EFFECT OF 2,4-D AND HEXAZINONE ON SOIL DEHYDROGENASE ACTIVITY IN SUGARCANE CULTIVATED SOILS IN NZOIA SUGARCANE PLANTATIONS

Njue, R.[1], Kiruki, S.[2], Muia, A.[3] and Ngigi, A.[4]

[1] Biochemistry and Molecular Biology Department, Egerton University, P. O. Box 536-20115, Egerton

[2] Physical Sciences Department, Chuka University, P. O. Box 109-60400, Chuka

[3] Biological Sciences Department, Egerton University P. O. Box 536-20115, Egerton

[4] Chemistry Department, Multimedia University of Kenya, P. O. Box 30305, Nairobi

Email: rnjue30@gmail.com

ABSTRACT

Herbicides have been used extensively all over the world and have become indispensable pact of high and cost effective agricultural production. The adverse effect of these herbicides is not only to their targets but also extend to non-targeted organisms. These effects may have detrimental impacts such as disruption of ecosystems, reduced soil health and fertility among other environmental hazardous. Dehydrogenase enzymes, which are intracellular enzyme in microflora of soil, play a key role in redox processes, especially in decomposition of organic matters. The aim of this experiments was to study the effects of 2,4-D and Hexazinone; commonly used herbicides to control weeds in sugarcane plantations in Nzoia sugar company nuclear estates, on the variation of dehydrogenase activity as an indicator of microbial activities on such soils. The soil for experiment were collected 0-10 cm depth using soil auger by random sampling method from three sites on each farm and a composite sample was prepared from the three sub-samples. Soil parameters such as pH, temperature, moisture content, N, P, K, Mg and Ca were analyzed. The experiments were conducted under the field conditions of soil samples collected from two farms which had history of application of the herbicides for five years and an out-grower farm as a control farm. The soils were spiked with the two xenobiotics at the field application rate and analyzed for the dehydrogenase activity for a period of seven days using TTC method and bacterial colony forming units using nutrient agar methods. Experiments showed that hexazinone and a boosting activity to the microbial activity as indicated by overall DHA activity of 16.375±1.822 in farm F139, 21.970±3.448 in farm F212, 113.45±15.453 in farm OGF. On the other hand, 2,4-D had suppressing effect on microbial activity as shown by DHA activity of 0.532±0.120 in farm F139, 0.541±0.139 in farm F212 and 6.594±1.175 in farm OGF. The noted results of DHA activity were in reference to untreated soils from the three farms which were 4.529±0.408 in farm F139, 6.103±0.341 in farm F212 and 21.578±3.234 in farm OGF. The two herbicides effects on the total microbial activity was also backed with bacterial density results which showed there was low bacterial count upon 2,4-D application 2.463±3.693*10^5 in untreated soil 2.487±5.607*10^5 in hexazinone treated soil and 2.007±4.194*10^4 in 2,4-D treated soil. All the farms had acidic soils with other parameters with the normal range.

Key words: Herbicides, 2,4-D, Hexazinone, enzyme activities, dehydrogenase.

INTRODUCTION

Microbial communities are vital for the normal operation of the ecosystem both in relation to direct interaction with fauna and in nutrients and organic matter cycling (Mandal *et al*., 2007). Soil enzymes, which are integral part of soil microbes, are important in their life processes, similarly in the soil they play significant role in maintaining the soil health and its environment (Das and Varma, 2011). The enzymatic activities in the soil are majorly of microbial community origin being derived from intracellular, cell-associated or free enzymes. Soil enzymes are a group of enzymes, which are found on soil microbial community and play a crucial role in maintaining soil ecology, physical and chemical properties, fertility and soil health (Adak *et al*., 2014; Das and Varma, 2011). The enzymes act as mediators and catalysts of important soil functions that include: decomposition of organic inputs; transformation of native soil organic matter; release of inorganic nutrients for plant growth; N_2 fixation; nitrification; denitrification; and detoxification of xenobiotics. In addition, soil enzymes have a crucial role in C (β-glucosidase and β-galactosidase), N (urease), P (phosphatase), and S (sulphatase) cycle (Martinez *et al*., 2010).

The amounts of the enzymes in soils vary, due to the fact that, soil may contain varying amount of organic matter content, composition and activity of the living organisms and intensity of biological processes (Makoi and Ndakidemi, 2008). Analysis of enzymes in soil provides essential information on biological processes taking place. Enzymes in the soil are sensitive to both anthropogenic and natural interferences and they can be used to elucidate any induced changes in soil ecosystem (Kizilkaya and Aşkin, 2007). For instance

some researches have revealed high dehydrogenase activities in soils collected from forests where there are less anthropogenic disturbances compared to areas like farms where there are frequent management activities (Kumar *et al*., 2013). Examples of the enzymes found in the soil include; amylase, arylsulphatases, b-glucosidase, cellulose, chitinase, dehydrogenase, phosphatase, protease, and urease released from plants (Das and Varma, 2011). Soil dehydrogenase enzymes are among the key enzymes involved in soil biochemical processes and maintaining soil biogeochemical cycles. Dehydrogenase enzymes belong to the oxidoreductases (EC 1.1.1) class of enzymes and catalyze the oxidation of organic compounds by separating two-H atoms.

The separated H atom is mostly transferred to nicotinamine adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADHP) (Wolińska and Stępniewska, 2012: Kumar *et al.*, 2013). Measurement of soil DHA was initiated by (Lenhard, 1956). Dehydrogenase enzymes activity measurement on soil has been used for decades and can be considered as the most important and sensitive indicator of soil microbial activity (Järvan *et al*., 2014). This is owing to the fact that, unlike other enzymes in soil, dehydrogenases are intracellular enzymes and occur in all viable microbial cells. The intracellular nature of the enzymes makes it a good parameter for the analysis of the viable cells in the soil. In addition, dehydrogenase enzymes are immediately degraded following cell death and therefore their detection is only in the living cells (Kizilkaya and Aşkin., 2007).

Its analysis gives a good correlation between biological activity in the soil and the microbial population at time of analysis (Kumar *et al.*, 2013). Measurement of dehydrogenase enzymes is based on the redox reaction process in which 2, 3, 5- triphenyltetrazolium chloride (TTC) is reduced to the creaming red-colored triphenylformazan (TPF). 2, 3, 5- triphenyltetrazolium chloride (TTC) act as an electron acceptor in anaerobic soil environment condition during the electron transfer process in ETC chains of Microbial. Subsequently TTC which is colorless water soluble is reduced by microbial dehydrogenase enzymes to TPF which is a red color water insoluble dye and can be quantified calorimetrically by visible light at 485 nm (Wolińska and Stępniewska ., 2012: Mambu., 2014).

