Original Research Article

The Effects of Abattoir Effluent on Soil Microbiological Properties and Vegetative Traits of Flint Maize

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Abstract

The effects of application of abattoir effluent on soil microbiological properties and vegetative traits of flint maize were investigated in pot experiments under screen-house conditions at Ambrose Alli University Teaching and Research Farm, Ekpoma, Nigeria. The effluent was applied to 10kg of soil at the rate of 0, 200, 400, 600 and 800ml per bag to give equivalents of 0, 20, 40, 60 and 80ml/kg of soil. The treatments were fitted into a complete randomized design (CRD) and replicated three times. The physical and chemical properties of the soil as well as the chemical properties of the effluents were determined before the experiment. The number and types of microorganisms present were also determined in the effluent and soil before and at 8 weeks after application. Growth parameters were taken at 2, 4, 6, and 8 weeks after planting (WAP). Results showed that the effluent was slightly acidic (pH = 6.7), while bacteria and fungi populations decreased in the soil at 8 weeks after application. The total viable bacterial counts in the effluent was 3.5×10^6 cfu/ml, while values in the soil before application and at 8 weeks after application were 5.5×10^6 cfu/g and 3.7×10^6 cfu/g respectively. For fungi in the effluent, a count of 1.2×10^6 cfu/ml was recorded while that in the soil before application was 8×10^5 cfu/g. At 8 weeks after application to the soil, a count of 2.0×10^6 cfu/ml was recorded. The bacteria isolated included *Bacillus subtilis*, *Klebsiella edwarsii* and *Proteus mirabilis*, while fungal isolates were identified as *Penicillum notatum*, *Aspergillus niger*, *Candida* spp. and *Trychophyton metagrophte*. Abattoir effluent significantly increased leaf area, number of leaves and plant height at all the WAP compared to the control.

Keywords: Abattoir effluent, Bacteria, Fungi, vegetative traits, Flint maize

Introduction

The use of organic manure has been an age long agricultural practice among subsistence farmers in Nigeria due to pressure from competing socio-economic demand for land. Lim *et al*. (1983) observed that high productivity of most cleared forest soils soon lose their natural fertility to rapid cultivation, hence, there should be need for the incorporation of cheap and readily available organic fertilizer.

Efforts have been made globally to protect the environment. In order to boost food production, many countries have resorted to the use of organic waste materials to supplement inorganic fertilizer application. Organic waste materials could be used as sources of nutrients in the soil, but the nutrient availability varies due to the type of material, weather, soil property and management practices (Rowe *et al*., 2003). The excessive reliance on inorganic fertilizer has not only caused the exhaustion of soil nutrient reserves but also resulted in soil health problems, affecting bio-ecosystems and increasing the cost of crop production (Singh, 1987). The ability to change the environment has increased faster than the ability to predict the effect of that change (Ogboghodo *et al*., 2004).

Strong emphasis has been laid on the sustainable use and management of available natural resources including wastes from agro-industries, organic wastes from processing industries, town refuse, and sludge as manure (McFarland *et al*., 1998). In different parts of the world, waste-water is the principal source of water for irrigating cropland. Most of the waste-water reuse sites in the United States today are in arid and semi-arid areas of the Western States (Tchobanoglous *et al*., 2003). Though effluents have a well-balanced ratio of nutrients, their nutrient level is very low, and as such, large quantities of effluents are required to meet the inorganic fertilizer supply (Wood *et al*., 1997).

Crops like maize grow very rapidly in soils rich in organic matter. Maize is considered as one of the most important annual crops across various parts of Nigeria. It is grown by many farmers as good sources of carbohydrates, minerals and some amino acid like lysine. The annual demand for maize keeps increasing and can no longer meet up with supply, and this has made farmers to adopt means of enhancing the productivity of the soil (Odedina, 2005). Flint maize (*Zea mays* indurata) is also known as Indian corn in most countries. The flint maize is yellowish with less soft starch than dent maize (Lincoln, 2009).

