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Impact of Different Doses of Agrochemicals on Soil Microbial Diversity and Crop Yield

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Abstract

The effects of agrochemicals pollution on soil nutrients, soil microbes as well as plants cultivated on the soil were studied. Insecticide and herbicide were used to impact on the soil samples across concentration gradients. Bean and maize seeds were planted in separate experiments to monitor the effects of the agrochemicals on the plants. The microbial diversity and the physicochemical characteristics of the soil before and after pollution were determined using standard methods. Chlorophyll contents of the leaves were also determined after planting. Results indicated that bacterial isolates such as *Enterococcus* sp 54(40%), *Staphylococcus* sp 8(13%), *Bacillus* sp 46(34%) and *Micrococcus* sp 15(11%) and fungal isolates such as *Penicillium* sp 41(34%), *Saccharomyces* sp 67(56%), Yeasts 4(3%), *Geotrichum* sp 6 (5%) and *Aspergillus* sp 1(1.8%) were recovered from treated and untreated soil with the percentages representing after planting. The total nitrogen (TN) and available phosphorus (AP) showed no significant differences before and after planting while the exchangeable potassium (EP) had significant increase recorded after planting. Chlorophyll contents in the leaf of the maize plant decreased with increase in the concentration of the pesticide. Plants generally had poor growth characteristics recorded by stem girth and length, as well as chlorophyll content.

Keywords: Agrochemicals, insecticides, herbicides

Introduction

Microorganisms being the primary soil decomposers also drives key ecosystem processes such as organic matter decomposition, nutrient cycling, etc. which in turn increases plant productivity (Paul *et al*., 2007). Modern agricultural practices globally uses a variety of agrochemicals which includes herbicides, insecticides, nematicides, fungicides etc. to optimize crop production (Das and Mukherjee, 2008). However, incessant application of agrochemicals can lead to soil pollution which threatens soil microbial processes, thereby affecting soil fertility (Lopez *et al*., 2002).

Federal Environmental Pesticide Control Act (FEPCA) defines pesticide as any substance or mixture of substances used for preventing, destroying, repelling or mitigating pests such as insects, rodents, weeds, bacteria, nematodes, fungus, and other terrestrial or aquatic microorganisms (Mishra *et al*., 2001).

The persistence of pesticides in nature is determined by physicochemical properties of the soil, nature of substrates and environmental degradation. Incessant application and biological active residues of agrochemical endanger non-target organisms. Some of these pesticides interferes in the molecular interaction between plants and N-fixing rhizobacteria thereby inhibiting the important process of biological nitrogen fixation. They also reduces the activities of soil enzymes which are key indicators of soil fertility. In addition, agrochemicals can affect some biological reactions such as denitrification, nitrification, mineralization of organic matter, methanogenesis etc. (Piao *et al*., 2001).

An ideal pesticide should be toxic only to the target organisms, biodegradable and should not leach into the groundwater. Unfortunately, this is rarely the case and the global use of pesticides in modern agriculture is of great concern (Bishnu *et al*., 2009). Because of their chemical compounds characteristics, pesticides may badly affect the proliferation of beneficial soil microorganisms and their associated biotransformation in the soil (Joergensen, 1995).
This study reports on the impact of different doses of agrochemicals on soil microbial density and crop yield.

Materials and Methods

Sample location and collection

The soil sample was collected from a farmland at Nekede, with GPS coordinates of $5^0 25^1$ 59.99" N Latitude and 7^0 01^1 60.00" E Longitude, Owerri West,Imo State Nigeria. The soil sample was a dark brown silt-loam soil collected from 15-30 cm below the soil surface using soil auger into a sterile polyethene bag and taken to the laboratory for analysis. Debris and other plant materials were removed manually before sieving the soil through a 2mm stainless steel sieve.

Physicochemical analysis of soil sample

The exchangeable cation of potassium (K), Phosphorous (P), and total nitrogen (N) content of the soil sample were determined according to standard methods (Udo *et al*., 2009).

Characteristics of Agrochemicals

Agrochemicals were obtained from a commercial supplier in Owerri Main Market. Two agrochemicals used include herbicide (Atrazine and S- metolachor) which contains 290g/litre S-metolachor and 370g/litre Atrazine with purity of 94% and pesticide (Deltamethrin) with the purity of 98%.