The purpose of this study was to analyze the spatial variation of application of two herbicides 2,4-D and hexazinone on soil microbial community using dehydrogenase as the indicator tool. Microbial biomass as mentioned earlier plays a critical role in biochemical transformation occurring in the soil (Masto *et al*., 2011). Several studies have been conducted on the effects of these two herbicides on other diversity of life. 2,4-D for example, has been linked with human health menaces such as non-Hodgkin's lymphoma (NHL) among farmers (Zahm, 1997; McDuffie *et al*., 2001), teratogenic, neurotoxic, immunosuppressive, cytotoxic and hepatoxic effects (Tuschl and Schwab, 2003). Hexazinone on the other hand, has been linked with adverse effects on aquatic lives (Baillie *et al*., 2015). However, there is limited information on the impacts that these two herbicides have on the soil microbial community.

MATERIALS AND METHODS

Study site

Soil samples for this study were collected from three selected farms, 212 (ELEV1458M N00^{o}34'06.2" E034^o 39'41.8"), 139 (ELEV1420M N00^{o}31'59.4" E034^o 40'45.2") and OGF farm (ELEV1454M N00^{o}31'51.1" E034^o 42'04.4") from Nzoia sugar company nuclear estate farms (which lies between 34^{o}50'49"E-35^{o}35'41"E longitudes and 0^{o}4'55"N-0^{o}20'11"S latitudes) in Western Kenya–Bungoma County. These farms have many water canals, which drain, into Kuywa river, which traverses through the farm and is one of the Nzoia river tributary. Nzoia river originates from Cherangani hills at a mean elevation of 2,300 m above sea level (asl) and drains into Lake Victoria at an altitude of 1000 m asl.

Nzoia river basin lies between latitudes 1º 30'N and 0º 05'S and longitudes 34º and 35º 45'E. It runs approximately south-west and measures about 334km with a catchment area of about 12,900km^2, and a mean annual discharge of 1777 x 106m^3/year. River Nzoia basin is home to more than 3 million people. The river is of international importance as it contributes enormously to the shared waters of Lake Victoria. Many other rivers feed the Nzoia river before it discharges into Lake Victoria (NRBMI 2006).

Soil sampling and preparation

The soil samples were collected from 0-10 cm using soil auger after removing the subsoil. Soil was collected in three sub-samples from each farm. The sub-samples were composited for each farm. The composites were dried in clear and sterile aluminium foils at room temperature. After recommended drying, the samples were crushed using motor and thimble to obtain sievable texture. The soils were sieved through 2 mm sieve to remove stones and other debris which were not required. The homogenized soils were kept at 4^{o}C for the subsequent experiments

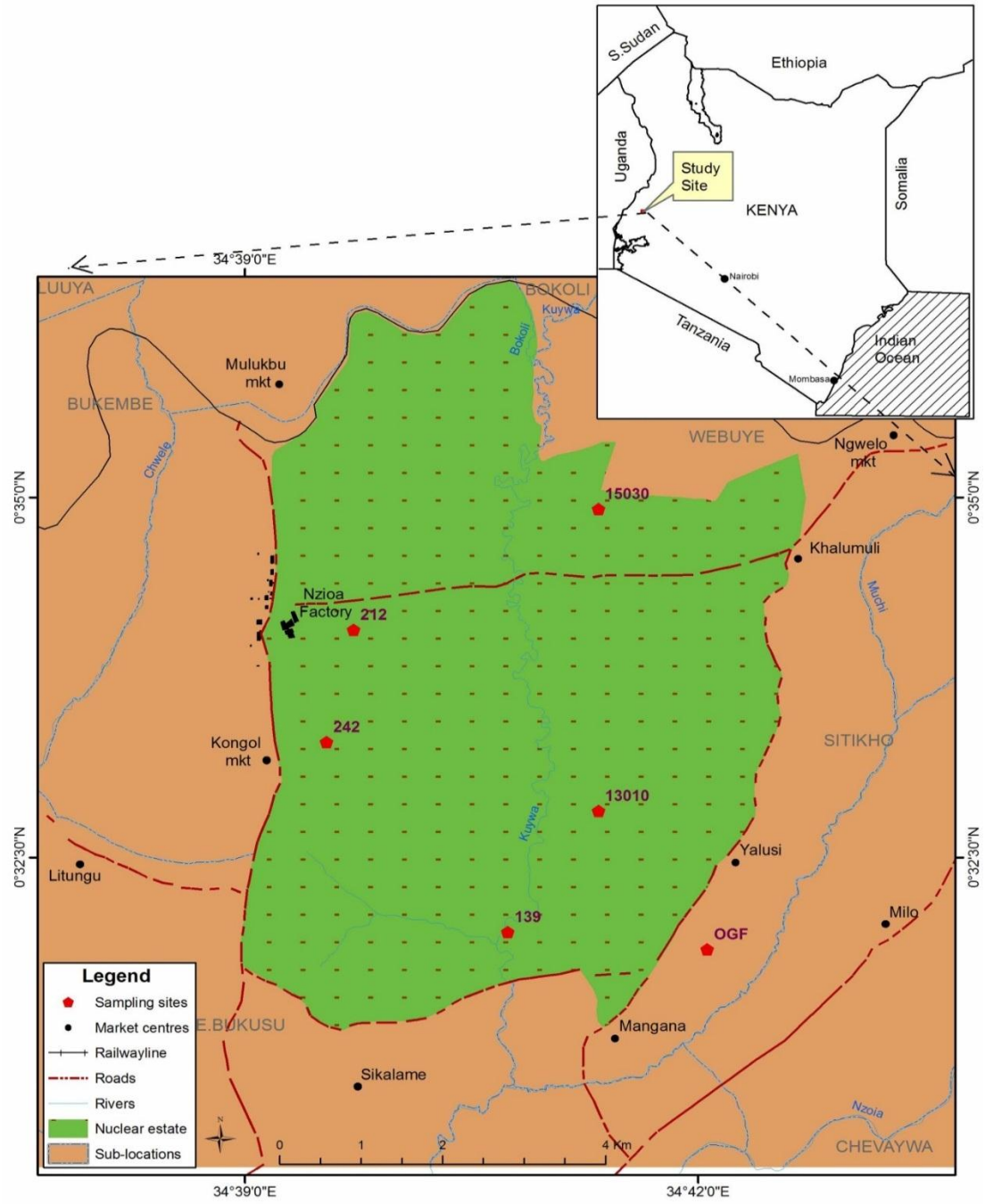

Figure 1: Geographical location of Nzoia Sugar Company farms in Bungoma County. (Courtesy of Mr. Geoffrey Maina, Cartographer, Department of Environmental Science, Egerton University, 2016).

