineral, vitamins and source of roughage in human diet. A 100g edible portion of okro has been reported to contain 90g of water, 2g of protein, 6g of carbohydrate,1g of fibre with traces of fat, vitamins A and C with traces of vitamin B, calcium, phosphorus and iron (Siemonsma and Hamon, 2002). Energy value of 140kg/100g; a comparable value to FAO recommended nutrient requirement is also reported. The seeds contain about 20% protein similar in amino acid composition of soya bean and 20% oil similar in fatty acid composition to cotton seed (Schipper, 2000). The oil fraction in seed includes oleic acid and linoleic acid. Okro also has medicinal and industrial potentials. In medicine, the mucilage is used as plasma replacement or blood volume expander (Siemonsma and Hamon, 2002). Leaves are used as a basis for poultices, as an emollient, surdorific antiscorbutic and also to treat dysuria (Siemonsma and Hamon, 2002). The fibres present are suitable for spinning into rope and in the manufacture of paper and cardboard. Locally in Nigeria, the fibres are braid together and used for fish line, game traps and sponges.

Materials and Methods

Plant Material

Dry capsules (fruits) of *Abelmoschus esculentus* (L) Moench. (dwarf species) were obtained from farmers in Ugoneki village near Benin City. The six (6) local accessions were identified based on fruit morphological characters outlined by Schippers (2000) which included length, tip curved or pointed, absence or presence of indentation at the base, base protruding or flat, number of ridges, ridges split above or below the dented base e.t.c. The six accessions were labeled as

S/N	Accession	Origin/ Source	Latitude & Longitude
1.	DEV/IKP-OHA/RAM 007 (DIOR – 007)	Ugoneki	06°13′ S, 06° 06′W
2.	DEV/IKP-OHA/RAM 008 (DIOR – 008)	Ugoneki	06°13′ S, 06° 06′W
3.	DEV/IKP-OHA/RAM 009 (DIOR – 009)	Ugoneki	06°13′ S, 06° 06′W
4.	DEV/IKP-OHA/RAM 010 (DIOR – 010)	Ugoneki	06°13′ S, 06° 06′W
5.	DEV/IKP-OHA/RAM 011 (DIOR – 011)	Ugoneki	06°13′ S, 06° 06′W
6.	DEV/IKP-OHA/RAM 012 (DIOR – 012)	Ugoneki	06°13′ S, 06° 06′W

Paint Waste Collection

The paint waste (water based) in solid form was obtained from a paint factory in Benin City.

Preparation and Contamination (Treatment) of Soil Samples

The soil used in the study was obtained as a composite (pooled) sample of top -soil (0 – 20 cm depth), from the University of Benin Teaching and Research Farm, Benin City. Eighty (80kg) kilogram quantity of soil was spread evenly on a flat surface as a bed. Paint waste (water-based, solid form) that was ground into powder 3.5 Kg, was evenly spread on top of the soil. The paint waste and soil were thoroughly mixed and spread out on the bed. It was then watered twice for a week and allowed to dry for another two weeks before placement in experimental pots (2kg each). The control soil treatment was prepared the same way except that paint waste was not added. A total of seventy two pots including control were prepared and observed under field condition.

Soil Chemical Determination

Samples of paint waste contaminated and uncontaminated (control) soil were analysed. The soil samples were air dried for one week and sieved using 180-µm sieve. One gram (1g) of soil samples was subjected to wet digestion using HF (hydrofluoric) acid for total elemental content. Standard methods were followed for the determination of N, organic C, P, Na, K and Mg. Elements like Zn, Fe, Cu, Ca, Mn, Cd, Co, Al, Cr, Ni and Pb were determined using AAS (flame atomic absorption spectroscopy). Soil pH of the samples were determined in distilled water and 0.01 M $CaCl_2$ in ratio 1:3 (soil : solution medium).

Planting and Thinning

After carrying out viability test, three seeds were sown in each pot at a depth of approximately 3 cm. The plants were watered once daily. The experiment was conducted between December 2003 and March 2004. The six accessions (6) had six replications each in control and paint waste contaminated soil. Thinning to one plant per pot was done after three weeks of planting.

Agronomic Characters Measurement

Number of Flower buds formed: Six weeks after planting (WAP), flower buds were observed in okro plants grown. The number of flower buds formed by each plant was recorded every fifth day till termination of experiment. This was used to calculate the average number of flower buds produced per plant of each accession after twelve weeks of growth.

