PH And Microbial Content of Soil; and Status of Reduced Glutathione (GSH) and Phosphatases of Soil Earthworm (*Apporectodea Longa*) From Glyphosate Impacted Field

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ABSTRACT: Glyphosate isa widely used herbicide for the control of annual and perennial weeds all over the world. In this study, the possible effect of glyphosate on pH of soil and microbial content, as well as reduced glutathione and phosphatase status of soil earthworm (*Aporrectodea longa*) from glyphosate impacted field were investigated. The site selected for this study was divided into five (5) sections. Section A was the control, (no water, no glyphosate), section B (impacted with the vehicle – H_2O), section C, (impacted with 1% (v/v)glyphosate in water), section D (impacted with 2% (v/v) glyphosate in water) and section E (impacted with 3% (v/v) glyphosate in water). Results showed that the impacted soil samples had lower pH values post-impact when compared to the control. The communities of microorganisms within the soil were found to be the same pre- and post-impact except for the sudden appearance of *Arthrobacter sp.*, *Streptococcus sp*. and*Aspergillusfumigatus*anddisappearance of*Proteus mirabilis* and*Saccharomyces sp.* No significant difference was observed between the amount of bacteria and fungi in the post-impact soil relative to the pre-impact soil. However there was an increase in bacteria population post-impact relative to pre-impact. Post-impact reduced glutathione (GSH) status of control, vehicle and 1% glyphosate had no significant difference relative to pre-impact. However, a significant decrease GSH status was observed at higher glyphosate concentration. The distribution of GSH was revealed in the head region of the earthworm. Glyphosate impact increases the activities of both acid phosphatase (ACP) and alkaline phosphatase(ALP) of soil *A. longa* when compared to the activities of *A.longa* from the pre-impact soil. This study has revealed that glyphosate can reduce the level of GSH and increase the activities of ACP and ALP in soil *A.longa.*

Key words: Glyphosate; Soil Microbial Community; Ecosystem; Earthworm; Phosphatases; Pesticides; Reduced glutathione; Round-up

INTRODUCTION

The communities of living things and the environment they live in constitute the ecosystems. The latter includes ponds, rivers, deserts, grasslands and forests.

In an ecosystem the organisms depend on each other for sustainable existence. The population of microorganisms, animals and plants that make up the community depend on each other for nutrient acquisition and cycling, such as nitrogen cycling (De Laender*et al*., 2010; Rockets, 2007; Johnsen*et al*., 2001). The nitrogen cycling may be affected if pesticides in whatever form, be it herbicides, rodenticides, insecticides etc, impact the bacteria and fungi communities in the soil (Lo, 2010).

Pesticides can affect individual plants and animals in two ways, directly or indirectly (Friberg-Jensen *et al*., 2003; Bohan*et al*., 2005; Kearns, 1998; Yardim and Edwards, 2002).

(1) Directly: They may cause injury or death after the plants and animals are exposed to pesticide directly. This may occur if the pesticide drifts onto the animal, the animal breaths in the pesticide or if the animal drinks or eats something that is contaminated. For plants, the roots may pick up pesticides in the soil. These exposures will cause direct effects.

(2) Indirectly: The second way pesticides may cause harm is by changing or killing something the animals need or impairing the uptake of what the plant depends on absolutely for survival. For instance, pesticides can affect an animal's food supply by killing certain plants and insects. The loss of plant cover may remove the shelter for animals. Plants could be affected if their pollinators or seed

dispersers are killed. In these ways the plants or animals in an ecosystem can be adversely affected indirectly.

A pesticide or contaminant must not necessarily kill an organism to create havoc in an ecosystem. The agent may have sub-lethal effects such as making the organism sick, changing its behavior or changing its ability to reproduce or survive stress.

The foregoing descriptions show that toxic agents can affect more than just the population of animals and plants that make up a community. The effects can be at the level of the ecosystem when basic processes like nutrient cycling or soil formation is affected. One herbicide that is in common use by farmers and non-farmers alike for the control of weeds in the farm or immediate vicinity of the home is GLYPHOSATE (Voetand Voet, 2011) which goes by the name "ROUND-UP". It is effective as a weed killer but it is conceivable that other organisms that make up the community, be they microorganisms (bacteria, fungi), burrowing insects and ants, as well as earthworms may be affected also, directly or indirectly. This may be physically via the soil or biochemically by way of analyte and/or enzyme changes.

The aim of the present study, therefore, was to ascertain what effects Glyphosate impact will have on soil pH, microbial population and status of selected analytes and enzymes in the earthworm species domicile in the impacted field.

MATERIALS AND METHODS

Study site: The study site is the field behind the new Biochemistry Department building (ETF 2004/2005). Behind the building is part of Uniben/UBTH perimeter wall. From the wall 12ft was measured which represent the width of the study area. The length was 50 feet. Lengthwise the study area was partitioned into 5 sections, each 10 feet in length. Section A was the control, (no water, no

glyphosate), section B (impacted with vehicle – H_2O), section C, (impacted with 1% (v/v) glyphosate in water), section D (impacted with 2% (v/v) glyphosate in water) and section E (impacted with 3% (v/v) glyphosate in water).