Preparation of Agrochemicals

Nine hundred milliliters (900 ml) of sterile distilled water was diluted with 100 ml of herbicide to a total volume of 1000 ml (10 %v/v) herbicide. This was repeated for the pesticides.

Processing of soil sample

Forty kilogram (40 kg) of soil sample was weighed into a nursery bag. This was replicated into thirty six (36) bags and labelled appropriately.

Preparation and Spiking of the Soil with Agrochemicals.

The nursery bags containing the soil were labelled and contaminated with the agrochemicals followed by planting of the seeds. The inoculation was done using the same concentration of agro-chemicals but different volumes in this order: 10 ml, 20 ml, 30 ml, 40 ml, and 50 ml.

Experimental Design of Agrochemicals and seeds

Chemical +	Seed Code	Number of bags
Herbicide + Beans	HB	6
Herbicide + Maize	HC	6
Pesticide + Beans	PB	6
Pesticide + Maize	PC	6
Pesticide + Herbicide +Beans	PHB	6
Pesticide + Herbicide +Maize	PHC	6
Soil + Seed (Control)	SO	1

Seed Sterilization and Planting

Thirty milliliters (30 ml) of hypo bleach was diluted in 70 ml of water, the solution was used to sterilize the seeds (beans and maize) by soaking in the solution for 30 min. The soil samples were watered, then the seeds were sieved out and planted.

Monitoring of Plant Growth Parameters

This was done after one week of planting. The leaf length, stem length and stem girth were measured for plants with visible growth. This was repeated weekly for a period of six weeks.

Determination of Wet and Dry Weight of Harvested Plant after Six Weeks

The plants representing the different agrochemical treatment were uprooted after six weeks and soils carefully removed. Plants from each soil sample were tied together and the sands on the roots were properly removed. The wet and dry plants were in triplicates with a Uniscope weighing balance and mean weights recorded.

Determination of Chlorophyll contents in leaf

Fresh leaves of the maize plants were harvested and cut into pieces and soaked in 50 ml of absolute ethanol and left overnight. The supernatant was withdrawn and absorbance was measured at six hundred and sixty four nanometer (664 nm) and six hundred and forty eight nanometer (648 nm) in a spectrophotometer.

Results

Table 1. Percentage (%) occurrence of bacterial and fungal isolates before and after planting

	Isolate Period	
Organism	Before	After
Bacteria	*Micrococcus* sp. 16(19)	*Micrococcus* sp. 15(11)
	Enterococcus sp. 16(19)	*Enterococcus* sp. 54(40)
	Bacillus sp. 36(44)	*Bacillus* sp. 46(34)
	Staphylococcus sp. 13(14)	*Staphylococcus* sp. 18(13)
Fungi	*Penicillum* sp. 26(24)	*Penicillum* sp. (41)
	Saccharomyces sp. 31(23)	*Saccharomyces* sp. 67(56)
	Aspergillus sp. 11(10)	*Aspergillus* sp. (0.8)
	Geotrichum sp. 7(6)	*Geotrichum* sp. 6(5)
	Fusarium sp. 11(10)	Yeasts 4(3)
	Streptomyces sp. 3(2)	
	Mucor sp. 18(16	

The figures are the total number each isolate occurred.
Numbers in parenthesis are percentage (%) of occurrence

Species of fungi belonging to the genus *Fusarium, Saccharomyces, Aspergillus, Penicillium, Mucor, Streptomyces* and *Geotrichum* were isolated from both the treated and untreated soils before and after planting. The results also showed that *Bacillus* and *Enterococcus* species dominated the bacterial isolates while *Saccharomyces* and *Penicillium* species dominated the fungal isolates. *Fusarium, Mucor* and *Streptomyces* species were not detected after planting (Table 1).