Isitekhale *et al*. (2006) observed that 100% germination of maize was obtained after polluting the soil with abbattoir effluent. Abhanzioya (2013) observed that abattoir effluent can be a good source of nutrients for the growth of maize as well as other crops. The application of abattoir effluent to soils has significant effects on soil microorganisms, soil physico-chemical properties, nutrient uptake and crop yield (Ogboghodo *et al*., 2001). Coker *et al*. (2001) identified seven pathogenic species of bacteria in abattoir effluents in South Western Nigeria. These species among others includes *Staphylococcus* and *Streptococcus* spp. Most researches are carried out using sweet maize but other varieties of maize are being neglected. Hence, the objectives of the study therefore, were to: assess the effects of abattoir effluents on the number and types of bacteria and fungi in a soil cultivated with flint maize, and evaluate the effect of abattoir effluent on the vegetative traits of flint maize in a screen-house.

Materials and Methods

Experimental site

The experiment was carried out in the screen-house of Ambrose Alli University Teaching and Research Farm, Ekpoma, Nigeria.

Planting material

The variety of maize used for planting was flint maize. This was obtained from the open market in Ekpoma.

Soil sample collection and preparation

Surface soil samples were collected at a depth of 0-15cm from a secondary forest in Ambrose Alli University, Ekpoma. These were bulked and mixed together to make a composite.

Experimental layout

Ten kilogram (10kg) of soil was weighed into separate polyethylene bags, and abattoir effluent was applied at rates of 0, 200, 400, 600 and 800ml per bag (which gave following rates 0, 20, 40, 60 and 80mlof abattoir effluent/kg of soil, with 0ml/kg serving as the control treatment).This was replicated three (3) times and laid out in a complete randomized design (CRD). The soil was left standing for two (2) weeks to allow proper mixture of soil with effluent before planting. Three (3) maize seeds were planted per polyethylene bag. The seedlings were later thinned to one plant per stand two weeks after planting, while watering was done twice every week. Soil samples were collected before application of abattoir effluent (for analyses of physico-chemical and macrobiological properties) and at eight (8) weeks after application of abattoir effluent (for macrobiological properties).

Data collection

The following plant parameters were taken at 2, 4, 6, and 8 weeks after planting (WAP): plant height, number of leaves, stem girth and leaf area.

Statistical analysis

The data obtained from the experiment were subjected to statistical analysis using analysis of variance (ANOVA) at a probability level of 5%.

Determination of Bacteria and Fungi Populations

The bacteria and fungi populations were determined using the pour plate method of serially diluted samples, and direct plating (Ogundama, 1989; Cowan and Steel, 1993; Cheesebrough, 2006). Observed colonies were subjected to several biochemical tests which included gram staining, motility, indole, and sugar fermentation. Fungi spp were identified using the methods of Jean *et al.* (1976).

Results and Discussion

The physico-chemical properties of the soils before application of abattoir effluent are shown in Table 1. The soil was moderately acidic, and the textural class was sand. At 2 weeks after planting (WAP), abattoir effluent had no significant effect on plant height, stem girth and number of leaves (Table 2). However, maize leaf area was significantly increased to 83.32cm^2 with the application of 60ml/kg of abattoir effluent compared to the control treatment and other treatments. The leaf area ranged from 20.32cm^2 with 40ml/kg application to 83.32cm^2 with 60ml/kg application.

At 4 WAP maize plant height, numbers of leaves and leaf area increased with increasing rates of abattoir effluent (Table 3). The highest plant height, number of leaves and leaf area of 55.73cm, 6.66 and 50.32cm^2 were obtained at the application of 80ml/kg, 40ml/kg and 60ml/kg of abattoir effluent respectively. The values were significantly ($p<0.5$) different from those of the control treatment. Though not significantly different, the highest stem girth was obtained at the rates of 60ml/kg and 80ml/kg while the lowest was observed in the control treatment.

At 6 WAP, abattoir effluent had no significant ($p>0.05$) effect on stem girth, but significantly increased plant height, leaf area and number of leaves (Table 4). Application of 80ml/kg abattoir effluent resulted in the highest plant height, leaf area, number of leaves and stem girth of 83.60cm, 127.79cm^2, 7.66 and 2.66cm respectively.

