

Evaluation of Antibiotics in Soil and *Panicum maximum* Around a Pig Manure Dumping Sites in the Derived Savannah Zone of Nigeria

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Abstract: Evaluation of antibiotics in soil and *Panicum maximum* around Pig manure dumping site was carried out. The samples (soil and *Panicum maximum*) were collected at distances of 0m, 30m and 60m and at a parallel of 2m at each distance to give three different locations per distance as replicates. Soils were collected to a depth of 15cm because grasses are fibrously rooted. Results revealed presence of Penicillin and Streptomycin in the grass and soil with the highest ($P < 0.05$) values at 60m distance and the lowest ($P < 0.05$) at 0m. Tetracycline was highest ($p < 0.05$) in 0m grass ($33.42 \times 10^{-7} \mu\text{g/kg}$) followed by 60m and then 30m. Chloramphenicol values observed to be 26.90, 28.76 and $23.86 \times 10^{-7} \mu\text{g/kg}$ were reported for 0, 30, and 60m respectively in the grass. In the soil samples, values for chloramphenicol were 12.83, 13.85 and $10.79 \times 10^{-7} \mu\text{g/kg}$ for 0, 30 and 60m respectively while tetracycline was observed to be highest ($P < 0.05$) at 60m with $15.91 \times 10^{-7} \mu\text{g/kg}$ followed by 0m and 30m with 14.33 and $12.37 \times 10^{-7} \mu\text{g/kg}$ respectively. The organic contents of the soil collected at the Piggery manure dumping site were all significantly ($P < 0.05$) influenced with the presence of the antibiotics. The organic carbon, organic matter and pH ranged from 1.03 – 1.28, 1.78 – 2.21 and 6.98 – 7.16 respectively.

Keywords: antibiotics, soil, *Panicum maximum*, pig manure, Nigeria

1. Introduction

Agricultural antimicrobial use (AMU) provides many benefits to livestock and producers including disease treatment, prevention, and growth promotion (12). This is a common practice in swine production as data from CIPARS in Canada indicated that 75% of the participating herds were exposed to at least one antimicrobial through feed, and 82% of the farmers incorporated antimicrobials into either the feed or water (Canadian Integrated Program for antimicrobial Resistance Surveillance (5). Sows and suckling piglets are more often treated with injectable antimicrobials while nursery and grow-finish pigs more often are administered antimicrobials as groups through feed or water (14;6).

Microbial resistance to antibiotics is a worldwide problem in human and veterinary medicine. (16) stated that the main risk factor for the increase in antibiotic resistance is attributed to the extensive use of antibiotics. This has led to the emergence and dissemination of resistant bacteria and resistance genes in animal and humans. The antimicrobial agents used in animal care are also significant, not only in increasing the resistance in animal pathogen, but also in bacteria transmitted from animals to human (3).

Additional concern has been raised with the role of pharmaceuticals as environmental contaminants. Among these are veterinary drugs, particularly antibiotic which ranked highest concerning possible adverse effects on the environment (4) and this has recently gained more attention. The main route of entry of veterinary antibiotics into the environment is via the application of manure of treated farm animals. (1) had earlier reported the presence of antibiotics (tetracycline, penicillin, streptomycin and chloramphenicol) in *Panicum maximum* and soil around poultry manure dumping site.

(11) and (18) reported that up to 80% of the orally administered antibiotics may pass through the animals unchanged and end up in manure and later find their way into the terrestrial environment when manure from antibiotic-fed animals is applied as a source of crop nutrients. (4) however observed that persistence of antibiotics in soil depends on many factors including soil type, climate, and class of antibiotics. (1) in their work stated that antibiotic manure-applied to land may leach to ground water or move to surface waters through surface runoff and this explained why at 60 m away from the dumping site, it was still found present. The present study aimed at examining the presence of antibiotics in soil and *Panicum maximum* around piggery manure dumping site.

2. Materials and Methods

2.1 Study Areas

Hamama piggery farm in Ogbomosho area of Oyo state Nigeria was considered for this study. Hamama farm has a pedigree of persistent piggery production over the years and its one of the known names in the livestock industry in the environs. The description of this ecological zone had earlier been reported by (13). It is located in the derived savanna zone of Nigeria and lies at approximately $8^{\circ}07'$ North of the equator and $4^{\circ}15'$ East of Greenwich Meridian. The climate is characterized by a fairly high uniform temperature (36.20°C), moderate to heavy seasonal rainfall of 1247mm annually and high relative humidity.

