Growth of Amaranth (*Celosia Spp.*) and Soil Microbial Changes as Influenced by Rubber Effluent and Poultry Manure in an Ultisol in the Lowland Forest Zone of Nigeria

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Abstract:- **This study was carried out at the University of Benin Teaching Research and Integrated Project (UBTRIP) Farm Site, Ugbowo Campus, Benin-City to evaluate the effect of rubber effluent and poultry manure on the growth response of amaranth as well as some microbial and chemicalproperties changes of the soil. The experiment was replicated three times with four treatments; 0ml, 5ml, 15ml and 30ml of rubber effluent and 1.92kg of poultry manure per plot (7m x 15m) for a period of eight weeks. The agronomic parameters measured were; number of leaves, plant height and leaf area. After 8 weeks of the treatment, the amaranth plants were harvested and soil samples collected from the treatment plots were analyzed for both its physical, chemical and microbial properties; Results obtained from the experiment showed that the soil pH increased from 4.84 in the control (0 ml) plots to a range of 4.98 – 5.09 in the rubber effluent treated plots. The N content increased from 0.18gkg^{-1} earlier determined in the initial soil test to 0.36gkg^{-1} in the controlled soil after the application of poultry manure and between 0.38 – 0.41gkg^{-1} in the effluent treated plots. The P, Mg and TOC were also enhanced by the effluent but had their highest amount at the 15 ml treated plots whereas; the Mn content was higher in the control plots. The growth of amaranth plantwas also affected by the rubber effluent treatments when compared with the control. The population of microorganisms increased in the test soil relative to the control as well as the introduction of new microorganisms into the environment in response to the application of rubber effluent. Only three organisms were isolated from the control sample; *Penicillium spp. Pseudomonas spp.*, and *Bacillus spp.* while *Penicillium spp.*, Mucor, *Culvulariaspp. Staphylococcus spp* and *Micrococcus spp* were isolated from the rubber effluent treated plots.**

Keywords: **Rubber effluent "Amaranth" Poultry manure" Microorganisms.**

I. INTRODUCTION

Soil is a major part of natural environment, along air and water, which are vital to the existence of life on the planet. Soil is the main stay of agriculture and horticulture, forming the medium in which growth and ultimately the yield of food producing crops occurs. Soil is increasingly being recognized as playing a fundamental role in the quality and distribution of our water supply.There is a direct impact of pollutants on minerals, organic matter and microbial community of soil (Aishwarya *et al.*, 2014). The discharge of industrial effluents especially without treatment may have profound influence on physico-chemical and biological properties of soil related to soil fertility (Aishwarya *et al.*, 2014).

Natural rubber processing sector is an industry which produces raw materials used for the manufacture of rubber industrial products. However, environmental damages generated from this sector could become big issues. Natural rubber processing sector consumes large volumes of water and energy and uses large amount of chemicals as well as other utilities. It also discharges massive amounts of wastes and effluents (Leong *et al*., 2003). Wastewater is an unavoidable by-product of rubber processing: whatever processing procedures are used for preparing productsfrom latex, there will always be an aqueous liquid as a by-product (Rungruang and Babel, 2008).

The major chemical component groups of natural rubber waste 'serum' shows that it consists mainly of nitrogenous compounds. In the waste water (effluent) the bulk of the nitrogen component consist mainly of ammoniacal nitrogen as a result of the use of ammonia in the preservation of the latex. The high level of NH_4^+ and other plant nutrients makes it a good medium for algal growth, thus resulting in the eutrophication of water bodies. The increasing global concern on the environment demands that wastes should be properly managed in order to minimize and possibly eliminate their potential harm to public health and the environment.

