South Asian Journal of Research in Microbiology

4(4): 1-13, 2019; Article no.SAJRM.50704

Bacteriological Assessment of Soil Treated with Pesticide and Herbicide in Birnin Kebbi Metropolis of Kebbi State, Nigeria

Joseph A. Famubo[1*] and Bunmi B. Oladunjoye[1]

[1]Department of Microbiology, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author JAF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BBO managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2019/v4i430114
Editor(s):
(1) Dr. Luciana Furlaneto-Maia, Lecturer, Department of Microbiology, Federal Technological University of Parana, Brazil.
(2) Dr. Eliton da Silva Vasconcelos, Department of Physiological Sciences, Federal University of Sao Carlos – UFSCar, Rod. Washington Luiz, Sao Carlos, Brazil.
(3) Dr. Chamari Hettiarachchi, Senior Lecturer, Department of Chemistry, University of Colombo, Sri Lanka.
(4) Dr. Osunsanmi Foluso Oluwagbemiga, Department of Biochemistry and Microbiology, University of Zululand, South Africa.
Reviewers:
(1) Ahmed Tabbabi, Jichi Medical University, Japan.
(2) P. Saravana Kumara, RVS College of Arts & Science, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/50704

Original Research Article

Received 20 July 2019
Accepted 21 September 2019
Published 30 September 2019

ABSTRACT

The present study was carried out on the effect of pesticides on soil microorganisms at half (x0.5) recommended rate (x1.0), and one and a half (x1.5). One commonly used insecticide Sniper as pesticide and herbicide Glyphosate were used on some physicochemical parameters and microbial populations. The mean value of pH for Sniper (x0.5) was 7.0; Sniper (x1.0) was 6.9; Sniper (x1.5) was 6.8; Glyphosate (x0.5) was 6.9; Glyphosate (x1.0) was 6.8: Glyphosate (x1.5) was 6.8 and for control soil was 7.3 respectively. The conductivity was ranged with a mean of 308.1 mS for Sniper x0.5, 410.3 mS for Sniper x1.0, 388.1 mS for Sniper x1.5, 197.8 mS for Glyphosate x0.5, 117.4 mS for Glyphosate x1.0, 223.85 mS for Glyphosate and 185.7 mS for the control soil. The soil organic matter was taken immediately after the treatments, and after the four weeks of treatment, the values were 1.50 g at week 0 and 0.72 g at week 4 for Sniper x0.5; 1.35 g at week 0 and 0.42 g at

**Corresponding author: Email: ayobreakthrough@gmail.com;*

week 4 for Sniper x1.0; 1.71 g at week 0 and 0.50 g at week 4 for Sniper x1.5; 1.21 g at week 0 and 0.75 g at week 4 for Glyphosate x0.5; 1.05 g at week 0 and 0.86 g at week 4 for Glyphosate x1.0; 1.67 g at week 0 and 1.01 g at week 4 for Glyphosate x1.5 and 1.90 g at week 0 and 1.45 g at week 4 for the control soil. A total of 8 bacteria species were identified, such as *Bacillus spp* (50%), *Lactobacillus spp* (8.3%), *Proteus spp* (5.6%), *Staphylococcus spp* (11.1%), *Actinomycetes spp* (8.3%), *Micrococcus spp* (2.8%), *Pseudomonas spp* (8.3%) and *Flavobacterium spp* (5.6%). The effect of these findings shows that pesticides might be affecting the soil microbial load by reducing it.

Keywords: Insecticide; herbicide; soil contamination; soil degradation; micro-organisms.

1. INTRODUCTION

Microorganisms affect the chemical exchange between roots and soil which act as a reservoir of nutrients. Soil organic matter is made up of organic compounds and includes remains of plants, animals and microbial material. A typical soil contents a biomass composition of 70% microorganisms, 22% macrofauna and 8% roots. A small part of the organic matter consists of living cells working to break down the dead organic matter. Soil types can be described as 3 main types namely sandy soil, clay soil and silt soil. The combination of two or more of these types can lead to the formation of a type of soil with a different texture e.g. loam soil [1].

Most pesticides are used in agriculture for one of the following purposes including protecting plants or plants products against all harmful organisms (e.g. fungicides), influencing the life processes of plants (e.g. PGRs), preserving plant products (e.g. fumigants), checking or preventing undesired growth or plants (e.g. herbicides), destroying undesired plants or plants or parts of plants (e.g. defoliants) [2].

Pesticides are also useful for maintenance of water reserves; treatment of large reserves of water, natural or artificial dams, ponds and pools, canals, pond etc. In public health, it is used in control of disease vectors such as malaria, dengue, Chagas disease, trypanosomiasis, schistosomiasis, leishmaniasis and typhus. It is also used in livestock and domestic care animals for disinfection of sheep and pets like dogs and cats. Pesticides are used for the treatment of structures: public and private buildings, offices, hospitals, hotels, cinemas, theatres, restaurants, school, supermarkets etc. In industries, pesticides are used in the manufacture of refrigerators, electrical equipment, paints, resins, adhesives, waxes, and liquid limpiamentales. In the food industry, it is used for preservation of fresh foods such as meat, pesacados etc [3].

Biodegradation of pesticides and herbicides is greatly influenced by the soil factors like moisture, temperature, pH and organic matter content. Optimum temperature, moisture and organic matter in the soil provide a congenial environment for the breakdown or retention of any pesticides added to the soil. Most of the organic pesticides and herbicides degrade within a short period (3-6 months) under tropical conditions. Not all pesticides and herbicides are biodegradable, and such chemicals shows complete resistance to biodegradation are referred to as being “recalcitrant” [4].