Number of Flowers formed: The flower buds opened into flowers at an average of 10 –11 days after formation. The number of flowers formed by each plant was counted and recorded to give the average number of flowers produced by plants of each accession.

Number of Fruits formed: The number of fruits formed was obtained by counting fruits on plants of each accession. This was used to calculate the average number of fruits produced by plants of each accession.

Number of days of 50% flowering: The number of days taken for 50% of plants of each accession to produce flowers was recorded for plants in both control and contaminated soils.

Fresh and Dry Weights Determination

Thirteen (13) weeks after planting (WAP), the plants were harvested for fresh weight determination. Fresh weights were measured using a top load weighing balance. The dry weights were determined after drying in an oven at 70˚C for 7 days until a constant weight was obtained. Fresh and dry weights of harvested fruits were also determined. Oven drying of fruits was done at 50˚C for two days until a constant weight was obtained.

Fe and Pb determination in plant tissues (root, stem and fruit)

Fe and Pb concentration in plant tissues were carried out using dried plant parts (fruit, stem, leaves and roots) of each accession in control and contaminated soils. The plant parts were ground using an electric powered grinding machine. One gram (1g) of each ground sample was put in a 50 ml porcelain beaker and placed in a muffle furnace for ashing at 550˚C for 5 hours. The dry ashed sample was dissolved in 20% concentrated nitric acid (HNO_3) and warmed on a hot plate for 15 minutes and stirred with a glass rod to enable it dissolve. The solution was allowed to cool and made up to 50 ml with distilled water. Metal concentrations (Fe and Pb) in plant tissue samples were determined by flame atomic absorption spectroscopy (AAS).

Ascorbic acid determination in dry fruits

Ascorbic acid content in dry fruits harvested from the six accessions in both control and paint waste contaminated soils were determined by titrimetric method using phenol indophenol dye method (AOAC, 1984).

Statistical Analysis

Data collected were analyzed using GENSTAT. Where significant F values were obtained, differences between individual means were separated using Duncan Multiple Range test (Steele and Torie, 1980).

Results

Soil Chemical analysis

The chemical analysis of uncontaminated (control) and paint waste contaminated soil samples is reported in Table 1. The values obtained for organic carbon, K, Ca, Na, Al and Mn were higher in paint waste contaminated soil. The values obtained for N, P, Fe, Zn, Cu, Co, Cd and Pb were higher in uncontaminated (control) soil samples. Two metals, Cr and Ni, were undetected in control soil but present in paint waste contaminated soil. The pH reading recorded for the soil samples is shown in Table 2. The paint waste contamination increased the pH in both media (distilled water and 0.01 M $CaCl_2$ solution).

Plant Growth

Table 3 shows the average number of buds, flowers and fruits per plant and number of days to 50 % flowering. While Table 5 shows the fresh and dry weights of harvested plants and fruits.

Average number of buds: The values obtained were higher in plants grown in uncontaminated soil as compared to paint waste contaminated soil. The highest and least values obtained per plant were 8 and 4 respectively. The result showed that some accessions produced more flower buds than others.

Average number of flowers: The average number of flowers per plant observed was higher in plants grown in control soil. The highest and least values recorded were 5 and 2 respectively.

Average number of days to 50 % flowering: The data showed that 50 % flowering did not occur at the same time in all accessions considered. Four accessions attained 50 % flowering earlier in control soil than in paint waste contaminated soil. The differences in average number of days to 50 % flowering between plants in control and test soils were 1 – 3 days.

Fresh and dry weights of fruits: Data obtained for fresh and dry weights of fruits for each accession is shown in Table 5. The highest and least fresh weights of fruit were obtained in accessions DIOR 012 and DIOR 007 respectively in control soil. For paint waste contaminated soil, the highest and least values were obtained in accessions DIOR 010 and DIOR 012 respectively. The mean dry weights obtained in control soil ranged from 1.93g – 2.69 g, while for contaminated soil was 1.04 g – 2.08g.