The low fertility status Utisols necessitated the need for external fertilizer input. The use of fertilizer (organic or inorganic) supplements the soil with nutrients, especially nitrogen for succulent green leafy growth (Emede*et al.*, 2012). The use of synthetic fertilizer to sustain crop production has been reported by several researchers to increase yield only for few years but not on long-term or sustainable basis (Ojeniyi, 2000). In highly weathered soils (e.g., utisols), the use of synthetic fertilizer has not been sustainable due to its induced soil acidity, nutrient imbalance and physical degradation leading to increase soil erosion coupled with the fact thatsynthetic fertilizers are usually not available and are always expensive for the resource-poor farmers. These stress the need and had also prompt many researchers to experiment for organic fertilizer (waste from farm animals) input to improve the physical, chemical and biological properties of the soil (Linger and Critchdey, 2007). Reports show that organic fertilizer is preferred to synthetic fertilizer as it improves the nutrient content better nutrient recycling, activating the microbial biomass and soil tilth (Emede*et al.*, 2012). Incorporating organic fertilizer into the soil as a cultural practice for crop production is expected to plays a direct role in plant growth as a source of all necessary macro and micronutrients in available forms during mineralization, thereby improving both physical and the biological properties of the soil.

Owing to the numerous importance of organic fertilizers and the scientist quest for more organic sources of fertilizer that will not only supply the needed nutrient for plant growth but that will also enhance the soil health and nutritional values of crops produced, hence: this investigation is aimed at determining the growth response of Amaranth in soils amended to rubber effluent and poultry manure (organic materials).

II. MATERIALS AND METHODS

The Study Area

This study was carried out at the University of Benin Teaching Research and Integrated Project (UBTRIP) Farm Site, Ugbowo Campus, Benin City. The site lies between latitude 06.39589^{0}N and longitude 005.63235^{0}E. The area is characterized by a tropical climate with an annual rainfall amount of 1900mm and mean annual temperatures ranging from 23^{0}C to 37^{0}C. The site is situated at the Rainforest belt of the humid tropics and southern ecological zone of Nigeria, with distinct dry and wet seasons. The dry season begins early November and ends by March, while the rainy season is from April to October. The rainfall pattern is bimodal with peaks in July and August; however there is a short spell (short day break) which is called “August-break” in mid-August, accompanied by few thunderstorms. The soils are derived from coastal plain sands (unconsolidated sands and sandy clay) and alluvial deposits.

Source of Materials

The rubber effluent (liquid waste) was obtained from Odia Farm, Ikpoba-hill, Benin City, Edo State, Nigeria. The poultry manure (cured state) was obtained from the University of Benin Animal Farm Project while the Amaranth seeds was obtained from the Crop Science Department of the University of Benin.

Field Layout / Operations

The field was laid out in a completely randomized designwith four treatments; 0ml, 5ml, 15ml and 30ml of rubber effluent which were replicated three times. The poultry manure which was applied as a basal amendment was applied at the rate of 1.92kg per plot (7m x 15m) which is an equivalent of 0.19 t/ha. Two weeks after the application of both amendments, amaranth seedlings were transplanted to the field. Agromonic parameters measured include: plant height, number of leaf and leaf area.

Soil / Plant Analysis

The land used for this investigation was previously cultivated solely of maize for about a year. Soil sampling from this field was made at 0 – 15cm depth before and after planting and wasthereafter analyzed for their physical, chemical and microbial properties.These soils were first air dried and sieved to 2 mm. Twenty (20) grams of each soil sample was weighed into a 100 ml beaker followed by the addition of 20 ml distilled water. The mixture was stirred intermittently for 30 minutes while the glass electrode pH meter was standardized with buffer 7.0 and 4.01. At the end of the 30 minutes, the suspension was read and recorded as pH (1:1) H_20. The particle size distributions of the soils were determined by the Hydrometer method (Gee and Or, 2002). Exchangeable bases (Ca, Mg, K and Na) were extracted with 1 N ammonium acetate (NH_40Ac). Exchangeable calcium and magnesium were determined by ethylene diamine-tetraacetic acid (EDTA) titration method while exchangeable potassium and sodium were estimated by flame photometry (Jackson, 1962). Exchangeable acidity was extracted with KCl (1 N) and measured titrimetrically according to the procedure of Maclean (1982). Soil organic carbon (SOC) was determined by Walkley and Black digestion method (Olsen and Sommers, 1982). Total Nitrogen was estimated by micro-Kjeldahl digestion method (Bremner and Mulvaney, 1982) while available phosphorus was determined by Bray 1 Method (Olsen and Sommers, 1982).