Concern for pesticide and herbicide contamination in the environment in the current context of pesticide use has assumed great importance [5]. The fate of the pesticides in the soil environment in respect of pest control efficacy, non-target organism exposure and off-site mobility has become a matter of environmental concern [6], potentially because of the adverse effects of pesticidal chemicals on soil microorganisms [7], which in turn might affect the soil fertility [8]. An ideal pesticide should have the ability to destroy target pest quickly and should be able to degrade to non-toxic substances as quickly as possible and not leach into groundwater. Pesticides are often applied directly to the soil. They might also reach the soil through application of foliage via spray drift, run-off, or wash-off vectors [9].

Indiscriminate, long term and over-application of pesticides have severe effects on soil ecology which lead to alterations in or the erosion of beneficial or plant probiotic soil microflora. Pesticides are widely used against a range of pests infesting crops. Globally, about 3×10^9 kg of pesticides is applied annually with a purchase price of nearly $40 billion each year [10]. The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminates the soil environment [11,12]. With the growing use of pesticides in contemporary

agriculture, the issue of the impact of these chemicals on the composition of soil microorganisms and the processes they direct has received more attention recently [13,4]. The applied pesticides might be harm the indigenous microorganisms, disturb the soil ecosystem, and might affect the human health by entering into the food chain. Adverse impacts of pesticides on soil microbial diversity and activities was described by several researchers [14,15,16,17].

Pesticides in soil undergo a variety of degradative, transport, and adsorption/desorption processes depending on the chemical nature of the pesticide [18] and soil properties [19]. Pesticides interact with soil organisms and their metabolic activities [20] and might alter the physiological and biochemical behaviour of the soil microbes. A microbial biomass is an important indicator of microbial activities and provides a direct assessment of the linkage between microbial activities and nutrient transformations and other ecological processes [21]. Several recent studies reveal the adverse impacts of pesticides on soil microbial biomass and soil respiration [22,5]. Generally, a decrease in soil respiration reflects the reduction in microbial biomass [23,24] or increase in respiration implies the enhanced growth of bacterial population [25].

Many modern chemical herbicides used in agriculture and gardening are specifically formulated to decompose within a short period after application. This is desirable, as it allows crops and plants to be planted afterwards, which could otherwise be affected by the herbicide. However, herbicides with low residual activity (i.e. that decompose quickly) often do not provide season-long weed control and do not ensure that weeds' roots are killed beneath construction and paving (and cannot emerge destructively in years to come). Therefore, it remains a role for weed killers with high levels of persistence within the soil [26].

2. MATERIALS AND METHODS

2.1 Collection of Sample

Ten kg of soil sample was collected from the Kebbi State University of Science and Technology agricultural field. Topsoil (0-20 cm deep) was collected after the weed and debris have been removed. The soil sample was sieved by passing through a 2.0 mm width mesh sieve to remove the plant debris and stones present in the soil. One kg of the soil sample was weighed into a plastic bowl that had been perforated under, to allow proper drainage of soil and aeration. This was replicated for six plastic bowls.

2.2 Sources of Pesticides

2.2.1 Insecticide (Sniper)

The chemical is dichlorvos and was obtained from a pesticide dealer shop in Birnin Kebbi, Kebbi State. Dichlorvos was marketed with the commercial name Sniper produced by Loveland products and supplied as 100 ml.

2.2.2 Herbicide (Glyphosate)

The chemical is Glyphosate and was supplied from the Department of Microbiology, Kebbi State University of Science and Technology, Aliero. Glyphosate is marketed with the commercial name Glyphosate and supplied as 1000 ml.

2.3 Experimental Setup

One kilogram (1 g) of the soil sample in each bowl was treated as described below:

i. BOWL A: Soil in bowl A was treated with DD Force at x0.5 of the manufacturer's average recommended rate of 1.0 ml in 100 ml distilled water per 1.0 kg of soil sample.
ii. BOWL B: Soil in bowl B was treated with DD Force at x 1.0 of the manufacturer's average recommended rate of 1.0 ml in 100 ml distilled water per 1.0 kg of soil sample.
iii. BOWL C: Soil in bowl C was treated with DD Force at x1.5 of the manufacturer's average recommended rate. To obtain this 1.5 ml in 100 ml distilled water was mixed with 1.0 kg of soil sample.
iv. BOWL D: Soil in bowl D was treated with Glyphosate at x 0.5 of the manufacturer's average recommended rate of 1.0 ml in 100 ml of distilled water mixed with the soil sample.
v. BOWL E: Soil in bowl E was treated with Glyphosate at x 1.0 of the manufacturer's recommended rate of 1.0 ml in 100 ml distilled water mixed with the soil sample.
vi. BOWL F: Soil in bowl F was treated with Glyphosate at x 1.5 of the manufacturer's recommended rate of 1.0

ml in 100 ml distilled water mixed with the soil sample.

vii. BOWL G: Soil in bowl G was treated with 100 ml distilled water into 1.0 kg of the soil sample to serve as the control.

2.4 Determination of Soil Organic Matter

Seven crucibles which had been cleaned were weighed and labelled according to the plastic bowls containing the soil samples. 5 g of soil was weighed from each sample and transferred into a crucible. The crucibles were placed over a Bunsen burner with the aid of a tripod stand covered with a net mesh and the soil was stirred over the fire for 15 minutes using a glass rod and a peg. The crucibles were removed and transferred to a desiccator to cool down, after which the soil organic matter was determined.