2.2 Sample Collection

Soil samples and *Panicum maximum* were collected for analysis at the dumping site around the farm. Soil was collected using soil auger while total plant of *Panicum*

maximum was collected using cutlass, and placed in nylon bag. The samples (soil and the forage) were collected at distances of 0m, 30m and 60m using measuring tape and at a parallel of 2m at each distance to give three different locations per distance as replicates. The soils were collected to a depth of 15cm because grasses are fibrously rooted.

2.3 Laboratory Procedure

2.3.1 Determination of the various antibiotics

a) Qualitative Test

A known weight of each sample was extracted with their corresponding solution and make up to 250ml volume with distilled water in a 250ml graduated flask. The mixture was placed in a steam bath for 10min till a corresponding colour precipitate begins to form (white, pink or red, bluish green and yellowish colour for tetracycline, chloramphenicol, penicillin and streptomycin respectively). This confirms the presence of antibiotics. (2)

b) Quantitation Test

The mixture above was filtered through a Whatman No. 42 filter paper into a 100ml volumetric flask and made up to mark with distilled water.

The filtrates were pipetted into a 100ml beaker, 5ml of freshly prepared 0.10% sodium solution added and allowed to stand for 3min. Standard solutions of various antibiotics of specific range in $\mu\text{g/ml}$ were prepared and treated like sample extract above. The Absorbencies of sample as well as standard were read against blank on a spectronic 21D spectrophotometer at a wavelength 510nm (2).

% Antibiotics.

$$= \frac{\text{Absorbance of sample} \times \text{Gradient Factor} \times \text{Dilution}}{10,000}$$

c) Statistical Analysis

All data collected were subjected to one way analysis of variance (ANOVA) using (17) software package. Differences between means were separated using Duncan multiple range test of the same package.

3. Results and Discussion

The presence of antibiotics in *Panicum maximum* around piggery manure dumping site is shown in Table 1. Amount of penicillin in the grass at 60m away from the dumping site was the highest with $41.63 \times 10^{-7} \mu\text{g/kg}$ followed by that of 30m and 0m which were $27.13 \times 10^{-7} \mu\text{g/kg}$ and $21.43 \times 10^{-7} \mu\text{g/kg}$ respectively. Streptomycin was also present and the percentage showed a similar trend with penicillin. These results could be attributed to the water run off due to the topography of the dumping site. The topography of the dumping site examined was steep, also the actual point of dropping the pig manure was rockier, and thus the soil there might have a poor absorbing ability of the antibiotics from the soil. It was also observed that the growth of *Panicum maximum* on the rockier part of the soil was not as prominent as the growth of the *Panicum maximum* down the slope. The rocky nature of the site does not allow for easy growth of the *Panicum maximum*. (4) observed that persistence of antibiotics in soil depends on many factors including soil type, climate, and class of antibiotics. Most antibiotics are biodegradable in soils but some of them have

a long shelf-life. Antibiotics in manure-applied to lands may leach to ground water or move to surface waters through surface runoff and this explained why at 60 m away from the dumping site more antibiotics was observed in the *Panicum maximum* than at the dumping point. Tetracycline was highest ($p < 0.05$) in 0m grass ($33.42 \times 10^{-7} \mu\text{g/kg}$) followed by 60m and then 30m. Chloramphenicol values observed to be 26.90, 28.76 and $23.86 \times 10^{-7} \mu\text{g/kg}$ were reported for 0, 30, and 60m respectively. (8) suggested that about 25% of the oral dose of tetracycline excreted in faeces and another 50%- 60% is excreted as an acute metabolite in urine. However once they are released to the environment, antibiotics can be transported either in a dissolved phase or adsorbs to colloids or soil particles into surface water and underground water (10) and a significant amount of this can still be present in soil after land preparation (9)