Determination of physicochemical characteristics

Soil temperature was measured and recorded in the field, during sampling, using a laser thermometer, Raytek® Model – RAYRPM30L2G (USA). The pH of the soil was measured in a soil water suspension (soil: water ratio of 1:2), by standard method described by Geotechnical Engineering Bureau (2007). 30 Grams of soil was weighed and put in a glass beaker and 30 mL of distilled water added to the sample and stirred. The sample was let to stand for one hour with stirring every 10 -15 minutes to allow pH of the soil slurry to stabilize. The pH meter was standardized by means of buffers of pH 4.0, 7.0 and 10. The pH reading was done by immersing the electrodes into the solution.

Soil moisture content was measured using the method described by Black (1965). 10 Grams of soil was weighed in aluminium tin and dried overnight in an oven at 105 ^{o}C. The weight of the dry soil sample was then recorded. The soil sample was returned in the oven, and dried further at the same temperature until no difference between any two consecutive weight measurements was recorded. The moisture content was calculated as the difference in the soil weight after drying and expressed in percentage.

The amount of Nitrogen (%) was analyzed using Kjeldahl technique (Anderson and InGram, 1994) and measured using FOSS TECATOR digester machine (2200 Kjeltec Auto Distillation, Sweden). The soil sample was air dried at room temperature (25 ^{o}C), ground and sieved using 2 mm sieve. A sample of 0.3 Grams of air dried soil was weighed into a test tube and 4.3 mL of digesting solution (selenium powder, hydrogen peroxide, H_2SO_4 and lithium sulphate) added. The test tubes were put in digestion block for 3 hours set at 360 ^{o}C to obtain clear solution (indicate complete digestion). The samples were allowed to cool at 25 ^{o}C for 30 minutes after which they were diluted to 100mL using 46% NaOH into a conical flask. 10mL aliquots of the samples and NaOH were taken and distilled. A recipient (1% boric) was prepared. 5 mL of the recipient was mixed with the sample and titrated against standardized acid (0.01 normal HCL) until the pink colour was noted. Volume of titres was recorded and the calculation done using the formula below:

$$\%\, Nitrogen = \frac{(a-b)N\times 0.014\times 100\times 100'}{a'\times 0.3} \quad (1)$$

Where a is volume of sample titre, b is black titre, N is normality of the acid used = 0.01 N HCL, 0.014 is molecular weight of N in a litre (1000 mL), 100 is the dilution factor, 100′ is conversion factor into percentage and a′ is mL of aliquot taken for analysis.
In order to analyze potassium, calcium and magnesium, the samples were prepared similar to those for nitrogen but no distillation was done. The amounts were determined using atomic absorption spectroscopy (AAS) (210 VGF AAS, USA). The quantification was done using calibration curves prepared using known amounts of standards compounds for K, Ca and Mg.

Phosphorous was analyzed using Mehlich method (Horneck *et al*., 2011). Thus, 5 g of air dried soil was weighed into 250mL conical flasks. 50 mL of extracting solution (H_2SO_4 and HCL) was added into the samples. Free activated charcoal was then added and the samples put in an electric shaker for 30 minutes. After shaking, the samples were allowed to settle for 10 minutes and then filtered and the filtrate collected. 5 mL of the filtrate was sampled for colour development using colour developer (vanadium and molybdate in the ratio 1:1). The colour was allowed to develop for 30 minutes and the readings were taken from spectrophotometer (UV-200-RS, USA) at 430 nm wavelength. The quantification was done using calibration curves prepared using known amounts of P standard compound.

Assessment of inhibition of 2,4-D and hexazinone to soil microorganisms

In order to confirm whether there was any toxicity of the two xenobiotics to the soil microorganism, colony forming unit (CFU) experiments were determined according to the method described by Curtis *et al.* (2000). To this end, fresh soil from a farm which had never received the two herbicides (OGF) for the control of weeds was used. Soil samples were randomly collected from three sites and mixed together to make a composite sample. The samples were sieved through < 2 mm sieve to remove any unwanted debris and to homogenize the soil.

The samples were then put on an aluminum foil at room temperature in three sub-sets. Two of the sub-sets were treated with 2,4-D and hexazinone at the field recommended rate and one of the sub-sets was used as the control. The samples were treated with water to moisture content (60% water holding capacity). The number of colony forming units in the soil samples was determined using serial dilution technique and the pour plate method. For enumeration of the bacteria, 1 g of soil sample was put in 9 mL distilled water and serially diluted to $\times 10^{-3}$. In order to enumerate the bacteria, 1 mL of the $\times 10^{-3}$ was then poured on sterile petri dish in which 15 mL of sterile molten nutrient agar was poured and the plates incubated at 30 ±1 ^{o}C. The effects of the pesticides on viable bacteria counts were monitored in the soils each day for seven days.

Determination of dehydrogenase activity

The dehydrogenase activity was determined according to the method described by Lenhard (1956) based on the reduction of 2,3,5-triphenyltetrazoliumchloride (TTC) to the creaming red colored formazan (TPF,). In this study 6 grams of homogenized soil sample from each farm was weighed in test tubes in triplicates. 1 ml of 50 µg/ml (field recommended application rate) hexazinone and 2,4-D was prepared and used to treat the samples in triplicates. 1 ml of 3% TTC solution was added to each sample and an addition 2 ml of deionized

distilled water. The test tubes were caped tightly to exclude the air since the activity of TTC is greatly affected by oxygen. The samples were incubated in dark at 30 (±1) ^{o}C for seven days with analysis of the tryphnylformazan (TPF) at each day. To the control replicates, 1 ml of 3% TTC was added with no addition of the two herbicides and subjected to the same conditions. The hydrolytic products of the TTC; triphenylformazan (TPF), was extracted by passing 50 ml analytical methanol through 4 µm cellulose glass microfilter paper on a vacuum pump until all the red color was completely collected. The collected red color formazan was analyzed spectrophotometrically at 485 nm using Genesys 10-S 10uv scanning model and the amount of the TPF expressed as µg TPF/g soil sample. The concentration of the TPF is a direct representation of the dehydrogenase activity (redox processes) taking place in that respective soil samples. The data are presented as means of triplicates samples and ±Std.Dev for each farm.

