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European Journal of Experimental Biology, 2016, 6(2):9-15

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Effect of atrazine and butachlor on some soil enzymes activities at different concentrations

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ABSTRACT

The effect of two herbicides (atrazine and butachlor) on soil phosphatase and urease activities was assessed every fourteen days over a period of seventy (70) days. Soil samples from Kogi State University farm were treated with herbicides at company recommended rates, concentrations above and below the recommended rates. Pseudomonas spp., Bacillus spp. and Flavobacterium spp. were the most frequently isolated bacteria while Aspergillus niger, Aspergillus flavus, Penicillium spp., Trichoderma spp., Mucor spp. and Fusarium spp. were the most frequently isolated fungi. As herbicide concentrations increased, decrease in phosphatase activities was observed. Atrazine-treated soils had more acid phosphatase activities than butachlor-treated samples, while butachlor-treated samples had more alkaline phosphatase activities than atrazine. As concentrations of the herbicides were increased, the urease activities increased correspondingly, with butachlor-treated soils having more urease activities than atrazine-treated soils. From this study, butachlor is more microbial and environmental friendly than atrazine. Atrazine was shown to be more toxic to microflora and enzyme activity in the soil than butachlor.

Key words: herbicides, microflora, atrazine, butachlor, concentrations

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INTRODUCTION

The basic principle of weed control is the recognition that a weed is plant species growing where it is not desired, or plant out of place, or plant that is more detrimental than beneficial [1]. The global drive for sustainable agriculture systems involves optimizing agricultural resources to satisfy human needs and at the same time maintaining the quality of the environment and sustaining natural resources [2]. In achieving this optimization, herbicide use is of great importance. Herbicides are substances or cultured biological organisms used to kill or suppress the growth of unwanted plants and vegetation [3]. During the past four decades, a large number of herbicides have been introduced as pre or post – emergent weed killers in many countries of the world [4].

In Nigeria, herbicides have since been effectively used to control weeds in agricultural systems [5]. As farmers continue to realize the usefulness of herbicides, larger quantities would be applied to the soil. But, the fate of these compounds in the soil is becoming increasingly important since they could be leached down in which case groundwater is contaminated or if immobile, they would persist on the top soil[6]. These herbicides could then accumulate to toxic levels in the soil and become harmful to microorganisms, plants, wildlife and man [7].

Microorganisms play important roles in soil processes, among which are the recycling of essential plant nutrients, humus formation, and pesticide detoxification [8]. Complete mineralization of herbicides by microorganisms involves oxidation of the parent compound to form carbon dioxide and water, a process that provides both carbon and energy for growth and reproduction of cells. Each degradation step in the pathway is catalyzed by a specific enzyme made by the degrading cell. Enzymes are most often found within a cell but are also made and released from the cell to help initiate degradation reactions. Enzymes found external to the cell are known as extra cellular enzymes. Herbicides must be broken down into smaller subunits outside the cell to allow transport of the smaller subunits into the cell. Degradation by either internal or extra cellular enzymes will stop at any step if the appropriate enzyme is not present. Lack of appropriate biodegrading enzymes is one common reason for persistence of herbicides [9].

Microbes mediate important biochemical transformations associated with nutrient cycling in soils. Since enzymes catalyze all biochemical transformations, measurements of soil enzyme activities are useful indicators of biological activity. Soil enzyme activity measurements have been used as indices of land quality and soil health as well as to understand how human activity is changing biogeochemical cycles in ecosystems. Biochemical reactions are brought about largely through the catalytic contribution of enzymes. Because of the complex and variable substrates that serve as energy sources for microorganisms soils are expected to contain a wide array of enzymes. The differences in the levels of enzymatic activity are caused primarily by the fact that every soil type is typified by its content of organic matter, its composition and the activity of its living organisms, and by the intensity of the biological processes[10]. The activity of soil enzymes is also important in making nutrients available for plants, an example of which is the mineralization of organic phosphorus compounds. From the total amount of phosphorus in agricultural soils only a small proportion of it is immediately available for plant uptake. Some part of the phosphorus in soils is bound organically. The mineralization of these organic fractions is of major importance and soil phosphatases have been accorded a major role in this mineralization process; namely, the catalysis of the hydrolytic cleavage of ester phosphate bonds [11]. Measurement of the level of urease activity in soil samples is a useful indicator of biological activity in herbicide-treated soil. According to Tabatabai and Bremner [12] some herbicides contain urea which acts as fertilizer or animal urine to the soil. This is then enzymatically hydrolysed by soil urease ($NH_2CONH_2 + H_2O = 2NH_3 + CO_2$), and it results in the release of ammonia and a slight rise in pH.

