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Investigation of Ecotoxicological Effects of Glyphosate (Herbicide) on Soil (Sandy Loam & Sandy Clay Loam) Physiochemical Properties and Microbial Diversity

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Abstract
This work showed that the adsorption of glyphosate depends on the textural class and amount of clays and concerntration of exchangeable cations in the soils. Organic matter had a secondary role in the adsorption of glyphosate on soils. This research was conducted to evaluate the influence of glyphosate-based herbicides systems on soil microbial activity. Soil was treated with commercial formulations of glyphosate (applied as Roundup). The soil microbial activity was measured by quantifying carbon and nitrogen mineralization, pH, concerntration of exchangeable cation, concerntration of exchangeable bases. Soil microbial biomass was determined using Nutrient culture media and total viable cell count method. Glyphosate applied at the recommended field rate to a sandy clay loam and a sandy loam non-agricultural soil resulted in few changes in their population but all the species (*Bacillus spp*, Mold, *Staphylococcus spp*, *Enterococci*, *Micrococcus spp*) isolated were maintained for the duration of the experiment. Total and culturable bacteria biomass, carbon utilization profiles were unaffected for the period of 21days after application of the herbicide. The result therefore suggest that, although the herbicide does not show any toxicity to the microbial community it however causes fluctuation in the microbial population
Keywords: Sandy loam, Sandy clay loam, Glyphosate, Microorganisms, Batch

INTRODUCTION

Glyphosate has been used for more than 35 years and is probably the world's most widely used herbicide. It is registered in more than 130 countries and is approved for weed control in more than 100 crops. The chemical revolution in agriculture of the 1950s was heralded as the panacea to any farming problems (Woodburn, 2000). The weeds and pests which had plagued farming for centuries would be eliminated and food would be cheap and plentiful for all. Glyphosate represents 60 per cent of global 'broad-spectrum' herbicide sales. It was developed by the US chemical company, Monsanto, in the early 1970s. Glyphosate is an herbicide. It is applied to the leaves of plants to kill both broadleaf plants and grasses. Glyphosate (N-Phosphonomethyl glycine) is a non-selective, post-emergent herbicide and broad-spectrum. It is useful on essentially all annual and perennial plants including grasses, sedges, broad-leaved weeds and woody plants. It can be used on non-cropland and among a great variety of crops (Tomlin, 2006). Glyphosate is usually formulated as an isopropylamine. In the last 50 years, agriculture has become increasingly dependent on artificial chemicals to control weeds, pests and diseases.

PROPERTIES OF GLYPHOSATE

Physical properties: Pure glyphosate is a colourless, odourless, crystalline solid with a melting point of 185 ºC and decomposes at 187 ºC producing toxic fumes including nitrogen oxides and phosphorus oxides. Solutions of the glyphosate salts are corrosive to iron or galvanized steel. At ambient temperatures, glyphosate is a white crystalline substance.

***Solubility*:** Pure glyphosate is slightly soluble in water (12 g/litre at 25 ºC), and is practically insoluble in most organic solvents. The alkali-metal and amine salts are readily soluble in water. ***Stability*:** Glyphosate formulations are stable for extended periods below 60 ºC.

***Vapour pressure*:** (Glyphosate isopropylamine salt) 2.1 x 10^{-3} mPa at 25 ºC or 1.58 x 10^{-8}mmHg

Chemical properties: In the crystalline form, glyphosate has both positive and negative regions of charge, indicated by the circled plus (+) and minus (-) signs in the schematic below. Such dipolar ion species are sometimes referred to as *zwitterions*. In aqueous solutions, the hydrogen atoms of the carboxylic acid (COOH) and phosphate (PO_4^3) groups may be associated.

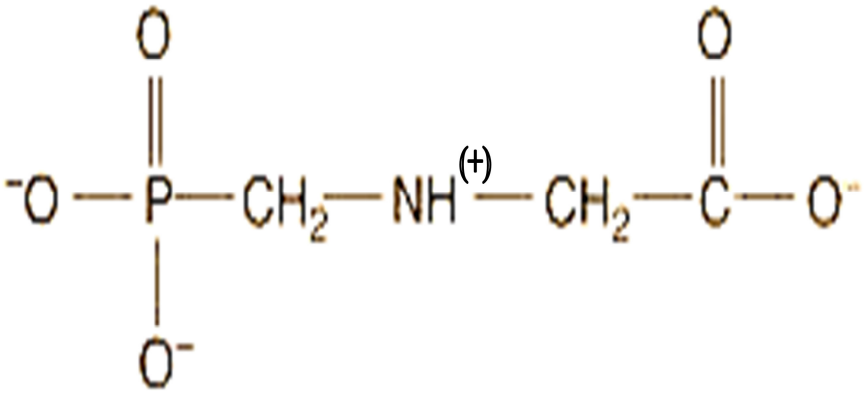

Figure 1: Chemical structure of glyphosate

FORMULATION OF GLYPHOSATE BASED HERBICIDE

Glyphosate is water soluble and binds tightly to soil. In addition to glyphosate, the formulation typically includes water and a surfactant system. In addition to being approved for use on land, it is approved for weed control in aquatic environments, including ponds and reservoirs, waterfall sanctuaries, and recreational waterways. Only a few herbicides have the favorable toxicological and environmental characteristics that allow them to be directly applied to aquatic vegetation. It is usually formulated as the isopropylamine or trimethylsulfonium salt of glyphosate. Other ingredients known as inerts or additives are also added to the formulation. A surfactant (wetting agent) known as polyoxyethylene amine (or POEA), which helps the active ingredient penetrate the plant surface, is usually added to glyphosate formulations. Other additives include sulphuric and phosphoric acids. The herbicidal action of glyphosate is also a threat to both targeted and untargeted species. The targeted ones are the grasses, some perennials and small woody plants that grow among the crop plant while the untargeted species includes the terrestrial (soil, earthworm, crop plants) and aquatic environment (aquatic flora, invertebrate fauna such as phytoplankton and zooplanktons , and vertebrates such as fishes). The farm crops, vegetation or forest reserve form the untargeted species. When the toxicity of herbicides is discussed, the focus is mostly on the active compound (in this case glyphosate acid or glyphosate salts). However, herbicides are formulated to increase their efficacy against target plants. Commercial glyphosate-based-herbicides contain other components, which are called inert ingredients. These inert ingredients are: mainly surfactants, solvents and antifoam compounds. Surfactant refers to chemicals that have pronounced surface activity in aqueous solutions that can decrease surface tension and perturb membrane permeability or transport function of membranes including permeability to glyphosate (Riechers *et al*., 1994). Antifoam compounds are chemical additives that reduce and hinder the formation of foam in industrial process liquids. Numerous contributions have demonstrated that inert ingredients in glyphosate formulations have several folds higher toxicity on non-target organisms than glyphosate alone (Folmar *et al*., 1997; Cruz *et al*., 2007; Cedergreen & Streibig, 2005). Therefore, glyphosate formulations are chemical mixtures and must be considered as mixtures in toxicity assessments. In this context, studies regarding specific toxicity or generalization about toxicology of inert ingredients (e.g. surfactants) must be conducted on glyphosate, inert ingredient and commercial formulation separately. The lack of such data will render any predictions about the effects of the formulations on glyphosate highly uncertain. Glyphosate concentration, as well as nature and concentration of inert additives, depend on commercial formulations and demonstrated in this work is its effect in physiochemical properties of soil and microbial diversity.