Quantification of red colour formazan of samples

In order to quantify the amount TPF present in the samples, a standard curve was prepared using standard formazan dye purchased from Kobian chemicals Kenya ltd. The varying concentration of 0 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml and 30 µg/ml were prepared in analytical grade methanol (Hurst *et al*., 2007). From these concentrations an aliquot of 1 ml was used to analyze the absorbance at 485 nm using UV-scanning spectrophotometer (Genesys 10-S 10uv scanning model (Austria); the same model used for sample analysis. The absorbance (A) readings versus the concentrations of the standards formazan were used to plot a standard curve (*fig. 2*) from which the formazan concentrations in samples were quantified.

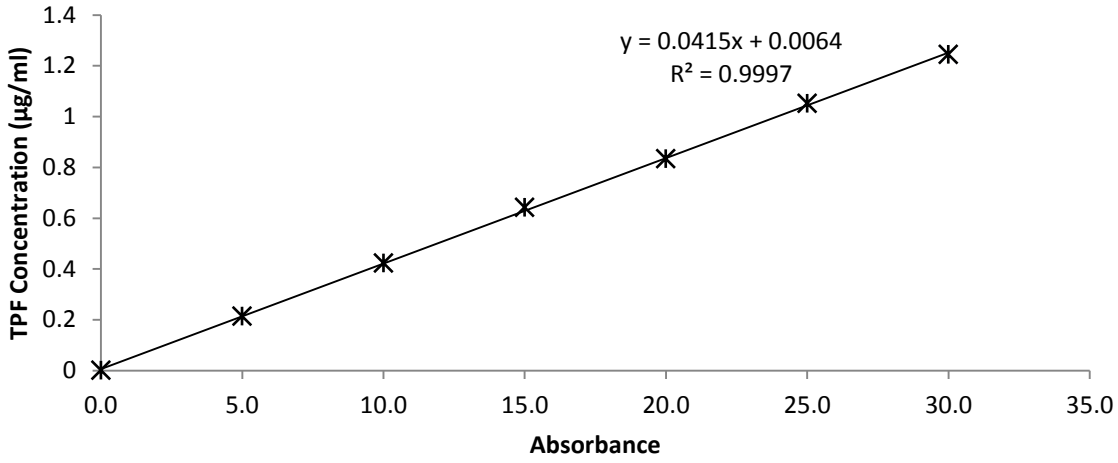

Figure 2: Linear standard calibration curve from standard formazan for quantification of samples formazan.

RESULTS

The values of TPF concentration obtained as well as bacterial colony forming units were subjected to analysis of variance (ANOVA) using SAS version 9.1 portable for windows and the means separated using the LSD. Microsoft Excel 2010.Ink was used to draw curves. The results are presented as a mean of triplicates samples and ± standard deviation.

The soil physicochemical parameters which includes nitrogen content, phosphorous, potassium, calcium, magnesium, pH, temperature and moisture content for the three farms where soil samples were collected were analyzed. Table 1 shows physicochemical parameters obtained from the two-selected pesticide treated farms and from one out grower farm (OGF).

Significant difference is at $P<0.05$, different letter assigned in the same column shows significance difference and same letter in the same column denote no significance difference.

Soils from all farms had medium range of soil N% ranging from 0.34±0.04 to 0.40±0.12, there was no significance difference ($P<0.05$) in the nitrogen percentage in the three farms. The phosphorous level was within the medium range within all farms. Farm OGF had higher level of P (31±0.34) compared to F212 and F139 which had 24.75±1.23 and 21.5±1.34 respectively. The level of P in OGF was statistically significant compared to farm 212 and 139, but there was no significance different in P level between farm F212 and F139. Though the potassium level was within the normal range among all the farms, these levels

differed greatly within the three farms, with farm F212 recording the highest level and farm F139 the lowest level. The calcium levels were below the normal range in all farms, with highest recorded in farm OGF and lowest in F139.

There was significant difference in calcium levels across all farms. Magnesium level was also within the medium range in all the farms with the highest being recorded in OGF and lowest in F139. These levels of magnesium statistically differed between OGF and the two farms; F212 and 139, but there was no difference in farm F212 and F139. All the farms had acid soils with pH ranging from 4.16±0.73, 4.94±0.53 and 5.04±0.61in OGF, F212 and F139 respectively.

Toxicity effects of hexazinone and 2,4-D on viable bacterial density

Colony forming units (CFUs) were enumerated after treating soil with hexazinone and 2,4-D. The results showed that there was gradual decrease in bacterial density as the days increased for 2,4-D treated soil samples. For the control soil samples (without treatment with either hexazinone nor 2,4-D) there was no significant difference (P<0.05) in CFUs for five subsequent days of incubation (2.49±5.03×10^5 g^{-1} soil to 2.41±3.18×10^5 g^{-1} soil). The suppression of the bacterial growth was observed in both hexazinone and 2,4-D with significantly higher suppression being noted in 2,4-D treated soils. In hexazinone amended soils, significant suppression was noted within the first and the next three days but there after bacteria continued to grow recording a significant increase between day 4 and day 7. There was significance (P < 0.05) decrease in CFUs in day one (2.53±5.13×10^5 g^{-1} soil) in hexazinone treated soils. This observed growth suppression effects lasted for a period of three days with a decline in CFUs reading of 1.22±2.89×10^5 g^{-1} soil. The suppression effect of hexazinone to bacterial growth was overcome on day four (1.89±8.29×10^5 ± g^{-1} soil), with an observed increased bacterial growth indicated by very high CFUs by day seven (3.51±6.69×10^5 g^{-1} soil), which was a significant (P<0.05) compared to the rest of the days.

There was suppression of bacteria growth by 2,4-D as noted in hexazinone. However, the adverse effects of 2,4-D were severe by 10 log units compared to hexazinone with the first day treatment recording being 1.88±7.45×10^5 CFUs g^{-1} soil. The suppression lasted for three days, with a decline to 1.33±1.33×10^4 g^{-1} soil. This was preceded with a recovery from suppression with highest recording of CFU noted in day seven (2.00±5.13×10^4 g^{-1} soil). Though, there was recovery of the microorganisms from day four up to day seven, there was no significance difference (P>0.05) in the recorded means for this period. Table 2 shows mean variation in colony forming units (CFUs) for soil samples treated with hexazinone and 2,4-D and a control soil sample with no treatment.

