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# Impact of Drifts Resulting from Pesticide Application on Soil Microorganisms around Waste Receptacles in Port Harcourt City, Nigeria

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## Authors' contributions

*This work was carried out in collaboration among all authors. Authors CLE and DNO designed the study, while author DM performed the statistical analysis, wrote the protocol and the first draft of the manuscript managed the analyses of the study and literature searches under the strict supervision of authors CLE and DNO. All authors read and approved the final manuscript.*

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## ABSTRACT

Pesticides are toxic substance used to reduce or kill pests but the deposits on soil environment can remain there for long period of time causing adverse effects on soil microorganisms which are responsible for soil health conditions. This study was carried out to determine the impact of pesticide drifts on soil microorganisms in a waste receptacle around Port Harcourt city. Soil samples were obtained from various depths around waste receptacles with hand auger using standard analytical procedures. Microbial analysis was done according to prescribed standard methods. Characterization and identification of the isolates were based on their cultural, morphological, and cellular characteristics. Results obtained showed that the bacterial isolates were identified as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas* sp and *Micrococcus* sp while fungal isolates include *Aspergillus niger*, *Penicillium* sp, *Fusarium siculi*, and *Aspergillus fumigatus*, *Aspergillus nidulas*, *Microsporium canis* and *Yeast*. The results of the microbial counts

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revealed that Total Heterotrophic Bacteria (THB) had  $2.08 \times 10^9$  cfu/g at a depth of 30-45 cm while Total Heterotrophic Fungi (THF) had  $6.0 \times 10^6$  cfu/g before application with a mean value of  $1.02 \times 10^9$  and  $2.8 \times 10^6$  cfu/g respectively while after application THB had  $4.1 \times 10^8$  and  $4.6 \times 10^8$  cfu/g for 0-15 and 30-45 cm respectively while the THF recorded  $1.0 \times 10^6$  and  $0.6 \times 10^6$  cfu/g for 0-15 cm and 30-45 cm respectively. However there was a drastic decrease in the number of microbes in the soils after pesticide application especially at the surface soil depth of 0-15 cm. This shows that the pesticides application affected microbial population by reducing their numbers in the soil and this may in turn affect soil health and physiological status of their habitat. It is therefore recommended that proper surveillance during pesticide application should be considered to avoid drift to non-target organisms and that concentrations of pesticides to be used should be taken into account to avoid reduction in the number of microorganisms in soils because of the vital roles they play in maintaining soil health.

*Keywords: Soil microorganisms; pesticides; waste receptacles; public health hazards.*

## 1. INTRODUCTION

Pesticides are chemicals that destroy, kill, restrain or prevent life forms that are of nuisance value in the environment [1]. However, they can also impact negatively on the environment causing pollution if use is not properly controlled [2]. Pesticide application involves drift to produce droplets which can either remain suspended in the air and or may be carried away by wind from the intended target area. Pesticide drift can cause physical movement of residues through the air at the time of application or soon thereafter from the target site to any non-or-off target site" [3].

Drifts may occur during the application thereby causing droplets or residues to migrate from the intended target site to cause some chemicals to turn into vapour because of its residual activity which may either move away from the target site during application, thereby, causing unwarranted exposure to people, animals, plants and property and the entire environment.

Waste receptacles have been known to harbour pests, microorganisms and also alter the aesthetics of the environment [4,5]. These pests and microorganisms enter into nearby homes, thus creating the need for disinfection and management of pest around the waste receptacles. When pesticides are applied around the waste receptacles, it leads to residues of pesticides drifting from the target area to nearby environments such as soil particularly because of the permeable nature of the soil thereby affecting

soil microorganisms which are indicators of soil health [6]. These microorganisms are responsible for maintaining the physiological status of the soil and when altered can lead to reduction in the functioning of soil. The pesticides may adversely affect the proliferation of beneficial soil microbes and their associated biotransformation process in the soil and inactivate nitrogen fixing and phosphorous- solubilizing microorganism [7]. They can also reduce activities of soil enzymes which are key indicators of soil health. These activities may result in the alteration of the number of soil microorganisms which eventually leads to the disturbance in soil ecosystem and loss of soil fertility [8]. The aim of this study therefore, was to determine the impact of drifts resulting from pesticide application on soil microorganisms around waste receptacles in Port Harcourt, Nigeria.