2.5 Determination of pH and Conductivity

The pH and conductivity of the soil were determined before and after the application of pesticides. The measurements were taken before and after the application of pesticides to a sample for four weeks having a week interval between each sampling.

To determine the soil pH and conductivity, 20 g of the soil sample was mixed in 40 ml of distilled water in a beaker and stirred thoroughly. The soil mixture was allowed to stand for 30 minutes with intermittent mixing. Allowing the coarse particles to settle, the pH and conductivity were taken by using a pH and conductivity meter respectively.

2.6 Sterilization of Materials

The laboratory materials used for these experiments such as test tubes, sterile water were sterilized first in an autoclave at 121℃ for 15 minutes at a pressure of 1 kg/cm square. Glass Petri dishes, pipettes were sterilized in an oven at 160°C for at least 3 hours. Inoculating loop and forceps were sterilized by passing them over a Bunsen burner flame until red hot and allowed to cool before use.

Bacteria were isolated by using the nutrient agar. The medium was prepared by following manufacturer's instruction. A known quantity of the medium was weighed and the instructed volume of distilled water was added to it and stirred to until completely dissolved and the flask was corked using cotton wool and covered tightly with aluminium foil before transferred into the autoclave for sterilization.

2.7 Preparation of Media (Nutrient Agar)

Nutrient agar powder (12.6 g) was suspended into a 500 ml conical flask, agar-agar was added to it to aid the solidification of the medium, and 450 ml of distilled water was added using a measuring cylinder. The conical flask was corked and sterilized at 121℃ for 15 minutes.

2.8 Bacterial Counting and Isolation

90 ml of distilled water was dispensed in 7 conical flasks and covered with cotton wool. Also, 9 ml of water was dispensed into test tubes and covered with cotton wool; it was autoclaved and allowed to cool. Serial dilution for each sample was done to the dilution 10^{-5}.

10 g each of the pesticide-treated soil sample and control sample was weighed into each of 90 ml sterile distilled water in conical flasks to give 10^{-1} dilution, using a sterile pipette under aseptic condition, further dilutions were made up to 10^{-5} by pipetting 1ml from the previous dilution into 9.0 ml of sterile water. One millilitre of each dilution at 10^{-4} and 10^{-5} of each sample was transferred aseptically using a sterile pipette into sterile petri dishes which had already been labelled appropriately and mixed with molten sterile nutrient agar using the pour plate method. Each dilution was duplicated. The petri dishes were swirled gently and allowed to set, which were then incubated at 37℃ for 24 hours and necessary observations were recorded.

2.8.1 Pure culture techniques

Distinct colonies were picked by using a sterile inoculating loop to be sub-cultured on sterile Petri dishes containing nutrient agar until a pure isolate was gotten. The pure isolate was transferred into a nutrient agar slant to prevent contamination. An agar slant medium prepared in McCartney bottles and sterilized at 121℃ for 15 minutes, and put in the slanting position to cool, so that when the agar sets it is in that form. The isolates were transferred aseptically and incubated at 37 ℃ for 24 hours to enter the reproductive stage, after which they were stored in the fridge to stop further growth of the microorganism and prevent overgrowth.

2.9 Bacterial Identification and Biochemical Test

2.9.1 Gram staining

This procedure was carried out by making a bacterial smear with a sterile inoculating loop on

a grease-free microscope slide and heat-fixed. Crystal violet was used to stain the smear and allowed to stand for 1 minute, after which the slide was flooded with water using a wash bottle with distilled water, excess water was drained off. Gram's Iodine which acts as a mordant is used to cover the smear and left for 1 minute, and then it was washed off with distilled water from a wash bottle. Alcohol is used to decolourize, the smear is covered in alcohol and left for 30 minutes after which it was washed off. Safranin is then used to stain the smear and left for 1 minute before washing off. The slide was blotted gently to dry and it was viewed with the microscope using x100 objective lens under oil immersion.

A purple colouration of the cells is an indication of Gram-positive bacteria while a red to pink colouration indicates Gram-negative bacteria.

2.9.2 Catalase test

A drop of 3% hydrogen peroxide (HO) is put on a grease-free microscope slide. Using a sterile inoculating loop, a loopful of the bacterial isolate is picked and dropped on the slide, and then the suspension is observed.

- Positive test result: is denoted by the gas formation in the form of bubbles.
- Negative test result: no gas formation.

2.9.3 Indole test

This is important in the differentiation of coliforms and depends on the production of indole from tryptophan by the organism. Peptone water was used and it was prepared according to the manufacturer's instruction. It was dispensed into test tubes at 5 ml each and was corked. The test tubes were autoclaved at 121℃ for 15 minutes, then allowed to cool before inoculating the organism into the medium and incubated at 37℃ for 3 days. After incubation 0.5 ml of Kovac's reagent was added and the test tube was shaken.

- Positive test result: a red or pink ring layer at the top.
- Negative test result: a yellow ring layer.

2.9.4 Methyl red test

It was used to detect the production of sufficient acid by fermentation of glucose. The test organism is inoculated into glucose phosphate broth (5 ml), which has been already prepared and sterilized in an autoclave. It was incubated at 37℃ for 3 days. After incubation 5 drops of 0.04% of methyl red is added and mixed. The result was read immediately.

- Positive test result: bright red colouration.
- Negative test result: bright yellow colouration.

2.9.5 Vogues proskauer test

It is a test for production of acetylmethylcarbinol or acetoin. The test organism was inoculated into glucose phosphate broth (5 ml), already prepared and sterilized. It was incubated at 37℃ for 3 days. After incubation 1 ml of potassium hydroxide (KOH) was added mixed then 1ml of α-naphthol was added and shaken for aeration.