MATERIALS AND METHODS

All reagents where of analytical reagent grade

MATERIALS: Hot air oven, Round bottom flask, Filter paper, Distill water, Glass stirrer, Wire loop, Incubator, Distiller, Aluminium foil, Petri dishes, Thermometer, Soil Hydrometer, Autoclave, Test tubes, Microscope, Beakers, Conical flask, Weighing Balance, Measuring cylinder, Micropipette, Measuring Cylinder, Glass pipettes, Spectrophotometer, pH Meter, Bijou bottles, Burette.

The three different soil samples were collected from different locations of non-agricultural activity in Nsukka with no known history of chemical treatment and a textural class test was performed to confirm they are sandy loam and sandy clay loam soil samples.

EXPERIMENTAL DESIGN: The soil samples (Sandy loam and sandy clay loam) were collected from a non-agricultural site which is believed not to have undergone any known chemical treatment in the past. The top soil

of about 2-20cm top soil was collected aseptically weighing about 2kg into pans. A microbial analysis (microbial load and microbial isolation) was carried out on this fresh soil sample and so was a physiochemical soil analysis (textural class, mechanical analysis in percentage, pH value, organic matter in percentage, exchangeable bases, available phosphorus in part/million).

The glyphosate herbicide was used to treat the soil and subsequent soil and microbial analysis was performed giving rise to groups:

Control or t0: Microbial analysis and soil physiochemical analysis before glyphosate herbicide treatment (0 days)

Batch one or t1: Microbial analysis and soil physiochemical analysis after 7days glyphosate herbicide treatment

Batch two or t2: Microbial analysis and soil physiochemical analysis after 14days of glyphosate herbicide treatment

Batch three or t3: Microbial analysis and soil physiochemical analysis after 21 days of glyphosate herbicide treatment

METHODS:

Calcium determination: Pipette 10ml of the leachaete into a 250ml conical flask. Add 20mL of distilled water. Add 20ml of 20% potassium hydroxide and a pinch of calcium indicator and titrate with 0.01M EDTA till the greenish pink colour disappears sharply at end point.

Na and K determination: Potassium and sodium are determined by running leachaete in a flame-photometer with standards of potassium and sodium.

Determination of concentration of exchangeable cation: 50mL of 0.1N KCl leachaete pipetted into a 250mL conical flask, 20mL of 20% formalin was added plus 3drops of 1% Phenolphthalein indicator and then mixture is titrated with 0.1N NaOH solution.

Phosphorus determination by Ascorbic Acid Method: Two grammes of soil sample were weighed accurately into a shake bottle. 20mL of 0.03N NH_4F in 0.1N HCl (Bray II) was added. The resulting mixture was shaked for 2minutes and filtered with Whatman filter paper. 2mL of filterate was pipetted into a long test tube and in it was added 1ml of 2.5% Ammonium Molybdate and 1ml of 2% Ascorbic acid and boiled in a water bath for 5minute until a blue colour developed. I took the absorbance readings at 882nM using a spectrophometer.

Organic Carbon / Organic matter determination: One gramme of soil was weighed into a 500mL conical flask. 10mL of 1N $K_2Cr_2O_7$ solution and 20mL of concerntrated sulphuric acid was added and allowed to cool for 30minnutes. 200mL of distilled water and 1g of NaF and 1% Diphenylamine inhibitor was also added. Blank is prepared the same way but with no soil sample and titrated with 1N $FeSO_4$. The titre value that was obtained was used to calculate for percentage carbon and for percentage organic matter.

Organic matter is given as:% Organic Carbon × 1.724

Nitrogen determination-Macro Kjeldahl Method: 5g of soil sample, 5g of catalyst mixture and 20mL of concerntrated H_2SO_4 were weighed into a kjeldahl flask. The mixture was digested on a macro-kjeldahl heater for about $1^{1}/_{2}$hour. It was allowed to cool for 6hours. Afterwards a 100mL of distilled water was added and top fluid decanted. Decant was titrated with 20mL of 2.5% Boric acid solution in a 100ml conical flask with 3drops of Bromocresol/methyl red indicator.

Distillation: 10mL of 45% Sodium hydroxide solution was added into a 500mL kjeldahl flask already containing 100mL of the digested sample. The mixtures were gradually fixed on the macro-kjeldahl distillation assembly and a receiving flask was also connected. 50mL distillate was collected and titrated with 0.05N HCl solution giving rise to a bluish solution; titration was continued until endpoint was achieved with characteristic brown colour.

PH determination: Step 1: Weigh 10g of soil in a 100mL beaker in duplicate. Add 25mL of distilled water in one beaker and 25mL of 0.1N KCl in other beaker, the mixtures are in a ratio of 1:2.5. Stir the suspension intermittently for 30minutes and take the pH value in a pH meter.

Bouywus Hydrometer Method of Particle size distribution (Mechanical analysis): 50g of soil samples, 50ml of Calgon (dispersing agent) were weighed into a shaking bottle followed by 200mL of tap water. Mixture was stirred and left for not less than 24hours (soaking). After 24hrs, the shake bottle was corked tightly and shaked reciprocally in a shaker for 30minutes. The residue from the shake bottle is transferred into a 1000mL measuring cylinder and made up to 1000mL volume with water. The hydrometer and thermometer is inserted in it and first and second hydrometer and thermometer readings were taken after 40 second. Decant by pouring out the water and replace with another. It was repeated until the supernatant is clear. Finally pour out the water and transfer the sand in a glass beaker, drain the beaker. The washed sand is dried in oven at 105^{o}C for 24hours, cooled and sieved with a 0.25mm sieve. The coarse sand was then weighed. The first and second readings of the soil hydrometer are then used to calculate the percentage clay, silt, fine sand and coarse sand of the soil sample.

