

Assessment of remediation Potentials of maize (*Zea mays*) on sites co-contaminated with Pb and antracene

Nwosu, O.U.¹; Nwoko, C.O.²; Agu, C.M.³; Njoku, P.C.⁴; Chigbo, C.O.⁵

¹Agricultural/Bioenvironmental Engineering Department, Imo State Polytechnic Umuagwo-Ohaji, Nigeria

²Environmental Technology Department, Federal University of Technology Owerri, Nigeria

³Crop Science Technology Department, Federal University of Technology Owerri, Nigeria

Correspondence Author's e-mail: nwosuoly@gmail.com

Abstract— *Phytoremediation is a promising technology for the remediation of sites co-contaminated with inorganic and organic pollutants. A pot experiment was conducted to evaluate the remediation potential of Z.mays in soil co-contaminated with Pb and antracene. Pristine sandy loam soils were polluted with Pb chloride salt and antracene at three different levels (50mg/kg of Pb, 100mg/kg of Pb, and 100mg/kg of Pb+100mg/kg of antracene) and laid out in completely randomized design with 3 replicates. Shoot dry matter weight was significantly reduced ($p \leq 0.05$) when compared with control treatments by 40% when exposed to 100mg kg⁻¹ of Pb. There was a 48% inhibition of shoot dry matter of Z.mays relative to control treatments when 100 mg Pb kg⁻¹ was mixed with 100 mgkg⁻¹ antracene. Root and shoot metal concentration in Zea mays increased with increasing concentration of Pb. The average Translocation Factor ($TF < 1$ (0.69) obtained suggests that Zea mays predominantly retains Pb in the root portion of the plant. There was a 5% increase in shoot Pb concentration when soil was contaminated with Pb and antracene. The extractable antracene decreased significantly ($p \leq 0.05$) in soil planted with Z.mays as well as in pots without maize plant. This accounted for 65 and 72% of antracene dissipation in planted soil and 40-46% dissipation in unplanted soil. This result suggested that Zeamays is a promising candidate for uptake Pb and dissipation of antracene in co-contaminated soils.*

Keywords— *Phytoremediation, Co-contamination, Antracene, Zeamays*

I. INTRODUCTION

Soil Contamination with heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) is one of the most profound threats to water and soil resources, as well as to human health. These contaminants are often generated by various agricultural and industrial processes, such as production of chemical fertilizers, burning of fossil fuel, petroleum spills, coal tar, and residues from

metalliferous mining (Nwoko,2010). Soil contamination by HMs and PAHs has increased recently as a result of social and economic development in Nigeria. Accumulation of these pollutants in soil is a major ecological concern as it has adverse effects on biota. Although it is not uncommon that metal and organic contaminants are both present in polluted sites (Chigbo *et al.*2013), environmental research has tended to focus on the remediation of single pollutants instead of tackling multiple contaminants. Frequent occurrence of co-contaminated soils in the environment reveal how important it is to find adequate remediation solutions. One in situ decontamination approach that shows promise for addressing both organic and inorganic contaminants is phytoremediation –the attenuation of pollution through the use of plants. This method imposes minimal environmental disturbance and offers economic, agronomic and societal benefits. Several researches has been carried out on phytoremediation of organic or inorganic contaminated media, and recently few studies have targeted co-contaminated soils (Ouvrard *et al.* 2011, Chigbo *et al.* 2013, Hechmi *et al.* 2013, Agnello *et al.*2015). The efficiency and processes of phytoremediation of organic pollutants co-existing with heavy metals is complicated and quite different from that in the single-pollutant system. Therefore, a more thorough understanding of the mechanisms by which metals affect the dissipation of organic pollutants in planted soils is needed. However, no previous study has addressed the subject of phytoremediation potential of *Zea mays* in Pb and antracene co-contaminated soils. This study was conducted to evaluate the effectiveness of *Z.mays* in its uptake, accumulation and dissipation of Pb and antracene in co-contaminated soil. Antracene was used as the model organic compounds in this study because antracene represent a class of organic compounds that are ever present in superfund sites while Pb is among the ten high priority contaminants. *Zea mays* was used because of its

desirable characteristics such as high shoot biomass, short life cycle, extensive tap root system and handling ease.

