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Research Paper



Effect of Bioremediation on early seedling growth of *Amaranthus hybridus* L. grown on palm oil mill effluent polluted soil

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Abstract

The effect of Bioremediation on Early Seedling growth of *Amaranthus hybridus* L. on oil palm effluent polluted soil was carried out in the field using perforated polythene bags. The soil samples were polluted with 0, 80, 120 and 240ml of oil palm effluent and amended with compost manure supplement (fermented oil palm spikelet) at 0, 40, 60 and 80 g respectively). The 0ml and 0g served as controls. The mean result obtained after 12 weeks showed that the addition of compost manure supplement (fermented oil palm spikelet) to the contaminated soil promotes growth of plant as there was increase in the growth parameters such as shoot height, petiole length, leaf area, number of nodes, number of branches, internode length, root length, and moisture content. This study recommends that contaminated soils should be amended with manure to improve the growth performance of plants.

Keywords: *Amaranthus, Bioremediation, Effluent, Palm Oil, Pollution and POME.*

1. Introduction

Palm oil is one of the most important vegetable oil in the world's oil and fat market. The extraction and purification processes generate different kinds of waste generally known as palm oil mill effluent (POME). The environmental impact of POME cannot be over emphasized (Igwe and Onyegbado, 2007). Palm oil mill effluent is the voluminous liquid waste that comes from the sterilisation and clarification sections of the oil palm milling process. The raw effluent contains 90-95% water and includes residual oil, soil particles and suspended solids (Heuzé *et al.*, 2012). Palm oil mill effluent is a highly polluting material and much research has been dedicated to means of alleviating its threat to the environment. Palm oil mill effluent is a highly polluting material, due to its high Biological Oxygen Demand, low pH and colloidal nature. Since the 1980-1990s (depending on the country), developments have taken place in the crude palm oil industry to make this industry environmentally more sustainable (Heuzé *et al.*, 2012). The techniques employed to remove oil pollutants from the environment include physical, chemical and biological methods (Atlas, 1981) (Wang *et al.*, 1994) (Solano-Serena *et al.*, 2000) (Obayori *et al.*, 2008). The physical and chemical methods hardly achieve complete elimination of oil from the environment. The use of chemical method involves application of expensive chemical dispersants, thereby introducing even more pollutants in the environment (Jain *et al.*, 1992). Bioremediation therefore, is indispensable as the most natural method to eliminate the bulk of oil contaminants from the environment (Obidi *et al.*, 2010). In this study, Oil palm spikelet which is relatively cheap and easy to obtain is used as a bioremediating agent to ameliorate oil palm effluent pollution.

2. Materials and methods

2.1. Collection of experimental materials

Seeds of *Amaranthus hybridus* used for this study were obtained from the matured plant in the Botanical Garden of the University of Uyo. The soil sample used in this study was obtained from the Department of Botany Postgraduate Research Farm, University of Uyo, Nigeria. The fermented oil palm spikelet used in this study for bioremediation treatment was obtained from an Oil Palm Mill in Ekpene Obom, Etinan Local Government Area, Akwa Ibom State, Nigeria.

2.2. Physiochemical analysis of the experimental soils

The physiochemical properties of the experimental soil were evaluated using Atomic Absorption. Spectrotophometer (AAS) (AOAC, 2005). The properties analyzed include: pH, Ec., organic matter, total nitrogen, available phosphorus, exchangeable calcium, Ex. Potassium. Ex. Acidity, ECEC, bases saturation, silt, clay, Ex. Magnesium, C/N ratio, organic carbon and particles size sand. This analysis was carried out on the unpolluted, polluted and the ameliorated soil samples.

2.3. Pollution of soil samples

Four kilograms (4 kg) of sterilized loamy soil were weighed using a Mettler (P-165) weighing balance into perforated polythene bags of 38 cm in width and 40 cm in length to enhance drainage. Each soil sample was thoroughly mixed with 80, 120 and 240 ml of oil palm effluent and incubated for three days (Udo and Oputa, 1984). The unpolluted soil sample served as a control for each treatment (Umoh and Esenowo, 1996).

2.4. Bioremediation treatment

Fermented oil palm spikelet was added to the polluted soils (80, 120 and 240 ml, at the rate of 40, 60 and 80 grammes respectively. The mixture of oil palm effluent, loamy soil and fermented oil

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palm spikelet were left undisturbed for three days to facilitate loosening of the soil constituents (Amadi *et al.*, 1992).

2.5. Planting of seeds

Seeds of *Amaranthus hybridus* were sown directly into perforated polythene bags filled with sterile sandy loam soil at the depth of 5 cm. The experiment was conducted in the field under normal environmental conditions.

