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Determination of Exchangeable Cations and Residual Concentration of Herbicide Treated Soils and Analysis of The In-Vitro Biodegradation of The Herbicides

Research Article

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Abstract

This study determined the effect of Atrazine, Xtravest, Gramoxone and Glyphosate on exchangeable cations and also analysed the in-vitro biodegradation of this herbicides as well as assayed for their residual concentration in soils. Exchangeable cations were analysed in atomic absorption spectrophotometer. Utilisation of herbicides was determined while herbicide degradation and residual concentration of herbicide were analysed using Gas Chromatography. K+, Ca2+, Mg2+, and Na+ declined while Fe2+ and Zn+ accumulated significantly. Bacteria and fungi significantly utilised herbicides as carbon source resulting in herbicide degradation. The lowest herbicide concentration of 118.55 ppm was obtained from atrazine inoculated with bacteria, while the highest herbicide concentration of 449.23 ppm was obtained from xtravest inoculated with fungi. Bacteria and fungi such as: B. subtilis, P. aeruginosa, P. florescences, P. putida, Actinomyces viscous, A. niger, A. tamarii, F. oxysporum, and P. chrysogenum were isolated in all the herbicide treated soils. Continuous herbicide treatment should be avoided because of their ability to persist in soils hence limiting essential nutrients available to plants. Indigenous microorganisms can be employed to remediate soils polluted by herbicides.
Keywords: Cation exchange, Bacteria, Fungi, Herbicide, Biodegradation

1. INTRODUCTION

The population is constantly being exposed to increased use of crop protection products. The continual protection of plants and manufacture of synthetic chemicals, such as herbicides, widely contributed to the regularity of the production. However, today the systematic use of herbicides is questioned, with the increasing awareness of the risks, which they can generate for all the components of the environment, even for man health (Tahar et al., 2017). Toxicological consequences due to the exhibition

in these thousand chemical components by means of the food, water and ground are alarming. The sustainable agriculture involves optimizing agricultural resources and at the same time maintaining the quality of environment and sustaining natural resources. In achieving this optimization, the soil microbial community composition is of great importance, because they play a crucial role in carbon flow, nutrient cycling and litter decomposition, which in turn affect soil fertility and plant growth (Chauhan et al., 2006; Tripathi et al., 2006), and hence occupy a unique position in biological cycles in terrestrial habitat. The soil microbial biomass is considered as active nutrient pool to plants and plays an important role in nutrient cycling and decomposition in ecosystem (De-Lorenzo et al., 2001).

Herbicides are applied to control weeds in the crop field have direct (or) indirect consequences on non-targeted organisms including soil microflora. After application of herbicides, microorganisms are able to degrade and utilize them as energy source for their metabolic activities and also for physiological processes. While processing these activities there may be a chance of change in soil physicochemical characteristics. Diversified effect of both toxic and as well as beneficial effect of herbicides on soil microorganisms and soil characteristics were studied in different ways in the recent past (Trimurtulu et al., 2015).

The continuous use of environmentally persistent herbicides and other synthetic agricultural chemicals posed great risks to soil and water contamination. A viable alternative pesticide that is equally effective and less harmful to the environment is in demand (Souza et al., 2012). The persistence of a pesticide in the soil is defined as the period or extension of time in which it remains active.

There is serious concern about the increased use of herbicides, which may cause (i) environmental hazards such as water table and water body contamination, (ii) biological disruption in crop field populations, and (iii) reduced efficiency as a result of increased population of soil microorganisms more efficient in herbicide degradation. The herbicide that are used frequently eventually reach the soil from the crop plants and accumulates in top 0-15 cm layer of soil, where the activities of microbes are found to be maximum. Herbicides in the soil affect the non-target and beneficial microorganisms and their activities which are essential for maintaining soil fertility (Sethi et al., 2015).

Atrazine is a selective pre- and post- emergence herbicide providing knockdown and residual action. It has low rate of volatilization from soil and is moderately persistent (half-life of ~60 days). It is more persistent in neutral and alkaline soils than in soils with low pH. It is moderately mobile and able to be leached through soils into groundwater (Kruger et al., 1996). Metolachlor is a selective herbicide used in the control of grassy weeds in the cultivation of corn, soybeans, peanuts, cotton and other crops. Metolachlor is often used in combination with other broadleaved herbicides (e.g. atrazine, metobromuron and propazine) to extend the spectrum of activity (Ayansina and Oso, 2006). Glyphosate is a broad-spectrum systemic herbicide. It is nonvolatile, it is strongly adsorbed by soil particles and is essentially immobile in soil (AATSE, 2002). Paraquat is a broad-spectrum herbicide that destroys plant tissue by contact action. It is highly soluble in water and because of its ionic properties is strongly adsorbed by soil particles and is essentially immobile in soil. The strong binding of paraquat to clay minerals is the factor most likely associated with its long half-life in soils (Clive, 2006).

Fertilizers, pesticides, herbicides, and some other materials applied to soil often contribute to water and air pollution. Therefore, soil is a key component of environment chemical cycles. Dissolved mineral matter in soil is largely present as ions. Prominent among the cations are H^+, Ca^{2+}, Mg^{2+}, K^+, Na^+ and usually very low levels of Fe^{2+}, Mn^{2+} and Al^{3+}. Multivalent cations and anions form ion pairs with each other in soil, solutions (Maynard, 2000). Hence this study determined the effect of selected herbicides (Atrazine, Xtravest, Gramoxone and Glyphosate) on exchangeable cations and also analysed the in-vitro biodegradation of this herbicides as well as assayed for their residual concentration in soils after treatment.

2. MATERIALS AND METHODS

Soil Sampling

Soil samples were collected from depths of 0–15 cm. Samples were then mixed and homogenized. After removing recognizable plant debris, samples were air-dried and sieved through a 2-mm mesh sieve.

Study Site

The present study was carried out in the agricultural field located at Oke Odo Street Ago-Iwoye, Nigeria. The soils had no prior pesticide treatment. The site (open field) was divided into fifteen plots (5 m2 each) of land. The experiment was made up of five treatments which include control (no-treatment) and four herbicide treatments (atrazine, xtravest, glyphosate and gramoxone).

Herbicides

The herbicides used in this work were provided from a local agricultural dealership store in Ibadan, Nigeria. They were; Xtravest {atrazine[1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine, C8H14CIN5] + metolachlor [2 -chloro -N- (2-ethyl-6-methylphenyl) -N- (1-methoxypropan-2-yl) acetamide], $C_{15}H_{22}CINO_2$, {27+15%} Suspo (SE)} (a product of Zhejiang Province Changxing First Chemical Co., Ltd., Xiaopu, Changxing, Zhejiang, China), Glyphosate [2,4-Dichlorophenoxyacetic acid, $C_3H_8NO_5P$] (Roundup, a product of Monsanto Europe S.A./N.V. Haven 627, Scheldelaan 460 2040 Antwerpen Belgium), Gramoxone [1,1-Dimethyl-4,4-bipyridinium dichloride, $C_{12}H_{14}CI_2N_2$] (Syngenta Crop Protection AG, Basle, Switzerland) and Atrazine [1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine, $C_8H_{14}CIN_5$] (Forward (Beihai) Hepu Pesticide Co. Ltd., 1, Quingshuijiang Liangzhou Hepu, Beihai, Guangxi, China, 536100).