Preparation of serial dilutions of the soil sample (Sandy loamy and Sandy clay loam): A set of dilutions (of

the soil samples) was prepared by initially diluting an aliquot of the sample (e.g.: soil), then diluting an aliquot of this dilution, and so on. In the course of this work we employed the log dilutions expressed thus: 1/10 = 10^{-1}, 1/100 = 10^{-2}, 1/1000 = 10^{-3} dilution factor etc.

Materials: 6 large test tubes, 1 bottle of sterile saline, 6 plates of Nutrient agar, Pipette tips, Pipettor, 1.0 gram each of the sandy loam and sandy clay loam soil was basically used.

Procedure: One gram of soil was added to tube 1, containing 9 ml of saline solution. (a) Soil in tube 1 was thoroughly vortex-mixed. (b) A tenfold serial dilution of the soil was made by transferring 1.0 ml of solution from each tube to the next one to achieve a final dilution of 1:1,000 in tube 3 because it gave countable colonies of microorganism when inoculated for growth. (c)An alcohol-flamed glass rod was used to spread the 1.0 ml of soil suspension on the surface of each of the agar plates in duplicates.

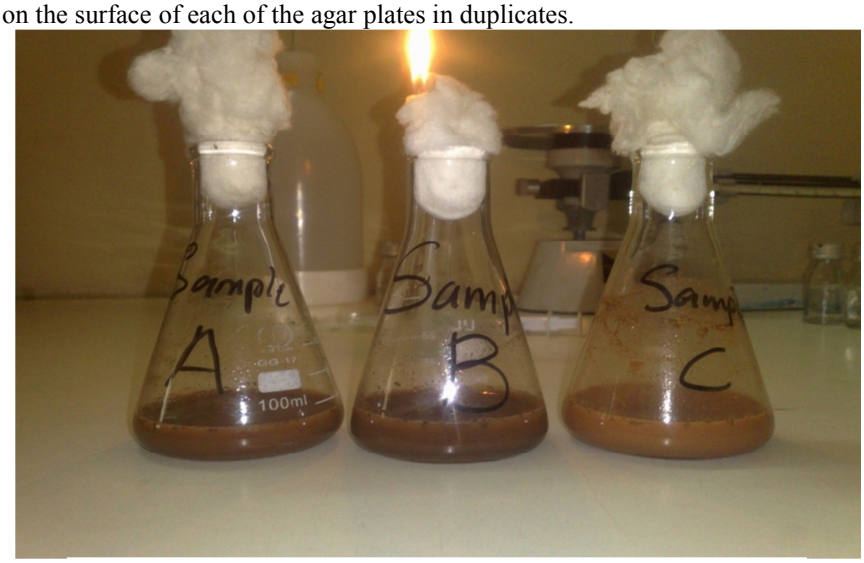

Figure 2: Soil samples in the tube 1

Key: Sample A: Sandy loam soil
Sample C: Sandy clay loam

Culture of sample from the serial dilution above for Total viable count: The method applied here is the surface viable plate count. This method is used due to the fact that the microorganisms that would probably be isolated from the top soil are facultative and obligate aerobes and so they are grown on the surface of the agar to enable aeration.

0.015ml of the inoculums was collected using a disposable pipette tip and its Pipettor

It was inoculated on the surface of the agar block and left that way for the surface viable microbial count**.** We incubated at 28^{o}C for 24 hours.

After incubation we checked the plates with the different inoculums from serial dilution 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} and found that the 10^{-3} serial dilution factor gave countable colonies. Then 10^{-3} d.f was adopted as the dilution factor throughout the work.

After which we divided the plates into quadrants and counted viable colonies and observed the colonial morphology on the other agar plates.

Procedure: The heat-fixed smear (as described above) of bacteria on a glass slide was

1. Stained for 1min with one of certain basic triphenylmethane dye (commonly crystal violet); most bacteria take up the dye.
2. The smear was then rinsed briefly under running water, treated for 1 minute with Lugol's iodine, and again rinsed with water.
3. Decolourization was then attempted by treating the smear with 70% ethanol. This is the critical step: solvent was allowed to run over the tilted slide until dye no longer runs freely from the smear (1–3 seconds), and the smear is immediately rinsed under running water; at this stage any Gram-negative bacteria in the smear will be colourless, while any Gram-positive cells will still be violet.
4. The smear was then counterstained for 30 seconds (e.g. with dilute carbolfuchsin) to stain any Gram-negative bacteria present in the smear. Finally, the slide was rinsed briefly under the tap, blotted dry, and examined under the microscope.

RESULTS AND DISCUSSION

DETERMINATION OF THE TOTAL VIABLE COUNT & MICROBIAL LOAD OF THE SANDY LOAM & SANDY CLAY LOAM SAMPLES

Table 1: Total Viable Count and Microbial Load of the Sandy Loam and sandy clay loam

Sample	Days	Total No. of Colonies	Mean No. of Colonies	CFU/ mL
Sandy Loam	**Control**	52	13	866,667
	t1	72	9	1,733,333
	t2	60	7.5	533,333
	t3	124	23	1,533,333
Sandy clay loam	**Control**	76	19	66,667
	t1	224	28	1,866,667
	t2	24	3	200,000
	t3	104	13	866,667

The table above contains the total population of cells isolated in the different batches of microbial isolation of the soil samples (control and treatments). t1- t3 represents the time dependent interval of 7, 14 and 21 days of diluted glyphosate herbicide treatment while the CFU/ mL i.e. colony forming unit of cells per/ mL gives an estimate of the microbial load of each soil sample.