Atrazine powder (2-chloro-4 (ethylamino) –6-isopropylamino-1,3-5-triazine) is a widely used 5-triazine herbicide. The percentage purity of the technical grade atrazine is 97%. The impurities include dichlorotriazine, tris(alkylamino) triazines and hydroxytriazines [13]. It is used as a selective pre- emergence herbicide in the control of broadleaf and grassy weeds in a variety of commercial crops as well as road side and fallow fields [14]. The recommended rate of atrazine powder is 3%w/v per kg of soil.

Butachlor liquid (N-(butoxy methyl 2-chloro-N-(2,6-diethylphenyl) acetamide) is a selective herbicide that controls most annual and perennial plants. It is a pre-emergence herbicide. It has a percentage purity of 94% [9]. The recommended rate of butachlor liquid is 15%v/v per kg of soil.

MATERIALS AND METHODS

2.1 Collection of Samples

Topsoil (0-15cm depth) was sampled from Kogi State University Staff agricultural farm, which has been under continuous cultivation of maize and cassava without any history of herbicide application. The soil samples were sieved through a 2.0mm dish mesh to remove stones and plant debris.

2.2 Soil Treatments with Herbicides

The herbicides used were purchased from a dealership store in Anyigba, Kogi State. The herbicides were atrazine (designated as “A”) and butachlor (designated as “B”). The recommended rate of atrazine powder is 3%w/v per kg of soil. The recommended rate of butachlor liquid is 15%v/v per kg of soil. One hundred ml (100ml) of each concentration was mixed thoroughly with 1kg of the soil sample. The set – ups were done in duplicates and incubated for 70 days. Samples were taken every 14 days and analyzed for microbial load.

2.3 Physicochemical Analysis

2.3.1 Soil**:** Water ratio of 1:1 was used to determine pH of herbicide-treated soils using pH meter.
2.3.2 Total Organic Carbon: Percentage organic matter was determined as described by Walky Black [15].

2.3.3 Microbial enumeration and identification

Nutrient agar was used for the enumeration of total heterotrophic bacteria by the pour plate method. Incubation was at 37^0C for 24h. Potato dextrose agar (PDA) was used for enumeration and isolation of fungi. Incubation was at 30^0C for 72h.

Bacterial isolates were characterized based on cultural characteristics, staining reactions and biochemical reactions. Identification was thereafter made with reference to Bergey's manual of systematic Bacteriology [16]. Fungal isolates were characterized as described by [17].

2.4 Soil Enzyme assay

2.4.1 Phosphatase assay: The assay method used was that of Tabatabai and Bremner [9]. The method used para nitrophenol- linked substrates and enzyme activity was determined by colorimetric measurement of para-nitrophenol released when soil is incubated in a buffered substrate solution.

For each sample, 15 g of soil sample was mixed with 100 ml of 0.05 M acetate buffer (pH 9.5 for alkaline phosphatase; pH 5.0 for acid phosphatase and all other enzymes) in 250-ml Nalgene bottles. Two milliliter aliquots of soil slurry were taken from each sample into polypropylene test tubes, which were kept chilled pending incubation. At each incubation 2 ml of substrate (5 Mmol para -nitrophenol -beta-D cellobioside) solution was added to each sample test tube. The tubes were then capped and placed on a rotary shaker and mixed for two hours during incubation at 25^0C. Following incubation, the tubes were centrifuged for 5 min, and 1 ml aliquot of clear supernatant was transferred from each tube to a 15 ml glass test tube containing 0.2 ml of 1 N NaOH, to stop the reaction and cause color change. The solution was brought to a final volume of 10 ml using deionized water, and light absorbance measured at 410 nm.

Sample and substrate controls were run during the incubation to control for color development due to the substrate or dissolved humic substances. Controls were made by mixing 2 ml of acetate buffer with 2 ml of soil slurry. The concentration of para- nitrophenol detected in samples after incubation was used to calculate the activity of phosphatase by subtracting the combined absorption results for the sample and substrate controls from the analytical samples.