- Positive test result: the presence of acetoin, a strong red colour develops within an hour.
- Negative test result: yellow colouration.

2.9.6 Citrate utilization test

It is to test the ability of an organism to utilize citrate as a sole carbon source for growth. It results in alkaline pH that turns the indicator from green to blue. The medium Simmon's citrate was prepared in a beaker on the hot plate and 5 ml of the medium was dispensed into test tubes and was corked with cotton wool, it was autoclaved at 121℃ for 15 minutes. The test organism was inoculated into the medium and incubated at 37℃ for 3 days.

- Positive test result: change in colour of medium from green to blue.
- Negative test result: no colour change in medium.

2.9.7 Motility test

The test is used to detect motile organisms, the ability for them to swim from the point of inoculation to another uninoculated surrounding medium. The test organism was inoculated into a sterile sloppy medium (semi-soft). The medium consists of agar to enable "swimming" through the medium which has already been prepared and sterilized. It was incubated at 37℃ for 3 days.

- Positive test result: the organism grows along the line of inoculation and into the surrounding medium.

- Negative test result: the organism grows only on the surface of the agar.

2.9.8 Sugar fermentation test

This test was carried out to show the microorganisms that are capable of metabolizing a large variety of sugars as carbon sources. Peptone solution of the desired sugars was used in the ratio of 3:1 and 2 ml of 0.01% phenol red were all dissolved in 100 ml of distilled water. 5 ml was dispensed into each test tubes and a Durham tube was inverted and inserted while making sure it had no gas bubbles in it so as not to alter the result, it was then plugged with cotton wool and autoclaved at 121℃ for 15 minutes. The test organism was inoculated into test tubes and incubated at 37 ℃ for 48 hours. Acid production is shown by a change in colour from red to orange or yellow. Gas production is shown by a displacement of the solution in the Durham tube by air.

3. RESULTS

3.1 Effects of Pesticides Treatment on Soil pH

The effect of pesticide treatment on the soil pH after three weeks of treatment is presented in Table 1. The insecticide Sniper treated soils had a decrease pH values at week 1 and the consequent weeks; at x0.5 the recommended rate there was a reduction in pH from 7.2 to 6.7, at the recommended rate from 7.1 to 6.5 and x1.5 the recommended rate from 7.0 to 6.3. Soils treated with the herbicide glyphosate had an initial increase in pH value at week 1 and decreased after, soils treated at x0.5 the recommended rate had a pH value from 7.2 to 6.4, at the recommended rate from 7.2 to 6.3 and x1.5 the recommended rate from 7.1 to 6.1.

3.2 Effects of Pesticide Treatment on Soil Conductivity

The effect of pesticide treatment on the soil conductivity after three weeks of treatment is presented in Table 2. Sniper treated soil at x0.5 the recommended rate gave an increased soil conductivity values 440.0 mS, 327.0 mS, 270.3 mS, and 195.0 mS, respectively. At recommended rates, the insecticide sniper treated soil had the same trend with values of 426.0 mS, 687.2 mS, 379.1 mS, and 148.7 mS, respectively and at x1.5 the recommended rate values of 369.0 mS, 622.0 mS, 305.0 mS and 256.4 mS, were gotten respectively showing an increase in soil conductivity. The same trend occurred with the herbicide glyphosate treated soils having values of 281.0 mS, 280.1 mS, 214.2 mS, and 120.1 mS, at the recommended rate plus half, 254.0 mS, 183.7 mS, 152.9 mS, and 119.0 mS, at the recommended rate and 250.0 mS, 214.3 mS, 192.6 mS and 134.2 mS at x0.5 the recommended rate.

Table 1. The effect of pesticide treatment on soil pH

Treatment/weeks	Week 0	Week 1	Week 2	Week 3	Mean
Sniper x 0.5	7.2	7.2	6.9	6.7	7.0
Sniper x 1.0	7.1	7.1	6.7	6.5	6.9
Sniper x 1.5	7.0	7.1	6.6	6.3	6.8
Glyphosate x 0.5	7.2	7.2	6.8	6.4	6.9
Glyphosate x 1.0	7.2	7.2	6.6	6.3	6.8
Glyphosate x 1.5	7.1	7.3	6.5	6.1	6.8
Control	7.4	7.5	7.2	7.1	7.3

Table 2. The effect of pesticide treatment on soil conductivity

Treatment/weeks	Week 0	Week 1	Week 2	Week 3	Mean
Sniper x 0.5	440.0	327.0	270.3	195.0	308.1
Sniper x 1.0	426.0	687.2	379.1	148.7	410.3
Sniper x1.5	369.0	622.0	305.0	256.4	388.1
Glyphosate x 0.5	250.0	214.3	192.6	134.2	197.8
Glyphosate x 1.0	254.0	183.7	152.9	119.0	117.4
Glyphosate x 1.5	281.0	280.1	214.2	120.1	223.85
Control	209.0	238.0	180.5	115.2	185.7

3.3 Effect of Pesticide Treatment on Soil Organic Matter

The effect of pesticide treatment on the soil organic matter is presented in Table 3. The organic matter values of the soil treated with pesticides and control soil were taken immediately after the treatment and after four weeks. The soil treated with the insecticide sniper had decreasing values from week 1 to week 3. At x0.5 the recommended rate the value decreased from 1.50 g to 0.72 g, at the recommended rate from 1.35 g to 0.42 g and x1.5 the recommended rate from 1.71 g to 0.50 g. The same trend occurred for the soil treated with glyphosate having value 1.21 g to 0.75 g at x0.5 the recommended rate, at the recommended rate from 1.05 g to 0.86 g and x1.5 the recommended rate from 1.67 g to 1.01 g.