Dehydrogenase activity results

Dehydrogenases are soil microbial enzymes involved in catalyzing degradation of organic matter in what is basically a redox process. Soil dehydrogenases are predominantly microbiological in origin and their activities depend on the conditions within the soil ecosystem. Therefore, a higher enzyme activity indicates a greater intensity of mineralization of the organic matter. Figure 3 shows comparison of TPF mean concentrations across incubation period (seven days) for the three farms 139, 212 and OGF without treatment with hexazinone or 2,4-D and upon treatment with the two herbicides.

The overall analysis of the TPF concentration showed that hexazinone treated soils had higher DHA activity across all farms compared to the other two treatments. Soil treated with 2,4-D recorded the least DHA activity in all farms. Table 3 shows the overall mean concentrations and the standard deviations for the TPF concentrations across the three farms 139, 212 and OGF without treatment with hexazinone and 2,4-D and after the treatment. In farm 139 there was a significant difference in enzyme activity for the two treatments and the control. Hexazinone treated soils performed best with 57% (16.38±1.82 µg/g soil) higher DHA activity as compared to the control which had 4.53±0.41 µg/g soil.

On the contrary, the soil sample treated with 2,4-D in farm 139 had higher inhibition of DHA in reference to the control samples, recording 78.98% (0.53±0.12 µg/g soil) decrease in DHA activity. In farm 212, there was also significant difference in DHA activity within all the treatments with soil sample treated with hexazinone having higher activity of DHA by 56.52% (6.10±0.34 µg/g soil) with reference to the untreated soil. On the other hand, 2,4-D had suppressing effects on DHA activity with a decrease of 83.71% (0.54±0.14 µg/g soil) with reference to the untreated soil sample. For the OGF farm, which had no history of herbicide treatment, DHA was significantly different for all the treatments. Hexazinone recorded the highest positive activity by 67.53% (113.45±15.45 µg/g soil) with reference to untreated soil sample, while 2,4-D had negative effects on DHA activity by 53.37% (6.59±1.18 µg/g soil) with reference to the untreated soil sample. From the experiment, there was clear evidence, of the negative effects on enzyme activity in all farms upon application of 2,4-D, while hexazinone had boosting effects on the enzyme activity in all evaluated farms.

Table 1: Physical-chemical parameters of soil samples from the three farms

Farms	% N	P ppm	K ppm	Ca ppm	Mg ppm	PH	Temp	Moisture Content
F212	0.38^{a}±0.02	24.75^{b}±1.23	153^{a}±0.41	602^{b}±0.23	116.8^{b}±1.37	4.94^{a}±0.53	21.67^{a}±0.23	20.41^{a}±1.23
F139	0.34^{a}±0.04	21.5^{b}±1.34	141^{c}±0.12	560^{c}±0.25	115.7^{b}±3.01	5.04^{a}±0.61	23.67^{a}±1.23	22.36^{a}±1.32
OGF	0.40^{a}±0.12	31^{a}±0.34	144^{b}±2.34	678.2^{a}±.1.57	135^{a}±2.47	4.16^{a}±0.73	22.33^{a}±1.20	22.49^{a}±2.12

Table 1: Densities of viable bacteria (CFUs g^{-1}) in soil recorded for seven consecutive days following herbicide treatment in an out-grower farm (OGF)

	Untreated soil	**Hexazinone treated soil**	**2,4-D treated soil**
Days	CFUs*10^5 g^{-1} soil	CFUs*10^5 g^{-1} soil	CFUs*10^5 g^{-1} soil
1	2.49±5.03^{abc}	2.53±5.13^{c}	1.88±7.45^{a}
2	2.54±4.67^{ab}	2.09±3.84^{d}	3.50±8.15*10^{4b}
3	2.56±0.88^{ab}	1.67±51.97^{e}	1.97±2.96*10^{4b}
4	2.63±6.64^{a}	1.89±8.29^{d}	1.33±1.33*10^{4b}
5	2.41±3.18^{abc}	2.61±6.00^{c}	1.67±2.03*10^{4b}
6	2.28±2.72^{c}	3.11±4.10^{b}	1.70±2.31*10^{4b}
7	2.33±2.73^{bc}	3.51±6.69^{a}	2.00±5.13*10^{4b}

Values are means ±SD, n = 3, Means followed by the same letter in the same column are not significantly different at 5% LSD.

Table 3. The overall mean concentrations and standard deviation of TPF in soils from the farms for the seven days treatments period

		139		**212**		**OGF**	
Treatment	**Replicates**	**Mean µg/g Soil**	**Std. Dev**	**Mean µg/g Soil**	**Std. Dev**	**Mean µg/g Soil**	**Std. Dev**
2,4-D	21	0.532^{c}	±0.120	0.541^{c}	±0.139	6.594^{b}	±1.175
Hexazinone	21	16.375^{a}	±1.822	21.970^{a}	±3.448	113.45^{a}	±15.453
Untreated	21	4.529^{b}	±0.408	6.103^{b}	±0.341	21.578^{b}	±3.234

Means followed by the same letter in the same column are not significantly different at 5% LSD