II. MATERIALS AND METHODS

Dry seeds of Maize (*Zea mays*) accession: ACR.91SUWANI-SRC1 were sourced from National Seeds Service, Umudike. The lead chloride salts was purchased from Finlab, Nigeria. Soils (0-20cm) depth with pH 4.8., organic matter 1.97%, CEC 640 Cmol/kg, moisture content 1.29% total nitrogen content 0.15%, sand 91%, silt 3.46% and clay 5.14% (sandy loam) were collected using soil auger from 3 selected sampling points from an agricultural field in Federal University of Technology Owerri.

2.1. Soil preparation / Soil spiking

The composite soil collected was finely sieved to pass through a 2mm sieve to remove foreign and coarse particles, air-dried and later packed into a total of 21 pots for spiking. Soil was spiked with anthracene by dissolving 100 mg of anthracene in 25 ml of acetone. 50 and 100 mg kg⁻¹ of Pb were prepared by dissolving 0.067g and 0.134 g of PbCl₂ and added singly in anthracene spiked soils. The spiked soil was thoroughly mixed by sieving and stored in a dark room for equilibration for 7 days before planting.

2.2. Planting

The experiment was laid out in a completely randomized design with 7 treatments and three replications. Pots spiked with anthracene and Pb were planted with maize seeds, pots without maize plant served as control to observe non-plant facilitated dissipation of anthracene. Pots were watered when required with tap water to maintain about 50% the soil moisture during plant growth, and the leachates from all pots were collected using the tray and returned to the soil. Plant samples were collected from the pots 70 days after planting, shoots were cut just above the soil surface and washed with deionized water. Each pot was emptied, and the roots separated from the soil by washing with running tap water. The roots were rinsed with deionized water three times to remove all soil particles. All samples were oven dried to constant weight at 80 °C for 48 h. The dried samples were weighed to determine dry matter yield which was used for plant analysis. In the case of vegetated pots, rhizosphere soil samples were taken. to collect rhizosphere soil, plant roots were strenuously shaken by hand, taking care of the roots integrity. The external soil not attached to roots was removed, while the soil in the close vicinity of roots was kept for the analyses. Samples were analyzed for Pb uptake by using Varian AA240 Atomic Absorption Spectrophotometer according to the method of APHA 1995 (American Public Health Association) and soil

anthracene dissipation was carried out by using Soxhlet extraction method (AOAC, 2000).

2.3. Assessment of Pb uptake and anthracene dissipation

To assess the efficiency of *Zea mays* in Pb uptake and in the removal of anthracene, the following parameters were determined: i). stover dry weight; Plant samples were divided into roots and shoots, rinsed, carefully blotted dry and weighed to determine dry matter yield. ii). Root: Shoot ratio (R:S); Calculated as the ratio between the dry weight of roots and the dry weight of shoots. iii). Metal concentration in plant tissues; Samples were prepared for analysis by digesting 0.2g of ground plant sample in 20ml of dilute sulfuric acid and heated on a digestion block for hours until a clear digest was gotten. The resulting digest was made up to 50ml with distilled water. Heavy metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer according to the method of APHA, 1995 (American Public Health Association). iv). Translocation factors (TFs) which is the transfer of metals from the roots to the aboveground parts was calculated as the metal in shoots to the metal in roots ratio and was expressed by the formula; $TF = C_{shoot}/C_{root}$.

v). Bioconcentration factors (BCFs) which compare the accumulation of metals in plants is calculated as the ratio between metal concentration in plant tissues and total metal initial soil concentration. BCFs expressed by the formula; $BCF_{shoots} = C_{shoot} / C_{soil}$. $BCF_{roots} = C_{root} / C_{soil}$. Where C_{shoot} and C_{root} are metal concentrations in the shoot (mg kg⁻¹) and root of plants (mg kg⁻¹), respectively, and C_{soil} is the metal concentration in the soil (mg kg⁻¹). vi). Soil PAH residual concentration and anthracene dissipation(%); This was calculated as the final soil residual anthracene concentration after G.C analysis. Anthracene dissipation (%) was calculated as; $100 \times \{ [M_i - M_s] / M_i \}$. Where M_s is the concentration of PAH in each treatment. M_i is the initial PAH concentration present in the soil (Chigbo *et al.* 2013).