Table 1: Chemical analysis of soil samples (uncontaminated and paint waste contaminated) before planting

Elements	%C	%N	P	K	Ca	Na	Al	Fe	Zn	Cu	Mn	Co	Cd	Pb	Cr	Ni
			◄--- ppm ---►													
Uncontaminated	0.85	0.60	135.01	642.5	868.3	682.5	5.0	1947	370.7	24.1	289.2	36.7	60.0	137.0	-	-
contaminated	2.43	0.50	105.0	707.6	918.3	884.5	15.0	1921	275.9	23.3	608.4	30.0	36.7	129.6	41.2	27.5

Table 2: Mean pH values of control and contaminated soil before planting

Soil sample	Medium	
	Distilled water	0.01M $CaCl_2$
Uncontaminated (control)	6.78	6.07
Contaminated	6.89	6.22

(soil : medium = 1:3)

Table 3: Average number of buds, flowers and fruits per plant, and number of days to 50% flowering of six local accessions of *A. esculentus* grown in paint waste contaminated soil

Accession	Average No. of flower buds / plant		Average No. of flowers / plant		Average No. of fruits harvested/ plant		Average No. of days to 50% flowering (DAP)	
	control	contaminated	control	contaminated	control	contaminated	control	contaminated
DIOR - 007	8	3	4	2	3	2	46	47
DIOR - 008	6	5	5	3	4	3	50	45
DIOR - 009	4	4	4	2	2	2	47	48
DIOR - 010	5	3	3	2	2	2	44	46
DIOR - 011	4	3	3	2	2	2	43	47
DIOR - 012	6	4	2	2	2	2	49	47

n = 6, DAP = days after planting

Table 4: Ascorbic acid content (mg/100g) of dried fruits of six local accessions of *A. esculentus* grown in paint waste contaminated soil

Accessions	Ascorbic acid (mg/100g) control	contaminated
DIOR - 007	1.95	3.39
DIOR - 008	2.13	0.80
DIOR - 009	1.58	3.41
DIOR - 010	2.48	7.12
DIOR - 011	3.28	2.79
DIOR - 012	1.79	1.51

Table 5: Fresh and dry weights (g) of harvested plants and fruits from six local accessions of *A. esculentus* grown in paint waste contaminated soil after 13 weeks of growth.

Accession No.	Treatment	Fruit fresh weight (g)	Fruit dry weight (g)	Plant fresh weight (g)	Plant dry weight (g)
DIOR 007	control	$10.59^{b,c,d} \pm 4.23$	$1.93^{a,b} \pm 0.96$	$15.92^{a} \pm 4.47$	$5.88^{b} \pm 1.82$
	contaminated	$7.89^{c,d,e,f} \pm 2.69$	$1.64^{b,c} \pm 0.69$	$11.52^{b,c} \pm 1.99$	$4.34^{c,d} \pm 0.75$
DIOR 008	control	$10.30^{b,c,d,e} \pm 4.51$	$2.20^{a,b} \pm 1.02$	$14.11^{a,b} \pm 4.80$	$4.96^{b,c} \pm 2.15$
	contaminated	$7.50^{d,e,f} \pm 3.18$	$2.08^{a,b} \pm 1.14$	$9.83^{c} \pm 3.89$	$2.91^{e} \pm 1.17$
DIOR 009	control	$14.21^{a} \pm 4.21$	$2.69^{a} \pm 0.80$	$16.31^{a} \pm 2.78$	$8.18^{a} \pm 1.76$
	contaminated	$7.19^{d,e,f} \pm 3.61$	$1.50^{b,c} \pm 0.99$	$10.45^{c} \pm 2.61$	$3.58^{d,e} \pm 1.00$
DIOR 010	control	$11.22^{a,b} \pm 3.52$	$2.22^{a,b} \pm 0.66$	$16.04^{a} \pm 3.65$	$5.33^{b,c} \pm 1.22$
	contaminated	$8.04^{c,d,e,f} \pm 3.77$	$1.04^{c} \pm 0.59$	$11.04^{c} \pm 2.61$	$3.57^{d,e} \pm 0.85$
DIOR 011	control	$11.61^{a,b} \pm 4.60$	$2.13^{a,b} \pm 0.89$	$15.45^{a} \pm 3.67$	$5.61^{b} \pm 1.20$
	contaminated	$6.98^{e,f} \pm 3.08$	$1.14^{c} \pm 0.37$	$10.50^{c} \pm 2.25$	$3.22^{d,e} \pm 0.74$
DIOR 012	control	$12.41^{a,b} \pm 4.43$	$2.42^{a} \pm 0.91$	$16.82^{a} \pm 3.65$	$5.46^{b,c} \pm 1.35$
	contaminated	$6.68^{f} \pm 3.16$	$1.08^{c} \pm 0.51$	$10.12^{c} \pm 2.83$	$3.70^{d,e} \pm 0.97$

Figures = Mean ± S.D., n=12 ; Figures in the same column with similar alphabets are not significantly different ($\alpha = 0.05$).