Table 1: Physico-chemical properties of the soil before application of abattoir effluent

Parameters	Values
pH	5.6
Organic carbon (g/kg)	10.1
Total Nitrogen (g/kg)	1.55
Exchangeable cations	
Calcium (cmol/kg)	3.50
Available P (cmol/kg)	8.20
Magnesium (cmol/kg)	2.05
Potassium (cmol/kg)	0.33
Sodium (cmol/kg)	0.40
Hydrogen (cmol/kg)	0.35
Aluminum (cmol/kg)	-
ECEC (cmol/kg)	5.86
Particle size	
Clay (g/kg)	4.50
Silt (g/kg)	5.50
Sand (g/kg)	90.50
Textural class	Sand

Table 2: Effects of abattoir effluent on mean vegetative traits of flint maize at 2WAP in the screen house

Treatment (ml/kg)	Plant height (cm)	Number of leaves	Stem girth (cm)	Leaf area (cm^2)
0	33.27	3.07	1.33	71.13b
20	33.60	4.00	1.07	20.32c
40	34.83	4.00	1.06	21.04c
60	36.03	4.00	1.16	83.32a
80	38.13	4.00	1.20	23.47c
LSD	NS	NS	NS	10.16

NS: Not Significant, LSD: Least Significant Difference;
Means in the same column with same letter are not significantly different at 5% level.

Table 3: Effects of abattoir effluent on maize plant height, number of leaves, stem girth and leaf area at 4 WAP in the screen house

Treatment (ml/kg)	Plant height (cm)	Number of leaves	Stem girth (cm)	Leaf area (cm^2)
0	44.66b	4.00b	1.50	23.19b
20	46.93b	5.66a	1.66	35.74ab
40	51.43a	6.66a	1.60	41.98a
60	52.70a	5.66a	1.70	50.32a
80	55.73a	6.33a	1.70	48.50a
LSD	8.16	1.00	NS	15.56

NS: Not Significant, LSD: Least Significant Difference;
Means in the same column with same letter are not significantly different at 5% level.

Table 4: Effects of abattoir effluent on maize plant height, number of leaves, stem girth and leaf area at 6 WAP in the screen house

Treatment (ml/kg)	Plant height (cm)	Number of leaves	Stem girth (cm)	Leaf area (cm^2)
0	45.33b	5.33b	1.53	44.13b
20	45.10b	7.33a	2.23	86.34abc
40	71.36a	7.00a	2.06	71.10c
60	73.73a	7.33a	2.23	103.50ab
80	83.60a	7.66a	2.66	127.79a
LSD	22.65	0.88	NS	55.40

NS: Not Significant, LSD: Least Significant Difference;
Means in the same column with same letter are not significantly different at 5% level.

At 8 WAP the highest leaf area, plant height and stem girth values were obtained from application of 80ml/kg abattoir effluent, and were significantly different from those of the control treatment

(Table 5). Number of leaves was significantly increased at 40ml/kg of the effluent. The increase in maize plant height, leaf area, number of leaves and stem girth indicates the organic fertilizer value of the applied abattoir effluent.

Table 5: Effects of abattoir effluent on maize plant height, number of leaves, stem girth and leaf area at 8 WAP in the screen house

Treatment (ml/kg)	Plant height (cm)	Number of leaves	Stem girth (cm)	Leaf area (cm^2)
0	64.33b	5.33b	1.33	82.52b
20	89.47a	7.00ab	2.20	140.22ab
40	91.67a	7.40a	2.60	143.59ab
60	93.10a	6.96ab	2.43	164.12a
80	106.70a	6.50ab	2.63	190.91a
LSD	1.55	1.75	NS	77.1

NS: Not Significant, LSD: Least Significant Difference;
Means in the same column with same letter are not significantly different at 5% level.

Table 6 shows the bacteria and fungi populations that were present in the effluent and soil before application of abattoir effluent. It was observed that few organisms were found in the effluent before it was applied to the soil. Fewer fungi but more bacteria were found in the soil before the application of abattoir effluent. This finding is not in agreement with Abhanzioya (2013) who reported that more organisms were found in the effluent before it was applied to the soil probably as a result of differences in the microbial load.