DETERMINATION OF ORGANISMS ISOLATED IN THE SOIL SAMPLES:

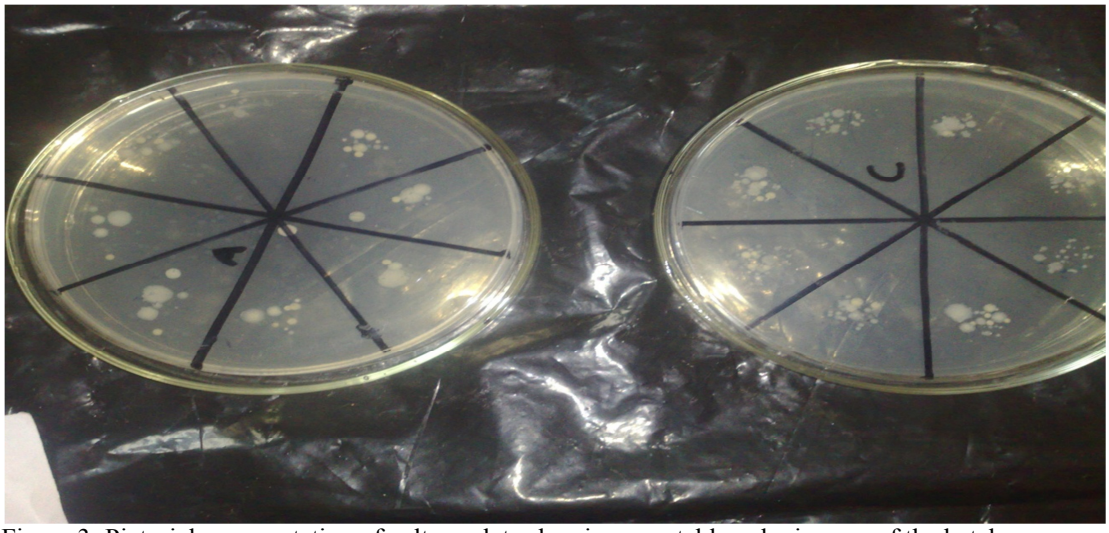

Figure 3: Pictorial representation of culture plate showing countable colonies one of the batches

Table 2: Organisms isolated from Sandy loam and Sandy clay loam

Isolates	Sandy loam soil	Sandy clay loam
	Stapylococcus aureus	*Micrococcus varians*
	Bacillus cereus	*Aeromonas spp*
	Bacillus subtilis	White mold
	White mold	*Bacillus pasteurii*
	Eschericia coli	*Eschericia coli*
	Staphylococcus epidermidis	*Bacillus subtilis*

The table above represents the microorganisms isolated in the control experiment which were also isolated until the end of the herbicide treatment. The only change experienced was differences in the population of cells in Colony Forming Unit per mL throughout the course of the work.

DETERMINATION OF THE MICROBIAL GROWTH CURVE FOR THE MICROORGANISMS ISOATED FROM SANDY LOAM SOIL SAMPLE

From the result plotted in the graph below the number of cells ranges from 533,333 to 1,733,333. The parameters of the graph are total number of cells in CFU/ mL against the batches (days). There was a time dependent increase in the total population of cells isolated in the control compared to the batch one (i.e. after 7days interval of glyphosate herbicide treatment). Total viable cells also decreased largely in the batch two (after 14days of herbicide treatment) and then again at the third batch isolation (after 21 days of herbicide treatment) there numbers increased gradually to almost the CFU like in the batch control.

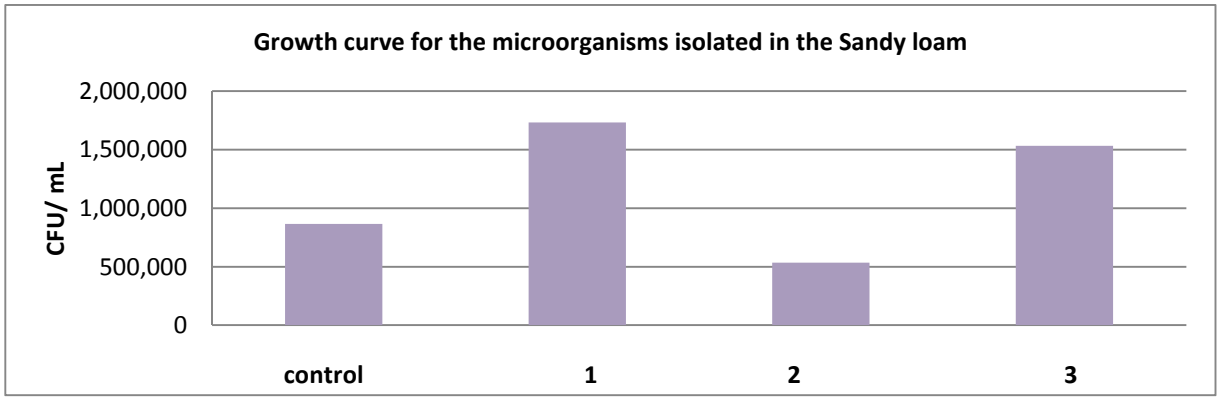

Figure 7: Growth curve for the microorganisms isolated from the sandy loam soil sample

DETERMINATION OF THE MICROBIAL GROWTH CURVE FOR THE MICROORGANISMS PRESENT IN SOIL SAMPLE (SANDY CLAY LOAM)

From the result plotted in the graph below the total number of cells range from 200,000 to 1,866,667. The parameters of the graph are total number of cells in CFU/ mL against the batches (days). There was a time dependent increase in the total population of cells isolated in batch control compared to the batch one (after 7days interval of glyphosate herbicide treatment). Total viable cells also decreased largely in the batch two (after 14days of treatment) and then again at the third batch isolation (after 21 days of herbicide treatment) there numbers increased gradually.

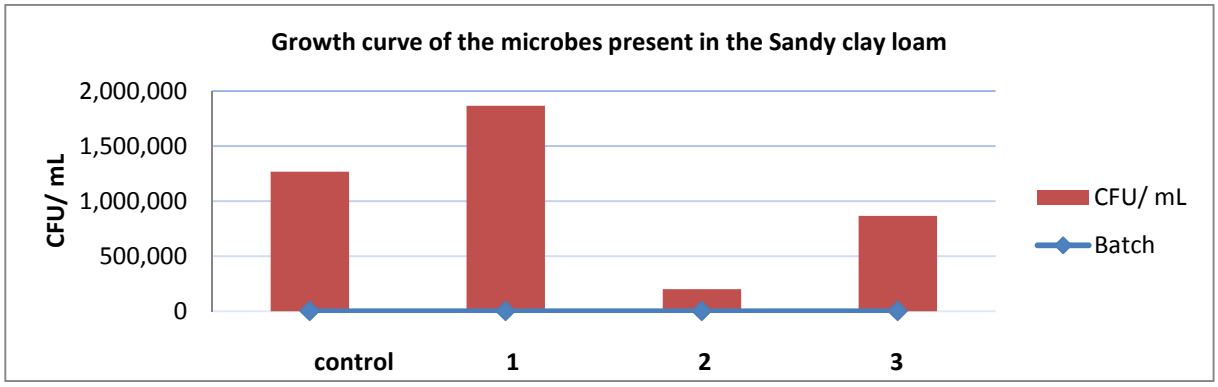

Figure 8: Growth curve for microorganism isolated from the Sandy clay loam sample

DETERMINATION OF SOIL PH IN H_2O AND KCL

The figure below is the graph of the pH in water and KCl of the soil samples (Sandy loam and sandy clay loam, their control and three different treatments which is time dependent). Result shows that the pH of the sandy loam soil in water ranges from 7.267-7.4 but in KCl it is 6.9-7.1 while the sandy clay loam in water ranges from 7.4-7.7 but in KCl it ranges from 7-7.4. There was not any significant difference between the controls of the sandy loam and sandy clay loam and their batches one –three in the pH values of the soil samples both in water and KCl ($p \geq 0.05$).