Table 3. The effect of pesticide on soil organic matter (g)

Treatment/week	Week 0	Week 4
Sniper x 0.5	1.50	0.72
Sniper x 1.0	1.35	0.42
Sniper x 1.5	1.71	0.50
Glyphosate x 0.5	1.21	0.75
Glyphosate x 1.0	1.05	0.86
Glyphosate x 1.5	1.67	1.01
Control	1.90	1.45

3.4 Effects of Pesticides on Bacterial Counts

The effect of pesticide Sniper and Glyphosate on bacterial counts at x0.5, the recommended rate and x1.5 the recommended rate is shown in Table 4. From the table, it can be observed that bacterial counts decreased steadily from week 1, with the insecticide sniper treated soil at x0.5 the recommended rate having values from 4.05 x 10^5cfu/ml to 3.0 x 10^5 and 1.60 x 10^5 to 1.21 x 10^5, at the recommended rate from 2.40 x 10^5 to 1.55 x 10^5 and 0.95 x 10^5 to 0.53 x 10^5 and at x1.5 the recommended rate from 1.90 x 10^5 to 1.80 x 10^5 and 1.11 x 10^5 to 0.8 x 10^5. The same trend of bacterial count decrease was seen to have occurred in soil treated with the herbicide glyphosate having values of 4.70 x 10^5 to 3.80 x 10^5 and 2.05 x 10^5 to 1.07 x 10^5 at x0.5 the recommended rate, at the recommended rate from 2.00 x 10^5 to 2.16 x 10^5 and 1.90 x 10^5 to 1.37 x 10^5 and at x1.5 the recommended rate the value of 2.95 x 10^5 and having an increase of 3.21 x 10^5 at week 1, then a decrease of 1.45 x 10^5 to 0.94 x 10^5 in the following weeks.

The bacterial count was higher in control soil compared to soils treated with pesticides.

3.5 Bacterial Isolations

The major bacteria isolated from soil treated with pesticides and control soil is seen in Table 6. A total of 36 bacteria were isolated from both the pesticide-treated soils and control soil, 10 of which were isolated from the control soil and 26 from pesticide-treated soils.

The most occurring bacteria isolated from the pesticide-treated soils and control soil were 18 *Bacillus spp* (50%). Other bacteria also isolated from the pesticide-treated soils and control soil include 3 *Lactobacillus spp* (8.3 %), 3 *Actinomycetes spp* (8.3%), 2 *Flavobacterium spp* (5.6%), 2 *Proteus spp* (5.6%), 4 *Staphylococcus spp* (11.1%), 1 *Micrococcus spp* (2.8%) and 3 *Pseudomonas spp* (8.3%). Details are presented in Table 5a and 5b.

Table 4. Bacterial counts (x105 CFU/ml) from soils treated with pesticides

Treatment/weeks	Week 0	Week 1	Week 2	Week 3	Mean
Sniper x 0.5	4.05	3.50	1.60	1.21	2.6
Sniper x 1.0	2.40	1.55	0.95	0.53	1.4
Sniper x 1.5	1.90	1.80	1.11	0.80	1.4
Glyphosate x 0.5	4.70	3.80	2.05	1.07	2.9
Glyphosate x 1.0	2.95	3.21	1.90	1.37	2.4
Glyphosate x 1.5	2.00	2.16	1.45	0.94	1.6
Control	5.10	4.60	6.00	1.55	4.3

Table 5a. Bacterial isolates from control soil

Bacterial isolates	Amount	(%)
Bacillus spp	4	40
Lactobacillus spp	2	10
Flavobacterium spp	1	10
Staphylococcus spp	2	20
Proteus spp	1	20
Total	10	100

Table 5b. Bacterial isolates from soil treated with pesticides

Bacterial isolates	Sniper	(%)	Bacterial isolates	Glyphosate	(%)
Bacillus spp	9	60	*Micrococcus spp*	1	9.1
Lactobacillus spp	1	6.7	*Actinomycetes spp*	2	18.2
Pseudomonas spp	3	20	*Staphylococcus spp*	2	18.2
Flavobacterium spp	1	6.7	*Proteus spp*	1	9.1
Actinomycetes spp	1	6.7	*Bacillus spp*	5	45.5
Total	15	100	Total	11	100