Soil Treatments

The treatments were carried out in triplicates, using the complete randomized block design for a period of 8 weeks; at company recommended rates of 4 l/h (at 350 ml in 15 l sprayer). The herbicides were applied successively to the soils every week to the 8th week. Soil samples were then collected every 2 weeks to the 8th week of treatment. Fifteen soil samples were collected in separate polyethene bags every 2 weeks to the 8th week of treatment. A total of sixty soil samples were collected transported immediately to the laboratory for analysis.

Mineral Analysis

To 2.0 g of soil sample, 30ml of IN NH4OAC (ammonium acetate solution) was added and the flasks were shaken on a mechanical shaker for 2 h. Ca2+, Mg2+, K+, Na+ in soil samples were determined using the methods of Blakemore et al. (1987) and USDA, SCS (1972, 1982). Total wet digestion of soil samples in Nitric/Perchloric acid mixtures at ratio 2:1 was used to extract the Fe2+ and Zn+ from soil samples. Concentrations of Ca2+, K+, Na+, Mg2+, Fe2+ and Zn+ were determined in Atomic Absorption Spectrophotometer fitted with a hollow cathode lamp and a fuel rich flame (air acetylene), using the methods of Blakemore et al. (1987) and USDA, SCS (1972, 1982).

Atomic Absorption Spectrophotometry

The atomic absorption spectrophotometer consisted of a premix burner (water cooled, fishtail type) with a 10 × 0.05 cm2 slot. In addition, a monochromator with a diffraction grating of 1800 grooves/mm, wavelength range of 190 - 900 nm, focal length of 450 nm and giving an average dispersion of 1.2 nm/mm was positioned. An air– acetylene flame was used with an oxidant pressure of 1.60 kg/cm2. As a source of radiation, neon-filled hollow cathode lamps were used. Firstly, a blank solution was run by the instrument to retrieve the background signals. Then standard solutions of each element were measured to optimize the instrument response, and finally sample solutions of soil samples were analyzed in order to determine the concentration of each mineral element. Lead, nickel, cadmium and chromium composition was determined by electro-thermal atomic absorption spectroscopy. The analysis of Ca2+, K+, Na+, Mg2+, Fe2+ and Zn+ was done by flame atomic absorption spectroscopy.

Determination of The Concentration of Residual Herbicides in Soils After Treatment Using Gas Chromatography

The modified method as described by Ayanthi et al. (2008) was used in sample preparation and extraction. The soil samples were homogenised. Extraction was carried out using 10g of the sample by adding acetone to sample bound water so that agglomeration of samples during extraction could be minimised, toluene was then added in equal volume of acetone and the samples were agitated using mechanical shaker. The samples were then filtered using vacuum filtration. Acetone was driven off leaving toluene in the mixture. Organic phase was drained using anhydrous sodium sulphate to remove the associated water during extraction. Methanol was added to dissolve the remaining analytes in the solid phase extracted. The filterate of the methanol and toluene phase was added together and then allowed to concentrate to about 2.0ml. The extract was loaded to pre-conditioned C-18 Mega Bond Elut cartridge. The analytes were eluted from the cartridge with ethyl acetate. The extract was collected and then concentrated with nitrogen stream to about 2.0 ml before gas chromatography analysis was used for the analysis. One micro-litre was injected into the Hewlett Packard Gas Chromatography (HP 6890 Powered with HP ChemStation Rev. A 09.01 [1206] Software).

Isolation of Herbicide Utilizing Bacteria and Fungi

Five grams of each soil sample was suspended in 250-ml Erlenmeyer flasks containing a mixture of 50 ml of mineral salts medium and 1 ml of each herbicide in separate flasks. This concentration was used because it is equivalent to the field application rate. The flasks were incubated on a rotary shaker (Gallenkamp, England) at 120 rpm for 7 days at 30°C. Isolation was then carried out using the spread plate method on the solid mineral salts medium with each herbicide added to separate plates. The plates were incubated at 30°C for 5 days for bacteria and 30ºC for 7days for fungi. Morphologically distinct colonies of indigenous microbial isolates of bacteria and fungi isolated on nutrient agar for bacteria and potato dextrose agar for fungi respectively were used in the herbicide utilization experiments. Identity of the bacterial isolates was affirmed after characterization by standard bacteriological methods (Holt et al., 1994; Cheesbrough, 1984) while the fungal isolates were identified using morphological and cultural characteristics.

Determination of the Abilities of Bacteria and Fungi to Utilize Selected Herbicides

The ability of microbial isolates (bacterial consortium and fungal consortium) to utilise herbicide substrates as carbon source (atrazine, xtravest, glyphosate and gramoxone) in pure cultures were determined in minimal salt medium (g/l) (Moneke et al., 2010). The components were dissolved in 1000ml distilled

water, homogenized on hot plate magnetic stirrer to form uniform solution for 30 min. The pH of the basal medium was adjusted to pH 7.2. The basal medium of 150 ml was dispensed into 250 ml Erlenmeyer flasks and herbicide substrates were introduced into each flask respectively at 100 ppm after sterilisation which was done separately in an autoclave at 121^{o}C for 15 min and cooled to ambient temperature. One ml aliquot of diluted overnight broth cultures of each test organisms ($\times 10^4$ cells/ml) were seeded into each flask respectively and the flasks were incubated in a gyratory shaker incubator at 150 rpm for a period of thirty days at 30^{o}C (Bacterial isolates used in this study were those that had the highest turbidity, while the fungal isolates used in this study were those that had the highest counts on each of the herbicides). Utilisation of herbicides by microbial isolates were evaluated by monitoring bacterial and fungal growth using viable count on nutrient and potato dextrose agars, fungal dry-weights, optical density of bacteria and pH.

Determination of *in-vitro* Biodegradation of Herbicides by Bacteria and Fungi

The ability of herbicide degrading bacteria and fungi to degrade pure herbicide substrates was tested in minimal salt medium in 100 ml flasks using the method of Moneke et al. (2010). The flasks were then autoclaved and inoculated with 1.0 ml portion of each isolate (except one which serves as control). The flasks were subsequently incubated in an orbital shaker incubator at 150 rev/min at 30^{o}C for 7days. Gas chromatography analysis was then used to determine the remaining herbicide after 7 days of incubation, in the orbital shaker incubator, using the method of Ayanthi et al. (2008) already described above.