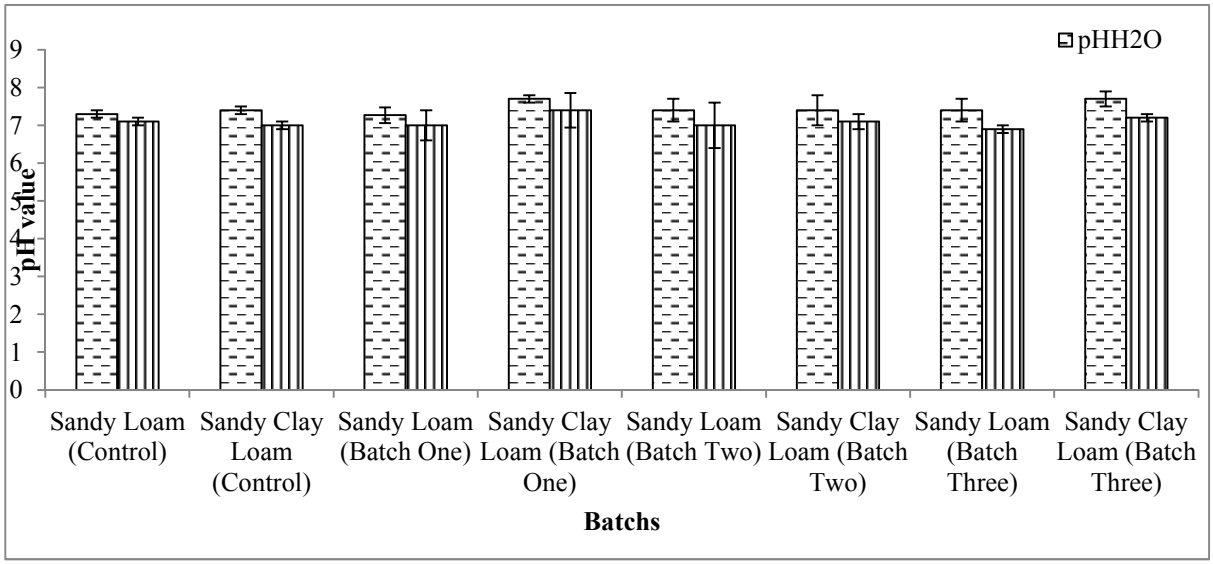

Figure 9: Combined graphical representation of the pH readings of the soil samples in H_2O and KCl

DETERMINATION OF THE SOILS ORGANIC COMPONENTS (C, O.M & N_2)

The figure below is the graph of the organic component (carbon, organic matter and nitrogen) of the soil samples (Sandy loam and sandy clay loam, their control and three different batch herbicide treatments which is time dependent). The concerntration of carbon ranges from 1.92 - 3.16%; 1.82 - 2.8, organic mater 3.3 - 5.2; 3.14 - 4.84 and nitrogen to be 0.154 - 0.686; 0.14 - 0.602 for sandy loam and sandy clay loam respectively. There was a significant decrease in the concerntration of carbon in the sandy loam (control) to that of sandy loam batch one ($p \geq 0.05$) unlike in two-three where there was no significant decrease ($p \geq 0.05$). The result also shows that there is a significant difference in the organic matter content between the two soil samples. After treatment of the sandy loam soil with glyphosate herbicide there was significant decrease in the percentage concerntration of organic matter in batch one unlike in batch two and three where there was no significant increase compared to the control. In sandy clay loam there was a significant decrease in batch two-three ($p \geq 0.05$) compared to the control unlike in batch one where there was least significance difference ($p \leq 0.05$). There is no significant difference in the concerntration of nitrogen in the two soil samples. Comparing the sandy loam (control) and batch one-two there was least significant difference in the % nitrogen unlike in the batch three where there was a significant increase ($p \leq 0.05$). In the sandy clay loam(control) and batch two there was least significant difference but no significant decrease in batch one ($p \leq 0.05$) and a significant increase in batch three ($p \leq 0.05$).

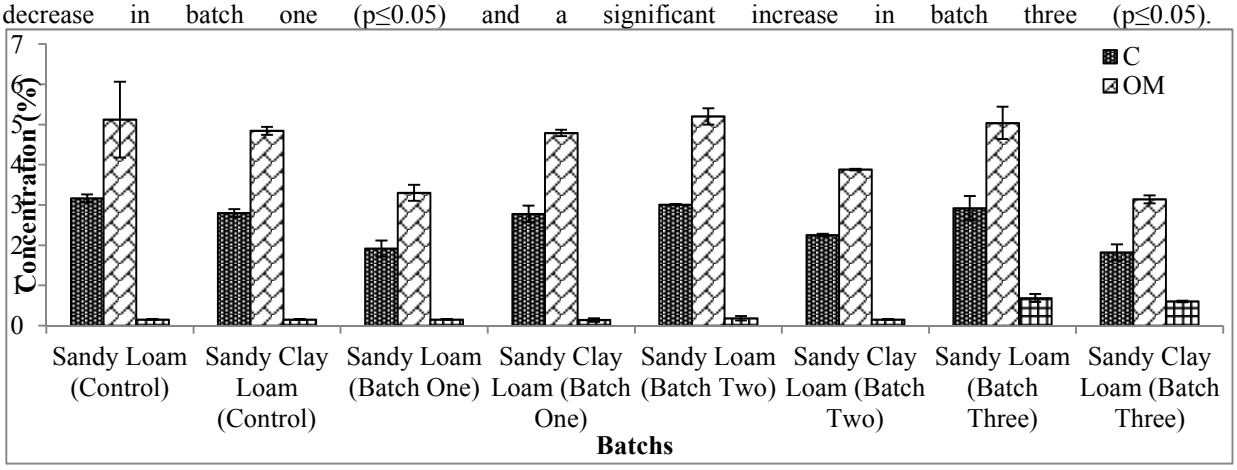

Figure 10: Combined graphical representation of the Organic components % concerntration of the soil samples (sandy loam and sandy clay loam)