Microbial Analysis

The serial dilution method was used to prepare the soil for determination of microbial propertiescoupled with series of biochemical tests (Coliform test, Methyl red test, Catalase test, Citrate utilization test, Coagulase slide test, Indole production test, Oxidase test, Urease test, the Vogues-proskauer test, Sugar fermentation test, Motility test, Spore stain) were used to identify the bacteria composition of the soil (Cowan and Steel 1970).

Statistical Analysis

The data generated from the experiment were subjected to ANOVA using a Genstat package and treatment means were compared using LSD at 5% level of probability.

III. RESULTS AND DISCUSION

Chemical and physical properties of rubber effluent / soil prior to amendment

The chemical properties of rubber effluent in Table 1 indicated that the effluent was slightly acidic (5.23).The chemical analysis of the effluent used in this experiment revealed that, the effluent contains some basic plant nutrients. This finding however aligned with the findings of Orhue and Osaigbovo, (2012). Who also reported rubber effluent tocontained N, P, organic carbon, K, Mg, Na and Ca. On the other hand, Table 2 shows the physical and chemical properties of the soil prior to amendment. Thesoil was slightly acidic (4.24) and classified as Ultisol and texturally sandy. Apart from Mg and P, the N, K and Cawere below the critical values of 1.5 - 2.0 g/kg (Sobulo and Osiname, 1985), 0.16 - 0.25 cmol/kg (Akinrindeand Obigbesan, 2000) and 2.50 cmol/kg (Akinrindeand Obigbesan, 2000) respectively. The properties of the soil used indicated that the soil is low in fertility, which is typical of anultisol as reported earlier by Ogunkunle (1993).

Table 1: Chemical Properties of the Rubber Effluent

Effluent Property	Mean Value
pH (1:1)	5.23
Total Nitrogen	2.30
Phosphorus mg/l	5.26
Organic carbon %	0.18
Potassium mg/l	11.30
Calcium mg/l	8.50
Sodium mg/l	1.22
Magnesium mg/l	2.86

Table 2: The physical and chemical properties of the soil before amendment and planting

Parameters	Value
pH (1:1)	4.24
H^+(cmol/kg)	0.20
Al (cmol/kg)	0.10
PO_4 (mg/kg)	22.83
N (g/kg)	0.18
TOC (g/kg)	19.50
SO_4 (mg/kg)	11.53
Mn (mg/kg)	22.64
Ca (cmol/kg)	2.28
Mg(cmol/kg)	0.99
Na(cmol/kg)	0.44
K(cmol/kg)	0.48
CEC(cmol/kg)	44.9
Clay (g/kg)	188.20
Silt(g/kg)	20.70
Sand (g/kg)	791.10

Influence of rubber effluent on height, number of leaves and leaf area of Amaranth

Table 3, 4 and 5 shows the influence of rubber effluent of the growth parameters of amaranth. The plant height, number of leaves and leaf area of the crops receiving rubber effluent treatment were higher than the control treatment. The increase in plant height, leaf area and number of leaves can be attributed to the presence of high soil nutrient content provided by the rubber effluent and poultry manure applied. This increase in growth parameters as a result of the treatment applied is similar to the earlier reports by Lim *et al.*, (1983) on oil palm, Tan *et al.*, (1975) on Nappier grass and Orhue *et al.*, (2005) on *Dialiumguineense* seedlings.