### 1.1 Description of Study Area

The waste receptacle is located at Market Junction by Ikwerre Road in Mile 4 Diobu of Port Harcourt city. The geographical positioning system is between latitudes  $4^{\circ}82'71''N$  and longitudes  $6^{\circ}98'14''E$  for waste receptacle and latitudes  $4^{\circ}82'69''N$  and longitudes  $6.98'99''E$  for control [Fig. 1].

The waste receptacle is situated close to markets, churches, schools, borehole, restaurant and shops. The vegetation consist mainly grasslands and surrounded by surface water with drainage system/gutters that drives the leachates into the surface water.

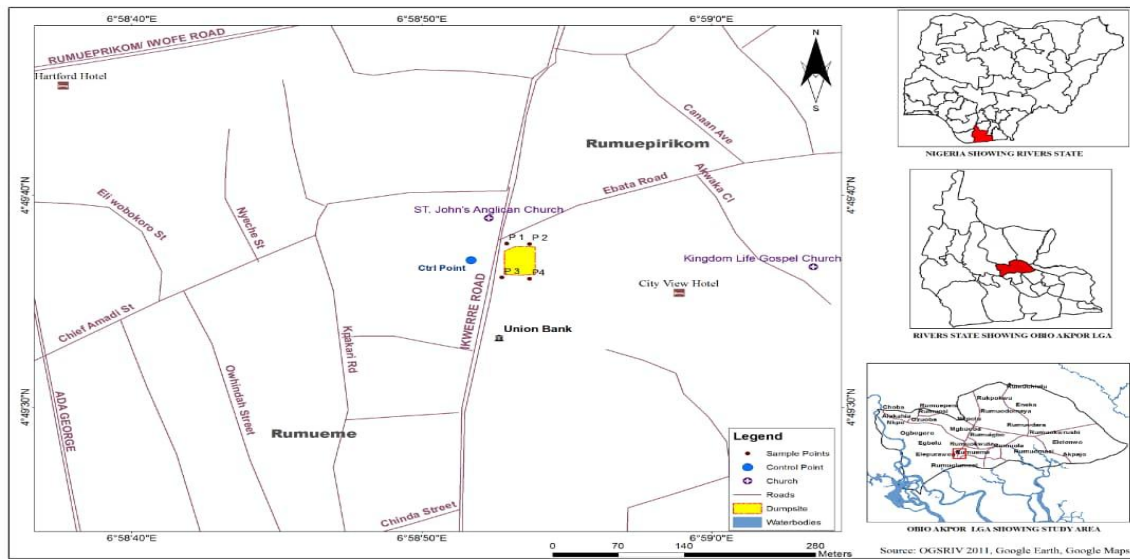


Fig. 1. Map showing Port Harcourt as the study Area

## 2. METHODOLOGY

### 2.1 Collection and Preparation of Samples

Samples were collected from the soil around the waste receptacle before application of pesticides. Samples were also collected 14 days after the application of pesticides to enable the percolation of pesticides into the soil and control samples were obtained 50 m away from the waste receptacle location. Ten grams [10 g] each of soil samples were obtained from different depths of 0 – 15 cm, 15 – 30 cm, 30 – 45 cm and 45 – 60 cm using a hand auger at four different points around the wastes receptacle. Samples were labelled A, B, C, and D while the control was CP1, CP2, CP3 and CP4. Using a hand-auger, approximately one inch of the top surface soil was discarded as this represented materials collected before penetration of the sample depth [9]. The same sample depth of soils from four different points around the waste receptacle were homogenized to form a composite soil mixture and were labelled P1, P2, P3 and P4 while that of the control was not homogenized. P1 represented homogenized soils from sample depths 0-15 cm from the four different points (A, B, C and D); P2 represented homogenized soils from sample depths 15-30 cm from the four different points (A, B, C and D); P3 represented homogenized soils from sample depths 30-45 cm from the four different points (A, B, C and D) and P4 represented homogenized soils from sample depths 45-60cm from the four different points (A,

B, C and D). A total of eight samples of soils (P1, P2, P3, P4, CP1, CP2, CP3 and CP4) for which each sample were aseptically transferred directly into well labelled sterilized glass bottle containers and capped secured tightly to minimize contamination and was taken to the laboratory in an ice-packed cooler for analyses.