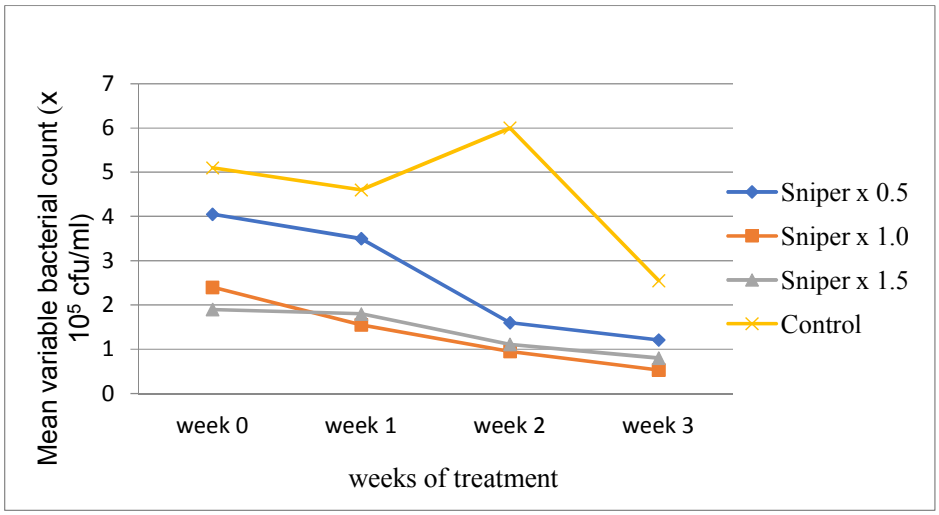

Fig. 1. Effect of the insecticide Sniper on mean bacterial counts

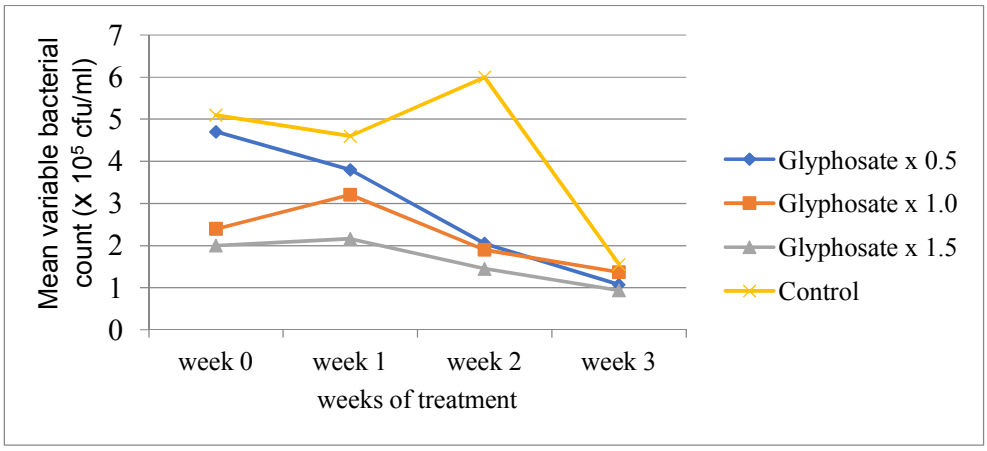

Fig. 2. Effect of the herbicide Glyphosate on mean bacterial counts

Table 6. Cultural, microscopic and biochemical characteristics of bacterial isolates from control soil and pesticides treated soil

Isolate code	Colour	Colony shape	Optical characteristics	Gram stain	Cell shape	Catalase	Indole	Methyl red	Vogues Proskauer	Citrate	Motility	Lactose	Sucrose	Glucose	Mannitol	Suspected organism
01	Cream	Circular	Translucent	+	Rod	+	-	-	+	+	+	-	-	A	A	*Lactobacillus spp*
02	Cream	Irregular	Opaque	+	Rod	+	-	-	-	+	+	-	A	AG	A	*Bacillus spp*
03	Cream	Circular	Opaque	+	Rod	+	-	-	+	+	-	-	AG	A	-	*Bacillus spp*
04	Yellow	Circular	Opaque	+	Cocci	+	+	-	-	+	+	-	A	A	A	*Staphylococcus spp*
05	Cream	Irregular	Translucent	+	Rod	+	-	+	+	+	+	AG	AG	AG	AG	*Actinomycetes spp*
06	Cream	Irregular	Opaque	+	Rod	+	-	+	+	+	+	-	A	A	A	*Bacillus spp*
07	Cream	Irregular	Opaque	-	Rod	+	-	+	-	+	+	AG	AG	-	AG	*Pseudomonas spp*
08	Cream	Irregular	Opaque	+	Rod	+	+	-	+	+	-	-	-	A	A	*Bacillus spp*
09	Cream	Circular	Opaque	+	Rod	+	-	-	+	+	+	AG	AG	AG	AG	*Bacillus spp*
10	Cream	Circular	Opaque	+	Rod	-	-	-	-	-	-	-	-	-	A	*Lactobacillus spp*
11	Cream	Circular	Opaque	+	Rod	-	-	-	+	+	+	-	AG	AG	AG	Bacillus *spp*
12	Yellow	Irregular	Translucent	+	Cocci	+	-	-	-	+	+	-	-	AG	AG	*Micrococcus spp*
13	Cream	Circular	Opaque	+	Rod	+	-	-	-	+	+	-	-	-	-	*Bacillus spp*
14	Cream	Irregular	Translucent	+	Rod	+	-	+	+	+	+	-	A	A	-	*Bacillus spp*
15	Cream	Circular	Translucent	+	Cocci	+	-	-	+	+	+	-	-	-	-	*Staphylococcus spp*
16	Cream	Irregular	Translucent	+	Rod	+	+	-	+	+	+	-	A	A	A	*Actinomycetes spp*
17	Cream	Circular	Opaque	+	Rod	-	-	-	-	+	-	-	-	A	A	*Lactobacillus spp*
18	Cream	Irregular	Translucent	-	Rod	-	-	-	-	+	+	-	-	-	-	*Proteus spp*
19	Cream	Circular	Opaque	+	Rod	-	-	-	-	+	+	AG	AG	AG	AG	*Bacillus spp*
20	Cream	Irregular	Translucent	+	Rod	+	-	+	+	+	+	-	AG	AG	A	Bacillus *spp*
21	Cream	Irregular	Opaque	-	Rod	+	-	+	-	+	+	AG	-	AG	AG	*Flavobacterium spp*
22	Cream	Irregular	Opaque	+	Rod	+	-	-	+	+	+	-	A	A	A	Actinomycetes *spp*

