Pathogens Effects of Herbicides (Atrazine, Gramaxone and Glyphosate) on Soil Physicochemical and Microbiological Properties

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Abstract: The studies on the effects of the herbicide (atrazine, glyphosate and gramaxone) on soil physico-chemical properties was carried out between the months of August 2022 to January2023. Two sites forest and a garden were selected in Federal Polytechnic Mubi, Adamawa State, Nigeria using randomized complete block design (RCBD). The herbicides had significantly increased the amounts of soil organic carbon, organic matter, pH, sodium, potassium, bulk density, particle density, percentage moisture, available phosphorus and percentage nitrogen at $p< 0.001$ level of confidence, at same time lowering the values of available phosphorus (6.27 ppm), magnesium (2.19 ppm), sodium (0.36 ppm), potassium (2.10 ppm), iron (0.25 ppm) and manganese (7.21 cmol k^{-1}). Bacterial species isolated at the study sites showed *Pseudomonas aeruginosa, Pseudomonas putida, Bacillus subtilis, Bacillus sphaericus, Staphylococcus aureus, Klebsiella, Escherichia coli, Azotobacter nigricans, Flavobacterium aquaticum, Micrococcus loteus, Salmonella typhimurium*, and *Proteus vulgaris.* Forest soil was found having the highest percentage of (52.75 %), while, the garden (47.25 %) of soil bacterial isolates. The herbicides caused a decrease in the soil bacterial counts and that gramaxone at higher dosage caused the highest decrease while glyphosate the least. Garden soil was found as having higher bacterial counts compared with the forest. Month of November/120th day ($157.5x10^{-3}$ ±13 cfu/g) had the highest counts, followed by August, while December and January had the lowest which may be attributed due to seasonal variation from rainy to dry season.
Key word: Herbicide, soil, biodegradation, absorption, bacteria

INTRODUCTION

In the history of agriculture, more time, energy and money have been devoted to weed control than any other agricultural activity. The focus was on controlling persistent perennial weeds such as thistles, and quack grass with inorganic compounds such as sodium chlorate, sodium arsenate, and sodium metaborate tetra hydrate (Souzaa *et. al*., 2024). High cost, high rates and re cropping issues limited their use to small patches (Holm and Johnson, 2009). Prior to the invention of herbicides, manual weeding by hand was the most widely used weed management technique in Nigeria. However, it takes a lot of time and effort, physically taxing, and often expensive (Imoloame, 2014). According to estimates, manual weeding consumed between 40 and 60 percent of the cost of production, and in addition to being expensive, labour availability was unpredictable, making timeless weeding challenging to achieve and increasing yield loss. In areas like Sub-Saharan Africa (SSA), which is made up of Nigeria, Ghana, Benin, and Senegal, where modern input uptake has historically been limited and crop yields low, farmers may benefit from using modern agricultural inputs like pesticides and fertilizers to increase productivity (Sheahan *et al.* 2017). Herbicide application is a vital strategy of weed control. The effects of these chemicals on the non-target soil microorganisms are very intense; have adverse impact on physicochemical parameters of the soil, which in turn affect soil fertility and plant growth (Tudararo-Aherobo and Ataikiru, 2020). The term "pesticide" specifically refers to chemical compounds that change the biological functions of living things considered to be pests, whether these are weeds, harmful plants, insects, or fungi (Damalas, 2009). Herbicides have many different brand names, which farmers are familiar with; grouping them together is the simplest method to identify them. phenoxys, atrazines, substituted urease, triazoles, arbamates, and bipyridiliums are the most significant and prevalent categories (Souza *et. al*., 2024). Pesticides can also be divided into categories based on their intended use, mode of action, duration of effect, or chemical function. Examples include insecticides (used to kill insects), herbicides (used to kill weeds), fungicides (used to kill fungus), and nematocides (used to kill nematodes) (Van Bruggen *et al.,* 2021). An ideal pesticide must not permeate into ground water, be recyclable, and harmful exclusively to the creature it is intended to kill. This is regrettably rarely the case, and the widespread usage of pesticides is concerning (Krishnasamy *et al.*, 2019). All agricultural toxins are dumped in the soil, which also acts as the primary habitat for the majority of microbial species, including bacteria, actinomycetes, and fungus, whose activities affect soil fertility (Tudararo and Ataikiru, 2020). The presence of organic carbon generally affects how most herbicides behave (Torrents and Jayasundra, 1997). The volatility of an herbicide is the rate at which it transforms from a liquid or solid into a gas which is determined by its vapor pressure. These herbicides are less likely to persist than herbicides with high vapor pressures because volatility rises with temperature (Wyskowska and Kitcharski, 2003). According to studies, the persistence of the chemical in the environment, the amount, the frequency of application, and the herbicide's toxicity all affect how contaminated the soil is with these chemicals (Tudararo and Ataikiru, 2020). Solubility, adsorption, volatility, and degradation are some pesticide qualities that have an impact on how quickly these chemicals reach groundwater. Atrazine, ($C_5H_{14}ClN_5$), is a chlorinated systemic selective herbicide that is used extensively throughout the world to eradicate weeds in crops including sugar cane, maize, cornifers, pineapples, and others (Febrina, *et al.,* 2017). It has a half-life of 13 to 261 days in soil, 100 days in rivers, and 10 days in sea water, making it extremely persistent in these environments (Jiangwei, 2019). The average half-life of atrazine in microbially active soil is 2.4 times shorter than in sterile soil. N-(phosphonomethyl) glycine, also known as glyphosate or Roundup, is a post-emergence, non-selective herbicide that is used to kill weeds (Van Bruggen *et al.*, 2021). The non-selective herbicide is frequently used (Chantana *et al*., 2016). The features of paraquat, a non-selective contact herbicide, were found in 1955 (Febrina *et al.,* 2017). Soil texture, soil permissibility, organic matter content, soil site conditions, depth to underground water, geologic and climatic conditions are among the soil characteristics that influence pesticide transport (Sebiomo *et al.*, 2020).