Fresh and dry weights of harvested plants: Data obtained for mean fresh weights of plants in control soil range from 14.11g – 16.82 g and in treated soil was 9.83 g – 11.54 g. Under control soil treatment, mean dry weights range from 4.96 g – 8.18 g and in treated soil, mean dry weights range from 2.91 g – 4.34 g.

Ascorbic acid content in dry fruits

Data obtained showed that the ascorbic acid content in dry fruits did not suggest a particular pattern (Table 4). The values showed that for three accessions, the fruits of plants grown in treated soil had higher ascorbic acid content than control.

Plant tissue concentration of metals – Iron and Lead

Figures 1 and 2 show the tissue concentration of Fe in plants grown in uncontaminated and paint waste contaminated soils (treatment) respectively.
Figures 3 and 4 show the tissue concentration of Pb in plants grown in uncontaminated and paint waste contaminated soils (treatment) respectively.

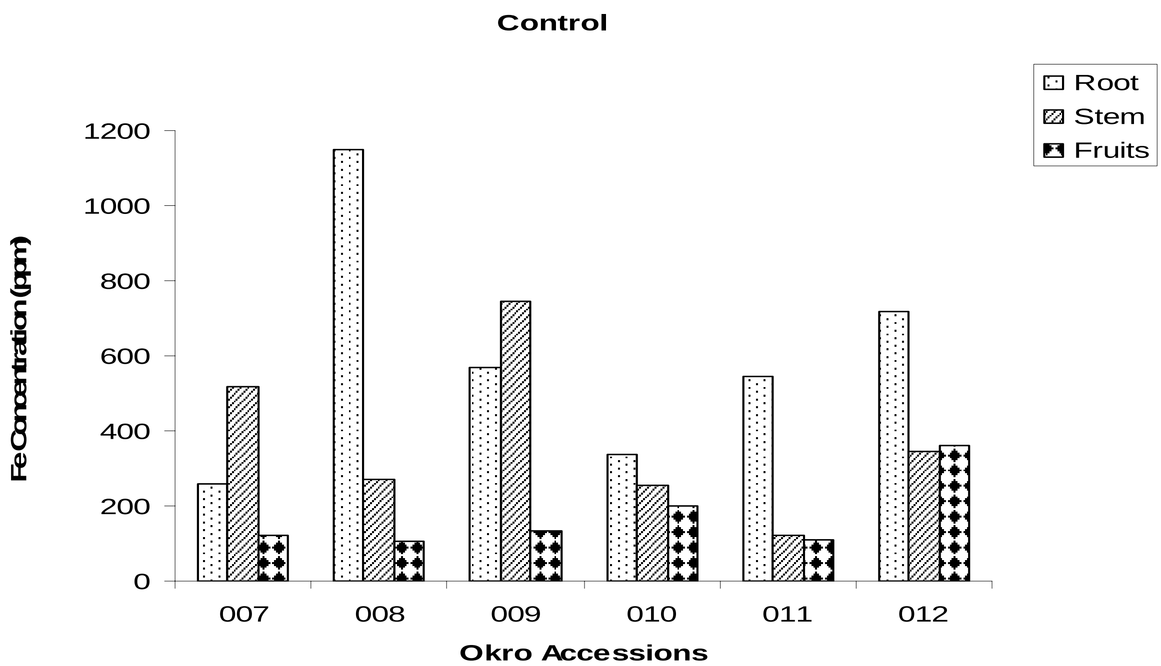

Figure 1: Fe concentration in tissues of plants grown in uncontaminated (control) soil