2.4. Statistical analysis

All data collected were subjected to statistical analysis using Statistical Package for Social Science (SPSS version 21 software package) (SPSS Inc, Chicago, IL, USA). Treatment effects were evaluated by analysis of variance (ANOVA). When a significant difference was observed between treatments, multiple comparisons were made by Tukey HSD test. Differences were considered significant at $p \leq 0.05$.

III. RESULT AND DISCUSSION

3.1. Growth response

Throughout the duration of the experiment, no visible toxic symptoms were observed in the test crop –maize plant. The shoot and root dry matter weight of *Z.mays* was affected by Pb and anthracene co-contamination. 50 mg kg⁻¹ and 100mg kg⁻¹ of Pb significantly decreased the shoot

dry matter of *Z.mays* by 28% and 40% respectively when compared with control treatments (Table 1). There was 48% inhibition of shoot dry matter of *Z.mays* relative to control treatments when 100 mg Pb kg⁻¹ was mixed with 100 mgkg⁻¹ anthracene.

Table.1: Plant dry matter yield of, *Z.mays* as affected by co-contaminated soil of Pb-anthracene 70 days after planting (DAP). Values are mean \pm SE, n=3

Treatments	Root weight	Shoot weight	Root/Shoot ratio
0 mg/kg Pb	0.43 \pm 0.09a	0.50 \pm 0.02a	0.86
50mg/kg Pb	0.30 \pm 0.01b	0.36 \pm 0.00b	0.83
100mg/kg Pb	0.28 \pm 0.01ab	0.30 \pm 0.00ab	0.93
100mg/kg Pb+PAH	0.24 \pm 0.01d	0.26 \pm 0.00c	0.92

Different letters within a column indicate a significant difference based on HSD ($p \leq 0.05$), n=3, PAH=100mg/kg anthracene.

3.2. Pb concentration in plants tissues

As the concentration of Pb in soil increased from 50 to 100 mg kg⁻¹, the shoot and root Pb concentration in *Z.mays* significantly increased with increasing concentration of soil Pb and increased with anthracene addition (Figures 1 and 2). When 50 and 100mg /kg Pb was added to soil, the shoot Pb concentrations for *Z.mays* was 5.0mg/kg for 50mg/kg Pb and 10.5mg/kg for 100mg/kg Pb respectively. The concentrations of Pb in

roots were 9.0mg/kg for 50mg/kg Pb and 14.5mg/kg for 100mg/kg respectively. *Z.mays* showed high accumulation of Pb in the roots and lower presence in the shoots, revealing, in general, poor metal translocation from roots to shoots. The joint contamination with Pb and anthracene had a significant effect on Pb concentration on *Z.mays*. The shoot Pb concentration in *Z.mays* increased with joint contamination with Pb and anthracene by 5% when compared with 100mg/kg Pb treatment.