2.6. Determination of growth parameters

The growth parameters such as plant height (seedling length), petiole length, leaf width and leaf length were measured using meter rule (in centimeter); internode number was also determined. The plants were uprooted during harvest and the root length was measured and moisture content determined.

2.7. Determination of leaf area

Leaf Area (LA) was determined by multiplying leaf length by leaf width with the correction co-efficient (r) which is 0.72 (Hoyt and Bradfield, 1962) (Udo and Oputa, 1984).

LA = L x W x r

Where L = leaf Length

W = Leaf width

r = correlation coefficient (0.72)

2.8. Statistical analysis

Results are expressed as mean ± Standard Error of Mean (SEM) of three replicates. Statistical significance between the different groups was determined by two-way Analysis of Variance (ANOVA) $P<0.05$ was considered as statistically significant (Ubom, 2004).

3. Results

The physiochemical properties of the soil analysis (Garden soil) indicated that the sandy loamy soil used in this study is rich in available phosphorus, effective cation exchange capacity and organic matter content. The garden soil (unpolluted soil) had a pH of 6.51. Exchange capacity, silt, clay, exchange Calcium, exchange acidity, exchange Magnesium and organic Carbon, were low while total Nitrogen, exchange Sodium, exchange Potassium and electrical conductivity were relatively low as shown on Table 1. Similarly, the amended soil was also rich in available Phosphorus and it showed slight stimulation in pH (6.81) with organic supplement (fermented oil palm spikelet). The organic matter content increased, while organic exchange Calcium, exchangeable acidity, silt and clay were low in the soil. Also, the total Nitrogen content (%), Sodium, Exchange Potassium and Electrical conductivity (EC) were low. the polluted soil imposes low availability of these physiochemical properties as indicated in Table 1.

The results obtained from the growth parameters taken revealed that seedling height of *Amaranthus hybridus* in polluted soil was 11.90 ± 0.21, 13.10 ± 2.11, 14.40 ± 0.22 and 13.13 ± 0.11 in 0ml, 80ml, 120ml, and 240 respectively. In the same manner, Leaf Area, number of nodes, number of branches, root length and moisture content showed total reduction when compared with the control except in petiole length which had 1.64 ± 0.41, 2.04 ± 0.13, 1.60 ± 0.3, 1.60 ± 0.00 and 2.03 ± 0.01 in 0ml, 80ml, 120ml and 240ml respectively Table 2.

Table 1: Physiochemical Properties of Experimental Soil

Soil properties	Garden soil	Polluted soil	Amended soil
Ph	6.81	6.60	6.81
Organic matter (%)	1.98	2.46	2.86
Total nitrogen (%)	0.07	0.03	0.04
Available P. (mg/kg)	36.77	36.00	38.88
Ex. Ca (mol/kg)	3.25	2.66	2.82
Ex. N (mol/kg)	0.07	0.02	0.04
Ex. K (mol/kg)	0.94	0.15	0.18
Ex. Acidity	3.58	2.30	2.10
ECEC (mol/kg)	6.85	4.43	5.43
Base saturation (%)	64.81	46.07	47.02
Silt (%)	4.00	2.40	2.80
Clay (%)	4.20	3.20	0.60
Ex. Mg (mol/kg)	1.30	0.60	0.89
Organic carbon	1.61	0.07	0.09
C/N ratio	91.67	94.69	98.89
Particle size sand (%)	91.80	93.80	94.00
Ec. (Gs/m)	0.068	0.0364	0.0467

Ex- Exchange
ECEC- Effective Cation Exchange Capacity
EC- Electrical conductivity
C/N- Carbon/Nitrogen ratio

Table 2: Effects of Palm Oil Mill Effluent Pollution of Soil on the Growth Parameters of *Amaranthus Hybridus* Seedlings grown for 12 weeks.

Conc. (ml)	Shoot height (cm)	Petiole length (cm)	Leaf Area (cm^2)	Number of nodes	Number of branches	Internode length (cm)	Root length (cm)	Moisture content (g)
0	11.90 ± 0.21	1.64 ± 0.41	5.04 ± 0.22	32.0 ± 0.00	24.0 ± 0.00	1.55 ± 2.11	11.90 ± 0.07	2.81 ± 0.08
80	13.10 ± 2.11	2.04 ± 0.13	4.86 ± 0.12	27.0 ± 0.00	21. 0 ± 0.00	2.14± 1.12	10.13 ± 0.24	2.60 ± 1.22
120	14.40 ± 0.22	1.60 ± 0.00	4.17 ± 0.11	14.0 ± 0.00	16.0 ± 0.00	1.72 ± 1.00	6.0 ± 0.28	1.82 ± 0.78
240	13.13 ± 0.11	2.03 ± 0.01	3.28 ± 0.01	9.0 ± 0.00	13.0± 0.00	1.53 ± 0.24	7.6 ± 0.05	1.50 ± 0.47

Data were processed and expressed as mean ± SD of three replicates.