Table 2: Physicochemical properties of soil samples before and after planting

	Concentration (%)									
	Before					After				
Plant/parameter	**10**	**20**	**30**	**40**	**50**	**10**	**20**	**30**	**40**	**50**
Pesticide + Beans (PB)										
Total nitrogen (g/kg)	0.10	0.12	0.06	0.09	0.11	0.10	0.02	0.12	0.14	0.12
Exchange potassium(mg/kg)	0.23	0.26	0.17	0.25	0.14	0.21	0.22	0.27	0.10	0.07
Available phosphorous (mg/kg)	0.50	0.48	4.09	12.06	0.32	16.12	15.95	19.64	7.71	0.4
Pesticide + Maize (PC)										
Total nitrogen (g/kg)	0.10	0.13	0.07	0.13	0.09	0.09	0.10	0.09	0.14	0.07
Exchange potassium(mg/kg)	0.06	0.10	0.14	0.21	0.10	0.12	0.07	0.24	0.14	0.06
Available phosphorous (mg/kg)	2.31	6.36	2.56	9.24	1.67	7.12	6.13	10.15	6.92	7.80
Herbicide + Bean (HB)										
Total nitrogen (g/kg)	0.12	0.14	0.08	0.10	0.07	0.07	0.08	0.03	0.12	0.11
Exchange potassium(mg/kg)	0.27	0.23	0.20	0.23	0.08	0.09	0.06	0.04	0.17	0.06
Available phosphorous (mg/kg)	0.33	0.93	3.36	4.12	10.13	5.10	7.80	16.82	17.56	1.10
Herbicide + Maize (HC)										
Total nitrogen (g/kg)	0.06	0.06	0.10	0.11	0.10	0.11	0.07	0.07	0.25	0.11
Exchange potassium(mg/kg)	0.15	0.17	0.13	0.12	0.24	0.29	0.20	0.19	0.14	0.15
Available phosphorous (mg/kg)	5.16	12.82	0.69	1.84	2.24	18.11	6.00	15.70	16.35	14.93
Pesticide + Herbicide + Beans (PHB)										
Total nitrogen (g/kg)	0.10	0.12	0.12	0.13	0.07	0.09	0.13	0.13	0.16	0.11
Exchange potassium(mg/kg)	0.29	0.24	0.25	0.20	0.19	0.18	0.12	0.26	0.25	0.23
Available phosphorous (mg/kg)	16.24	0.21	9.61	12.11	15.23	7.65	2.51	15.75	16.70	15.40
Pesticide + Herbicide + Maize (PHC)										
Total nitrogen (g/kg)	0.09	0.09	0.10	0.12	0.10	0.08	0.09	0.13	0.17	0.15
Exchange potassium(mg/kg)	0.09	0.18	0.25	0.22	0.21	0.24	0.13	0.12	0.26	0.09
Available phosphorous (mg/kg)	1.76	6.76	9.63	7.37	2.55	17.79	8.67	18.24	14.36	14.06
Control (SO)										
Total nitrogen (g/kg)	0.10					0.09				
Exchange potassium(mg/kg)	0.12					0.16				
Available phosphorous (mg/kg)	0.16					19.10				

The results of the physicochemical tests indicates that the soil quality was negatively affected. The increased volume of pesticides to soil samples reduced the microbial diversity drastically (Table 2).

Table 3: Effect of pesticide on the growth of maize

		Pesticide [PC (%)]				
Weeks	**Parameter (cm)**	**0**	**10**	**30**	**40**	**50**
1	Leaf length	-	-	-	-	-
	Stem length	4	6	6	-	-
	Stem girth	0.4	0.6	0.4	-	-
2	Leaf length	6	11	13	-	-
	Stem length	5	7	7	4	4
	Stem girth	0.4	0.6	0.3	0.5	0.6
3	Leaf length	9	15	13	4	-
	Stem length	8	8	8	6	6
	Stem girth	1.0	1.0	1.8	0.7	0.5
4	Leaf length10	20	20	13	4	
	Stem length8	5	10	8	8	
	Stem girth1.1	1.2	1.3	0.8	0.7	
5	Leaf length	13	21	20	15	14
	Stem length	10	9	13	13	9
	Stem girth	1.6	1.6	1.6	1.6	1.1
6	Leaf length	19	26	28	28	17
	Stem length	10	10	14	13	19
	Stem girth	1.6	1.3	1.2	1.1	1.3

KEY: Control – 0

Table 3 shows the effects of pesticide application on the growth parameters of maize plant over 6 weeks. It was observed that at week 1, there was an increased in seed dormancy caused by higher concentration of the pesticide at 40-50%. Despite the fact that the dormancy was overcome, the plants had an etiolated growth that is characterized by an increase in leave length with slender stems. The control plant had a normal growth with a large stem size, broad and short leaves. The growth pattern was seriously affected by increased concentration of the insecticide.