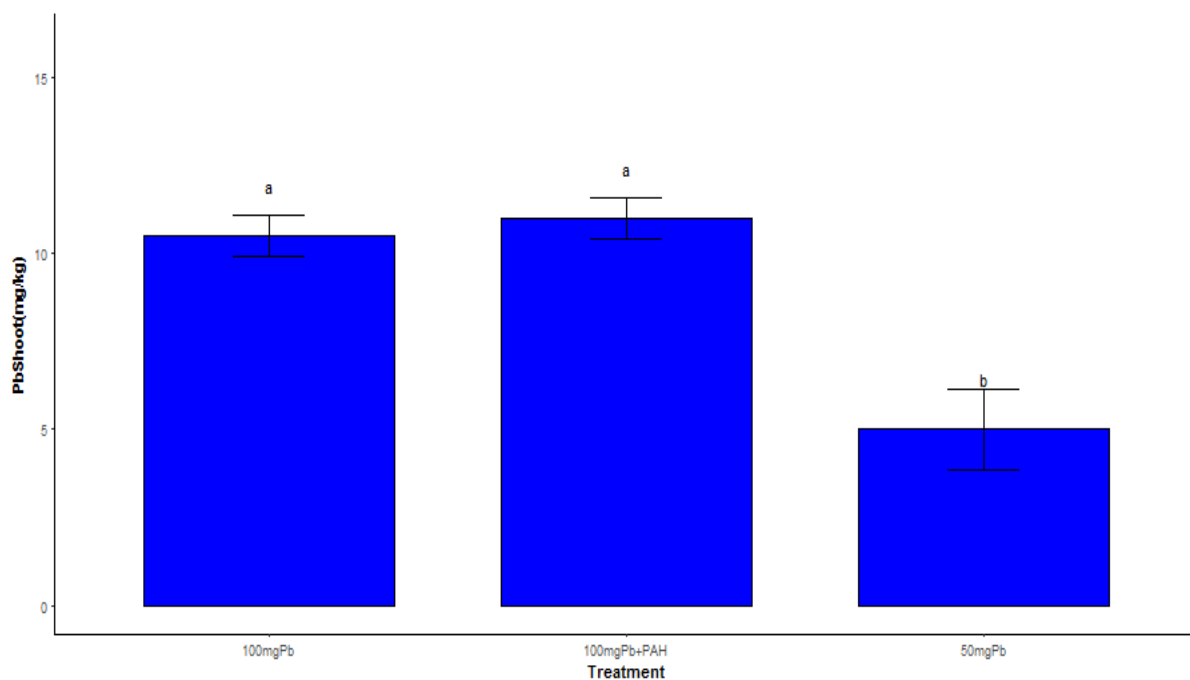


Fig.1: Shoot Pb concentration of *Zeamays* influenced by 50mg /kg Pb, 100mg Pb/kg and 100mg Pb+100mg/kg anthracene treatments after 70 days of growth. Bars indicate means \pm SE, n=3. Different letters indicate significant difference (TukeyHSD, $p \leq 0.05$).

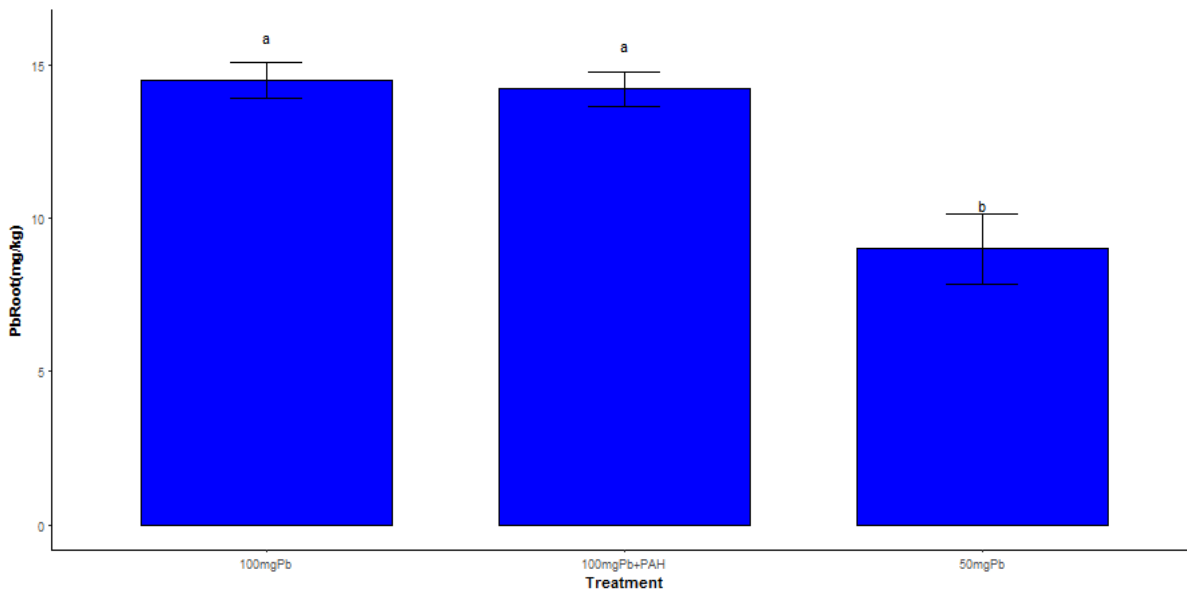


Fig.2: Root Pb concentration of *Zeamays* influenced by 50mg /kg Pb, 100mg Pb/kg and 100mg Pb+100mg/kg antracene treatments after 70 days of growth. Bars indicate means \pm SE, n=3. Different letters indicate significant difference (TukeyHSD, $p \leq 0.05$).