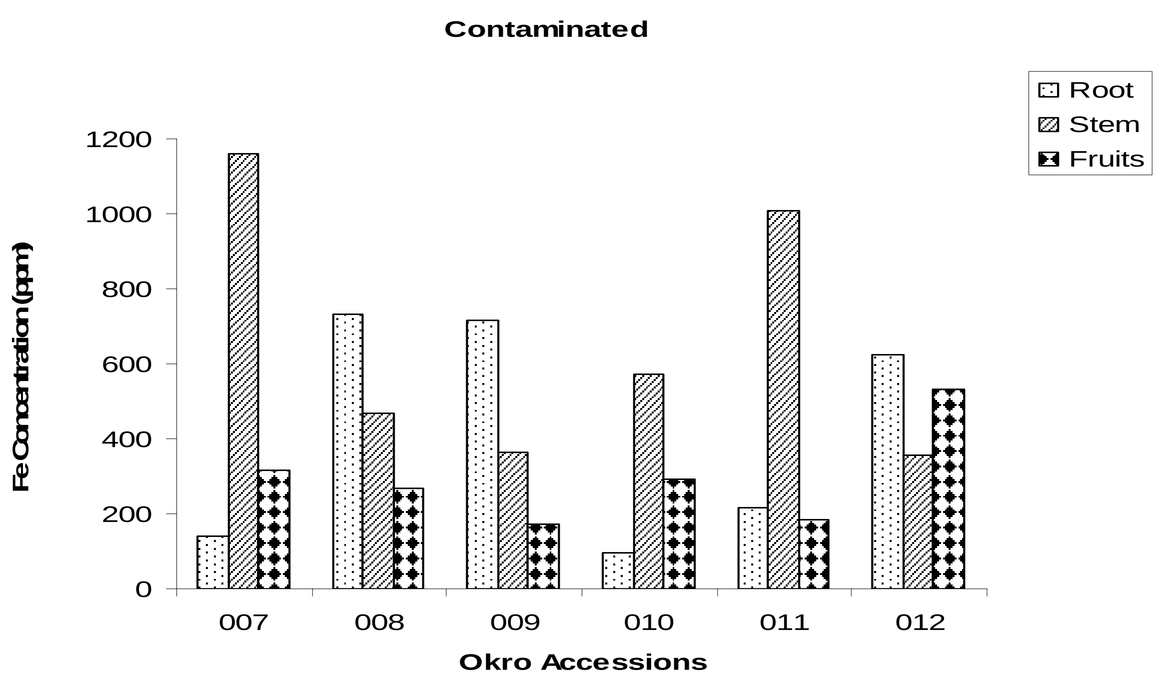

Figure 2: Fe concentration in tissues of plants grown in paint waste contaminated soil

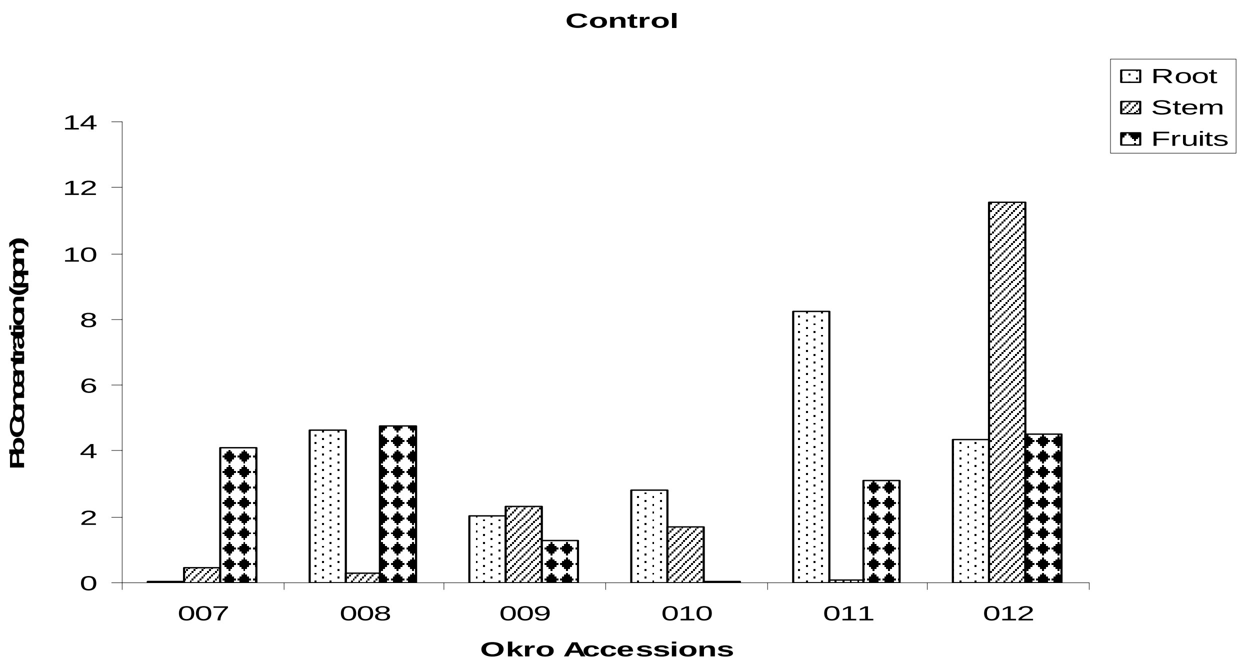

Figure 3: Pb concentration in tissues of plants grown in uncontaminated (control) soil