MATERIALS AND METHODS

Description of the Study Area: The soil samples were collected in two distinct places that is a biological garden and forest area of Federal Polytechnic Mubi, Mubi North Local Government Area in Adamawa State, Nigeria. The Department of Biological Science's Biological Garden and Forest (which have never been treated with pesticides).

Sample Collection: Soil samples were collected following the composite method as described by Jaiswal, 2003. About 10 g of soil was collected after removal of debris at the depth of 0-30 cm using soil auger and placed in polythene bags. The samples were placed in the refrigerator. Petri dishes, conical flasks distilled water, plate count agar were used for bacterial culture and enumeration using colony counter. Herbicides were obtained ADADP stores, Yola. Soil analysis was carried out using pH meter, Flame Photometer, Kjedahl apparatus, Atomic Absorption Spectrometer

Experimental Design and Soil Sample Treatment: The soil plots from the two study locations (the Forest and the Biological Garden), were subjected to a randomized complete block design (RCBD) in the experimental layout. The test land was divided into equal sections that were each one meter square (1 $m^{2)}$ in size. For suitable spaces (alleys) between plots, one meter (1 m) of free ground was left on either side of each plot (Figure 2). Each of the farmlands under study was divided into one-meter-square (1 m^2) subplots, with a one-meter gap of open space between each subplot to produce gaps. Atrazine was measured and diluted in 5.6 milliliters (ml) of distilled water to a concentration that was twice the manufacturer's concentration (6 kg/ha) and sprayed on subplot one. Subplots 2 and 3 were treated with the same concentrations by mixing 0.3 milliliters of gramaxone or glyphosate with 5.6 milliliters of distilled water. Subplot 4 was left untreated and served as the control. Atrazine, gramaxone, or glyphosate were each diluted with 0.6 ml each of the three herbicides with 5.6mls of distilled water before being sprayed on subplots 5, 6, and 7. In each of the subplots, soil samples were taken before and after the treatment for a total of six months, and were then taken to the microbiology laboratory Adamawa State University, Mubi for analysis.

Determination of Physicochemical Properties of Soil samples: The soil particle size distributions was determined using the hydrometer method of soil mechanical analysis to separate the soil sample into sand, silt and clay particles (Jaiswal, 2003). Soil pH, presence of organic matter, organic carbon, soil nitrogen, bulk density, particle density, porosity, moisture content were determined also as described by Jaiswal (2003). Soil exchangeable bases such as calcium, magnesium were determined using Atomic Absorption Spectrophotometer(AAS), model; sp-AA3604F produced by Drawell Scientific while sodium and potassium using Flame Photometer model; PFP7 by Janway with their appropriate lamps as described by Jaiswal (2003).

Heterotrophic Bacterial Counts and Biochemical Characterization of Bacterial Isolates: Using the pour plate procedure as outlined by Cheesbrough (2006), 0.1 ml of the 10^{-4} soil dilution was pipetted into triplicate plate count agar plates. The inoculated plates were then kept in an incubator for 24 to 48 hours at 37ºC. Discrete colonies that appeared on the plates were then counted using a colony counter (√Henry and Paul (1971) and Cheesbrough (2006) and the results were multiplied by the reciprocal of the dilution factor. The colonies were expressed as colony forming units (cfu/g) per gram of soil. Biochemical tests such as Grams staining reactions, catalase, Indole, motility, Voges Proskuer, methyl-red, urease, and citrate tests were carried out to identify the bacterial isolates as described by Cheesbrough (2006), and the results compared with a standard table based on Bergeys Manual of Determinative Bacteriology by Bergey and John (1994).