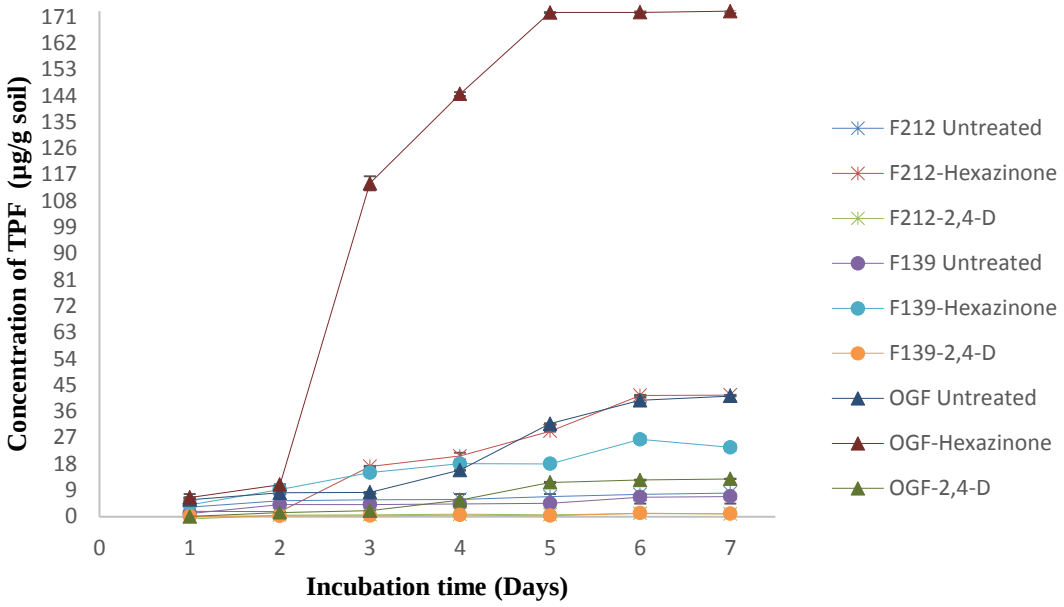

Figure 3. TPF concentration in soils with and without the addition of herbicides in the farms OGF, 212 and 139. Data points represent means for three replicates

DISCUSSION

Soil physicochemical parameters

Soil physical-chemical parameters are important tools in biodegradation experiments since they can be used as inferences for the outcomes of such experiments. As the mineralization of the pesticides depends on microorganisms, their activity largely depends on these environmental factors (Shahgholi, and Ahangar, 2014). These soil properties can also be used to explain some soil anomalies that may be observed during analysis. The soil pH ranged from 4.16 to 5.05 indicating all the farms had acidic soil. Biodegradation of some of the pesticides have been found to be slow at pH above 6 and optimum at pH below 5 (Schoenholtz *et al*., 2000). However, the pH impact is relative to the individual compound being degraded and the potential organism which degrade it.

The soil phosphorous for five farms were within the medium range (20-40 ppm) with only one field with excess of 144 ppm (>100) (Horneck *et al*., 2011). The nitrogen content was also very low (<5%) (Galloway, 2010). Similarly, the K content was extremely low for all the farms (<150 ppm) and magnesium being with the medium range (60–300 ppm) (Horneck *et al*., 2011). Low level of phosphorous, potassium, calcium and nitrogen may be attributed to acidity of soil (Locascio, 2000; Schoenholtz *et al*., 2000). Other factors that may lead to low nutrient content in soil are vegetation cover, agricultural activities such as application of fertilizer and clearing of vegetation by burning which are common practice in Nzoia sugar company nuclear estates (Ezeigbo *et al*., 2013). Total nitrogen may also be low in acidic soils since in acid conditions, there is unavailability of NH_4^+ (Medinski, 2007).

The soil temperature ranged from 21-27^{o}C. The major factor that affect the soil temperature is the weather condition such as sun heating which also affect the moisture content (the moisture content ranged between 16 and 22 %) as well (Ezeigbo *et al*., 2013). The temperatures of soil play a major role in the degradation of the pesticides. It has been reported that most of the degradation of pesticides tends to increase with increase in temperature between 10 to 45^{o}C (Rani and Sud, 2015). Soil physicochemical properties such as temperature, humidity, and moisture content affect the rate of decomposition of herbicides in soil (Milosevia and Govedarica, 2000).

According to Shahgholi and Ahangar (2014), soil moisture content is very crucial to the degradation process. Water acts as the solvent for the pesticides and determines its availability for the microorganism. Dry soil tends to have slow biodegradation compared to wet soil. In water logged soil, anaerobic degradation has been found to take place as opposed to aerobic degradation since there is limitation of oxygen entry to the soil. However, high moisture content may

accelerate or hinder the degradation depending on the subject pesticide. On other hand, long term application of pesticides may have adverse effects on some of the soil physical chemical constituents. For instance, application of some of pesticides may lead to alteration of nitrogen (N_2) fixing organisms such as *Rhizobium*, *Azotobactera* and *Azospirillum* (Omakor, Onyido and Buncel, 2001). It may also affect cellulolytic and phosphate solubilizing microorganisms (Gulhane, Gomashe and Sunderkar, 2015).

DHA activity and viable bacterial counts

Soil enzymes have been for long used as sensitive indicator of soil ecological disturbances in natural and agricultural ecosystems (Badiane *et al*., 2001; Sannino and Gianfreda, 2001). DHAs are key enzymes in the soil microbial respiratory processes and hence a good tool to assess microbial activity upon subjection of soil to pesticides (Cycon *et al*., 2010). According to Mambu (2014), DHA activity is higher in low doses application of pesticides and lower in high doses pesticides applied areas. In the case of current study, this could be one of the major reasons for the low overall DHA activity recorded in farm 139 and 212 untreated soil samples, which were on frequent application of the two herbicides compared to the higher activity recorded in farm OGF which had never been applied with two herbicides. Secondly, it has been found that inorganically fertilized soils have low level of DHA activity compared to soils fertilized organically using animal manure or compost (Monkiedje, 2006).

The results from this study showed drastic decrease in DHA activity across all farms upon treatment with 2,4-D as compared with untreated (control) soil samples. Hexazinone application boosted DHA activity in all the farms. The activity of the DHA enzymes was highly recorded in OGF farm in all experimental treatments. Initially upon the application of the two herbicides, 2,4-D and hexazinone, there was a lag phase in both pesticides, which took approximately two and half days. This could be attributed to the toxicity of the two herbicides towards the soil microorganisms. Pesticides cause respiration inhibition in their initial application stages. The effects are then recovered depending on whether the microorganisms are able to metabolize the respective compounds for their physiological needs (Radivojević *et al*., 2008). Thereafter there was exponential increase in activity of DHA especially in hexazinone treated soils. This could have been due to recovery of microbial population and enzymes activity after initial inhibition due to microbial adaptation to the chemicals or due to degradation thus being used by microorganisms as sources of carbon or nitrogen.