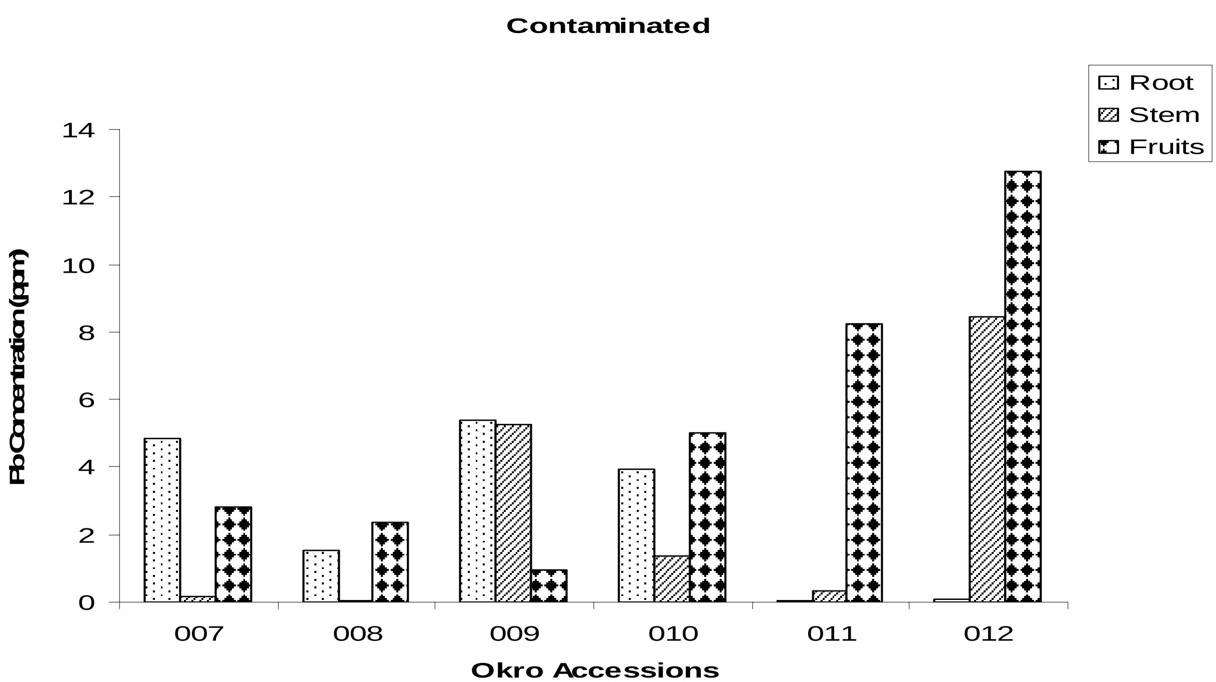

Figure 4: Pb concentration in tissues of plants grown in paint waste contaminated soil.

Discussion

The result of soil analysis for element concentrations in both control and paint waste contaminated soils (Table 1) indicated higher concentration of some elements such as Pb and Fe in control soil. Control and contaminated soil had pH ranges from 6 – 7. The contaminated soils had pH values closer to neutral than control soil treatments. Acidic conditions favour nutrient absorption and availability of some heavy metals (Wong *et. al.,* 2001).

Fresh weight of fruits of the six local accessions of *A. esculentus* exhibited a strong response to the treatment. The fruits from plants grown in control soil gave weight values that were significantly higher than those from plants in contaminated soil (P = 5%) and further comparison among the accessions showed significant differences. Higher percent moisture contents of fruits harvested were estimated for the control plants than for contaminated plants. The contaminant in the soil may be responsible for the low water content of the fruits harvested from plants grown in the contaminated soil. Similar significant differences were observed in fresh and dry weights of harvested plants in both soil types (Table 5) even though the control plants gave higher weight values.