RESULTS

Effects of Herbicides on Soil Physicochemical Properties

The findings obtained on the effect of the three herbicides (atrazine, gramaxone and glyphosate) on soil physicochemical properties (Table 1) showed no significant effect of increase or reduction at $p< 0.001$ level of confidence on soil particle size distribution (sand, silt and clay), magnesium, porosity and manganese on all the three herbicides treated soils. Gramaxone treated soil showed slight increase of silt (20.20 %), while atrazine treated soils had the highest amounts of clay (7.84%) and sand (72.68%). The herbicides had caused a significant increased at $p< 0.001$ level of confidence on soil organic carbon, organic matter, pH, sodium, potassium, bulk density, particle density, percentage moisture, available phosphorus and percentage nitrogen. Percentage organic matter (1.90 %) was the highest in glyphosate and lowest at atrazine treated soil. Organic carbon (1.17%) were recorded highest at atrazine treated soils while gramaxone treated soil had the least (1.17%). The present findings is in agreement with those of Blu *et al*. (2019) and Sebiomo and Banjo, 2020 observed that the values of potassium, magnesium, calcium, percentage organic matter, percentage organic carbon and percentage nitrogen were higher in herbicide treated soil than the control. The analysis of variance (ANOVA) interaction on the effects of the herbicides, its concentration, site of application and the control on soil physicochemical properties (Table 2) were found to have no significant difference on soil particle size distribution (sand, silt and clay), bulk density, particle density, percentage moisture content ,soil porosity, magnesium and potassium. The herbicides, its concentration, site of application and the control were found to have increased the amounts of soil organic carbon, soil pH, calcium and zinc, soil organic matter, percentage nitrogen, percentage moisture, available phosphorus, sodium, iron, cupper and manganese at $p < 0.01$ compared with the control and is in conformity with results obtained by Sebiomo and Banjo, 2020. The soil textural class for both forest and garden were sandy loam which shows appreciable amount of sand. Blu *et al*. (2019) attributed to the predominance of sand as a result of the humid tropical nature of Nigerian soils. Control soils were found having the highest percentages of organic carbon and organic matter in forest (1.61 %, organic matter 2.35 %) and (garden organic carbon 1.57 %, organic matter (2.72 %), while gramaxone treated with 6 kg/ha. had the lowest (O C. 0.46 % and O M 1.54%). According to Landon (1991) organic carbon above 20 % is rated as very high, 10-20 % as high, 2-4 % low and less than 2 % as very low. Thus, percentage organic carbon in both the herbicide treated and control soils are very low. This could be attributed to poor root growth of weeds due to their suppression by herbicides. Available phosphorus, sodium, potassium, iron, zinc, copper and manganese in control soils of both forest and garden soil were found significantly lower at $p > 0.01$ confidence level than herbicide treated sites. Forest control had high values of available phosphorus (6.27 ppm), magnesium (2.19 ppm), sodium (0.36 ppm), potassium (2.10 ppm), iron (0.25 ppm) and manganese (7.21 cmol k^{-1}) compared to garden and herbicide treated soils (Table 2). This observation is in agreement with the findings made by Sebiomo and Banjo (2020), who reported significant increase in iron, zinc, potassium, available phosphorus, sodium and manganese concentrations in soils treated with atrazine, primextra, glyphosate and gramaxone as compared with the control. Effect of period of treatment monitored for six months from August to January (30^{th} to 180^{th} day) on the soil physicochemical properties showed that at 99% confidence level of significant effect on all the other soil properties except on soil particle size distribution (Table 3). Highest values of organic carbon and organic matter were recorded in the month of December (150^{th} day after herbicide treatment) (O C 1.91 %) and (O M 2.88 %) and the lowest in September (60^{th} day) which agrees with the findings made by Rhoda *et al*. (2009) in which the authors asserted that long term use of herbicides may result to depletion of these micro and macro elements from the soil. Singh (2002) rated percentage nitrogen (< 0.15% as low, 0.2% as medium and > 0.2 % as high). Available phosphorus was also found in low quantities at both sites with average mean of 8.95 ppm as the highest in gramaxone treated soil which can be considered as low based on Singh (2002) rating (when phosphorus is < 8 ppm low, 8 – 20 ppm medium, > 20 high).

Isolation and characterization of bacterial isolates from herbicide treated and control soils

The bacterial species isolated at the study sites (Table 4) showed *Pseudomonas aeruginosa, Pseudomonas putida, Bacillus subtilis, Bacillus sphaericus, Staphylococcus aureus, Klebsiella, Escherichia coli, Azotobacter nigricans, Flavobacterium aquaticum, Micrococcus loteus, Salmonella typhimurium*, and *Proteus vulgaris.* Sebiomo and Banjo (2020) isolated *P. aeruginosa, P. putida, B. subtilis P. vulgaris, P. florescence* bacteria and some fungi in herbicide treated soils in Ijebu Ode, Nigeria, and also Muhiuddin *et al*. (2017) obtained similar organisms as observed in the present study. All the bacterial isolates were found widely distributed at the forest as well as garden soils and also in atrazine, gramaxone and glyphosate treated soils with exception of

Flavobacterium aquaticum (found only at the garden) and *Proteus vulgaris* (absent at the forest treated with atrazine). Forest soil was found having highest percentage of (52.75 %), while, the garden (47.25 %) of soil bacterial isolates. Ijah *et al*. (2021) found that the bacteria are not evenly distributed at the treated sites and the control which is in agreement with the present findings and also that of Nwadike, and Jibola-Shittu (2020). Percentage abundance of each bacterial isolate from each of the two study sites (Table 4) revealed *Pseudomonas aeruginosa, Pseudomonas putida,* and *Bacillus subtilis* as the most common bacterial species. Findings obtained and analyzed using ANOVA showed no significant differences in the frequency distribution of *Pseudomonas aeruginosa, Pseudomonas putida, Flavobacterium aquaticum, Klebsiella, Bacillus sphaericus, Bacillus subtilis and Salmonella typhimurium* in both atrazine, glyphosate and gramaxone treated soils. Significant differences at 95% confidence level were found in the frequency of distribution in the herbicide treated soils on Escherichia coli, *Micrococcus loteus, Azotobacter nigricans, Staphylococcus aureus* and *Proteus vulgaris* (Table 5). Highest frequency of distribution of *B. subtilis, E. coli, P. aeruginosa* (17.5) and *Klebsiella* (17.0) were found in atrazine treated soils. The lower values were recoded for *P. vulgaris, M. loteus and A. nigrican* in gramaxone treated soils with each having 2.5 frequency of distribution. P. vulgaris was completely absent in forest atrazine treated soils but present in all garden soils which can be attributed to the inhibition of the organisms by atrazine and its ability to develop resistance to the herbicides when continually in use on the site. Nwadike and Jibola-Shittu (2020) had similar results with the present findings in which they observed species of *Proteus vulgaris* and *Acinetobacter* were isolated only on in soils with lower dosages of herbicides and not found in higher dosages. The authors suggested that higher herbicide dosages may lead to disappearance of some species

Effects of Herbicides on Total Heterotrophic Bacterial Counts (THBC)