2.4.2 Urease enzyme assay: Into a 50ml volumetric flask, 5g of soil sample was placed. Then, 0.2ml of toluene and 9ml of tris(hydroxymethyl)aminomethane (THAM) buffer were added. The flask was swirled for a few seconds to mix the contents. After this, 0.2 M urea solution was added and the flask was swirled again for a few seconds. The flask was stoppered and incubated at 37^0C for 2 hours. After 2 hours, the stopper was removed and 35ml of KCl-Ag_2SO_4 solution was added. The flask was swirled for a few seconds and allowed to stand and allowed to cool to room temperature. The content was made up to 50ml volume by adding potassium chloride and silver sulphate (KCl-Ag_2SO_4) solution and the flask was stopperd and inverted several times to mix the contents. To determine ammonium in the resulting soil suspension, a 20ml aliquot of the suspension was pippetted into a 100ml distillation flask designed for use with the steam distillation apparatus. The ammonium-N released was determined by steam distillation of the aliquot with 0.2g of MgO for 3.3 min[9].

2.4 Statistical Analysis

Data generated from the study were subjected to analysis of variance (ANOVA) and the student's statistical t-test.

RESULTS AND DISCUSSION

3.1 Results

Table 1: Acidic Phosphatase activity of soil samples (ug p-nitrophenyl/2h)

Samples	Day 0	14	28	42	56	70	Mean	**Samples**	Day 0	14	28	42	56	70	Mean
	Atrazine-treated soil samples						Mean		Butachlor-treated soil samples						Mean
1%w/v	0.025	0.026	0.03	0.024	0.022	0.014	0.06±0.01	5%v/v	0.024	0.025	0.026	0.021	0.015	0.015	0.02±0.01
2%w/v	0.023	0.024	0.029	0.022	0.018	0.012	0.05±0.01	10%v/v	0.018	0.02	0.022	0.018	0.015	0.012	0.020±0.004
3%w/v	0.021	0.023	0.024	0.02	0.015	0.011	0.04±0.01	15%v/v	0.014	0.015	0.016	0.014	0.012	0.008	0.010±0.003
4.5%w/v	0.018	0.019	0.024	0.017	0.013	0.011	0.03±0.01	22.5%v/v	0.009	0.009	0.012	0.009	0.007	0.005	0.010±0.003
6.8%w/v	0.015	0.016	0.022	0.013	0.01	0.01	0.02±0.01	33.8%v/v	0.007	0.007	0.011	0.004	0.003	0.002	0.010±0.004
10.1%w/v	0.014	0.015	0.02	0.011	0.009	0.008	0.01±0.01	50.6%v/v	0.004	0.004	0.009	0.002	0.001	0.001	0.003±0.003
15.2%w/v	0.012	0.012	0.018	0.009	0.004	0.007	0.01±0.01	75.9%v/v	0.003	0.003	0.007	0.001	0.001`	0.001	0.003±0.003
Control	0.026	0.027	0.032	0.025	0.022	0.019	0.03±0.01								

Table 2: Alkaline Phosphatase activity of soil samples (ug p-nitrophenyl/2h)

Samples	Day 0	14	28	42	56	70	Mean	**Samples**	Day0	14	28	42	56	70	Mean
	Atrazine-treated soil samples								Butachlor-treated soil samples						
1%w/v	0.029	0.030	0.028	0.025	0.022	0.020	0.03±0.004	5%v/v	0.033	0.033	0.033	0.030	0.025	0.022	0.05±0.005
2%w/v	0.025	0.025	0.023	0.020	0.018	0.018	0.02±0.003	10%v/v	0.028	0.029	0.028	0.029	0.024	0.020	0.04±0.004
3%w/v	0.023	0.023	0.021	0.017	0.017	0.015	0.02±0.003	15%v/v	0.025	0.025	0.024	0.022	0.020	0.019	0.03±0.003
4.5%w/v	0.019	0.020	0.019	0.018	0.016	0.015	0.01±0.002	22.5%v/v	0.024	0.025	0.021	0.023	0.021	0.017	0.03±0.003
6.8%w/v	0.016	0.017	0.015	0.015	0.016	0.013	0.02±0.001	33.8%v/v	0.022	0.023	0.020	0.017	0.015	0.012	0.02±0.004
10.1%w/v	0.012	0.012	0.013	0.012	0.011	0.01	0.01±0.001	50.6%v/v	0.019	0.020	0.018	0.014	0.01	0.009	0.01±0.005
15.2%w/v	0.009	0.01	0.009	0.008	0.006	0.005	0.008±0.002	75.9%v/v	0.018	0.018	0.015	0.013	0.013	0.01	0.01±0.003
Control	0.035	0.036	0.037	0.033	0.029	0.026	0.08±0.005								