Statistical Analysis

The data were statistically analysed, with SPSS 20 software, using a one-way analysis of variance (ANOVA). Means were compared at 5% level of significance using Duncan's multiple range test.

3. RESULTS

Pseudomonas spp and *Bacillus* spp were found to be of common occurrence in herbicide treated soils. *B. subtilis, P. aeruginosa, P. florescences, P. putida* and *Actinomyces viscous* were isolated in all the herbicide treated soils. Meanwhile fungi such as *Aspergillus, Fusarium* and *Penicillum* species were of common occurrence in all the herbicide treated soils. *A. niger, A. tamarii, F. oxysporum,* and *P. chrysogenum* were isolated in all the herbicide treated soils.

Microbiological and Physicochemical Properties of Soil at the Experimental Site

Presented in Table 1 are the microbiological and physicochemical properties of the soil at the experimental site before treatment with herbicides. Table 2 shows the effect of herbicide treatment on exchangeable Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Fe^{2+} and Zn^{+} in soil samples.

Table 1: Microbiological and physicochemical properties of soil at the experimental site

Soil properties	Values
Soil type	Ferric luvisols
Bacterial count (CFU/g$\times 10^5$)	9.70
Fungal count (CFU/g$\times 10^5$)	1.10
Actinomycetes count (CFU/g$\times 10^5$)	0.80
Total nitrogen (%)	0.12
Available phosphorus (ppm)	10.0
Organic carbon (%)	1.42
Sodium (Na) (cmolkg^{-1})	0.42
Potassium (K) (cmolkg^{-1})	0.44

Calcium (cmolkg^{-1})	4.62
Magnesium (Mg) (cmolkg^{-1})	1.41
Iron (Fe) (cmolkg^{-1})	1820
Zinc (Zn)(cmolkg^{-1})	19
Soil electrical conductivity (µS/cm)	250
pH	6.80
Soil moisture (%)	18.32
Sand (%)	67.6
Silt (%)	20
Clay (%)	12.4

Exchangeable Cations (cmolkg-1) of Soil Samples Obtained at the 2nd, 4th, 6th, and 8th Weeks of Herbicide Treatment

Exchangeable Na^+ declined considerably in atrazine, gramoxone and xtravest treated soils from the 2nd to the 8th week. Glyphosate treated soils had the highest exchangeable Na^+ content of 0.67 cmolkg^{-1}at the 6th week, while soils treated with gramoxone recorded the lowest exchangeable Na^+ value of 0.19 cmolkg^{-1} at the 8th week. In glyphosate treated soils exchangeable K^+ accumulated from the 2nd (0.78 cmolkg^{-1}) to the 6th (1.31 cmolkg^{-1}) weeks of treatment. Glyphosate treated soils had the highest exchangeable K^+ value of 1.31 cmolkg^{-1} at the 6th week while gramoxone treated soil samples recorded the lowest exchangeable K^+ value of 0.1 cmolkg^{-1} at the 8th week of treatment (Table 2). Soils treated with atrazine recorded the highest exchangeable Ca^{2+} value of 8.44 cmolkg^{-1} at the 6th week of treatment while soils treated with glyphosate and gramoxone had the lowest calcium content value of 3.27 cmolkg^{-1} at the 4th week of treatment (Table 2). Statistical analysis showed that treatment with the herbicide types resulted in significant changes at the 2nd ($p\leq0.001$), 4th ($p\leq0.004$), 6th ($p\leq0.000$) and 8th ($p\leq0.000$) weeks, in values of exchangeable Mg^{2+}. Glyphosate treated soils had the lowest exchangeable Fe^{2+} value of 2643 cmolkg^{-1} while gramoxone treated soils had the highest exchangeable Fe^{2+} value of 5862 cmolkg^{-1} at the 2nd week. Soils treated with glyphosate had the highest exchangeable Zn^+ value of 81.77cmolkg^{-1} at the 8th week of treatment. Glyphosate and xtravest treated soils had the lowest value of 29 cmolkg^{-1} at the 2nd week of treatment (Table 2).

Table 2: Exchangeable cations (cmolkg^{-1}) of soil samples obtained at the 2nd, 4th, 6th, and 8th weeks of herbicide treatment

		2ND WK	4TH WK	6TH WK	8TH WK
Na	CON	0.480±0.017^{bc}	0.550±0.075^{b}	0.440±0.015^{b}	0.280±0.017^{b}
	GLY	0.550±0.003^{c}	0.560±0.015^{b}	0.670±0.031^{c}	0.430±0.019^{c}
	ATR	0.420±0.009^{a}	0.260±0.009^{a}	0.260±0.018^{a}	0.220±0.006^{a}
	GRA	0.480±0.007^{b}	0.360±0.006^{a}	0.300±0.015^{a}	0.190±0.015^{a}
	XTR	0.490±0.015^{b}	0.330±0.015^{a}	0.280±0.009^{a}	0.210±0.003^{a}
K	CON	0.480±0.023^{b}	0.460±0.006^{c}	0.600±0.029^{b}	0.420±0.009^{b}
	GLY	0.780±0.012^{c}	0.880±0.009^{d}	1.310±0.047^{c}	0.800±0.032^{c}
	ATR	0.280±0.000^{a}	0.190±0.015a	0.270±0.006^{a}	0.120±0.020^{a}
	GRA	0.510±0.012^{b}	0.230±0.021^{b}	0.200±0.015^{a}	0.100±0.007^{a}
	XTR	0.500±0.015^{b}	0.430±0.000^{c}	0.250±0.021^{a}	0.140±0.012^{a}
Ca	CON	4.730±0.121^{a}	4.140±0.020^{c}	7.680±0.277^{c}	6.730±0.064^{b}
	GLY	4.960±0.037^{a}	3.270±0.038^{a}	5.650±0.165^{a}	6.070±0.035^{a}
	ATR	4.710±0.055^{a}	3.320±0.064^{a}	8.440±0.009^{d}	6.620±0.118^{b}