Results of one way Analysis of Variance (ANOVA) (Figure 3) plotted on the effects of herbicides on total heterotrophic bacterial counts showed that the control soil had highest bacterial counts ($273.03x10^3$ ±32 cfu/g), followed by glyphosate at 3 kg/ha (300 ppm) (161.0 $x10^3$ ±57 cfu/g) and gramaxone at 6 kg/ha (600 ppm) ($44.39x10^3$ ±22 cfu/g) having the least. This is line with findings made by Mazhari and Ferguson (2018), Sebiomo *et al.* (2020) and also Nwadike and Jibola-Shittu (2020) in which the authors reported higher bacterial counts in glyphosate than in gramaxone. Period of soil treatment with the herbicide (Months/ Days) showed the month of November/120th day ($157.5x10^3$ ±13 cfu/g) having the highest HBC counts, followed by August/30th day ($133.0x10^3$ ±72 cfu/g), while December and January/150th and 180th days had the lowest ($97.376x10^3$ ±57 cfu/g) and ($103.776x10^3$ ±645 cfu/g) respectively (Figure 4). Garden soil was found to have a higher THBC ($130.6.5x10^3$ ±19 cfu/g), while forest had ($115.78x10^3$ ±66 cfu/g). Garden soil was found as having higher bacterial counts compared with the forest (Figure 5). Mazahri and Ferguson (2018) had similar results with the present findings, and observed that the effect of the herbicides gramaxone and glyphosate led to reduction in bacterial population and colony counts were dependent on concentration of the herbicide as also was observed by Nwadike and Jibola-Shittu (2020). Chantana *et al.* (2016) reported that gramaxone is toxic to soil microorganisms and was implicated in reduction of bacterial population which also agrees with the present findings. Adomako and Ayeampong (2016) also reported reduction in bacterial population when treated with gramaxone. Mazhari and Ferguson (2018) reported that glyphosate treatment was found to cause an increase in bacterial population.