Table 6: Bacteria and Fungi Population/Identification of Abattoir Effluent and Soil before the Application of Abattoir Effluent

Type of Sample	Population	Bacteria Identification	Population	Fungi Identification
*Effluent (Cfu/ml)	3.5 x 10^6	*Proteus mirabilis* *Pseudomonas spp*	1.2 x 10^6	*Penicillum notatum;* *Aspergillus niger*
Soil (Cfu/g)	5.5 x 10^6	*Klebsiella edwarsii* *Bacillus spp* *Clostridium spp*	8 x 10^5	*Candida spp;* *Penicillum notatum* *Aspergillus*

*pH 6.7

Table 7 shows the bacteria and fungi populations that were present in the soil after the application of the effluent. It was observed that the population of bacteria decreased with increased rate of abattoir effluent application with 60ml/kg having the highest population. The bacteria present were *Bacillus subtilis, Klebsiella edwarsii* and *Proteus mirabilis,* while the fungus present was *Trychophyton metagrophyte*. These findings are contrary to what were reported earlier by Ogboghodo *et al*. (2006) and Abhanzioya *et al*. (2016) who observed that the number of the different microorganisms increased as the levels of application of the effluent to the soil increased at 8 weeks after application.

Table 8 shows that *Klebsiella edwarsii* was motile and positive for mannitol and maltose fermentation but negative to lactose, glucose, and sucrose utilization (with no gas production), and indole tests. *Bacillus subtilis* was motile and positive to glucose (with no gas production) and mannitol tests but was negative to indole and sucrose tests. Also *Proteus mirablis* was motile but negative to glucose, lactose, maltose, sucrose, mannitol (with no gas production) and indole tests.

Table 7: Bacteria and Fungi Population of Soil at 8 Weeks after Application of Abattoir Effluent

Treatment(ml/kg)	Cfu/ml	Bacteria Identification	Cfu/ml	Fungi Identification
0	3.0×10^6	*Bacillus subtilis*	10×10^5	*Trychophyton metagrophyte*
20	3.7×10^6	*Bacillus subtilis* *Klebsiella edwarsii*	18×10^5	*Trychophyton metagrophyte*
40	3.6×10^6	*Bacillus subtilis* *Klebsiella edwarsii*	8×10^5	*Trychophyton metagrophyte*
60	3.8×10^6	*Bacillus subtilis* *Klebsiella edwarsii*	6×10^5	*Trychophyton metagrophyte*
80	3.6×10^6	*Bacillus subtilis* *Proteus mirabilis* *Klebsiella edwarsii*	20×10^5	*Trychophyton metagrophyte*

Table 8: Morphological and Biochemical Characteristics of Isolates

Isolates	Gram reaction	Morphological characteristics	Biochemical characteristics of isolates							Identified bacteria
			Maltose	Glucose	Sucrose	Mannitol	Lactose	Indole	Motility	
A	Positive, rod-shaped	*Rough, rapidly spreading, gram positive, mucoid*	+	+	-	+	+	-	+	*Bacillus subtilis*
B	Negative, rod-shaped	*Mucoid, flat with irregular edges*	+	-	-	+	-	-	+	*Klebsiella edwarsii*
C	Negative, rod-shaped	*Milky, dried, flat with rounded edges*	-	-	-	-	-	-	+	*Proteus mirabilis*

Key: + = positive; - = negative

Summary and Conclusion

Microbiological assessment of abattoir effluent on vegetative traits of flint maize was investigated at the screen-house of Ambrose Alli University, Teaching and Research Farm, Ekpoma, Nigeria. Application of abattoir effluent significantly increased maize plant height, stem girth, number of leaves, and leaf area. A few fungi and more types of bacteria were present in the soil before application of abattoir effluent to the soil. At eight (8) weeks after application, the population of bacterial and fungal species decreased in the soil, with *Klebsiella edwarsii*and *Bacillus subtilis* dominating. At eight (8) weeks after application, 80 ml/kg soil led to more types of bacteria and highest population of fungi. Hence, abattoir effluent at the rate of 80ml/kg is recommended for maize production. Furthermore, 80ml/kg soil gave the highest significant increase of the vegetative traits (growth parameters).

Conclusively, further studies should be carried out for a longer duration in order to know if these organisms that were absent at eight (8) weeks after application would reappear, because these organisms e.g. *Pennicillum* and *Aspergillus* enhance nitrogen and phosphorus fixation thereby increasing soil fertility for sustainable crop production.

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