	GRA	5.130±0.121^{a}	3.270±0.038^{a}	6.790±0.105^{b}	6.080±0.064^{a}
	XTR	4.100±0.078^{a}	4.010±0.009^{b}	6.860±0.032^{b}	6.310±0.055^{a}
Mg	CON	1.480±0.003^{c}	0.970±0.072^{b}	1.990±0.006^{c}	1.800±0.020^{c}
	GLY	1.430±0.010^{bc}	0.940±0.012^{b}	2.720±0.092^{d}	1.930±0.037^{d}
	ATR	1.400±0.012^{b}	0.780±0.061^{a}	1.520±0.006^{b}	1.360±0.050^{a}
	GRA	1.490±0.026^{c}	0.770±0.012^{a}	1.140±0.064^{a}	1.380±0.040^{a}
	XTR	1.330±0.029^{a}	1.040±0.000^{b}	1.430±0.069^{b}	1.500±0.028^{b}
Fe	CON	1956±182^{a}	3976±183^{a}	5031±223^{c}	4990±491^{a}
	GLY	2643±44^{a}	4049±104^{a}	4283±85^{ab}	4926±6^{a}
	ATR	4087±441^{b}	4463±60^{b}	3888±165^{a}	4605±77^{a}
	GRA	5862±512^{c}	4579±68^{b}	4698±34^{bc}	5348±204^{a}
	XTR	5216±379^{c}	4666±65^{b}	5002±55^{c}	4933±150^{a}
Zn	CON	28.670±0.880^{a}	40.670±2.030^{a}	69.670±0.880^{d}	90.000±2.020^{e}
	GLY	29.000±0.580^{a}	41.670±1.450^{a}	63.000±0.580^{c}	81.770±1.590^{d}
	ATR	31.000±0.580^{a}	62.670±2.030^{b}	57.000±2.000^{b}	61.270±1.300^{b}
	GRA	44.670±1.450^{b}	62.770±0.720^{b}	46.930±1.440^{a}	44.270±0.720^{a}
	XTR	29.000±1.730^{a}	67.770±1.010^{c}	65.000±0.000^{c}	73.000±1.150^{c}

CON=Control, GLY= Glyphosate, ATR= Atrazine, GRA= Gramoxone, XTR= Xtravest, WK=Week. Columns with values that have the same letter show that there are no significant differences($p \geq 0.05$) between the values; columns with values that have different letters show that there is a significant difference ($p \leq 0.05$) between the values.

Changes in Viable Counts ([CFU/g] $\times 10^5$) of Bacteria During Biodegradation

There were significant increases ($p \leq 0.000$) in viable counts from the 2nd to the 12th day and at the 14th day ($p \leq 0.001$) at the 2nd week. Glyphosate had the highest viable count value of 10.80×10^5 CFU/g at the 6th day of incubation, while xtravest recorded the lowest viable count value of 6.37×10^5 CFU/g at the 14th day (Table 3). Xtravest had lower viable count values between the 8th and 14th day compared to the control. Although all viable count values at the 2nd week dropped from the 6th to the 14th day. Glyphosate recorded the highest viable count of 12.76×10^5 CFU/g at the 10th day of incubation (Table 3). Control soil samples had lower viable count values than those of the herbicides.

Changes in Optical Density of Bacteria During Biodegradation

Optical density values increased significantly ($p \leq 0.000$) from the 2nd to the 14th day at the 2nd week. Gramoxone had the lowest optical density value of 0.885 and 0.955 at the 2nd and 14th days of incubation respectively at the 8th week herbicide types had significant effect ($p \leq 0.000$) on optical density values from the 2nd to the 14th day. Optical density values for control and glyphosate reduced from the 2nd to the 14th day. Xtravest had the highest optical density value of 0.974 at the 14th day of incubation. Atrazine had the lowest optical density value of 0.913 at the 2nd day of incubation, while at the 14th day of incubation glyphosate had the lowest optical density value of 0.929 (Table 4).

Table 3: Changes in viable counts ([CFU/g] $\times 10^5$) of bacteria during biodegradation

		2^{ND}	4^{TH}	6^{TH}	8^{TH}	10^{TH}	12^{TH}	14^{TH}
2^{ND}WK	CON	8.17±0.033^{a}	8.57±0.033^{a}	8.75±0.003^{a}	7.37±0.088^{a}	6.63±0.133^{a}	6.84±0.003^{b}	6.76±0.019^{ab}
	GLY	10.33±0.067^{e}	10.65±0.029^{e}	10.80±0.000^{e}	9.60±0.000^{d}	8.20±0.118^{c}	7.85±0.087^{d}	7.38±0.053^{c}
	ATR	9.83±0.033^{d}	10.03±0.033^{d}	10.41±0.007^{d}	8.20±0.000^{b}	7.34±0.125^{b}	7.20±0.153^{bc}	7.13±0.167^{bc}
	XTR	8.65±0.047^{b}	8.72±0.017^{b}	8.94±0.020^{b}	7.28±0.600^{a}	6.53±0.203^{a}	6.43±0.203^{a}	6.37±0.233^{a}
	GRA	9.33±0.067^{c}	9.81±0.007^{c}	9.90±0.000^{c}	8.50±0.000^{c}	7.66±0.030^{b}	7.54±0.037^{cd}	7.43±0.033^{c}
4^{TH}WK	CON	8.33±0.133^{a}	8.73±0.133^{a}	9.00±0.115^{a}	6.83±0.015^{a}	6.83±0.015^{a}	6.73±0.015^{b}	6.67±0.037^{c}
	GLY	9.97±0.033^{c}	10.30±0.058^{d}	10.52±0.044^{d}	9.38±0.028^{d}	7.88±0.061^{c}	7.45±0.024^{c}	7.17±0.033^{d}
	ATR	9.81±0.007^{c}	10.03±0.033^{c}	10.41±0.007^{d}	9.22±0.012^{d}	8.55±0.029^{d}	8.37±0.033^{d}	8.03±0.033^{e}
	XTR	8.41±0.007^{a}	8.87±0.067^{a}	9.22±0.017^{b}	8.13±0.033^{c}	7.23±0.088^{b}	6.89±0.047^{b}	6.37±0.035^{b}
	GRA	9.10±0.058^{b}	9.38±0.017^{b}	9.67±0.017^{c}	7.57±0.145^{b}	7.10±0.153^{b}	6.43±0.120^{a}	6.10±0.058^{a}
6^{TH}WK	CON	7.33±0.033^{a}	7.43±0.033^{a}	7.57±0.033^{a}	7.63±0.033^{a}	7.70±0.058^{a}	7.57±0.033^{a}	7.37±0.033^{a}
	GLY	11.33±0.176^{d}	11.41±0.174^{d}	11.50±0.173^{d}	11.60±0.173^{d}	11.67±0.145^{d}	11.57±0.145^{d}	11.3±0.058^{d}
	ATR	10.13± 0.133^{c}	10.23±0.088^{c}	10.33±0.088^{c}	10.40±0.100^{c}	11.43±0.033^{d}	11.33±0.067^{d}	11.03±0.033^{d}
	XTR	8.87±0.037^{b}	8.87±0.033^{b}	9.20±0.058^{b}	9.30±0.000^{b}	9.43±0.033^{b}	9.17±0.033^{b}	8.80±0.200^{b}
	GRA	10.10±0.153^{c}	10.20±0.153^{c}	10.37±0.088^{c}	10.43±0.067^{c}	10.53±0.067^{c}	10.10±0.058^{c}	9.30±0.058^{c}
8^{TH}WK	CON	7.50±0.000^{a}	7.57±0.033^{a}	7.67±0.033^{a}	7.83±0.033^{a}	7.83±0.033^{a}	7.67±0.033^{a}	7.37±0.033^{a}
	GLY	12.23±0.145^{e}	12.37±0.145^{e}	12.47±0.133^{e}	12.67±0.033^{e}	12.77±0.033^{e}	12.53±0.033^{e}	12.17±0.033^{d}
	ATR	10.57±0.088^{c}	10.60±0.058^{c}	10.70±0.000^{c}	10.87±0.033^{c}	11.00±0.058^{c}	10.67±0.176^{c}	9.67±0.176^{c}
	XTR	9.33±0.067^{b}	9.57±0.088^{b}	9.67±0.088^{b}	9.80±0.058^{b}	9.83±0.033^{b}	9.60±0.000^{b}	9.07±0.033^{b}
	GRA	10.83±0.033^{d}	10.90±0.000^{d}	11.00±0.000^{d}	11.47±0.033^{d}	11.60±0.058^{d}	11.00±0.000^{d}	9.90±0.058^{c}