Secondly this can also be due to microbial multiplication on increased supply of nutrients available in form of microorganisms initially killed by herbicides as evidenced in other studies (Vandana *et al*., 2012; Latha and Gopal., 2010). According to Milosevia and Govedarica (2000), some microorganisms are able to metabolize herbicides immediately they are applied to the soil; however, there is secondary population of microbial community which may take a number of days before they can adapt to metabolize xenobiotics since enzymes responsible are inducible enzymes. The DHA activity increase upon treatment of the soil sample with hexazinone was also supported by viable bacterial enumeration which showed that there was increased CFU compared to untreated soil sample. Study by Rahman *et al*. (2005) on impact of herbicide oxadiazon on microbial activity showed the herbicide was able to increase the enzyme activity in soil. Other studies that have shown the boosting ability of herbicides to soil microbial activity is that of Haney *et al*. (2000) and Araujo *et al*. (2003) that showed glyphosate was able to increase soil microbial activity.

For 2,4-D treatments, the detracting affect was prolonged to approximately four days after which the activity was increased but at very low intensity as compared to the hexazinone treatment. The low DHA activity in 2,4-D treated soil samples was also supported by low viable bacterial density. These effects of 2,4-D to the DHA activity are similar with the findings by Mohiuddin and Mohammed (2014), who found that 2,4-D had inhibitory role on enzymes activity in soil for a period of 20 days and a decrease in inhibition of DHA was noted in 21st day following pesticide application. These findings also reported negative effects of 2,4-D on DHA activity in the initial application to control weeds in agricultural soils particularly in groundnuts cultivated soil (Hussain *et al*., 2009). The DHA activities recorded initially before the application of the two herbicides in this study were extremely higher in OGF farm than in 212 and 139.

This difference in DHA activity could be ascribed to the fact that soil enzymes activities are very sensitive to both natural and anthropogenic disturbances (Kumar *et al*., 2013). For instance, in this study, OGF farm was used as a control farm in which there was no history of herbicides application. Moreover, another factor which may contribute to the low DHA activity in 212 and 139 compared to the high activity in the untreated OGF farm is the regular use of tractor ploughing. This being one of the common anthropogenic disturbances experienced in farm 212 and 139, it may cause reduction in organic matter content in soil due to interference with the accumulation of crop residues in

soil top layer and this may cause a reduction in microbial activity (Roldan *et al*., 2005).

Soil microbial activity is more vigorous on soil rich in organic matter, and this could explain why there was high activity of DHA activity in OGF as compared to 212 and 139. Besides, herbicides decomposition also depends on the organic matter in the soil, this is apparently because of vigorous microbial activities (Baboo *et al*., 2013). This factor can also explain why there was high activity of DHA in OGF farm when treated with hexazinone and 2,4-D as compared to other farms. However, the amount of the applied herbicides may also have a great effect on the amount degraded per given time and also the residue effect of the herbicide detectable in soil (Ngigi *et al*., 2014).

CONCLUSION

The indiscriminate use of herbicides to control weeds has become a matter of environmental concern due to their adverse effects on the soil microbial diversity which consequently alter the soil fertility. This study was conducted to study the effects of 2,4-D and Hexazinone on soil dehydrogenase enzyme. The study confirmed that 2,4-D has great negative impact on the soil microbial activity at its recommended field rate while Hexazinone had boosting effects on the enzyme activity at the recommended field rate. This therefore, raises an alarm on the appropriate field rate at which the 2,4-D should be applied. Although the use of herbicides is important as it offer cheap and effective way of weed control, application should be considered due to the detrimental effects they pose to the untargeted organisms.

ACKNOWLEDGEMENTS

This work was supported by National Commission for Science, Technology and Innovation, Kenya (grant number: NCST/RCD/ST&I/WS/4th CALL/035). We are grateful to Egerton University for providing facilities and consumables for the study. Appreciation to Nzoia Sugar Company for allow us to carry out this study in their farms.

REFERENCES

Adak, T., Singha, A., Kumar, K., Shukla, S.K., Singh, A., and Kumar Singh, V. 2014. Soil organic carbon, dehydrogenase activity, nutrient availability and leaf nutrient content as affected by organic and inorganic source of nutrient in mango orchard soil. Journal of Soil Science and Plant Nutrition, 142:394-406.

Anderson, J.M., and InGram, J.S. 1994. Tropical soil biology and fertility: A handbook of methods. Soil Science, 1574, 265.

Araujo, A.S.F., Monterio, R.T.R. and Abarkeli, R. B. 2003 Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere, 52:799-804.

Baboo, M., Pasayat, M., Samal, A., Kujur, M., Maharana, J.K., and Patel, A.K. 2013. Effect of four herbicides on soil organic carbon, microbial biomass-C, enzyme activity and microbial populations in agricultural soil.

Badiane, N.N.Y., Chotte, J.L., Pate, E., Masse, D. and Rouland, C. 2001. Use of soil enzyme activities to monitor soil quality in natural and improved fallows in semi-arid tropical regions. Applied Soil Ecology, 183:229-238.

Baillie, B.R., Neary, D.G., Gous, S., and Rolando, C. A. 2015. Aquatic fate of aerially applied hexazinone and terbuthylazine in a New Zealand planted forest. Journal of Sustainable Watershed Science and Management, 21:118-129.

Black C.A. 1965. Methods of soil analysis; Part 1 physical and mineralogical properties. American Society of Agronomy, Madison, Wisconsin USA.

Curtis, T.P., Sloan, W.T. and Scannell, J.W. 2002. Estimating prokaryotic diversity and its limits. Proceedings of Natural Academic Sciences USA, 99:10494-10499.

Cycoń, M., Piotrowska-Seget, Z., and Kozdrój, J. 2010. Dehydrogenase activity as an indicator of different microbial responses to pesticide-treated soils. Chemistry and Ecology, 264:243-250.

Das, S.K., and Varma, A. 2011. Role of enzymes in maintaining soil health. Soil enzymology, pp. 25-42. Springer Berlin Heidelberg.

Ezeigbo, O.R., Okike-osisiogu, F. U., Ihemanma, C. N., and Agomoh, N. G. 2013. Microbiological effects of gas flaring on Agricultural soil at Izombe flow station, Imo State, Nigeria. Journal of Biology, Agriculture and Health, 3:15.

Galloway, W.E. 2010. Soil nitrogen content. How to build the nitrogen content of your soil in a safe, organic way. Retrieved 23rd March, 2017, http://www.rodalesorganiclife.com/soil-nitrogen-content.

Gulhane, P.A., Gomashe, A.V., and Sunderkar, K.M. 2015. Influence of pesticides on nitrogen fixing bacteria. Int J Tech Res Appl, *3*4:157-160.

Haney, R.L., Senseman, S.A., Hons, E.M. and Zuberer, D.A. 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science 48:89-93.