Table 3: Urease activity of soil samples (mg NH^{+}_{4}/2h)

Samples	Atrazine treated soil samples Day							Samples	Butachlor treated soil sample Day						
	0	14	28	42	56	70			0	14	28	42	56	70	
1%w/v	500.0	580.0	441.0	592.0	604.0	505.0	537±64.8	5%v/v	500.0	582.0	480.0	581.0	599.0	507.0	541.5±51.4
2%w/v	500.0	579.0	470.0	584.0	599.0	509.0	540.2±53.7	**10**%v/v	500.0	582.0	498.0	588.0	597.0	512.0	546.2±47.4
3%w/v	500.0	578.0	501.0	584.0	594.0	511.0	544.7±45.0	15%v/v	500.0	585.0	521.0	589.0	596.0	518.0	551.5±42.9
4.5%w/v	500.0	578.0	523.0	582.0	592.0	512.0	547.8±40.5	22.5%v/v	500.0	586.0	560.0	590.0	594.0	524.0	559.0±39.0
6.8%w/v	500.0	579.0	530.0	583.0	586.0	514.0	548.7±38.5	33.8%v/v	500.0	588.0	570.0	591.0	592.0	525.0	561.0±39.2
10.1%w/v	500.0	579.0	574.0	581.0	582.0	515.0	555.2±37.3	50.6%v/v	500.0	588.0	576.0	591.0	591.0	526.0	562.0±39.2
15.2%w/v	500.0	580.0	582.0	580.0	581.0	516.0	556.5±37.9	75.9%v/v	500.0	590.0	582.0	592.0	590.0	527.0	563.5±39.8
Control	500.0	580.0	430.0	600.0	605.0	502.0	536.2±70.0								

Table 4 : Bacteria isolated from herbicides-treated soil samples

Control soil	Atrazine-treated soil	Butachlor-treated soil
Bacillus cereus	*Bacillus cereus*	*Bacillus cereus*
Flavobacterium sp.	*Pseudomonas sp*	*Pseudomonas sp.*
Actinomycetes sp.		*Flavobacterium sp.*
Proteus sp.		
Staphylococcus aureus		
Leuconostoc sp.		
Pseudomonas sp.		

Table 5: Fungi isolated from herbicides-treated soil sample

Control soil	A-treated soil	B-treated soil
Aspergillus flavus	*A.flavus*	*Fusarium sp.*
Aspergillus niger	*A. Niger*	*Aspergillus niger*
Fusarium sp.	*Penicillium sp.*	*Penicillium sp.*
Penicillium sp.		*Mucor sp.*
Trichoderma sp.		*Trichoderma sp.*
Rhizopus sp.		
Mucor sp.		

DISCUSSION

3.2.1 Effects of Herbicides on Soil Phosphatase Activity

(i) Acid Phosphatase Activity

For atrazine, the mean of the acidic phosphatase activity ranged from 0.06±0.01 for 1%w/v (lowest concentration) to 0.01±0.01 for 15.2%w/v (highest concentration) as shown in Table 1 while that of the recommended rate was

0.04±0.01 at 3%w/v. At lower concentrations, the mean of acidic phosphatase ranged from 0.06±0.006 (1%w/v) to 0.05+0.006 (2%w/v). For butachlor, the mean of the acidic phosphatase ranged from 0.02±0.01 (5%v/v, lowest concentration) to 0.003±0.003 (75.9%v/v, highest concentrations) while the recommended rate was 0.01±0.003 at 15%v/v. Results obtained from the mean acidic phosphatase of herbicide-treated soils at recommended, higher and lower rates showed a continuous decrease in phosphatase activity of soil samples compared to the control which had a mean of 0.03±0.05. Treatment of soil samples at concentrations higher than the recommended rate resulted in lower level of acidic phosphatase compared to soils treated at recommended rate. Also, treatments at lower concentration rates did not have any significant difference ($p > 0.05$) from the control even after 70 days of incubation.