### 2.2 Microbiological Analysis

One gram each of the soil samples were aseptically transferred into a sterile test-tube containing 9.0 ml diluents (normal saline) as described by Akinde and Obire [10], and [11]. Subsequently, six fold ( $10^{-6}$ ) serial dilutions were prepared from the  $10^{-1}$  dilution. A 0.1 ml aliquot of  $10^{-4}$  dilution was aseptically spread on solidified nutrient agar (NA) (Oxoid CM3) plates for bacteria and on Sabouraud Dextrose Agar (SDA) plates for fungi isolates. The inoculated plates were incubated at 37°C for 24 to 48 h and 25°C for 5 days respectively after which the plates were examined [12], for growth. Both bacterial and fungal counts were measured and expressed as colony forming units (cfu) per gram of soil sample. Discrete colonies were sub-cultured onto NA plates and SDA medium for the development of pure isolates, which was stored on slants for subsequent characterization and identification on the basis of their cultural, morphological, and cellular characteristics. The presumptive identification of isolates was done with reference to Bergey's Manual of Determinative Bacteriology [13].

### 3. RESULTS AND DISCUSSION

The results of the microbial counts for total heterotrophic bacteria and fungi before and after application are present in Table 1. THB recorded  $2.08 \times 10^9$  cfu/g at a depth of 30-45 cm while THF had  $6.0 \times 10^6$  cfu/g before application with a mean value of  $1.02 \times 10^9$  and  $2.8 \times 10^6$  cfu/g respectively. THB had for the surface soil of 0-15 cm recorded  $1.28 \times 10^9$  cfu/g and THF had  $1.9 \times 10^6$  cfu/g before application. However, after application of pesticide on the soil THB had  $4.1 \times 10^8$  and  $4.6 \times 10^8$  cfu/g for 0-15 and 30-45 cm respectively while the THF recorded  $1.0 \times 10^6$  and  $0.6 \times 10^6$  cfu/g for 0-15 cm and 30-45 cm respectively [Table 1].

The mean total heterotrophic bacteria (THB) counts in soils before application of pesticide show that  $1.02 \times 10^9$  cfu/g was obtained before application while  $3.82 \times 10^8$  cfu/g was recorded after application. The control was  $2.7 \times 10^9$  cfu/g and this was higher than the value obtained before and after application (Table 1). This showed that pesticide application on soil environment reduced the bacterial population comparatively to the control where there were no application of the chemical. However, from this study, pesticides application affected the soil microbial population, by reducing the number of microorganisms and this may in turn affect soil health and physiological status of their habitat. This result corroborates with [14] who observed that the application of pesticides inhibits or kill certain group of microorganisms. Furthermore, [15] reported in their study that application of pesticides also led to a major decline in the bacterial population which may lead to a decline in the soil fertility. Majority of microorganisms that are found in these waste receptacles derived their nutritional requirement from the

biodegradation of the waste materials; which accounts for the high bacterial growth profile [16]. In this study, high bacterial count occurred before the application of pesticides and reduced after the application of pesticides. The microbial population is considered as an indicator of soil health [16] and a useful indicator for the improvement or mineralization of the soils [17].

The mean counts for total heterotrophic fungi (THF) in soils before application of pesticides show that  $2.8 \times 10^6$  cfu/g was obtained, while  $6.8 \times 10^5$  cfu/g was recorded after application. There was a reduction in THF as a result of pesticides that were applied around waste receptacles and this process caused a reduction in the number of fungal counts thereby affecting the microbial population as well as affecting the soil health. This report is in consonance with [18] who observed similar trends on the effect of pesticides on soil microbial spectrum where soil microbes were reduced drastically due to injection of some quantities of pesticides, resulting in the extinction of some microbes particularly *Heruncola grisea* and *Alternaria terins* in the soil.