Table 3: Plant Height Measurement

Treatments	**Plant Height (cm)**						
	2WAS	**3WAS**	**4WAS**	**5WAS**	**6WAS**	**7WAS**	**8WAS**
Control (0ml)	3.03a	4.49a	10.00b	21.50a	30.00ab	36.00a	40.00a
Trt 1 (5ml)	3.02a	5.13a	11.40a	22.00a	35.00ab	40.00a	48.00b
Trt 2 (15ml)	3.03a	5.20a	11.40a	24.00a	37.00a	42.00ab	50.00ab
Trt 3 (30ml)	3.10a	5.30a	11.41a	23.00a	40.00a	47.00ab	52.00ab
$Lsd_{(0.05)}$	**0.06**	**0.20**	**0.40**	**0.49**	**3.40**	**4.20**	**5.1**

- Mean with same letters are not significantly different
- WAS = Weeks after sowing.

Table 4: Number of Leaves

Treatments	**Number of Leaves**						
	2WAS	**3WAS**	**4WAS**	**5WAS**	**6WAS**	**7WAS**	**8WAS**
Control (0ml)	2.3a	6.50a	13.00a	19.0a	24.00 a	26.00a	27.00a
Trt 1 (5ml)	2.3a	6.00a	18.0 a	20.0a	24.00a	27.00ab	30.00b
Trt 2 (15ml)	3.00a	7.00a	15.00a	22.0ab	25.00ab	28.00b	31.00bc
Trt 3 (30ml)	2.5a	7.00a	16.00a	23.0ab	26.00ab	29.00b	30.00c
$Lsd_{(0.05)}$	**0.29**	**2.20**	**0.21**	**1.00**	**0.73**	**2.30**	**1.76**

- Mean with same letters are not significantly different
- WAS = Weeks after sowing

Table 5: Leaf Area Measurement

Treatments	Number of Leaves						
	2WAS	3WAS	4WAS	5WAS	6WAS	7WAS	8WAS
Control (0ml)	2.30a	6.50a	13.00a	18.60a	24.00 a	25.50a	26.50a
Trt 1 (5ml)	2.30a	6.00a	18.0 a	19.50a	24.25a	27.00ab	29.50b
Trt 2 (15ml)	2.50a	7.00a	15.10a	22.05ab	25.00ab	28.25b	30.55bc
Trt 3 (30ml)	2.45a	6.50a	16.08a	23.10ab	26.27ab	29.25b	30.01c
$Lsd_{(0.05)}$	**0.29**	**2.20**	**0.21**	**1.00**	**0.73**	**2.30**	**1.76**

- Mean with same letters are not significantly different
- WAS = Weeks after sowing.

Microbial properties of soil before and after field experiment

Table 6: shows that the initial bacterial mean counts was $1.1X10^6$ in the soil sample, the autochthonous bacteria in the soil sample were *Pseudomonans sp. Klebisellasp* and *Bacillus Sp*. whereas, at the end of the experiment, only three organisms were isolated from the control plot that received only the basal treatment (poultry manure) and the organisms were; *Pseudomonas spp., Penicillium spp.* And *Bacillus spp* with mean population of 1.2×10^6, 1.8×10^6 and 1.2×10^6cfu/g respectively. Of the three autochthonous (indigenous)soil organisms associated with the soil prior to amendment, it was observed that the poultry manure amended soil (control plot) supported two of these autochthonous (*Pseudomonas spp.*, and *Bacillus spp*) soil organisms. While *Penicilliumspp*, *Staphylococcus aureus,* Mucor, *Culvulariaspp* and *Micrococcusspp* were isolated from the rubber effluent treated soil.More so, there was an increase in the microbial population and activities with every increase in the amount of effluent application with the 30ml treated plots having the highest microbial isolates and population for most of the organisms identified. These results further suggest that the concentration of the rubber effluent resulted in a significant change in the microbial species that became dominant in terms of their population in the soil. This finding however aligned with the work of Ingham (2000) whose investigation revealed that, different and specific soil organisms occur where they can find a suitable pH, appropriate food supply, space, nutrient, and moisture.