(ii) Alkaline Phosphatase Activity

The mean of the alkaline phosphatase activity in atrazine-treated soil ranged from 0.03±0.004 (1%w/v, lowest concentration) to 0.008± 0.002 (15.2%w/v) as given in Table 2. At lower concentrations, the mean of the alkaline phosphatase ranged from 0.03±0.004 (1%w/v) to 0.02±0.003 (2%w/v). For butachlor-treated soil, the mean of the alkaline phosphatase ranged from 0.05±0.005 (5%v/v) to 0.01±0.003 (75.9%v/v). At lower concentrations, the mean of the alkaline phosphatase ranged from 0.05±0.005 (5%v/v) to 0.04±0.004 (10%v/v).

Results obtained from phosphatase activity of herbicides-treated soils at recommended, higher and lower rates showed a continuous reduction in the phosphatase activity compared to the control which was 0.08±0.005. Treatments at higher concentrations resulted in much lower phosphatase activity compared to soils treated at recommended rate. Treatments at concentrations lower than the recommended rate had no significant differences ($p > 0.05$). Generally, butachlor-treated soil samples had more alkaline phosphatase activity than atrazine-treated samples. Atrazine-treated samples, on the other hand, had more acid phosphatase activity than butachlor.

3.2.2Effect of herbicides on soil urease activity

The mean of urease activity in atrazine-treated soil ranged from 537±64.8 for 1%w/v (lowest concentration) to 556.5±37.9 for 15.2%w/v (highest concentration) as in Table 3. At lower concentrations, the mean ranged from 537.0±64.8 (1%w/v) to 540.2±53.7 (2%w/v). For butachlor, the mean ranged from 541.5±51.4 (5%v/v, lowest concentration) to 563.2±39.8 for (75.9%v/v, highest concentrations). Results obtained from the mean of herbicides treated soils at recommended, higher and lower rates showed a continuous increase in urease activity of soil samples compared to the control which had a mean of 536.2±70.0 (figure 6). Treatment of soil samples at concentrations higher than the recommended rate resulted in slightly higher level of urease activity compared to soils treated at recommended rate. Treatments at lower concentrations did not have significant difference ($p > 0.05$) from the control. However, butachlor-treated soil samples had higher urease activity than atrazine-treated samples all through the 70 days of incubation. It was observed that the applied herbicides had no negative effect on the urease activities of the soil. Instead the applied herbicides acted as a urea fertilizer.

3.2.3 Bacterial and fungal species isolated from herbicide-treated soil sample

Bacillus sp., Flavobacterium sp., Actinomycetes sp, Proteus sp., Staphylococcus aureus, Leuconostoc sp. and *Pseudomonas sp.* were bacteria isolated from the control (Table 4). *Bacillus sp.* and *Pseudomonas sp.* were the bacteria isolated from atrazine-treated soil samples. Bacteria isolated from butachlor-treated soil were *Bacillus sp.,Pseudomonas sp.,* and *Flavobacterium sp.* These were possibly the bacteria that were able to tolerate the herbicide.

The fungi isolated from the control soil sample were *Aspergillus flavus, Aspergillus niger, Fusarium sp., Penicillium sp., Trichoderma sp., Rhizopus sp., and Mucor sp.* (Table 5). Fungi isolated from the atrazine-treated soil were *Aspergillus flavus, Aspergillus niger,* and *Penicillium sp.* These were the fungi capable of tolerating the atrazine present in the soil sample. These microorganisms were isolated at the following concentrations control, 1%w/v, 3%w/v, 4.5%w/v, and 15.2%w/v .For butachlor-treated soil samples the concentrations were the control, 5%v/v, 15%v/v, 22.5%v/v and 75.9%v/v, respectively.

Fungi isolated from the butachlor treated soil were *Fusarium sp., Aspergillus niger, Penicilium sp., Mucor sp.*, and *Trichoderma sp.* as shown in Table 5. Herbicide treatments also resulted in the elimination of some microbial species. *Pseudomonas sp. Bacillus sp.* and *Flavobacterium sp.* were the most frequently isolated bacteria from herbicide treated soils. Bacteria eliminated by the herbicides were *Actinomycetes sp., Proteus sp., Staphylococcus aureus, Leuconostoc sp.,* While *A. niger, A.Flavus, Penicillium sp., Trichoderma sp., Mucor sp., Fusarium sp.* were

the most frequently isolated fungi from herbicide treated soils. Fungus eliminated by the herbicides was *Rhizopus sp.*

Atrazine had a more significant effect on fungal counts than butachlor ($P<0.05$), because it caused more reduction in mean viable fungal counts than butachlor. The application of herbicides to the soils led to a significant drop in enzyme activity compared with untreated control soil samples. Obtained results indicated that soils treated with atrazine had the lowest enzyme activity, while soils treated with butachlor had the highest enzyme activity. In literature, same effects on several soil enzymes are reported[18, 19, 20]. Atrazine was found to inhibit both phosphatase and urease activities in sandy loam soil[21] but others reported no effect on soil enzyme activities [22, 23]. Reduced enzymatic activities were also found[20, 21] in studies on the interference of atrazine with phosphatase, dehydrogenase, urease and esterase activity of soil.