However, the bacterial isolates that were identified from soils treated with pesticides around waste receptacles and their frequency of occurrence were *Staphylococcus aureus* (15.4%) *Bacillus subtilis* (15.4%) *Bacillus megaterium* (15.4%), *Pseudomonas* sp (15.4%), *Micrococcus* sp (15.4%), *Bacillus cereus* (7.7%), *Bacillus licheniformis* (7.7%) and *Escherichia coli* (7.7%) (Table 2) but were not regular after application of pesticides on the soils. However, before the application of pesticides, microorganisms such as *Bacillus cereus*, *Bacillus licheniformis* and *Escherichia coli* were isolated from the soils. This shows that pesticide application the

**Table 1. Microbial counts of isolates from Soil treated with pesticides**

Pesticide application	Sample Depths	MICROBIAL THB (cfu/g)	COUNTS mean counts (cfu/g)	THF (cfu/g)	Mean counts (cfu/g)
Control	CP1 (0 – 15 cm)	$2.83 \times 10^9$	$2.7 \times 10^9$	$2.4 \times 10^6$	$2.1 \times 10^6$
	CP2 (15 – 30 cm)	$2.63 \times 10^9$		$1.8 \times 10^6$	
Before	BP1 (0 – 15 cm)	$1.28 \times 10^9$	$1.02 \times 10^9$	$1.9 \times 10^6$	$2.8 \times 10^6$
	BP2 (15 – 30 cm)	$0.55 \times 10^9$		$2.6 \times 10^6$	
	BP3 (30 – 45 cm)	$2.08 \times 10^9$		$6.0 \times 10^6$	
	BP4 (45 – 60 cm)	$0.20 \times 10^9$		$1.8 \times 10^6$	
After	AP1 (0 – 15 cm)	$4.1 \times 10^8$	$3.82 \times 10^8$	$1.0 \times 10^6$	$6.8 \times 10^5$
	AP2 (15 – 30cm)	$3.7 \times 10^8$		$0.4 \times 10^6$	
	AP3 (30 – 45cm)	$4.6 \times 10^8$		$0.6 \times 10^6$	
	AP4 (45 – 60cm)	$2.9 \times 10^8$		$0.7 \times 10^6$	

**Table 2. Biochemical characteristics of bacterial isolates from soil samples treated with pesticides**

Isolates	Colonial/morphology	Gram Reaction	Cell Morphology	Oxidase	Catalase	Methyl Red	VP	Indole	Citrate	Triple Sugar Iron	Motility	Sucrose	Glucose	Fructose	Lactose	Probable identity	F	F%
1	Gold-yellow, Small, Moist, Opaque round colony	+	Cocci	+	+	+	+	+	-	A	+	-	A	A	A	<i>Staphylococcus aureus</i>	2	15.4
2	Milk color, irregular, Opaque, undulated colony	+	Rod	-	+	+	+	-	+	A	+	-	A	-	-	<i>Bacillus subtilis</i>	2	15.4
3	Light green, Round, Moist, Opaque colony	-	Rod	+	+	+	-	-	-	-	+	-	-	-	-	<i>Pseudomonas sp.</i>	2	15.4
4	Yellow small, round, opaque, colony	+	Cocci	+	+	+	-	+	+	-	+	-	A	A	A	<i>Micrococcus sp.</i>	2	15.4
5	Milk color, irregular, opaque, rough surface, large colony	+	Rod	+	+	+	-	+	+	-	+	A	A	A	-	<i>Bacillus cereus</i>	1	7.7
6	Milk color, large, flat edged, moist, Opaque colony	+	Rod	-	+	+	-	+	-	-	+	-	A	A	-	<i>Bacillus megaterium</i>	2	15.4
7	Milk color, dry surface, filamentous, raised, opaque colony	+	Rod	+	+	+	+	-	-	-	+	A	A	A	A	<i>Bacillus lichenformis</i>	1	7.7
8	Large, Circular, convex, greyish white, moist, smooth and opaque colony	-	Rod	+	+	+	-	+	+	A	-	-	A	A	A	<i>Escherichia coli</i>	1	7.7

Key: - = negative, + = positive, A = Acid, F= Frequency of occurrence, VP= Vogues Proskauer