3.3. Translocation and Bioconcentration factor (TF&BCF) of Pb

With single contamination of Pb at 50mg kg⁻¹ the TF values for *Z.mays*, was 0.56 which increased by 22% as the concentration of Pb in soil increased to 100mg kg⁻¹. Co-contamination treatment significantly increased the TF values by 8% when compared to single treatments with 100 mg kg⁻¹ Pb (Table 2). The interactive effect of Pb

and antracene on the BCF_S and BCF_R are shown in Table 2. The BCF_R value was much higher than the BCF_S value under both single Pb exposure as well as when antracene was added. The BCF_S values for 50mg/kg Pb was 0.1. The BCF_S values increased by 5% when the Pb concentration was increased to 100mg/kg. With co-contamination of Pb and antracene, the BCF_S value significantly increased by 5%.

Table.2: Translocation and Bioconcentration factor of *Z.mays* as affected by single Pb and co-contamination of Pb and antracene 70 days after planting.

Treatments	TF	BCF _S	BCF _R
0 mg/kg Pb	0.8 \pm 0.00a	0.002 \pm 0.01a	0.003 \pm 0.01a
50mg/kg Pb	0.56 \pm 0.04a	0.1 \pm 0.02a	0.18 \pm 0.01bcd
100mg/kg Pb	0.72 \pm 0.04a	0.105 \pm 0.02a	0.15 \pm 0.01ab
100mg/kg Pb+PAH	0.78 \pm 0.04c	0.11 \pm 0.02a	0.14 \pm 0.01d

Different letters within a column indicate a significant difference based on HSD ($p \leq 0.05$). Values are mean \pm SE, n=3. PAH=100mg/kg antracene.

3.4. Antracene removal from soil

Extractable antracene decreased significantly ($p \leq 0.05$) in soil planted with *Z.mays* as well as in the pot without maize plant 70 days after planting (Figure 3). This accounted for 65% to 72% of antracene dissipation in planted soil and 40% to 46% dissipation for unplanted soil. In the presence of *Z.mays* the extractable antracene remained at 35mg kg⁻¹ for 100 mg kg⁻¹ antracene contaminated soil. However in the soil without plants, the

extractable antracene was 60mgkg⁻¹. Pb increased the dissipation rate of antracene in Pb- antracene co-contaminated soil. Results showed that the addition of Pb to antracene contaminated soil reduced the residual antracene concentration in soil. The addition of 100 mg kg⁻¹ of Pb to 100 mg kg⁻¹ antracene contaminated soil significantly reduced the residual antracene from 60mg/kg⁻¹ to 54mg kg⁻¹.