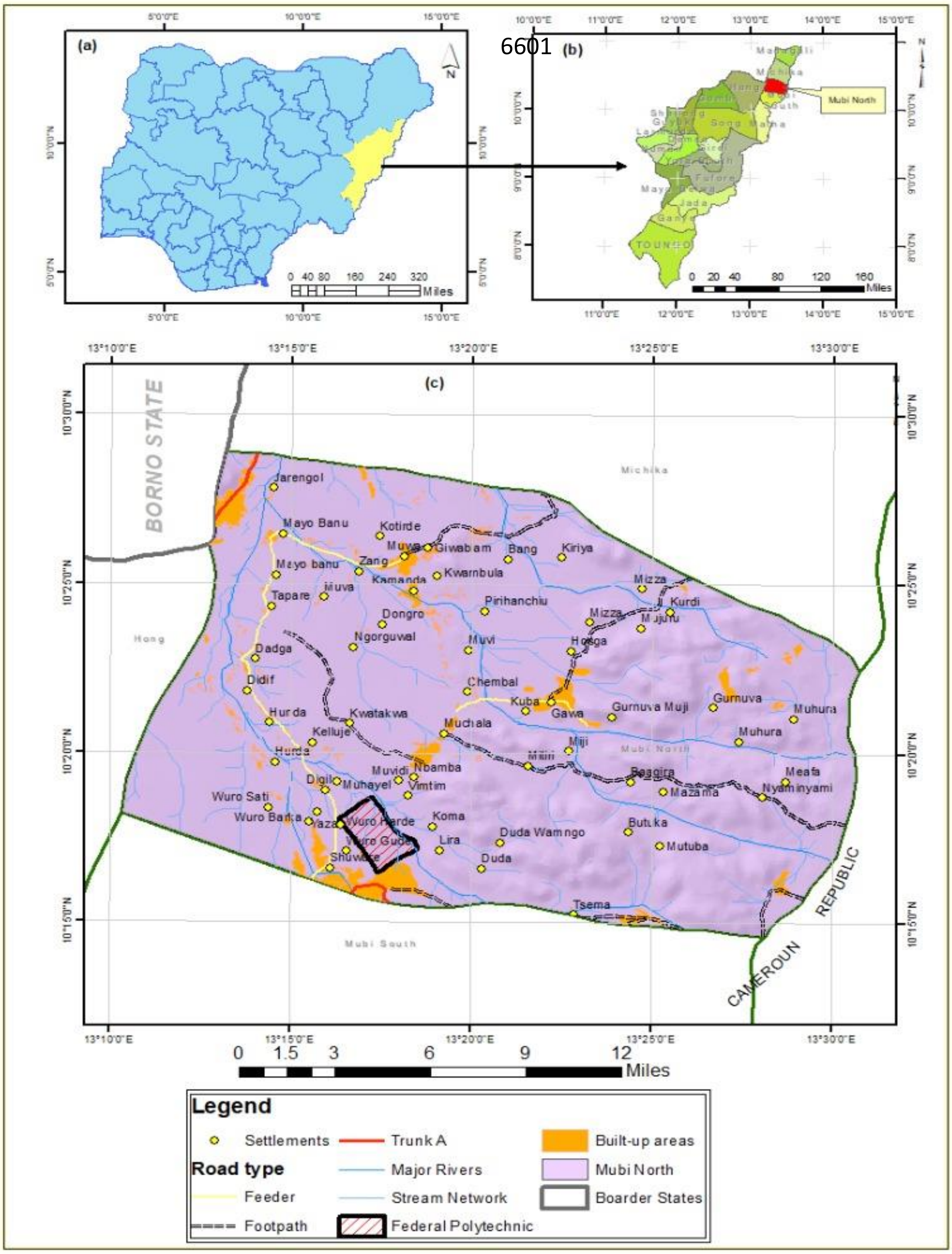

Figure 1: Map of Mubi and Study Area

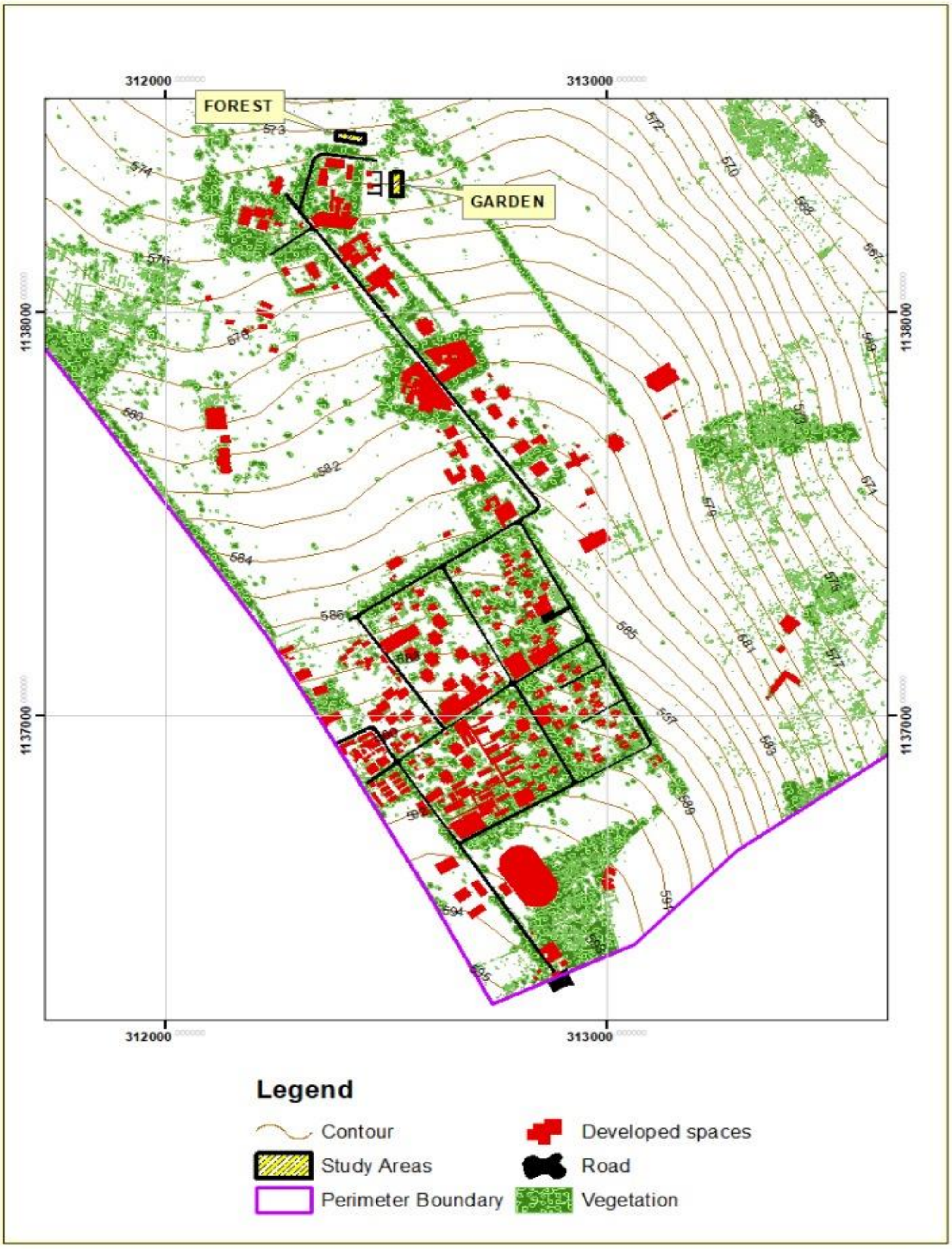

Figure 2: Study sites (Adebayo and Tukur, 1999)

Table 1: Effect of Herbicides on Physicochemical Properties of Soil samples

Soil Properties	Atrazine	Herbicides Glyphosate	Gramaxone	±SEM	LOS
Silt (%)	1 9.67	19.96	20.30	0.42	NS
Clay (%)	7.84	7.73	7.25	0.43	NS
Sand (%)	72.68	70.28	71.97	1.57	NS
Organic Carbon (%)	1.17^{a}	1.16^{b}	1.15^{b}	0.14	***
Organic Matter (%)	2.15^{a}	1.90^{b}	1.73^{c}	0.02	***
Nitrogen (%)	0.45^{a}	0.37^{c}	0.38^{b}	4.11	***
Available Phosphorus(ppm)	8.92^{a}	8.57^{b}	8.95a	0.06	***
Bulk Density (gcm^{-3})	0.31^{a}	0.31^{a}	0.25^{b}	2.10	***
Particle Density (gcm^{-3})	1.89^{b}	1.97^{a}	1.79^{c}	0.03	***
Moisture (%)	2.52^{a}	2.42^{a}	2.32^{c}	0.03	***
Porosity (%)	0.16^{a}	0.15^{b}	0.16^{a}	1.57	NS
pH	6.18^{b}	6.22^{b}	6.31^{a}	0.03	***
Magnesium (ppm)	3.04	3.05	3.05	0.04	NS
Sodium (ppm)	1.02	1.49	1.26	0.26	***
Calcium (ppm)	3.42a	3.40a	3.07b	0.02	***
Potassium (ppm)	0.33b	0.45a	0.33b	0.12	***
Iron (ppm)	8.00c	8.20a	8.07b	0.02	***
Zinc (ppm)	4.19b	4.34a	4.13b	0.05	***
Cupper (ppm)	0.17c	0.18b	0.24a	3.62	***
Manganese (ppm)	8.87	8.99	8.69	0.03	NS