Table 4: Wet and dry weight of whole plants

PC	**WET WEIGHT (gm)**	**DRY WEIGHT (gm)**	**WATER CONTENT (gm)**
0	20.54	11.46	9.08
PC_{10}	13.79	5.35	7.94
PC_{30}	9.63	1.19	7.94
PC_{40}	10.30	3.96	6.42
PC_{50}	10.77	4.44	6.33

KEYS: Control – 0
Pesticide in maize - PC

Table 4 shows the wet and dry weights of whole maize plants after harvest. The results showed that the pesticide treated soils had negative effects on the weights and water contents of the whole plants as seen in the differences that occurred between the control and test experiments.

Table 5: Chlorophyll Content of the Leafs of Maize Plants in Pesticide impacted Soil Sample

SAMPLE	ABSORBANCE(nm)	
PC	664	648
0	2.032	2.187
10	1.834	2.038
30	1.064	1.025
40	0.717	1.026
50	0.477	0.371

KEYS: 0 - Control
PC – insecticide in maize

The effect of the pesticide on the chlorophyll content of the plant is shown in Table 5. Chlorophyll contents decreased with increase in concentration of the pesticides.

Discussion

Agrochemicals are the major soil, water and air pollutant and as a result, there is growing concern throughout the world at the way man is damaging his environment by injudicious use of pesticide to overcome problem of controlling insects, diseases, weeds etc. The microbes play an important role in the soil ecosystem, and their functions are very crucial in nutrient cycling and decomposition (Lorenzo *et al*., 2001).The increased use of agrochemicals in agricultural soils causes the contamination of the soil with toxic chemicals (Muñoz-Leoz *et al*., 2013). Despite this, some organisms have the capacity to resist concentrations of these chemicals to a particular thresholds. Moulds, yeasts and certain bacteria are dominant with this trait. In this work, after planting, the microbial community assessed revealed *Enterococcus* sp 54 (40 %), *Staphylococcus* sp 8(13 %), *Bacillus* sp 46 (34 %) and *Micrococcus* sp 15 (11 %) while the fungal isolates include *Penicillium* sp 41(34 %), *Saccharomyces* sp 67 (56 %), Yeast 4 (3 %), *Geotrichum* sp 6(5 %) and *Aspergillus* sp 1(0.8 %). *Fusarium, Mucor* and *Streptomyces* species were not detected upon analysis after planting which indicates their inability to resist concentrations of the agrochemicals used. This research has demonstrated that agrochemicals has significant effects on the soil nutrients, as well as the survival of plants in the polluted soil. In addition, previous researchers reported that the persistence of agrochemicals in soil results to chemical degradation of the soil (Kamrin, 1997; Gupta, 2001).Depending on several factors (e.g., pesticide composition, soil type, soil physicochemical and biological properties), most of these chemicals frequently have slow rates of degradation in the soil environment. Consequently, repeated application of agrochemicals can ultimately lead to their accumulation at concentrations detrimental to soil microorganisms (Munier-Lamy and Borde, 2000; Rice *et al*., 2002).

The chlorophyll content of the plant was also used as a yardstick to access the health of the plant. It was observed that higher chlorophyll content was seen in the control plant than all test plants at different concentrations. In addition, the chlorophyll content showed a decrease with increase in concentration of the pesticide.

Conclusion

Some organisms have the capacity to resist concentrations of agrochemicals to a particular thresholds. Moulds, yeasts and certain bacteria are dominant with this trait. Results shows that*Fusarium, Mucor* and *Streptomyces* species were not detected after planting which indicates their inability to resist concentrations of the insecticides and herbicides used. This work has demonstrated that agrochemicals has significant effects on the microbial population, soil nutrients, as well as the survival of plants in the polluted soil. The fate of pesticides in the soil and the transport processes depend on the cumulative effects of the pesticide's characteristics (e.g., absorptivity, solubility, volatility and degradation rate), the soil's characteristics (e.g., texture and organic matter), the application methods used (e.g., aerial or ground) and the site conditions (e.g., topography, weather and irrigation).

Recommendations

Based on the conclusion, to minimize damage due to pesticide application to soil ecosystems, the following precautionary measures should be adopted: pesticides with least hazard should be applied in minimum effective dose; application should be limited to target area; adequate and proper information on the use of agrochemicals should be provided to farmers.

It is also recommended that a socioecological studies be carried out in communities where there has been regular use of agrochemicals to avert the negative effects associated with improper use. In addition, molecular studies on resistant and recalcitrant organisms will help to strengthen knowledge about the spectrum of activities of pesticides.

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