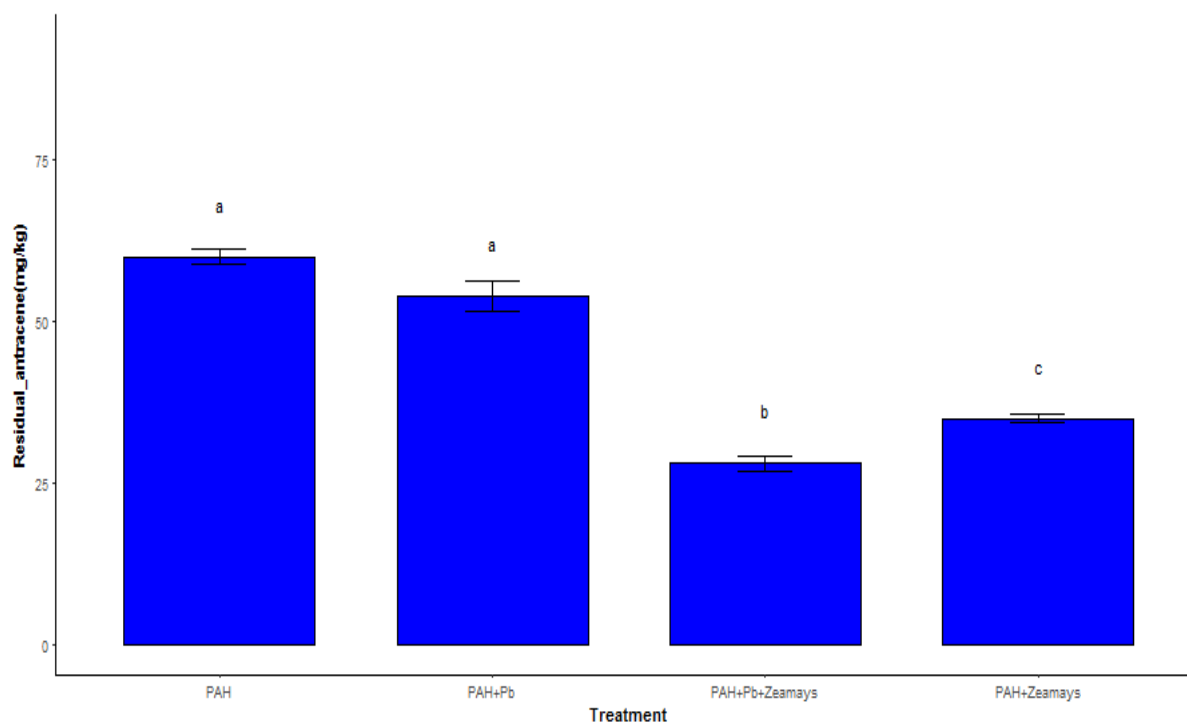


Fig.3: Residual anthracene concentration in non-planted soil and in soil planted with *Z.mays* after 70 days in anthracene contaminated soil. Bars indicate means \pm SE, $n=3$. Different letters indicate significant difference (TukeyHSD, $p \leq 0.05$).

IV. DISCUSSION

4.1. Interaction of Pb and anthracene influencing plant growth

The simultaneous presence of heavy metals together with polyaromatic hydrocarbons contributed more to plant toxicity. It appears that the plants roots were more sensitive than shoots to the toxic effect exerted by the co-contaminated soil as demonstrated by the greater negative impact on root biomass than on shoot biomass. The decrease of the root dry biomass of the plants could be as a result of the direct contact between polluted soil and the root surface which may have contributed to the root sensitivity (Kummerová *et al.* 2012). Other authors (Seth *et al.* 2011 and Chigbo *et al.* 2013) also found reductions of dry matter production in many plant species as a function of the application of increasing doses of metal in experiments with soil and nutrient solution. Chigbo *et al.* (2013) showed that PAHs might affect the plants indirectly by reducing water and nutrient availability to plants in polluted soil leading to reduction in dry matter production. This suggests a synergistic effect of metals and PAH in co-contaminated soils. Mechanisms underlying heavy metal and polyaromatic hydrocarbon phytotoxicity may be related both to direct effects on plant physiology (*e.g.* cell membrane disruption, damage of photosynthetic apparatus) or indirect ones such as, altering the biological, chemical and physical properties of the soil in which plants grow (Kabata-Pendias, 2011).

4.2. Pb concentration and accumulation as affected by co-contamination

The observed higher concentration of Pb in *Zeamays* could be as a result of co-contamination with anthracene which can change the extent of Pb uptake by plants or change the Pb solubility. For example, Mucha *et al.* (2005) observed that plants can release organic compounds which may complex metals and therefore change the availability of metals. It is possible that anthracene might control the release of Pb ligands that are capable of forming bioavailable Pb complexes. Increased metal availability, especially an increased uptake by plants in the presence of metal complexes has been found (Degryse *et al.* 2006). Alternatively as explained by Alkio *et al.* (2005), PAH may passively penetrate the root cell membranes without any carrier which can therefore facilitate the penetration of metal or metal complexes into the cell. Lin *et al.* (2008) observed a reduction in the root concentration of Cu in *Z.mays* with Cu-pyrene co-contamination. The penetration of anthracene to root cell membranes could be the reason for the observed reduction in root concentration of Pb and the observed increase in the shoot concentration of Pb with the addition of anthracene in this result. When pyrene was co-contaminated with cadmium, Zhang *et al.* (2009) suggested that the reduction in cadmium uptake with increased pyrene concentration could be as a result of the competition for adsorption between the co-existent pyrene and cadmium. In contrast, Almeida *et al.* (2009a)