Key: $^{a, b, c}$ Means in the same row bearing different superscript (s) is significantly ($P < 0.05$) different, SEM = Standard error of the mean, LOS = Level of significant, NS = Not significant, * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$ (SPSE, 2012)

Table 2: Interaction effects of herbicides, its concentration and site on soil physicochemical properties

Soil Properties	Forest C *C*F	A*3* F	A*6* F	L*3 F	L*6* F	G*3* F	G*6* F	C*C *G	A*3* G	Garden A*6* G	L*3* G	L*6* G	G*3* G	G*6* G	± SEM	LOS
Silt (%)	20.21	19.20	18.35	19.20	19.05	19.05	19.10	20.20	19.75	19.05	19.75	19.00	17.45	18.15	1.08	NS
Clay (%)+	4.15	3.70	4.85	4.05	4.20	3.90	4.85	4.16	7.80	3.40	7.58	4.20	4.05	6.10	0.07	NS
Sand (%)	75.65	77.05	75.40	76.95	76.95	77.05	76.05	75.65	72.80	77.55	72.45	76.85	77.00	75.75	1.96	NS
Organic Carbon (%)	1.61^{a}	0.6^{cd}	1.30^{ab}	0.60^{def}	0.54^{def}	0.60^{def}	0.46^{ef}	1.57^{a}	0.78^{cde}	0.71^{cde}	0.78^{cd} e	0.49ef	0.53def	0.63def	0.16	*
Organic Matter (%)	2.35^{b}	1.89^{c}	2.25b	0.97f	0.93fg	1.87c	1.54g	2.72a	1.33de	1.27e	1.34de	0.83fg	0.75d	0.89fg	0.10	***
Nitrogen (%)	0.52^{cd}	0.39^{cde}	0.30b	0.39^{cde}	0.38^{cde}	0.39^{cde}	0.42^{cd}	0.41^{c} d	0.32ef	0.42^{cd}	0.32^{ef}	0.40cd	0.42cd	0.44c	0.04	***
Available Phosphorus(ppm)	6.27	11.20	13.64	11.21	10.66	11.11	12.56	6.16	11.23	10.24	11.23	11.68	11.17	11.48	0.84	NS
Bulk Density (gcm^{-3})	0.88^{ab}	0.87b	0.87b	0.87b	0.87b	0.88ab	0.87b	0.88ab	0.88ab	0.87b	0.88ab	0.87b	0.87b	0.88ab	9.13	NS
Particle Density (gcm^{-3})	2.19	2.17	2.17	2.46	2.18	2.13	1.57	1.70	2.23	2.18	2.19	2.19	2.13	2.18	0.22	NS
Moisture (%)	5.15	4.85	5.05	4.75	4.90	4.75	5.15	5.15	4.90	5.00	4.85	4.95	4.75	4.15	0.20	NS
Porosity (%)	0.42	0.44	0.41	0.38	0.41	0.41	0.44	0.42	0.45	0.41	0.42	0.41	0.41	0.41	0.02	NS
pH	7.15	6.3^{ab}	6.15^{b}	6.35^{ab}	6.2^{b}	6.35^{ab}	5.35^{ab}	6.95	6.16^{b}	6.35^{ab}	6.15^{b}	6.30^{ab}	6.35^{ab}	6.45^{a}	0.11	*
Magnesium (ppm)	2.19	2.65	2.65	2.75	2.75	2.65	2.40	2.20	2.45	2.40	2.45	2.55	2.72	2.90	0.13	NS
Sodium (ppm)	0.36	0.55^{cd}	0.25^{f}	1.30^{a}	1.35^{a}	0.25^{f}	0.75^{b}	0.32	0.35^{bf}	0.45^{de}	0.75^{bf}	0.40ef	0.35ef	0.65bc	0.07	***
Calcium (ppm^{1})	2.10^{de}	2.25de	3.35a	3.15a	2.35	2.45e	2.30de	2.13de	2.65^{b} c	2.45^{be}	2.70b	2.40cde	2.35de	2.45bcd	0.14	*
Potassium (ppm)	0.25	0.35	0.25	0.45	0.30	0.25	0.35	0.20	0.35	0.35	0.35	0.30	0.55	0.50	0.10	NS
Iron (ppm^{1})	3.69^{b}	3.71b	5.65^{a}	3.72b	3.70b	3.78b	3.70b	3.56b	3.92b	3.73b	3.93b	3.66b	3.57b	3.73b	0.65	***
Zinc (ppm^{-1})	7.71^{bc}	6.79f	7.71^{bc}	6.85f	7.70bc	7.84b	7.27e	7.37e	7.37e	7.84b	7.37e	8.24a	7.58c	7.56cd	0.07	*
Cupper (ppm^{1})	0.12^{ef}	0.15^{de}	0.15^{de}	0.14ef	0.16^{cd}	0.14ef	0.15^{4ef}	0.11b	0.16^{ef}	0.17^{c}	0.14ef	0.17c	0.16cd	0.13ef	8.17	***
Manganese ($cmolk^{-1}$)	7.21^{bcd}	8.32^{cd}	8.27^{bcd}	7.84^{d}	9.04^{a}	8.52^{ab} c	7.24e	7.15^{e}	8.28bc	8.95^{a}	8.28bc	8.21e	8.54abc	8.72ab	0.26	***

Key: $^{a, b, -- g}$ Means in the same row bearing different superscript (s) is significantly ($P< 0.05$)different, SEM= Standard error of the mean, LOS = Level of significant, NS = Not significant, * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$, A= Atrazine, L= Glyphosate, G = Gramaxone, F= Forest, G = Garden, Control = (0kg), 3= 3kg, 6= 6kg(SPSE, 2012).