Table 6. The Initial and Post Treatment Soil Microbial (Bacteria) Properties

Isolates	Initial	0ml	5ml	15ml	30ml
		Mean Population in Cfu/g			
Pseudomonas sp	1.2×10^6	1.2×10^6	-	-	-
Klebisellasp	1.1×10^6	-	-	-	-
Bacillus Sp.	1.1×10^6	1.2×10^6	-	-	-
Culvulariasp		-	-	-	1.1×10^6
Mucor		-	1.0×10^6	1.2×10^6	2.1×10^6
Micrococcusspp		-	-	2.6×10^6	2.8×10^6
Penicilliumsp		1.8×10^6	1.8×10^6	-	-
Staphylococcus aureus		-	1.6×10^6	1.6×10^6	1.1×10^6

Physicochemical properties of soil after field experiment:

The post-trial properties of the soil are shown in Table 7. The soil N, P, Naand CEC increased slightly with increasing levels of rubber effluent while soil pH, organic carbon, K, Mg, and Caalso showed an increase though not in a steady order with increasing rubber effluent treatments but were slightly lower in the rubber effluent amended soils.

The increase in soil N, P, and Nacontent of the soil can be attributed to the serum properties of the effluent as earlier reported by Orhue and Osaigbovo (2012). This increase in soil nutrient content especially in PO_4, N, Ca and Mg further confirms that applying effluent is not problematic especially when the rate of application is geared to supply nutrients at a level corresponding to those in inorganic fertilizers normally applied to promote satisfactory crop performance and that controlled application of effluent and poultry manure causes no detrimental changes to soil physical and chemical properties, rather it improves soil fertility and has no apparent adverse effect on the environment.

Table 7 Physicochemical properties of soil after field experiment

Treatment	pH	H^+	AL^{3+}	PO_4	N	SO_4	Mn	Ca	Mg	Na	K	CEC	TOC	CLAY	SILT	SAND
		---cmol/kg----		mg/kg	g/kg-----------------------------cmol/kg-----------------------								-------------------g/kg----------------			
0ml	4.84a	0.63a	0.30a	22.24b	0.36a	13.69a	38.58a	4.45a	0.65a	0.41a	0.24a	5.75a	3.63a	13.21a	1.06a	85.75a
5ml	5.09a	0.47a	0.20a	34.02ab	0.38a	14.63a	15.94a	5.13a	1.98a	0.42a	0.26a	7.80a	4.01a	12.88a	1.16a	85.96a
15ml	5.09a	0.47a	0.21a	41.88a	0.41a	18.07a	30.09a	5.81a	2.36a	1.21a	0.35a	9.163a	4.15a	9.03a	0.98a	86.65a
30ml	4.98a	0.47a	0.27a	38.84a	0.39a	12.54a	28.52a	6.32a	1.19a	0.99a	0.38a	8.41a	4.01a	15.86a	1.54a	82.6b

IV. CONCLUSION

This experiment revealed that the controlled application of rubber effluent has a positive effect on the growth of the amaranth (*Celosia* spp) plant with increased plant height, higher number of leaves and leaf area than the control. Although the results did not show a steady increase in soil nutrient with increasing levels of rubber effluent but the basic soil nutrients were relative higher in the effluent treated soil than the control. Therefore, it could be concluded that rubber effluent has a potential value as organic fertilizer for the growth of amaranth. While on the other hand, the rubber effluent treated soils though had the highest microbial population; it however had a negative effect on the soil autochthonous (indigenous) organisms. The experiment revealed that the application of rubber effluent completely led to the displacement of the indigenous organisms which are considered more relevant in the biochemical reactions that occurs in the soil as supported by many authors and researchers.

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