DETERMINATION OF CONCERNTRATION OF EXCHANGEABLE BASES (Na, Ca, Mg, K)

The figure below is the graph of the exchangeable bases component of the soil samples (Sandy loam and sandy clay loam, their control and three different batch treatments which is time dependent). For sodium concerntration in both soils there is no significant difference in the concerntration of sodium in both controls and also in between the controls and there three batches for the different soils. For the potassium concerntration, there is the

least significant difference between the both controls. In the sandy loam (control) and sandy loam (batch one-three) there is no significant difference between them ($p \geq 0.05$) and also in sandy clay loam (control) compared with sandy clay loam batch one-three ($p \geq 0.05$).

The result of the calcium concerntration for the sandy loam (control) compared with sandy loam (batch one-three) shows a significant increase, and significant decrease in batch three ($p \leq 0.05$). And the sandy clay loam (control) compared to sandy clay loam batch (two-three) shows a significant increase. ($p \leq 0.05$) unlike in batch one where there was no significant increase ($p \geq 0.05$). Magnesium concerntration in the sandy loam and sandy clay soil have the least significant difference. Comparing the sandy loam (control) and batches; there is a significant decrease in the magnesium concerntration of the sandy loam (batch one-two) ($p \leq 0.05$) and no significant decrease in the sandy loam (batch three) ($p \geq 0.05$). Comparing the results of the sandy clay loam (control) and sandy clay loam (batch one & two) there is no significant decrease unlike in sandy clay loam (batch two) with least significant difference.

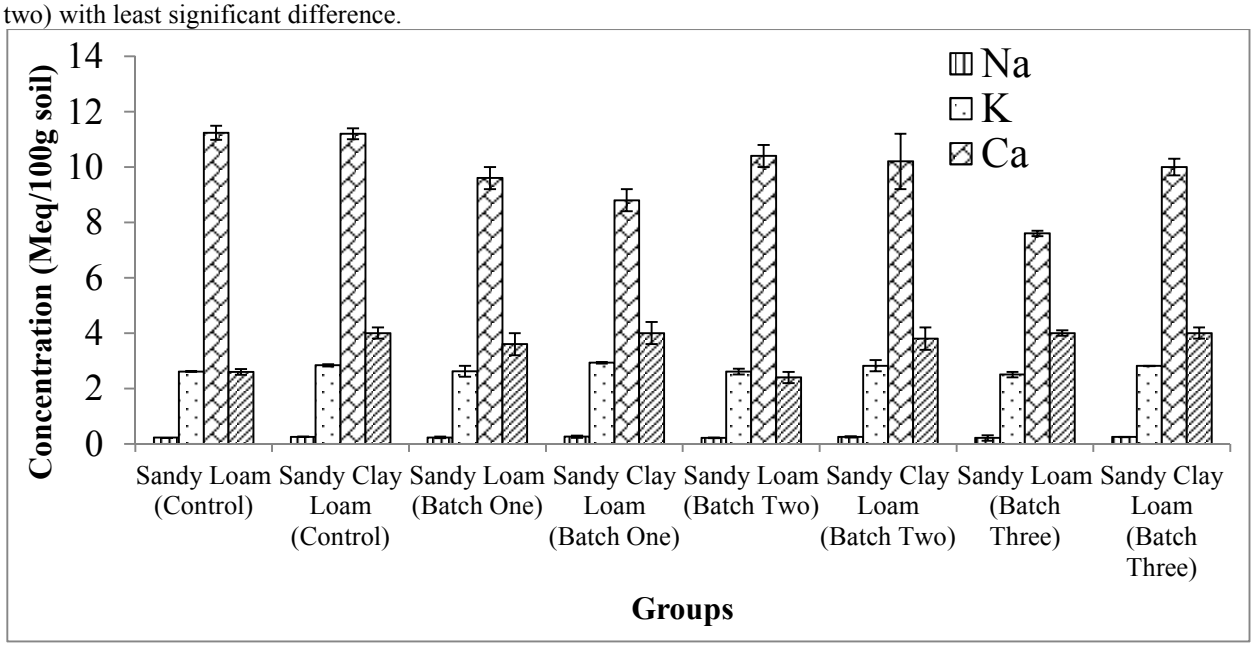

Figure 11: Graphical representation of the % concerntration of the exchangeable bases for the soil samples (sandy loam and sandy clay loam)

DETERMINATION OF CONCERNTRATION OF EXCHANGEABLE CATIONS

The figure below represents the graph of the concerntration of exchangeable cations (Sandy loam and sandy clay loam, their control and three different treatments which is time dependent herbicide treatment. The CEC for sandy loam ranges from 40-68 unlike the sandy clay loam which ranges from 48-68 Meq. There was no significant difference between the concerntration of exchangeable cations in the sandy loam and the sandy clay loam ($p \leq 0.05$) unlike comparing sandy clay loam and sandy loam there was significant decrease in the concerntration of CEC Meq/100g soil ($p \geq 0.05$). There was a significant increase in the CEC between the control for the sandy loam and its batch one-three ($p \leq 0.05$) and also the same for the sandy clay loam control and its batch one-three.