Table 3: Effect of Herbicides Period of Treatment (Months) on Physicochemical Properties of Soil

Physicochemical Properties	Months August	September	October	November	December	January	± SEM	LOS
Silt (%)	19.52	17.95	20.03	22.78	20.03	21.21	0.59	NS
Clay (%)	4.36	5.66	7.12	9.29	10.62	8.68	0.610.1	NS
Sand (%)	76.13	71.23	72.89	68.49	71.12	69.96	2.23	NS
Organic Carbon (%)	1.07b	1.12	1.07b	1.27b	1.91	1.18b	0.19	***
Organic Matter (%)	1.92b	1.32	1.76a	2.03a	2.88b	1.80c	0.03	***
Nitrogen (%)	0.33	0.46a	0.45a	0.39c	0.35d	0.33	5.82	***
Available Phosphorus(ppm)	9.12b	8.83c	9.70a	8.45d	8.47d	8.30d	0.09	***
Bulk Density (gcm^{-3})	0.82	0.57	0.45	0.19	0.21	0.10	2.98	***
Particle Density (gcm^{-3})	2.20a	2.22a	1.90b	1.72c	1.82b	1.42d	0.04	***
Moisture (%)	4.98a	4.47b	2.56c	1.57d	0.72e	0.22	0.04	***
Porosity (%)	0.41a	0.25b	0.25b	0.01c	0.14c	0.21c	2.22	***
pH	6.24b	6.25b	6.12c	6.11c	6.31ab	6.39a	0.04	***
Magnesium (ppm)	2.44e	2.52e	2.84d	3.08c	3.50b	3.91a	0.05	***
Sodium (ppm)	0.50	1.32	1.03	1.18	1.82	1.69	0.37	NS
Calcium (ppm)	2.62f	3.18e	3.34d	3.45c	3.54b	3.63a	0.03	***
Potassium (ppm)	0.29d	0.34cd	0.36bc	0.39ab	0.42a	0.42a	0.02	***
Iron (ppm)	7.38d	7.73c	8.16b	8.19b	8.53a	8.57a	0.03	***
Zinc (ppm)	3.65d	4.04d	4.23b	4.31b	4.52a	4.59a	0.07	***
Cupper (ppm)	0.17cd	0.15e	0.16de	0.18bc	0.19b	0.34a	5.12	***
Manganese (ppm)	7.64f	8.49e	8.99d	9.16c	9.59a	9.26b	0.04	***

Key: [a, b, c] Means in the same row bearing different superscript(s) is significantly (P< 0.05)different, SEM= Standard error of the mean, LOS = Level of significant, NS = Not significant, * = P<0.05, ** = P<0.01, *** = P<0.001(SPSE, 2012).

Table 4: Effects of herbicides on soil bacterial percentage abundance

Bacteria	% abundance in Forest Atrazine	Gramaxone	Glyphosate	% abundance in Garden Atrazine	Gramaxone	Glyphosate
p. aeruginosa	18(14.28)	18(14.63)	18(16.07)	18(13.43)	18(12.08)	18(13.63)
P. putida	17(12.49)	17(13.82)	16 (14.28)	13(9.70)	14(9.39)	15(11.36)
B. subtilis	18(14.28)	18(14.63)	18(16.07)	18(13.43)	18(12.08)	17(12.87)
B. sphaericus	14(11.11)	13(12.19)	12(10.71)	12(8.95)	10(6.71)	15(11.36)
F. aquaticum	0(((0.00)	0((0.00)	0(0.00)	8(5.97)	12(8.05)	11(8.33)
S.typhimurium	3 (2.38)	2(1.62)	3(2.67)	5(3.73)	8(5.37)	5(3.78)
E. coli	18(14.28)	16(13.01)	11(9.82)	18(13.43)	18(12.08)	13(9.84)
Klebsiella sp	17(12.49)	18(14.63)	17(15.18)	18(13.43)	17(11.41)	18(13.63)
M.loteus	5(3.96)	6(4.87)	4(3.57)	6(4.47)	5(3.35)	2(1.51)
P. vulgaris	0(0.00)	2(1.62)	2(1.78)	10(7.46)	7(4.70)	6(4.54)
S. aureus	9(7.14)	7(5.69)	7(6.25)	11(8.21)	13(8.79)	6(4.54)
A.nigrican	7(5.55)	6(4.87)	4(3.57)	7(5.22)	9(6.04)	6(4.54)
Total	126	123	112	134	149	132
Freest Total/Percentage=361(47.25 %)				Garden =403 (52.75 %)		

Table 5: Effect of Herbicides on Frequency of Bacterial Isolates

Bacterial Isolates	Herbicides			±SEM	LOS
	Atrazine	Glyphosate	Gramaxone		
Pseudomonas aeruginosa	17.50	17.50	17.50	0.50	NS
Flavobacterium aquaticum	5.75^{b}	5. 25^{a}	6.00^{a}	0.41	NS
Escherichia coli	17.50^{a}	16.50^{a}	11.50^{b}	0.50	***
Klebsiella sp	17.00^{a}	17.00	17.00	0.50	NS
Micrococcus loteus	5.00^{a}	5.00^{a}	2.50^{b}	0.50	**
Azotobacter nigricans	6.50^{a}	6.00^{a}	2.50^{b}	0.50	**
Pseudomonas putida	15.00	14.50	14.50	0.76	NS
Bacillus sphaericus	12.50	12.00	13.00	0.50	NS
Staphylococcus aureus	9.500^{a}	9.500^{a}	6.00^{b}	0.50	***
Bacillus subtilis	17.50	17.50	17.00	0.50	NS
Salmonella typhimurium	3.50	4.50	3.50	0.50	NS
Proteus vulgaris	4.70^{a}	4.00^{a}	2.50^{b}	0.46	***