**Table 3. Characteristics of fungi from soils treated with pesticides**

Isolates codes	Morphological characteristics	Microscopic characteristics	Probable organisms	F	F%
1.	Black cotton growth, white boarder with dark brown reverse side.	Septate hyphae, road head conidia and spores	<i>Aspergillus niger</i>	2	18.2
2.	Green cotton radial growth with white boarder and dark brown reverse	Non septate branding hyphae and chain-like conidia. No spores	<i>Penicillium sp.</i>	2	18.2
3.	Brown cotton growth with brown reverse	Septate hyphae, round head conidia spores	<i>Aspergillus nidulas</i>	1	9.1
4.	White fluffy colony with yellow reverse side	Branding septate hyphae, no conidia head, no spores.	<i>Fusarium siculi</i>	2	18.2
5.	White fluffy growth with white reverse side	Septate branding hyphae, no conidial head, spores present	<i>Microsporium canis</i>	1	9.1
6.	Green colony with white boarder ad dark brown reverse side	Septate hyphae, columnar head, spores present. Vesicle present.	<i>Aspergillus fumigatus</i>	2	18.2
7.	Mucoid, milk colour opaque, elevated round colony	Cocoid shape	Yeast	1	9.1

Key: F%= Frequency of occurrence

occurrence and therefore reduces the capacity of the soil microbes in degrading waste materials. According to Handa *et al* [7], the pesticides may adversely affect the proliferation of beneficial soil microbes and their associated biotransformation process in the soil and also inactivate nitrogen fixing and phosphorous-solubilizing microorganisms. Pesticides application may also inhibit or kill certain group of microorganisms and outnumber other groups who were able to survive the chemical application by releasing them from competition in such environments. The observation corroborates with that of [19] who identified the presence of species of *Bacillus*, *Staphylococcus* and *Klebsiella* from a wastes dumpsite located at Eagle Island in Rivers state. The absence of some bacteria in the soil raises concern as these bacteria have been associated with soil health resulting in increases in soil fertility [20]. Also the findings corroborates with [6] who observed that pesticides can also reduce activities of soil enzymes which are key indicators of soil health. These microbes produce enzymes like DNase, hyaluronidase, staphylokinase, staphylolysin, streptokinase among others that help degrade waste materials at waste receptacle sites [18].

The distribution and frequency of occurrence of bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas sp*, *Micrococcus sp.*, *Bacillus cereus*, *Bacillus licheniformis* and *Escherichia coli* present in the waste dumpsites as observed in this study was

not surprising. However others organisms such as coliforms, faecal coliforms like *Escherichia coli*, *Pseudomonas sp* and *Salmonella sp* have been reported to associate with waste dumpsites or found in sewage [21].

The fungal isolates that were identified from soils treated with pesticides around the waste receptacles with their corresponding frequency of occurrence were *Aspergillus niger* (18.2%) *Penicillium sp* (18.2%), *Fusarium siculi* (18.2%) and *Aspergillus fumigatus* (18.2%), *Aspergillus nidulas*, *Microsporium canis* and *Yeast* had 9.1% respectively (Table.3). However, the fungal isolates in the soils before and after application of pesticides around waste receptacles were *Aspergillus niger*, *Penicillium sp.*, *Fusarium siculi* and *Aspergillus fumigatus* while *Aspergillus nidulas*, *Microsporium canis* and *Yeast* were absent after application. Fungi are important decomposers in the soil food web and help bind physically soil particles together thereby creating a condition of stable aggregate that increases water infiltration and soil water holding capacity [16]. A reduction in the number of fungal isolates may affect decomposition of organic matter and also cause a reduction in the binding of soil particles together. The frequency of occurrence of these fungal isolates have been reported earlier [22]. The high fungi counts observed in the control soil samples before application of pesticides may result from complex substrates of plant origin that are present in the soil suitable for agriculture.

#### 4. CONCLUSION

Pesticides have been widely applied in the management of pests around waste receptacles in Port Harcourt. Application of pesticides drifts may occur which adversely affects the ecosystem and also affects non-target organisms. The study revealed that there were reductions in total heterotrophic bacteria and total heterotrophic fungal counts after the application of pesticides when compared to soils that were not treated with pesticides. This reduction may affect soil health around waste receptacles which may affect soil fertility and the associated microorganisms which are involved in biogeochemical cycles. Their absence or decrease in the number of microorganisms in such ecosystems may disrupt nitrification and denitrification and associated roles they in maintaining soil health.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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