In amended soil, the results showed that the growth parameters tested were stimulated above the control , seedling height was 11.90 ± 0.27, 20.71 ± 0.20, 18.60 ± 0.10 and 18.10 ± 0.00 in 0ml, 80ml, 120ml, 240ml. Other parameters showed the same increase except in moisture content which showed stimulation in 240ml above lower concentration, i.e. 2.81 ± 0.00, 3.87 ± 0.21, 3.98 ± 0.81 and 4.00 ± 0.78 in 0, 80, 120, and 240ml respectively. It was also observed that, the higher the concentration of POME pollution, decrease in growth parameters was observed as indicated on Table 3.

Table 3: Effects of Bioremediation Treatment on the Growth Parameters of *Amaranthus Hybridus* Seedlings grown for 12 Weeks.

Conc. (ml)	Shoot height (cm)	Petiole length (cm)	Leaf area (cm^2)	Number of nodes	Number of branches	Internode length (cm)	Root length (cm)	Moisture content (g)
0	11.90 ± 0.27	1.64 ± 0.00	5.04 ± 0.08	32.0 ± 0.00	24. 0 ± 0.00	1.55 ± 0.28	11.90 ± 0.56	2.81 ± 0.00
80	20.71 ± 0.20	2.84 ± 0.28	10.52 ± 0.22	38.0 ± 0.00	32.0 ± 0.00	2.62 ± 0.71	18.8 ± 0.18	3.87 ± 0.21
120	18.60 ± 0.10	2.88 ± 0.24	8.42 ± 0.11	29.0 ± 0.00	29.0 ± 0.00	1.98 ± 2.00	17.2 ± 0.00	3.98 ± 0.81
240	10.10 ± 0.00	2.60 ± 0.00	6.81 ± 1.28	26.0 ± 0.00	27.0 ± 0.00	1.82 ± 1.87	15.8 ± 1.22	4.80 ± 0.78

Data were processed and expressed as mean ± SD of three replicates.

4. Discussion

Palm oil effluent like any other oil products adversely affects the growth and performances of plants as indicated in the results got-

ten from this study. The effect of palm oil mill effluent on the seedling height observed here were similar to those reported on the effect of spent engine oil on *Amaranthus hybridus* (Odjegba and Sadiq, 2002). Njoku *et al.* (2008) reported same observation on the effect of crude oil on the growth of *Glycine max* and *Lycopersicon esculentum.* Adedokum and Ataya (2007) also showed that treatment of soils with crude oil, automotive gasoline oil and spent engine oil significantly affect the time of germination, percentage germination, plant height, leaf production and biomass of *Vigna unguiculata* delaying germination and growth rate. Similarly, Okon *et al.* (2012) concluded that at high concentrations of crude oil, growth yield and tubers production of *Manihot esculenta* were hindered but organic supplements (chicken droppings) were found to reduce the toxic effects of crude oil thereby stimulating the yield of cassava.

The leaf surface area determines in large part the amount of carbon gained through photosynthesis and the amount of water lost through transpiration and ultimately the crop yield (Kathirvelan and Kalaiselvan, 2007). Therefore, the reduction observed in this study implies that there would be lower contact of photosynthetic efficiency of the plant as much of the solar energy emitted by sun would not be absorbed by plant for photosynthesis. This can lead to low performance of the plant (*Amaranthus hybridus*) with subsequent low availability of food and poor economy due to low sales of such plants products. Availability of nitrogen in the soil directly affects the relative growth rate of plants (Walker *et al.*, 2001). Since oil products are known to reduce nitrogen availability (Agbogidi *et al.*, 2007), this could be the cause of adverse effect on the plant growth parameters with increase in the palm oil effluent. The growth parameter in this study showed a significant ($P<0.05$) stimulation with the addition of nutrient supplements because it has been shown that plants grow slower under low nutrient (Grotkopp and Rejmanek, 2007). Proper growth of cultivated plants depends on the content of nutrients in the soil (Wyszkowski and Zoilkowska, 2008). Okon *et al.* (2013) also reported that nutrient amendment in poor soil successfully improved the soil quality, germination and yield component of two varieties of Okra (*Abelmoschus esculentus*); they added that the use of organic manure is therefore necessary in poor nutrient soils.

5. Conclusion

This study showed that palm oil mill effluent contamination of soil may result in low soil fertility. As shown in this study, this effect can be remedied by the addition of compost manure supplements especially fermented oil palm spikelet and the quantity of the supplement added has significant effect on the remediation process of the soil. Moreover, it can be deduced from this research that plants can perform relatively better in palm oil effluent polluted agricultural soil if such soil is amended with reasonable quantity of manure.

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