Measurement of the level of phosphatase activity in soil samples is a useful indicator of biological activity in herbicide-treated soil. In this study, Treatment of soil samples at concentrations higher than the recommended rate resulted in lower level of acidic phosphatase compared to soils treated at recommended rate. Also, treatments at concentrations lower than recommended rate did not show significant difference from the control at ($P < 0.05$). Generally butachlor-treated soil samples had more alkaline phosphatase activity level than atrazine. Atrazine-treated samples, on the other hand, had more acid phosphatase activity levels than butachlor. According to Bremner and Tabatabai[9] in the presence of alkaline phosphatase microorganisms are capable of mineralizing organic compound (herbicides) more readily than in the presence of acid phosphatase. This probably explains why the microorganisms were able to strive better in butachlor than in atrazine. Phosphatase activity was generally very high in control than in treated soil samples, for both atrazine and butachlor-treated soil samples. During the first four (4) weeks, the level of acidic phosphatase increase gradually in both control and treated soil samples and decrease rapidly in subsequent weeks. The increase might be due to increase in microbial populations with the capability of utilizing the herbicides as carbon source [24, 25]. The level of acidic phosphatase decreases as the concentration of the herbicides increased. Group of researcher has also reported that this decrease may be due to an increase in metabolic activity with herbicide concentration and with incubation time [26, 27]. The activity level of acidic phosphatase in soil treated with atrazine was higher than the activity in butachlor-treated soil. Consequently, alkaline phosphatase activity was higher in soil treated with butachlor compared to atrzine-treated soil. The difference in behaviour between the two herbicides in soil explained the differences in phosphatase activity. Phosphatase activity in soil treated with either herbicide showed correlation with microbial population in which lower microbial population was observed at the higher herbicide concentrations. Earlier reports have shown that herbicides may enhance or inhibit soil enzyme activities [28, 8]. They showed that after an initial inhibition, there was a consistent increase in phosphatase activity in soil treated with barban. In contrast, atrazine significantly reduces soil enzymatic activity readings than control [28].

Measurement of the level of urease activity in soil samples is a useful indicator of biological activity in herbicide-treated soil. Treatment of soil samples at concentrations higher than the recommended rate resulted in higher level of urease activity compared to soils treated at recommended rate. Treatments at concentrations lower than recommended rate did not show significant difference from the control ($P < 0.05$). Generally. butachlor-treated soil samples had more urease activity than atrazine-treated soil. According to Tabatabai and Bremner [9] some herbicides contain urea which acts as fertilizer or animal urine to the soil. This is then enzymatically hydrolysed by soil urease ($NH_2CONH_2 + H_2O = 2NH_3 + CO_2$), and it results in the release of ammonia and a slight rise in pH. Butachlor contains more urea than atrazine hence the butachlor-treated soils had higher urease activity than their counterpart [14]. Herbicides caused reduction in the mean viable counts of the microflora with atrazine-treated soil samples having a more significantly reduced count. Also some of the heterotrophic microorganisms were eliminated by the herbicide. Butachlor-treated soil samples were found to be insignificantly different from the control.

Compared to the control, as the concentrations of the herbicides increased, the phosphatase activities reduced consistently. Atrazine-treated soils had more acidic phosphatase activities than butachlor-treated soil samples, while butachlor-treated soil samples had more alkaline phosphatase activities than their counterparts. The concentrations of the herbicides increased, the urease activities increased consistently. Butachlor-treated soils had more urease activities than atrazine-treated soils.

CONCLUSION

From the results, it appears that the herbicides were inert to urease-producing microbes and their activity. Phosphatase activity by the microbes as indicated by the release of p-nitrophenyl, was affected by the herbicides by causing a significant reduction in the activity, especially in the atrazine-treated soils. The atrazine had more impact on soil microbial activity.

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