Isolate code	Colour	Colony shape	Optical characteristics	Gram stain	Cell shape	Catalase	Indole	Methyl red	Vogues Proskauer	Citrate	Motility	Lactose	Sucrose	Glucose	Mannitol	Suspected organism
23	Cream	Irregular	Opaque	+	Rod	-	+	+	+	+	-	-	-	A	-	*Bacillus spp*
24	Cream	Irregular	Opaque	+	Rod	+	+	-	+	+	+	-	A	A	A	*Bacillus spp*
25	Cream	Irregular	Opaque	+	Rod	+	-	-	+	+	+	-	-	-	-	*Bacillus spp*
26	Cream	Circular	Opaque	-	Rod	+	-	+	-	-	+	-	-	-	A	*Pseudomonas spp*
27	Cream	Irregular	Translucent	+	Rod	+	-	-	+	+	-	AG	AG	AG	AG	*Bacillus spp*
28	Cream	Circular	Opaque	+	Cocci	+	-	-	-	+	+	-	-	-	-	*Staphylococcus spp*
29	Cream	Irregular	Opaque	+	Rod	+	-	+	+	+	+	A	A	A	A	*Bacillus spp*
30	Yellow	Circular	Opaque	+	Cocci	+	-	+	-	+	+	-	A	A	A	*Staphylococcus spp*
31	Cream	Circular	Opaque	+	Rod	+	+	-	-	+	+	-	A	-	-	*Bacillus spp*
32	Cream	Circular	Opaque	-	Rod	-	-	-	+	+	-	-	A	-	-	*Proteus spp*
33	Cream	Irregular	Translucent	-	Rod	+	-	-	-	+	+	-	AG	AG	AG	*Flavobacterium spp*
34	Cream	Circular	Opaque	-	Rod	+	-	-	-	+	+	AG	AG	-	AG	*Pseudomonas spp*
35	Cream	Circular	Opaque	+	Rod	+	-	-	-	+	+	-	-	-	-	*Bacillus spp*
36	Cream	Irregular	Opaque	+	Rod	+	-	-	+	+	-	-	-	A	-	*Bacillus spp*

4. DISCUSSION

The primary objective by using pesticides in the fields and the environment is to achieve control of crop pests and disease vectors. This has been a deliberate human effort in a search for increasing agricultural yields and improving public health [27]. However, application of these pesticides might affect the soil microflora.

Degradation of pesticides is usually a combination of several processes, including microbial degradation and chemical hydrolysis and is also influenced by some physicochemical properties such as temperature, pH and carbon and nitrogen source [28].

The result shows that the application of pesticide leads to a decrease in organic matter. It shows that treatment at x0.5, x1.0 and x1.5 the recommended rate resulted in the reduction of organic matter between week 0 to week 4 when compared with control soil, which agrees with the report of Lotter, et al., 2003 endorsed a similar content of organic matter in the soil increases the amount of pesticide that will leave the area of application, because organic matter binds to helps breakdown pesticides. It has also been reported by [29] and [30] that the retention of glyphosate increases when the soil pH decreases allowing the adsorption of organic matter. There was a reduction in soil pH after week 1 from the neutral level to a slight acidic pH that could affected the microbial activity in pesticides degradation as stated by [31]. pH of the soil affects the degradation rate and suggested that the soil pH most competent for the best grade of degradation is around pH 7.

Result revealed a decrease in bacterial counts at each week at x0.5, x1.0 and x1.5 the recommended rates. It was observed that bacterial counts were higher in soils treated with Glyphosate in compared to Sniper treated soils at x0.5, x1.0 recommended rates and at higher concentrations. It was also reported that higher concentrations of glyphosate and sniper resulted in reduction of microbial counts as seen in Glyphosate treated soil with 2.16x105 cfu/ml at week 1 when compared to treatment at recommended rate which was 3.2 x105 cfu/ml which is in agreement with the reports of [26] that disruptions in microbial counts, activities and species can be caused by application of pesticides at greater or higher concentrations than the recommended doses.

From the results obtained in this research, pesticide applications have effects on soil organic matter, soil pH and conductivity Pesticides concentration also suppress microbial counts which in turn affect the soil fertility because microorganisms in the soil increase the soil fertility.

5. CONCLUSION

It is concluded that pesticides are major soil, water and air pollutant. There is a growing concern throughout the world, human being is damaging his environment by injudicious use of pesticides to overcome the problem of controlling insects, diseases etc. In spite of the importance of pesticide used in agriculture, indiscriminate use of pesticide can lead to soil degradation as well as damaging the environment. Precautionary measures can be taken in this regard such as limiting the area of application to target areas only to avoid excessive drift-off, not exceeding recommended application rates, selection of pesticides and methods that are least hazardous should be effectively carried out. Proper education on the use of pesticides in agriculture should be given to farmers for betterment of the environment and sustainability.

COMPETING INTERESTS

Authors have declared that no competing interests exist, but rather the research was a collective effort of all the authors. The authors were not funded or influenced by any detergent company. The choice of detergents chosen for the study was made by the authors because these detergents are commonly used in our country.