CON=Control, GLY= Glyphosate, ATR= Atrazine, GRA= Gramoxone, XTR= Xtravest, WK=Week. Columns with values that have the same letter show that there are no significant differences($p \geq 0.05$) between the values; columns with values that have different letters show that there is a significant difference ($p \leq 0.05$) between the values.

Table 4: Changes in optical density of bacteria during biodegradation

		2^{ND}	4^{TH}	6^{TH}	8^{TH}	10^{TH}	12^{TH}	14^{TH}
2^{ND}WK	CON	0.882±0.001^{a}	0.886±0.001^{a}	0.891±0.001^{a}	0.893±0.001^{a}	0.894±0.001^{a}	0.896±0.001^{a}	0.898±0.001^{a}
	GLY	0.941±0.001^{b}	0.945±0.001^{c}	0.956±0.001^{c}	0.961±0.001^{c}	0.971±0.001^{d}	0.976±0.002^{c}	0.984±0.003^{c}
	ATR	0.894±0.001^{a}	0.903±0.003^{b}	0.924±0.000^{b}	0.941±0.001^{b}	0.961±0.001^{c}	0.970±0.002^{c}	0.985±0.003^{c}
	XTR	0.954±0.002^{c}	0.965±0.001^{d}	0.975±0.001^{d}	0.983±0.001^{d}	1.043±0.001^{e}	1.045±0.001^{d}	1.046±0.001^{d}
	GRA	0.885±0.008^{a}	0.887±0.001^{a}	0.892±0.001^{a}	0.895±0.001^{a}	0.917±0.003^{b}	0.938±0.004^{b}	0.955±0.003^{b}
4^{TH}WK	CON	0.881±0.001^{a}	0.888±0.000^{a}	0.893±0.001^{a}	0.895±0.001^{a}	0.895±0.001^{a}	0.898±0.001^{a}	0.910±0.006^{a}
	GLY	0.945±0.001^{c}	0.947±0.001^{c}	0.961±0.002^{c}	0.965±0.001^{c}	0.975±0.001^{d}	0.977±0.001^{d}	0.981±0.001^{d}
	ATR	0.897±0.001^{b}	0.909±0.003^{b}	0.928±0.001^{b}	0.945±0.001^{b}	0.963±0.001^{c}	0.967±0.002^{c}	0.969±0.002^{c}
	XTR	0.956±0.002^{d}	0.956±0.002^{d}	0.977±0.001^{d}	0.986±0.000^{d}	1.047±0.001^{e}	1.138±0.001^{e}	1.143±0.002^{e}
	GRA	0.887±0.007^{ab}	0.887±0.007^{a}	0.894±0.001^{a}	0.896±0.001^{a}	0.925±0.004^{b}	0.931±0.004^{b}	0.939±0.003^{b}
6^{TH}WK	CON	0.885±0.001^{a}	0.887±0.001^{a}	0.890±0.001^{a}	0.892±0.001^{a}	0.898±0.001^{a}	0.993±0.001^{c}	1.086±0.031^{a}
	GLY	0.946±0.002^{d}	0.949±0.003^{d}	0.952±0.003^{d}	0.954±0.003^{c}	0.960±0.003^{c}	0.965±0.002^{ab}	1.357±0.027^{b}
	ATR	0.907±0.004^{b}	0.915±0.001^{b}	0.920±0.001^{b}	0.930±0.001^{b}	0.942±0.001^{b}	0.971±0.004^{b}	1.426±0.003^{c}
	XTR	0.964±0.002^{e}	0.967±0.001^{e}	0.968±0.001^{e}	0.972±0.001^{d}	0.976±0.001^{d}	0.988±0.001^{c}	1.465±0.002^{c}
	GRA	0.934±0.007^{c}	0.937±0.007^{c}	0.939±0.006^{c}	0.936±0.003^{b}	0.943±0.003^{b}	0.956±0.004^{a}	1.330±0.006^{b}
8^{TH}WK	CON	0.980±0.000^{e}	0.980±0.000^{e}	0.977±0.002^{e}	0.970±0.000^{d}	0.970±0.000^{d}	0.961±0.001^{d}	0.961±0.001^{d}
	GLY	0.951±0.001^{c}	0.950±0.000^{c}	0.950±0.000^{c}	0.941±0.001^{b}	0.938±0.001^{b}	0.931±0.001^{a}	0.929±0.001^{a}
	ATR	0.913±0.004^{a}	0.917±0.002^{a}	0.925±0.001^{a}	0.927±0.001^{a}	0.931±0.001^{a}	0.935±0.001^{b}	0.938±0.000^{b}
	XTR	0.968±0.001^{d}	0.963±0.001^{d}	0.964±0.000^{d}	0.966±0.000^{c}	0.969±0.001^{d}	0.971±0.000^{e}	0.974±0.001^{e}
	GRA	0.941±0.001^{b}	0.940±0.000^{b}	0.942±0.000^{b}	0.943±0.001^{b}	0.945±0.001^{c}	0.945±0.000^{c}	0.949±0.001^{c}

CON=Control, GLY= Glyphosate, ATR= Atrazine, GRA= Gramoxone, XTR= Xtravest, WK=Week. Columns with values that have the same letter show that there are no significant differences($p \geq 0.05$) between the values; columns with values that have different letters show that there is a significant difference ($p \leq 0.05$) between the values.