Key: $^{a, b, c}$ Means in the same row bearing different superscript (s) is significantly (P< 0.05)different, SEM= Standard error of the mean, LOS = Level of significant, NS = Not significant, * = P<0.05, ** = P<0.01, *= P<0.001 (SP

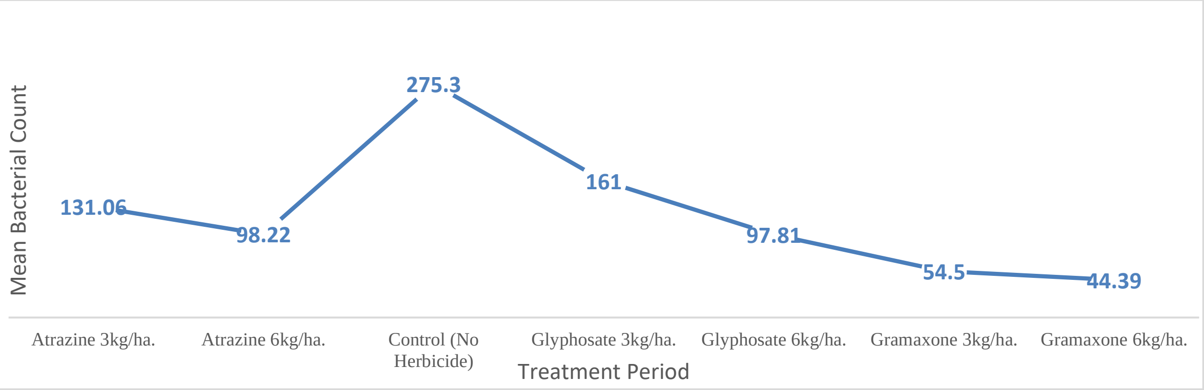

Figure 3: Effects of Herbicide Dosage on Soil Heterotrophic Bacterial Count

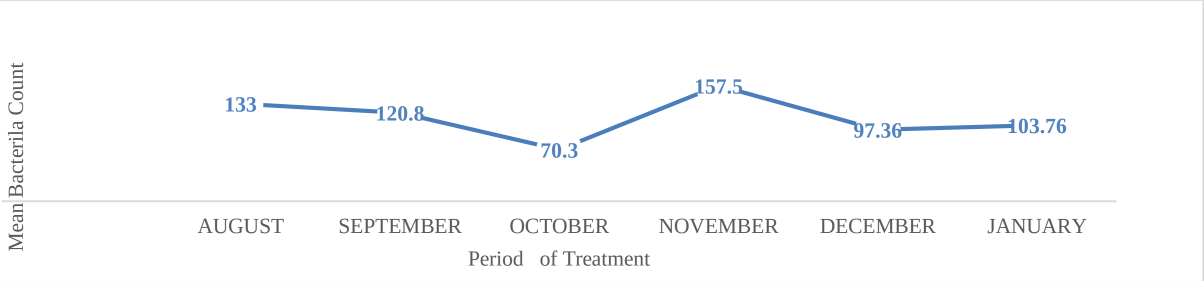

Figure 4: Effects of Herbicide Period of Treatment on Soil Heterotrophic Bacterial Count

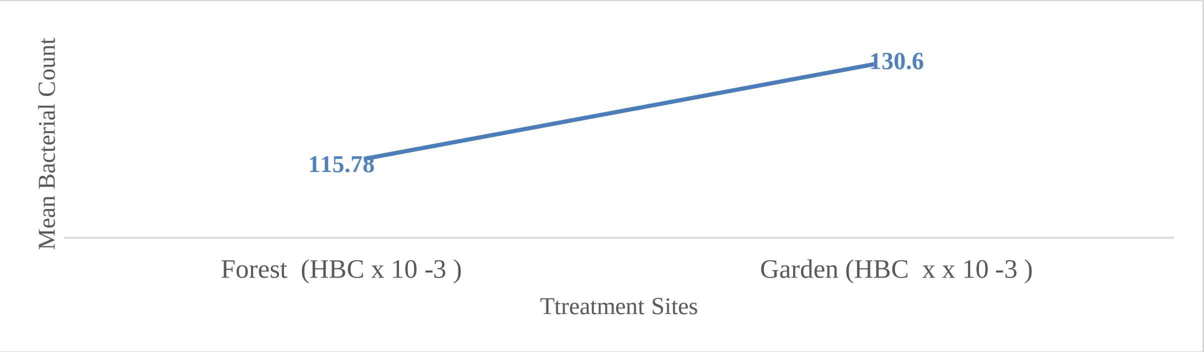

Figure 5: Effects of Herbicide Site of Treatment on Soil Heterotrophic Bacterial Count

CONCLUSION

The herbicides had significantly increased the amounts of soil organic carbon, organic matter, pH, sodium, potassium, bulk density, particle density, percentage moisture, available phosphorus and percentage nitrogen at $p< 0.001$ level of confidence, at same time lowering the values of available phosphorus (6.27 ppm), magnesium (2.19 ppm), sodium (0.36 ppm), potassium (2.10 ppm), iron (0.25 ppm) and manganese (7.21 cmol k^{-1}). The herbicides caused a decrease in the soil bacterial counts and that gramaxone at higher dosage caused the highest decrease while glyphosate the least. Garden soil was found as having higher bacterial counts compared with the forest. Period of soil treatment showed the month of November/120th day ($157.5x10^{-3}$ ±13 cfu/g) having the highest counts, followed by August, while December and January had the lowest which may be attributed due to seasonal variation from rainy to dry season and also the ability of bacteria to become adapted with the herbicides presence.

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