Horneck, D.A., Hart, J.M., Topper, K., and Koepsell, B. 2011. Methods of soil analysis used in the soil testing laboratory at Oregon State University: Agricultural Experiment Station, Oregon State University.

Hussain, S., Siddique, T., Saleem, M., Arshad, M., and Khalid, A. 2009. Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. Advances in Agronomy, 102:159-200.

Järvan, M., Edesi, L., Adamson, A., and Võsa, T. 2014. Soil microbial communities and dehydrogenase activity depending on farming systems. Plant, Soil and Environment, 6010:459-463.

Kizilkaya, R., and Aşkin, T. 2007. The spatial variability of soil dehydrogenase activity: a survey in urban soils. Agriculturae Conspectus Scientificus, 721:89-94.

Kumar, S., Chaudhuri, S., and Maiti, S.K. 2013. Soil dehydrogenase enzyme activity in natural and mine soil: A review. Middle-East Journal of Scientific Research, 137:898-906.

Latha, P.C. and Gopal, G. 2010. Influence of herbicides on cellulolytic, proteolytic and phosphate solubalizing bacteria. International Journal of Plant Protection 31:83-88.

Lenhard, G. 1956. The dehydrogenase activity in soil as a measure of the activity of soil microorganisms. Z. Pflanzenernaehr. Dueng. Bodenkd., 73:1-11.

Locascio, S.J. 2000. The Fertile Triangle. The Interrelationship of Air, Water, and Nutrients in Maximizing Soil Productivity. HortTechnology, 101:232-232.

Makoi, J. H., and Ndakidemi, P. A. 2008. Selected soil enzymes: Examples of their potential roles in the ecosystem. African Journal of Biotechnology, 73.

Mambu, S.M. 2014. Soil dehydrogenase activity: A comparison between The TTC and INT methods. A review. Jurnal Ilmiah Sains, 142:87-94.

Mandal, A., Patra, A. K., Singh, D., Swarup, A. and Masto, R.E. 2007. Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Bioresource Technology, 9818:3585-3592.

Martinez-Salgado, M.M., Gutiérrez-Romero, V., Jannsens, M., and Ortega-Blu, R. 2010. Biological soil quality indicators: A review. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology.

Masto, R.E., Ahirwar, R., George, J., Ram, L.C., and Selvi, V.A. 2011. Soil biological and biochemical responses to Cd exposure. Open Journal of Soil Science, 101:8.

McDuffie, H.H., Pahwa, P., McLaughlin, J.R. 2001. Non-Hodgkin's lymphoma and specific pesticide exposures in men: Cross-Canada study of pesticides and health. Cancer Epidemiology Biomarkers Preview, 1011:1155-63.

Medinski, T. 2007. Soil chemical and physical properties and their influence on the plant species richness of arid South-West Africa Doctoral dissertation, Stellenbosch: University of Stellenbosch.

Milosevia, N., and Govedarica, M. 2000. Effect of some herbicides on microbial properties of soil. In: Proceedings of the 1st European conferences on pesticides and related organic micropollutants in the environment, Ioannina, Greece, 61-62.

Mohiuddin, M., and Mohammed, M.K. 2014. Research article fungicide carbendazim and herbicides 2, 4-D and atrazine influence on soil microorganisms and soil enzymes of rhizospheric soil of groundnut crop.

Monkiedje, A., Spiteller, M., Fotio, D., and Sukul, P. 2006. The effect of land use on soil health indicators in peri-urban agriculture in the humid forest zone of southern Cameroon. Journal of environmental quality, 356:2402-2409.

Ngigi, A., Getenga, Z., Boga, H. and Ndalut, P., 2014. Isolation and identification of hexazinone-degrading bacterium from sugarcane-cultivated soil in Kenya. Bulletin of Environmental Contamination and Toxicology, 923:364-368.

NRBMI, 2006. Nzoia River Basin management initiatives NRBMI report: A public private partnership between water resources management authority and civil society, learning institutions and communities. Strategic plan for 2006-2011.

Omakor, J.E., Onyido, I., and Buncel, E. 2001. Mechanisms of abiotic degradation and soil–water interactions of pesticides and other hydrophobic organic compounds. Part 3. Nucleophilic displacement at the phosphorus centre of the pesticide fenitrothion [O, O-dimethyl O-3-methyl-4-nitrophenyl phosphorothioate] by oxygen nucleophiles in aqueous solution: α-effect and mechanism. Journal of the Chemical Society, Perkin Transactions 23:324-330.

Radivojević, L., Gašić, S., Šantrić, L., and Stanković-Kalezić, R. 2008. The impact of atrazine on several biochemical properties of chernozem soil. Journal of the Serbian Chemical Society, 7310:951-959.

Rahman, M., Song, K.S., Rhee, I.K., and Kim, J.E. 2005. Impact of herbicide oxadiazon on microbial activity and nitrogen dynamics in soil environment. Journal of Applied Biological Chemistry, 484:187-192.

Rani, S., and Sud, D. 2015. Effect of temperature on adsorption-desorption behaviour of triazophos in Indian soils. Plant Soil Environ, 611:36-42.

Roldán, A., Salinas-García, J.R., Alguacil, M.M., Diaz, E., and Caravaca, F. 2005. Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions. *Geoderma*, 1293:178-185.

Sannino, F., and Gianfreda, L. 2001. Pesticide influence on soil enzymatic activities. Chemosphere, 454:417-425.

Schoenholtz, S.H., Van Miegroet, H., and Burger, J.A. 2000. A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. Forest ecology and management, 1381:335-356.

Shahgoh, H., Ahangar, A.G. 2014. Factors controlling degradation of pesticides in the soil environment. Agricultural Science and Development, 3:273-278.

Tuschl, H., and Schwab, C. 2003. Cytotoxic effects of the herbicide 2,4-Dichlorophenoxyacetic acid in HepG2 cells. Food and Chemical Toxicology, 413:385-393.

Vandana, L.J., Rao, P.C., and Padmaja, G. 2012. Effect of herbicides and nutrient management on soil enzyme activity. New Facets of 21 st Century Plant Breeding, 51:51.

Wolińska, A., and Stępniewska, Z., 2012. *Dehydrogenase activity in the soil environment*. INTECH Open Access Publisher.

Zahm, S.H. 1997. Mortality study of pesticide applicators and other employees of a lawn care service company. Journal of Occupational Environmental Medicine, 39:1055-67.