Changes in Fungal Counts ([CFU/g] ×10^4) During Biodegradation

Treatment with the herbicide types at the 2nd week showed significant changes ($p \leq 0.009$; $p \leq 0.005$; $p \leq 0.004$; $p \leq 0.000$; $p \leq 0.001$) in fungal counts at the 5th, 10th, 15th, 20th and 25th days respectively (Table 5). Fungal counts increased from the 5th day to the 20th day and dropped on the 25th day. xtravest had the lowest fungal count of 3.30× 10^4 CFU/g at the 5th day. At the 8th week, significant changes ($p \leq 0.001$; $p \leq 0.002$; $p \leq 0.001$; $p \leq 0.005$; $p \leq 0.002$) in fungal counts occurred at 5th, 10th, 15th, 20th and 25th days of incubation respectively. Fungal counts increased from the 5th to the 15th days and then decreased from the 20th to the 25th day. Xtravest had the highest fungal count value of 1.17× 10^5 CFU/g at the 15th day, while at the 5th day gramoxone had the lowest fungal count value of 4.00× 10^4 CFU/g. At the 25th day gramoxone recorded the lowest fungal count value of 3.30× 10^4 CFU/g.

Changes in Fungal dry-weights During Biodegradation

In Table 6, at the 2nd, 4th and 8th weeks, fungal dry weight values increased from the 5th to the 20th days and then dropped at the 25th day. At the 2nd week, fungal dry weight showed significant changes ($P \leq 0.000$) in values from the 5th to the 25th day (Table 6). At the 20th day xtravest recorded the highest fungal dry weight value of 0.020 g. At the 25th day gramoxone recorded the lowest fungal dry weight of 0.012 g. There were significant changes ($p \leq 0.000$) in fungal dry weight values from the 5th to the 25th days at the 8th week. Atrazine had the highest fungal dry weight value of 0.022 g at the 20th day while gramoxone and glyphosate had the lowest value of 0.012 g at the 5th day. At the 25th day gramoxone had the lowest value of 0.012 g (Table 6).

Table 5: Changes in fungal counts ([CFU/g] ×10^4) during biodegradation

		5TH	10TH	15TH	20TH	25TH
2ND WK	CON	4.30±0.033^a	5.70±0.033^{ab}	8.00±0.058^a	9.30±0.067^{ab}	8.70±0.033^a
	GLY	5.70±0.033^b	7.70±0.033^c	10.70±0.088^b	13.70±0.033^c	11.00±0.058^b
	ATR	4.30±0.033^a	6.30±0.033^{ab}	8.30±0.033^a	10.30±0.033^b	8.70±0.033^a
	XTR	3.30±0.033^a	5.30±0.033^a	6.70±0.033^a	8.70±0.033^a	7.70±0.033^a
	GRA	4.30±0.033^a	6.70±0.033^{bc}	8.00±0.000^a	12.70±0.033^c	10.30±0.033^b
4TH WK	CON	3.30±0.033^a	5.70±0.033^{ab}	6.70±0.033^a	4.70±0.033^{bc}	3.00±0.000^{ab}
	GLY	4.70±0.033^b	6.00±0.000^{bc}	6.30±0.033^a	4.00±0.000^{ab}	2.30±0.033^a
	ATR	4.30±0.033^b	5.70±0.03^{ab}	7.00±0.058^{ab}	3.30±0.033^a	2.70±0.033^a
	XTR	5.70±0.033^c	7.00±0.058^c	8.00±0.000^b	5.70±0.033^{cd}	4.30±0.033^c
	GRA	3.00±0.000^a	4.70±0.033^a	6.70±0.033^a	5.00±0.000^d	3.70±0.033^{bc}
6TH WK	CON	4.00±0.000^{ab}	4.70±0.033^a	4.70±0.033^b	4.30±0.033^b	2.70±0.033^a
	GLY	5.00±0.000^{bc}	5.30±0.033^{ab}	5.70±0.067^{ab}	4.00±0.000^{ab}	2.30±0.033^a
	ATR	5.30±0.067^c	5.70±0.120^{ab}	6.00±0.115^a	3.30±0.033^a	1.70±0.033^a
	XTR	6.70±0.033^d	7.00±0.058^b	6.70±0.067^c	4.70±0.033^b	2.70±0.033^a
	GRA	3.00±0.000^a	4.00±0.000^a	4.30±0.033^{ab}	4.00±0.000^{ab}	2.00±0.000^a
8TH WK	CON	5.70±0.033^b	6.70±0.033^{ab}	7.00±0.058^a	4.30±0.033^a	3.70±0.033^a
	GLY	6.70±0.033^b	7.70±0.033^b	8.30±0.067^a	6.30±0.067^{ab}	4.70±0.033^{ab}
	ATR	6.70±0.088^b	7.70±0.088^b	8.70±0.088^a	7.30±0.088^{bc}	5.70±0.067^{bc}
	XTR	8.70±0.033^c	10.30±0.067^c	11.70±0.033^b	8.70±0.033^c	6.30±0.033^c
	GRA	4.00±0.000^a	5.70±0.033^a	6.70±0.033^a	5.70±0.067^{ab}	3.30±0.033^a

CON=Control, GLY= Glyphosate, ATR= Atrazine, GRA= Gramoxone, XTR= Xtravest, WK=Week. Columns with values that have the same letter show that there are no significant differences($p \geq 0.05$) between the values; columns with values that have different letters show that there is a significant difference ($p \leq 0.05$) between the values.