The Presence of Antibiotics in soil around Piggery manure dumping site was as shown in Table 2 below. Penicillin was found present in all the samples. It increased as the distance increase while value for 60m ($22.12 \times 10^{-7} \mu\text{g/kg}$) was significant ($P < 0.05$). The least was however found in 0m. The amount of Penicillin found in the piggery manure dumping site is relatively higher and at different distance compared with the work of (1) variation could be due to the quantity of antibiotics which will depend on the specie of animal involved and the retention ability of the specie of the animal concerned. Also usage rate and land absorption ability could also be a factor as opined by (4). Higher values were observed in streptomycin in the soil at 60m ($12.03 \times 10^{-7} \mu\text{g/kg}$) and the least was observed at 0m ($5.37 \times 10^{-7} \mu\text{g/kg}$). Generally low values of streptomycin in the soil agreed with the findings of (1) which concluded that it was as a result of the low frequency of usage by farmers. The value recorded for tetracycline was observed to be highest ($P < 0.05$) at 60m followed by 0m and 30m (15.91, 14.33 and $12.37 \times 10^{-7} \mu\text{g/kg}$) respectively. The values for chloramphenicol were 12.83, 13.85 and $10.79 \times 10^{-7} \mu\text{g/kg}$ for 0, 30 and 60m respectively. (8) observed that many antibiotics are not completely absorbed in the gut, resulting in the excretion of the parent compound and its breakdown metabolites, however, all these could be excreted to the environment (7).

Table 3 revealed the organic content of soil collected at piggery manure dumping site. The 0m sample of the soil has highest ($p < 0.05$) percentage of organic carbon with value 1.28%, 60m has the lowest value with 1.03%. The % organic carbon value for 30m was 1.03%. The 0m sample was significant ($P < 0.05$) with 2.21% for the % organic matter. 30m and 60m were 1.78% and 2.16% respectively. The length of time that introduced organism can persist in the soil varies with temperature, moisture, pH and the indigenous community present. The higher percentage of organic carbon and organic matter at 0m distance could have been attributed to effect of time of deposition as reported by (15) who studied the persistence of culturable antibiotics resistant bacteria in heavily manured soil and five months later observed that the proportion of resistant bacteria had returned to levels within the range of non – manure control samples. However, (9) concluded that the longer antibiotics persist in the soil in an active form the greater the potential for native soil bacteria population to be affected.

4. Conclusion

The utilization of antibiotics in farms needs to be regulated. It has been observed that animals do not fully absorb these antibiotics which are then deposited on lands. These can be transported to man through ruminant animal grazing the forages or to soil water. All these leads to more resistance of humans and animals grazing the forages to the efficacy of the antibiotics

Table 1: The presence of antibiotics in Panicum maximum around piggery manure dumping site

Antibiotics x 10 ⁻⁷ µg/kg	0m	30m	60m SEM
Penicillin	21.43 ^c	27.13 ^b	41.63 ^a 3.01
Streptomycin	4.73 ^c	12.13 ^b	13.59 ^a 1.40
Tetracycline	33.42 ^a	25.49 ^c	27.10 ^b 1.21
Chloramphenicol	26.90 ^b	28.76 ^a	23.8 ^c 0.71

^{abc} mean values in the same row are significantly different (P<0.05)

Table 2: The Presence of Antibiotics in soil around Piggery manure dumping site

Antibiotics x 10 ⁻⁷ µg/kg	0m	30m	60m SEM
Penicillin	13.52 ^c	14.47 ^b	22.12 ^a 1.36
Streptomycin	5.37 ^c	6.14 ^b	12.03 ^a 1.05
Tetracycline	14.33 ^b	12.37 ^c	15.91 ^a 0.51
Chloramphenicol	12.83 ^b	13.85 ^a	10.79 ^c 0.45

^{abc} mean values in the same row are significantly different (P<0.05)

Table 3: The organic content of soil collected at Piggery manure dumping site

Parameters	0m	30m	60m SEM
% Organic carbon	1.28 ^a	1.26 ^b	1.03 ^c 0.04
% Organic matter	2.21 ^a	1.78 ^c	2.16 ^b 0.07
pH	6.98 ^c	7.05 ^b	7.16 ^a 0.03

^{abc} mean values in the same row are significantly different (P<0.05)

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