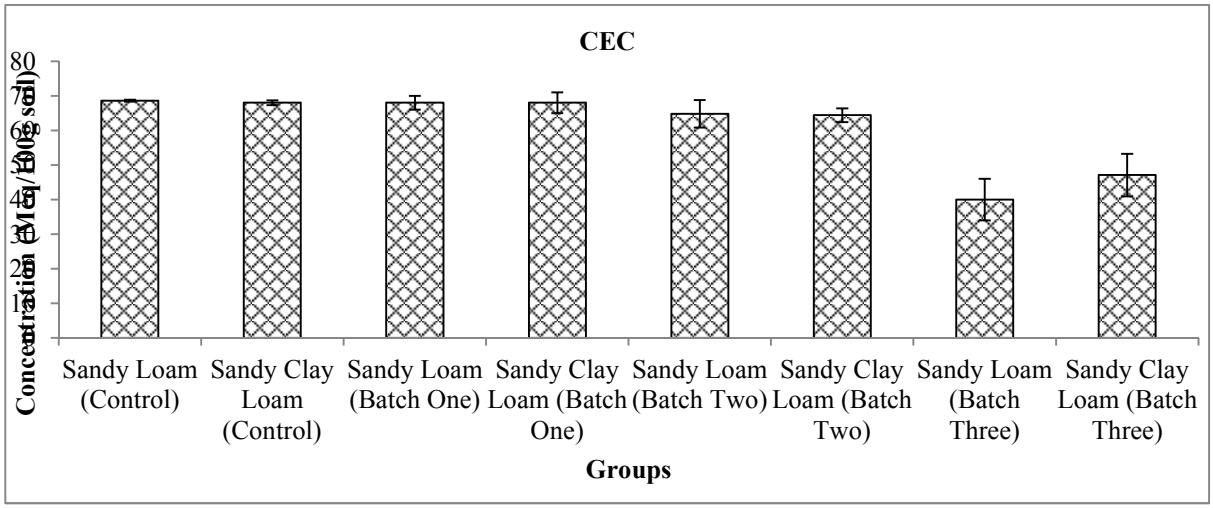

Figure 12: Graphical representation of the concerntration of exchangeable cations in Meq/100g of sandy loam and sandy clay loam soil.

DETERMINATION OF CONCERNTRATION OF AVAILABLE PHOSPHORUS

The figure below represents the graph of the available phosphorus of the soil samples (Sandy loam and sandy clay loam, their control and three different treatments which is time dependent). It's concerntration in the sandy loam ranges from 46.2-58.1 unlike in sandy clay loam which ranges from 67.62-133.37.In the control, sandy clay loam contains more phosphorus than the sandy loam. When comparing the sandy loam (control) and (batch one to three) there was no significant decrease in the levels of phosphorus ($p \geq 0.05$). In sandy clay loam (control) there was a significant increase the concerntration of phosphorus in batch one and two ($p \leq 0.05$), whereas a significant decrease in batch three ($p \geq 0.05$).

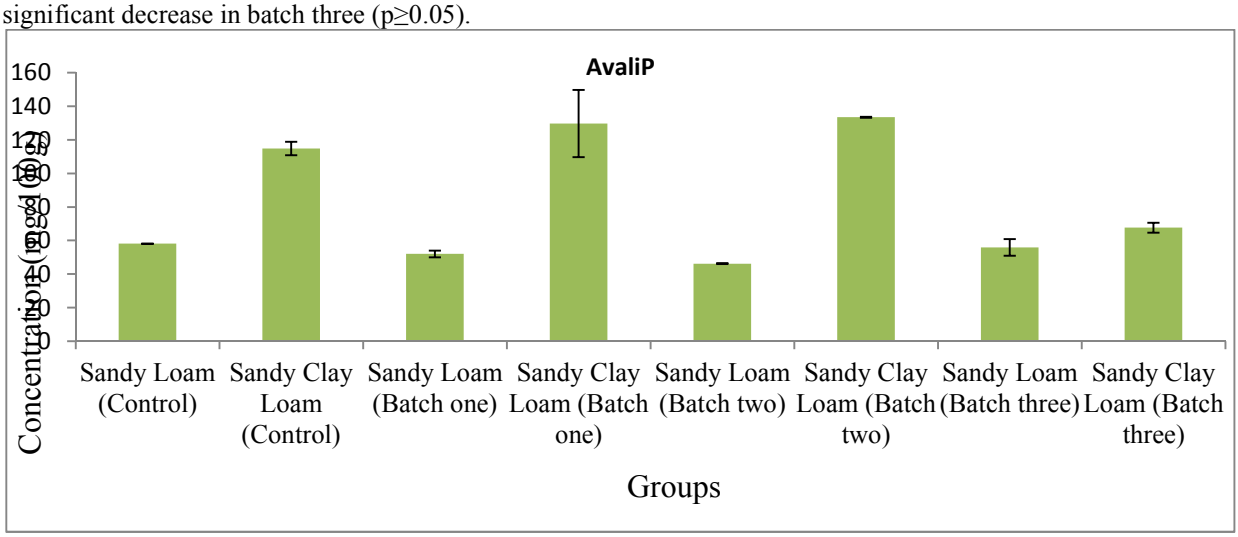

Figure 13: Combined graphical representation of the Concerntration of available phosphorus in the soil samples (sandy loam and sandy clay loam)

DETERMINATION OF HYDROGEN ION CONCERNTRATION

The figure below is the graph of the $[H^+]$ of the soil samples (Sandy loam and sandy clay loam, their control and three different treatments which are time dependent). The $[H^+]$ ranges from 1.2-3.1 and 1.2-2.8 for the sandy loam and the sandy clay loam respectively. At the introduction of glyphosate, in the sandy loam and sandy clay loam there was a significant increase in the concerntration of $[H^+]$ in the batch one to three when compared to their control ($p \leq 0.05$).

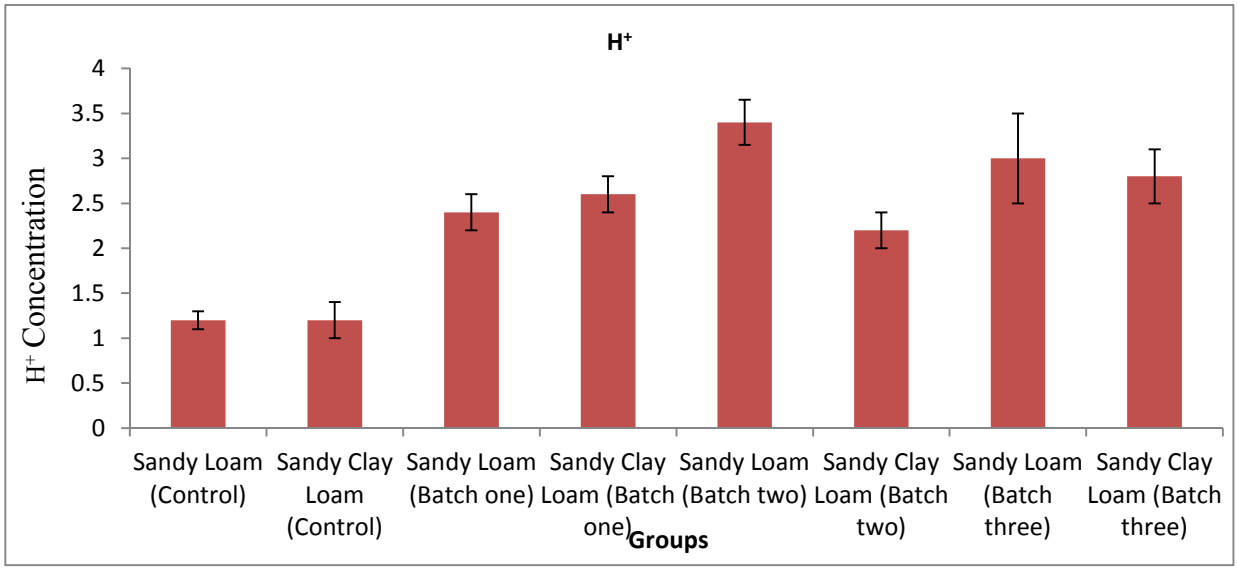

Figure 14: Combined graphical representation of the [H^+] of the sandy loam and the sandy clay loam sample