Table 6: Changes in fungal dry-weights during biodegradation

		5TH	10TH	15TH	20TH	25TH
2ND WK	CON	0.010±0.000^{b}	0.012±0.000^{b}	0.014±0.000^{b}	0.015±0.000^{b}	0.015±0.000^{bc}
	GLY	0.012±0.000^{c}	0.015±0.000^{c}	0.016±0.001^{c}	0.018±0.001^{cd}	0.016±0.000^{cd}
	ATR	0.013±0.000^{cd}	0.015±0.001^{c}	0.016±0.001^{c}	0.017±0.001^{c}	0.015±0.001^{b}
	XTR	0.014±0.000^{d}	0.015±0.000^{c}	0.017±0.000^{c}	0.020±0.000^{d}	0.017±0.000^{d}
	GRA	0.008±0.000^{a}	0.010±0.000^{a}	0.012±0.000^{a}	0.014±0.000^{a}	0.012±0.000^{a}
4TH WK	CON	0.011±0.000^{a}	0.014±0.000^{a}	0.016±0.000^{a}	0.017±0.000^{a}	0.013±0.001^{a}
	GLY	0.014±0.000^{b}	0.017±0.001^{b}	0.019±0.001^{a}	0.020±0.000^{bc}	0.014±0.001^{a}
	ATR	0.015±0.000^{c}	0.017±0.000^{b}	0.019±0.001^{a}	0.023±0.000^{c}	0.019±0.000^{b}
	XTR	0.024±0.000^{d}	0.026±0.001^{c}	0.030±0.001^{b}	0.032±0.002^{d}	0.021±0.000^{c}
	GRA	0.013±0.000^{b}	0.015±0.001^{ab}	0.019±0.002^{a}	0.019±0.001^{ab}	0.015±0.001^{a}
6TH WK	CON	0.013±0.000^{b}	0.014±0.000^{ab}	0.015±0.001^{ab}	0.014±0.000^{c}	0.010±0.000^{c}
	GLY	0.013±0.000^{bc}	0.014±0.000^{bc}	0.015±0.000^{ab}	0.013±0.000^{b}	0.010±0.001^{c}
	ATR	0.014±0.000^{c}	0.015±0.000^{c}	0.016±0.001^{b}	0.013±0.001^{b}	0.009±0.001^{c}
	XTR	0.018±0.000^{d}	0.018±0.000^{d}	0.020±0.000^{c}	0.016±0.000^{d}	0.006±0.001^{b}
	GRA	0.011±0.000^{a}	0.013±0.000^{a}	0.014±0.000^{a}	0.010±0.000^{a}	0.004±0.000^{a}
8TH WK	CON	0.012±0.000^{a}	0.013±0.000^{a}	0.014±0.000^{a}	0.015±0.000^{a}	0.012±0.000^{a}
	GLY	0.015±0.001^{b}	0.017±0.000^{b}	0.019±0.000^{c}	0.021±0.001^{b}	0.016±0.000^{b}
	ATR	0.016±0.001^{bc}	0.019±0.001^{c}	0.021±0.000^{d}	0.022±0.001^{b}	0.020±0.001^{c}
	XTR	0.018±0.001^{c}	0.019±0.001^{c}	0.020±0.001^{d}	0.021±0.001^{b}	0.019±0.001^{c}
	GRA	0.012±0.000^{a}	0.014±0.001^{a}	0.016±0.000^{b}	0.014±0.000^{a}	0.012±0.000^{a}

CON=Control, GLY= Glyphosate, ATR= Atrazine, GRA= Gramoxone, XTR= Xtravest, WK=Week. Columns with values that have the same letter show that there are no significant differences($p \geq 0.05$) between the values; columns with values that have different letters show that there is a significant difference ($p \leq 0.05$) between the values.

Residual Concentration of Herbicides Obtained from Soil Samples

Fig.1 shows the residual the residual concentration of herbicides obtained from herbicide treated soils. Residual concentration of herbicides increased significantly ($p \leq 0.024$) from the 2nd week to the 8th week of treatment. There was also significant difference ($p \leq 0.001$) in residual concentration values of the herbicide types. The highest residual concentration value of 9.94×10^{-1} ppm was recovered from glyphosate treated soils at the 8th week of treatment, and peak detection time of 11.37 mins. Also, at the 2nd, 4th and 6th weeks of treatment it was noted that glyphosate treated soils had the highest residual concentration values of 4.13×10^{-1} ppm, 6.05×10^{-1} ppm, and 8.62×10^{-1} ppm respectively (at peak detection times of 12.41 mins, 12.30 mins and 17.53 mins respectively) compared to other herbicide treated soils. The lowest residual concentration of 2.53×10^{-1} ppm (atrazine) was obtained from atrazine treated soils at the 2nd week of treatment at peak detection time of 14.95 mins, while also at the 8th week of treatment atrazine treated soils had the lowest concentration of atrazine (6.67×10^{-1} ppm), at peak detection time of 15.26 mins compared to other herbicides at the 8th week of treatment.

Biodegradation of Herbicides by Bacteria and Fungi

In Figure 2, the concentrations of the control were higher than the concentrations of the bacteria and fungi inoculated herbicides. There was significant reduction ($p \leq 0.001$) in the herbicide concentrations after degradation by bacteria and fungi. All herbicides inoculated with bacteria were found to contain lower concentrations of the corresponding herbicide compared to those that had been inoculated with fungi after 7 days of incubation on rotatory shaker incubator. The bacteria and fungi inoculated into each of the herbicides caused sharp reduction in herbicide concentrations compared to the control. The lowest herbicide concentration of 118.55 ppm was obtained from atrazine inoculated with bacteria, while the highest

herbicide concentration of 449.23ppm was obtained from xtravest inoculated with fungi at the 7th day of incubation.

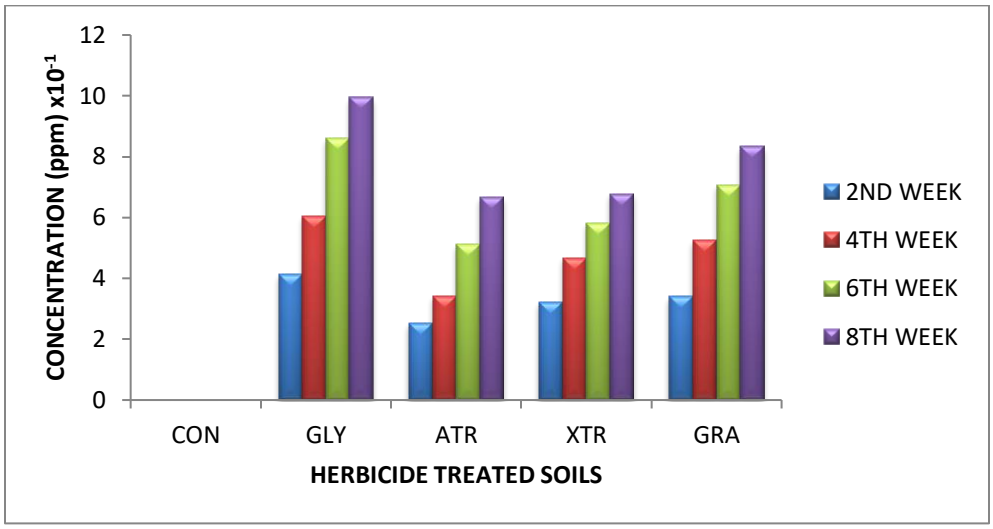

Figure 1: Residual concentration of herbicides obtained from soil samples.