The agronomic characters observed in this study showed, for example, that the average number of buds and flowers produced per plant grown on control soil is higher than those grown on paint waste contaminated soil (Table 3). From the data obtained, not all flower buds produced developed into flowers and then fruits as observed for plants in control and contaminated soils. Percentages of flower buds that developed into fruits on plants grown in control soil for all accessions were 38%, 67%, 50%, 40%, 50% and 34% for DIOR 007, 008, 009, 010, 011 and 012 respectively. It was observed that flower buds that failed to develop into fruits shriveled between the period of formation and appearance on the plant and fruit development. The cause of this is yet to be understood. Communication of this observation with other colleagues indicates that it is a common syndrome with *A. esculentus*. This syndrome has economic implication to the farmer. In addition, two accessions flowered earlier in contaminated soil. This may indicate that the stress produced by the contamination may have induced early flowering in some of the accessions. Abiotic stress has been reported to induce early flowering in plants (Vwioko and Fashemi, 2005). A comparison of the ascorbic acid content (mg/100g) fruits of plants grown on both control and contaminated soils does not show any significant difference (Table 4).

Iron is the fourth most abundant element in the earth crust (5%) and is unavailable to plants because of its ability to become insoluble (Lepp, 1981). Iron solubility is largely determined by the pH and oxidation-reduction potential of the substratum (Snowden and Wheeler, 1993). But the six local accessions of *A. esculentus* grown on both control and contaminated soils showed considerable uptake of Fe. Data obtained for tissue concentration of Fe were less than the soil concentration. It can be seen in Figures 1 & 3 that the concentration of Iron in fruits of plants grown on contaminated soil were higher than plants grown on control soil. For instance, in DIOR 007, Fe concentration in fruits of control plants was 38% of the Fe concentration in fruits of plants grown in contaminated soil. Also, the Fe concentration in fruit was 23% and 46% of Fe concentration in stem and root respectively, for plants (DIOR 007) in control soil. With reference to its concentration in fruits, the accessions can be divided to two groups. Group 1 includes DIOR 007 and 008 with 38-39% of values obtained for fruits of plants in contaminated soil. Group 2 include DIOR 009, 010, 011 and 012 with 60-69% of values obtained for fruits of plants in contaminated soil. Also, fruits seem to have accumulated less amount of iron than other plant tissues. Although, the pattern of absorption of Fe is not consistent in all the six local accessions, from the results we observe the ability of *A. esculentus* to take up Fe considerably from the paint waste contaminated soil and its translocation to the fruits. Baligar and Duncan (1990) reported that some plants carry out processes that enhance availability, uptake and transport of Fe which include increased ability of roots to reduce Fe^{3+} to Fe^{2+} and increases in production of citrate in root sap.

Pb absorption by the six local accessions of *A. esculentus* does not follow a regular pattern as shown on Figure 4. Fifty eight percent of plants in both control and paint waste contaminated soils contained higher concentration of Pb in root as compared to stem. Pb concentration in fruits is considerable and does not agree with the report by Pichtel *et al.,* (1999). They stated that Pb is immobilized in the root after absorption. The results obtained for tissue concentration of Pb showed that there was translocation of Pb from the root after absorption through stem to edible parts (fruits). Highest concentration of Pb obtained for all the tissues analysed was 9% of the Pb concentration in control soil. Pb concentration in fruits of all accessions grown in control and contaminated soils divided the accessions into two groups again. Accessions within the first group were DIOR 007, 008 and 009 which gave values of Pb concentration in fruits of control plants as 146-202% of fruits of plants in contaminated soil. And the second group comprises accessions DIOR 010, 011 and 012 which gave values of Pb concentration in fruits of control plants as 1-38% of fruits of plants grown in contaminated soil. The values obtained as tissue concentration of Pb in the plants were very low (less than one hundredth of values obtained for soil concentration of Pb). This may be linked to the presence of anions such as phosphate and sulphate, which reduces Pb uptake (Lepp, 1981). Sharma and Dubey (2005) reported that Pb content in plant organs tends to decrease in the following order: roots>leaves>stem>inflorescence>seeds. However, this order can vary with plant species (Antosiewicz, 1992, cited by Sharma and Dubey, 2005) as has been demonstrated by *A. esculentus.* Pb is recorded to cause imbalance of mineral nutrients in growing plants (Sharma and Dubey, 2005) but a direct relationship cannot be drawn on its effect on the ascorbic acid content of the fruits of *A. esculentus* grown on contaminated soil.

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