observed an increased accumulation of Cu by roots of *Halimione portulacoides* with the addition of PAHs. They suggested that PAHs could have altered the way the plants influenced Cu solubility and sorption. Of recently, researchers have demonstrated that even at low dose, the combination of heavy metal and PAH could strongly cause oxidative stress and cellular organelle deformation in plant resulting in the suppression of heavy metal uptake (Li *et al.*2010). In heavy metal pollutant combined system, previous studies have shown that biodegradation of organic contaminants is often severely inhibited by toxic metals such as Cd. In some cases the addition of some metals has been observed to stimulate microbial activity. Chigbo *et al.*(2013) reported that the addition of some metals at low levels stimulated biodegradation. The efficiency and mechanisms of phytoremediation of organic pollutants co-existing with heavy metal is complex and quite different from that in the single-pollutant system. Therefore a more thorough understanding of the processes by which metals affect the dissipation of organic pollutants in the planted soils is needed.

4.3. Phytoremediation potential of *Zeamays*

Plants with TF values >1 is classified as high efficiency plants for metal translocation from the roots to shoots. Wei *et al.*(2009)suggested that plants species with TF values >1 actively take up metals from the soil and accumulate them in their above ground parts, therefore they could be good phytoremediators. It is important to know that plant species with higher BCF values combined with a lower TF values can also be suitable for phytoremediation of soils contaminated with heavy metal. In the present study TF and BCF were calculated to better evaluate the potential of *Zeamays* for phytoremediation purposes. The TF values for *Zeamays* was low (<1), revealing low mobility of metals towards aboveground tissues, while immobilization of heavy metals in roots were favored. The great capacity of Pb accumulation in the roots and the low translocation of Pb to the shoots in *Zeamays* (Table 2) suggest that these species have great potential for remediation of Pb in the soil (Oladele *et al.*2018).

4.4. Anthracene dissipation in soil

The residual anthracene in co-contaminated soil planted with *Z.mays* was significantly ($p \leq 0.05$) lower than in the unplanted co-contaminated soil. This shows the benefit of vegetation in anthracene contaminated soils. This result is in agreement with other research. For example Lin *et al.* (2008) showed that the residual pyrene in soil planted with *Z.mays* was significantly lower than in non-planted soil with the initial pyrene concentration up to 500mg kg⁻¹. The high removal rate of anthracene in the presence of *Z.mays*, in anthracene contaminated soil could also be

related to the rhizospheric microbes that plays an important role in degradation of organics. For example Sun *et al.* (2010) showed that the dissipation of pyrene was higher in soil amended with root exudates than in soil with growing root of *L. perenne* releasing organic substances. An interactive effect of heavy metals and PAHs on the degradation of PAHs can either cause a negative or positive effect depending on the type and concentration of both PAHs and heavy metals (Khan *et al.* 2009). For example, Khan *et al.* (2009) showed that Pb can increase the dissipation rate of pyrene in Pb- pyrene co-contaminated soil and an enhanced bacterial community was detected in soil. The increased concentration of dissipated anthracene in the presence of Pb could be linked to change in microbial activity as well as the composition of the microbes. As suggested by Olsen *et al.* (2003), the distinction in the quality or quantity of nutrients released by plants root exudates as well as dead roots could lead to differences in microbial PAH degradation. These differences could be either positive or negative.

V. CONCLUSION

The present study explores the phytoremediation potential of *Zea mays* in Pb and anthracene co-contaminated soil. There was reduction in the shoot and root dry weight of *Zea mays* in both single Pb and co-contamination with anthracene. The shoot and root Pb concentration in *Zea mays* significantly increased with increasing concentration of soil Pb and increased with anthracene. The concentration of Pb in shoots were lower than in roots and the T.F values were lower than 1.0 for all treatments. Plants grown in co-contaminated soil had higher T.Fs than those in single contaminated soil. The dissipation of anthracene was enhanced by vegetation both in single Pb and co-contamination with anthracene. Pb also enhanced the dissipation of anthracene in Pb-anthracene co-contaminated soil. Hence, *Zeamays* could be used for phytoremediation of Pb-anthracene co-contaminated soil.

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