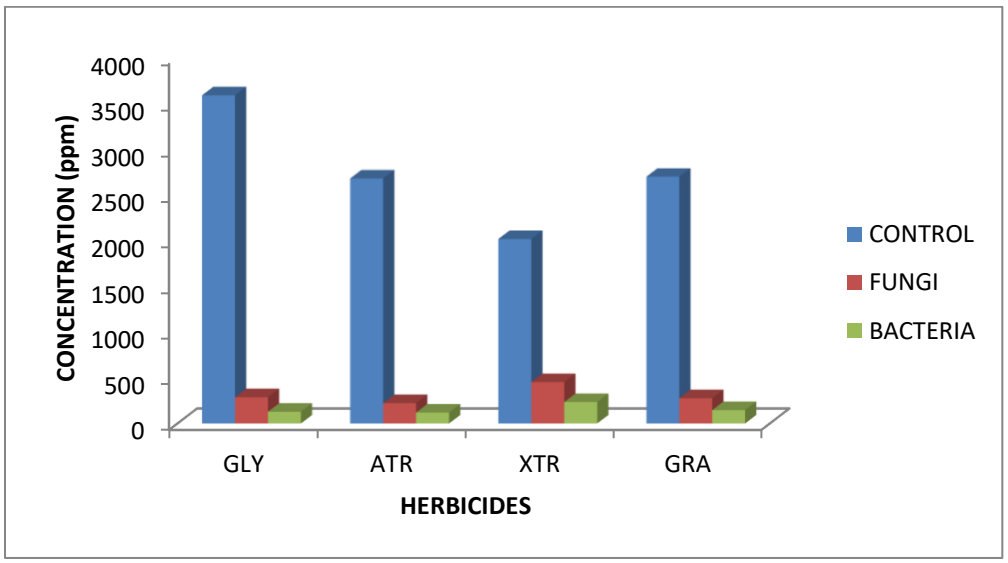

Figure 2: Biodegradation of herbicides by bacteria and fungi.

4. DISCUSSION

In this study Zn and Fe cations accumulated in soils after treatment with herbicides. Similar observation were also reported by Abah et al. (2012). The levels of Fe and Zn recorded in this study were found to be above critical limits of 10-20 mg/kg Fe and 60-400 mg/kg Zn which cause phytotoxicity in plants (FAO/WHO, 1976). This might be due to the chelation ability of these herbicides (allowing them to accumulate in this form in soils) where they form complexes with these metal co-factors essential for enzyme activities thus immobilizing them in soil reducing their availability to plants. Iron deficiency chlorosis is also becoming increasingly prevalent in cropping systems receiving frequent or prolonged applications of glyphosate (Ozturk et al., 2008). Cations in the soil solution are bonded to the surface of clay minerals by electrostatic interactions and can return in solution by the substitution of other cations or by dilution. The most representative exchange cations are K^+, Ca^{2+}, Mg^{2+}, and Na^+ (Blasioli et al., 2011).

The strong negative correlation of exchangeable Ca^{2+} with residual concentration of herbicides indicates that as the herbicides accumulated in the soils, as a result of successive treatment, there was concomitant reduction in exchangeable Ca^{2+} in the soils. The accumulation of K^+ in glyphosate treated soils occurred because K^+ is a constituent component of glyphosate chemical compound ($C_3H_7KNO_5P$). The reduction in concentration of some soil minerals (K^+, Ca^{2+}, Mg^{2+}, and Na^+) in this study might have occurred as a result of leaching of the minerals in solution and the degradation of the herbicides consequent upon the utilisation of these soil minerals by soil microbes and also their resultant uptake by plants. Benzon et al. (2015) reported that the data on available P, exchangeable Mg, Na, Ca, and K. CEC showed no significant difference among treatments. They also reported that relatively lower values were obtained compared to the control. Cucci et al. (2015) reported that the variations in available phosphorus and exchangeable potassium were negligible and not statistically different between the various treatments.

Herbicides used in this study persisted in soils upon successive treatments with the herbicides. GC analysis used in this study revealed that the herbicides accumulated in the soils throughout the period of treatment. This might be as a result of strong bonding interactions between the herbicides and soil organic matter resulting in their accumulation in soils. Glyphosate showed more persistence in soils as a result of its strong binding interaction with organic matter. Jilani and Khan (2004) reported that the residues of an applied pesticide may remain in the environment for variable periods of time.

The bacterial and fungal isolates used in the determination of the time utilisation of the herbicides showed appreciable growth in culture medium containing the herbicides as carbon source. The differences observed in the growth of the isolates in the media are indications of the differences between the organisms in tolerating the herbicides. This study showed that the bacterial and fungal isolates grew maximally on all the herbicides. The utilisation of herbicides in minimal salt medium have shown that none of the bacterial and fungal isolates exhibited lag phases, because the microorganisms used in this study are indigenous to the soil from which they were obtained and consequently have adapted to the herbicides used in treatment. Xtravest and glyphosate were better utilised by the indigenous bacteria and fungi in minimal salt medium compared to other herbicides tested in this study. According to Andy et al. (2017), microbial degradation of paraquat involves the action of some fungi and bacteria organisms. The genera of organisms that degrades paraquat include fungi: *Rhizopus*, *Penicillium*, *Aspergillus* and *Mucor* species and sole source of carbon and energy revealed that the isolates utilized paraquat at different rates. This indicates the varying ability of microorganisms to breakdown paraquat. In the work of Moneke et al. (2010), of the seven bacterial species they identified, two (*Acetobacter* sp. and *P. fluorescens*) were selected for further biodegradation studies based on their short lag phase and rapid utilisation of glyphosate. Many *Pseudomonas* species have been used extensively in the degradation and metabolism of glyphosate. Cheloufi et al. (2017) state that, glyphosate and 2.4-D have a negative effect on production of P_2O_5 and NO_3 - in two Soils Types of the Bou Namoussa irrigable perimeter (Algerian Extreme Northeast), while herbicide 2.4-D exerts a more depressive action than that of Glyphosate towards microflora in both related soils from the point of view of texture and structure, loamy ground and other sandy in the irrigable perimeter of Bou Namoussa. Inhibition by these two herbicides decreases the two microbial activities concerning mineralisation of assimilable phosphorus and organic nitrogen in nitrate. The sharp reduction in herbicide concentrations after 7 days of incubation caused by the indigenous bacterial and fungal consortium indicates high rate of degradation by the indigenous bacteria and fungi, hence pointing to the ability of the microorganisms to use herbicides as carbon source. The bacterial consortium degraded herbicides faster than the fungal consortium because of their rapid rate of proliferation in aquatic environments. Bacteria degraded atrazine faster than any of the other herbicides used in this study. Moreno *et al.* (2007) reported that after 16 days of incubation there was, at a maximum, 50% of the added atrazine remaining. Shaner and Henry (2007) reported that there was approximately a 3- to 5- fold difference between the rates of degradation in the rapid assay compared to field dissipation.

5. CONCLUSION

There were considerable reductions of exchangeable Na^+, K^+ and Mg^{2+} in soils after herbicide treatment. The indigenous bacteria and fungi were able to utilize the herbicides as carbon source and consequently degrading them *in-vitro*. Continuous herbicide treatment should be avoided because of their ability to accumulate and persist in soils resulting in soil pollution and limiting essential nutrients available to plants. Indigenous microorganisms can be employed to remediate soils polluted by herbicides. Future studies on acceleration of biodegradation rates by indigenous microbial consortium should be examined.

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