DISCUSSION

The result of glyphosate application on soil has been shown to penetrate the upper 2 mm of the soil (Haney et al. 2000) at recommended field application rates. Single application or short term studies of this soil in the lab have shown that microbial biomass is unaffected or stimulated which agrees with the findings of Hart and Brookes; Haney *et al*. (2000). In similar, short term applications, glyphosate even at rates significantly above recommended rates generally show minimal or transitory effects on soil biology. The adsorptive capacity of a soil is determined by its clay and organic matter content. For most soils, organic matter is responsible for the majority of herbicide binding. Soil pH influences binding of herbicides that are classified as basic chemical compounds (versus acidic or non-ionic compounds). These molecules have a neutral or positive charge depending on the soil pH. In neutral or basic soils (pH ≥ 7) a basic herbicide will have a neutral charge, whereas under acidic soil conditions (pH < 7) the herbicide takes on a positive charge. Due to the positive charge on the molecule in acid soils, basic herbicides are more tightly bound to soil colloids in soils with a low pH.

Thus from the result of this work, the organic component of the soil samples which includes the organic matter, carbon and nitrogen content shows there is a significant difference between the carbon content of sandy loam and sandy clay loam controls. There was a significant decrease in the concerntration of carbon in the sandy loam (control) to that of sandy loam batch one ($p \geq 0.05$) unlike in two-three where there was no significant decrease ($p \geq 0.05$). The higher reading for the carbon component of the soil was as a result of the fact that in the second batch of both the sandy loam and sandy clay loam the population of microorganisms decreased must and consequent upon this least amount of carbon was used up at this time. The result also shows that there is a significant difference in the organic matter content between the two soil samples. After treatment of the soils with glyphosate herbicide there was significant decrease in the percentage concerntration of organic matter in batch one unlike in batch two and three where there was no significant increase compared to the control. In sandy clay loam there was a significant decrease in batch two-three ($p \geq 0.05$) compared to the control unlike in batch one where there was least significance difference ($p \leq 0.05$) when the microbes have not resorted to it as a carbon source. There is no significant difference in the concerntration of nitrogen in the two soil samples. Comparing the sandy loam (control) and batch one-two there was least significant difference in the % nitrogen unlike in the batch three where there was a significant increase as a result of build up on the concerntration of glyphosate which normally contains nitrogen through the amino acid it contains known as glycine. In the sandy clay loam(control) there was a negligible change in the concerntration of nitrogen between the control and one and but a significant increase in batch three ($p \leq 0.05$).

The figure 8 representing the graph of the pH in water and KCl of the soil samples showed that there are variations in the graph which does not follow a definite pattern and shows that glyphosate does affect the pH of the soil samples, thus the pH of the soil can be affected by others external or internal factors.

The figure 11 represents the graph of the concerntration of exchangeable cations for the soil samples. There was a significant increase in the CEC for the sandy loam and sandy clay loam soil samples which did not have any direct effect on the microbial load on other isolation batches except in the isolation after 14days herbicide treatment which showed an increase in CEC and decrease in the cell number which may have contributed directly or indirectly to this decrease in total viable count.

The figure 12 representing the graph of the available phosphorus of the soil samples (Sandy loam and sandy clay loam, their control and three different treatments which is time dependent). Phosphorus in the soil can be free or bound. There is more bound phosphorus in the soil than is the free ones. This bound phosphorus is freed by the action of soil microorganisms. In the control for the sandy clay loam, it contains more phosphorus than the sandy loam. At the introduction of glyphosate (which normally contains phosphorus) in batch one there was an increase in the levels of phosphorus in the sandy clay loam but cannot ascertain exactly why there was a decrease in the sandy loamy, but the soil may have components in it that bound the phosphorus introduced and due to fact that there was increase in microbial load; the cells may have used it for their cell wall phospholipids.

The figure 13 representing the graph of the $[H^+]$ of the soil samples showed that at the introduction of glyphosate herbicide that there was a significant increase in the concerntration of $[H^+]$ in the batch one to three when compared to their controls ($p \leq 0.05$) but the microorganism were able to undergo a transitory change from high numbers to lower numbers and high numbers again even with the accumulation of high concerntration of hydrogen ions. Microorganisms can be grouped into acidophiles and basophiles. Since all the isolates where retained from the beginning of the work until the end I can deduce that most of the microorganisms are acidophiles and where able to withstand the high hydrogen concerntration and so the increase in hydrogen ion concerntration had little or no effect on the microorganisms present in the soil.

CONCLUSION

Earlier stated, short term applications, of glyphosate even at rates significantly above recommended rates generally show minimal or transitory effects on soil biology which was typical of this work. There were surprisingly few differences in microbial characteristics between the two soils, especially given their differences in chemical and physical properties. Sandy loam soil with relatively higher organic matter content and glyphosate herbicide binding capacity (shown with the higher concerntration of carbon) in comparison sandy clay loam soil is lesser developed to herbicide binding as a result of lesser organic matter than the sandy loam. Nevertheless, most of the microbial indices were comparable between soils whether glyphosate was added or not. Carbon utilization was an exception. As a consequence, the bacterial biomass increased within 1 week of application but after 2 week of application it decreased largely as a result of used up available nutrients but finally in the 3rd week the microbial community started to utilize glyphosate herbicide as a source of nutrients and their numbers gradually increased again. Culturable bacteria also comprised a greater proportion of the total population following the addition of diluted glyphosate, and were responsible for greater carbon utilization of glyphosate. No major changes in microbial community structure assessed by carbon utilization, in these soils following the addition of the recommended field-rate concentration of glyphosate. This finding complements previous results from an array of soils and vegetation types that glyphosate has a benign non-target effect on soil microbial activity.

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