REFERENCES

1. Monkiedje A, Ilori MO, Spiteller M. Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. Soil Biol. Biochem. 2002;34:1939–1948.
2. EPPO. European and Mediterranean plant protection organization. Harmonized classification and coding of the uses of plant protection products; 2015. (Retrieved 25 June, 2015)
3. García J. Introducción a los plaguicidas. Universidad Estatal a Distancia, San José, Costa Rica. 1997;450. Spanish.
4. Baxter J, Cummings SP. The degradation of the herbicide bromoxynil and its impact

on bacterial diversity in a top soil. J. Appl. Microbiol. 2008;104:1605–1616.
5. Zhu G, et al. Microbial degradation of fipranil in clay loam soil. Water Air Soil Pollution. 2004;153:35-44.
6. Hafez HFH, Thiemann WHP. Persistence and biodegradation of diazinone and irnidacloprid in soil. Proc. XII Symp. Pest. Chem., Congress Centre Universita Cattolica, Via Emilia Parmense 84, Piacenza. 2003;35-42.
7. Araujo ASF, Monteiro RTR, Abarkeli RB. Effect of glyphosate on the microbial activity of two Brazillian soils. Chemosphere. 2003;52:799-804.
8. Schuster E, Schronder D. Side-effects of sequentially-applied pesticides on non-target soil microorganisms: Field experiments. Soil Biol. Biochem. 1990;22:365-373.
9. Racke KD, Coasts CD. Pesticides in the soil microbial ecosystem in enhanced biodegradation of pesticides in environment (Racke, K.D and coasts, J.R. Eds). Symposium 198th of American Chem. Sept 10-15, 1989 ACS Washington, DC. 1990;1-12.
10. Pan-UK. Current pesticide spectrum, global use and major concerns; 2003. (Retrieved 14 February, 2008)
11. Pimentel D. Amounts of pesticides reaching target pests: Environmental impacts and ethics. J. Agric. Environ. Ethics. 1995;8:17–29.
12. Carriger JF, Rand GM, Gardinali PR, Perry WB, Tomkins MS, Ferrandez AM. Pesticides of potential ecological concern in sediments from South Florida canals: An ecological risk prioritization for aquatic anthropods. Soil Sed. Contam. 2006;15:21-45.
13. Andrea MM, Peres TB, Luchini LC, Pettinelli A. Jr. Impact of long-term pesticide application on some soil biological parameters. J. Environ. Sci. Health B. 2000;35:297–307.
14. Niewiadomska A. Effect of carbendazim, imazetapir and thiram onnitrogenase activity, the number of microorganisms in soil and yield of red clover (*Trifolium pretense* L). Pol. J. Environ. Stud. 2004;13:403–410.
15. Ingram CW, Coyne MS, Williams DW. Effects of commercial diazinon and imidacloprid on microbial urease activity in soil. J. Environ. Qual. 2005;34:1573–1580.
16. Wang MC, Gong M, Zang HB, Hua XM, Yao J, Pang YJ, Yang YH. Effect of methamidophos and urea application on microbial communities in soils as determined by microbial biomass and community level physiological profiles. J. Environ. Sci. Health B. 2006;41:399–413.
17. Littlefield-Wyer JG, Brooks P, Katouli M. Application of biochemical fingerprinting and fatty acid methyl ester profiling to assess the effect of the pesticide Atradex on aquatic microbial communities. Environ. Pollut. 2008;153:393–400.
18. Laabs V, Wehrhan A, Pinto A, Dores E, Amelung W. Pesticide fate in tropical wetlands of Brazil: An aquatic microcosm study under semi-field conditions. Chemosphere. 2007;67:975–989.
19. Weberet JB, Wilkerson GG, Reinhardt CF. Calculating pesticide sorption coefficients (K sub(d)) using selected soil properties. Chemosphere. 2004;55:157–166.
20. Singh BK, Walker A. Microbial degradation of organophosphorus compounds. FEMS Microbiol. Rev. 2006;30:428–471.
21. Schultz P, Urban NR. Effects of bacterial dynamics on organic matter decomposition and nutrient release from sediments: A modeling study. Ecol. Model. 2008;210:1–14.
22. Pampulha ME, Oliveira A. Impact of an herbicide combination of bromoxynil and prosulfuron on soil microorganisms. Curr. Microbiol. 2006;53:238–243.
23. Chen SK, Edwards CA, Subler S. Effect of fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. Soil Biol. Biochem. 2001a;33:1971–1980.
24. Klose S, Ajwa HA. Enzymes activities in agricultural soils fumigated with methyl bromide alternatives. Soil Biol. Biochem. 2004;36:1625–1635.
25. Haney RL, Senseman SA, Hons FM, Zuberer DA. Effect of Glyphosate on soil microbial activity and biomass. Weed Sci. 2000;48:89–93.
26. Moorman TB, Bello. A review of pesticides effects in microorganisms and microbial processes related to soil fertility. Journal of Crop Production. 2000;2:14-23.
27. Helweg C, et al. Fate of pesticides in surface waters, laboratory and field experiments. Ministry of Environment, Danish Environmental Protection Agency. Pesticides Research No. 68; 2003.

28. Gunther AF, Gunther JD. Residues of pesticides and other contaminants in the total environment. Residue Reviews, 1st Edn., Springer, New York, ISBN-10: 978-0-387-05864-1; 1973.
29. Calvet R. Adsorption of organic chemicals in soils. Environmental Health Perspectives. 1989;83:145-177.
30. Mercadier C, Vega D, Bastide J. Iprodione degradation by isolated soil microorganisms. FEMS Microbiol. Ecol. 1997;23:207–215.
31. Muller K, Magesan GN, Bolan NS. A critical review of the influence of effluent irrigation on the fate of pesticides in soil. Agric. Ecosyst. Environ. 2007;120:93-116.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/50704