SoilCollection

The site made up of sandy loamy soil, was used for this study. The soil was collected from the top to a depth of 8-12 cm (topsoil) and 13-25 cm depth (subsoil). Sufficient portion of the bulked soil, top and bottom, were taken to the laboratory for baseline physico-chemical (microbial and pH) analysis to obtain the pre impact values and was repeated a week after impacting the field with glyphosate. Soil samples collection was repeated a week after impacting the field with glyphosate to obtain the post impact values.

EarthwormCollection

Aporrectodea longa (A.longa) earthworms were collected from each portion by digging with shovel to an average depth of 22cm and handpicked. The collection was limited to a particular area to reduce variability (Cholewa*et al*., 2006). The worms were adults with clitella with average life weight of 0.36g. Worms were stabilized in soil collected from the same site for at least 24 hours before use.

EarthwormIdentification

Aporrectodea longa (A.longa) are species of earthworm ecologically relevant to southern part of Nigeria. *A.longa*represents epigeic (macrophagous topsoil) species which lives among litters and close to the surface. The nomenclature adopted is according to Sims and Gerrard (1985) and Nature Watch (2003). *A.longa*also called **blackhead** worm, has a dark anterior segment with a red-violet posterior segment. The length varies from 9-15cm long, its clitellium is less than 2cm anteriorly, saddle shaped and not flared. Prostomium is problobus (segments 153-164), tubercular puberstalis is bar-shaped and genital tumescence alternates in pairs (Nature watch, 2003). The species was

authenticated by Dr. Alex Enuneku, Department of Animal and Environmental Biology, Faculty of life sciences, University of Benin.

Soil pH Determination

Pre- and post-impact soil pH values were determined by weighing out 5g of each soil sample suspended in 12.5ml each of 1M potassium chloride (KCl) and distilled water (H_2O). The pH values for both top- and sub-soils were taken using pH meter.

Soil Microbial Analysis

Pre- and Post-impact soil samples (top- and sub- soils respectively) were taken from each section of the study site to the laboratory for both total heterotrophic bacterial and fungal counts.

Preparation of culture media

All media were prepared according to manufacturers' instruction. The media used in this study are Nutrient agar and potatoes dextrose agar.

Isolation and enumeration of microorganisms

This was carried out by applying methods of Cheesbrough (2000). Ten grams (10 g) of the soil samples (pre- and post- impact) were weighed using an analytical balance and mixed with 90 ml of sterile distilled water to form the stock suspension. A 1.0 ml of the stock suspension was transferred into a sterile test tube containing 9.0 ml of sterilized distilled water. This process was repeated for other sterilized test tubes so that at the end, dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions were obtained. A 0.1 ml from 10^{-1}, 10^{-3}, 10^{-5} dilution was then plated out by pour plate method on nutrient agar and potato dextrose agar. The nutrient agar plates and the potato dextrose agar plates were incubated at room temperature 28 ± 2 ^{0}C for 72 hours. After incubation discrete colonies of culture on nutrient agar and potato dextrose agar plates were counted and the unit expressed in cfu/g.

Characterization and Identification of Bacterial and Fungal Isolates

Bacterial isolates were characterized and identified after studying their Gram reaction as well as morphological characteristics. Other tests performed were spore formation, motility, and catalase production as well as citrate utilization, oxidative/fermentative utilization of glucose, indole production, urease and coagulase production and sugar fermentation. The tests were carried out according to the methods described by Cheesbrough (2005).Fungal isolates were examined macroscopically and microscopically using the needle mounts technique. Their identification was performed according to the scheme of Larone (1986).

Sample preparation

Collected earthworms were kept in perforated plastic containers on wet filter paper for 2-3days for them to egest the soil from their digestive tract. Experiments were done on clitellate, adult animals. Animals were cleaned, weighed and dissected on ice. Fragments representing anterior (Head), medial (Mid) and posterior (Tail) part of the body were taken from individuals weighing more than 0.2g for further preparation. Samples were homogenised in 0.9% Normal saline. The homogenates were centrifuged at relative acceleration 4000g × 20mins. Aliquots of this fraction were frozen in microtubes at -70^{o}C until they were used as material for Antioxidant status and Enzymatic assays.

Antioxidant Status and Enzyme Activities

Reduced glutathione concentration was determined after protein precipitation with Trichloroacetic acid (TCA). Glutathione reacts with Ellman's reagent (5, 5'- dithiobis- (2- nitrobenzoic acid) or DTNB), cleaving the disulfide bonds to give 2- nitro -5- thiobenzoate (TNB), which ionizes to the

TNB^{2-}dianion in water at neutral and alkaline pH; a yellow coloured product which absorbs at 412nm.

The activities of acid and alkaline phosphatases were assayed for according to methods described in Teco Diagnostics manual.

Statistical Analysis

Data were expressed as Mean ± SEM. All statistical analyses were conducted with the software SPSS (version 16) for Windows. One-way ANOVA was applied to assess the significant difference ($p \leq 0.05$) among groups. Least Square Difference was used for multiple comparisons while Student (Independent-Sample) T Test was used to check the significant difference between pre- and post-impact status of each parameter. Differences between means were considered significant at $P \leq 0.05$.

RESULTS

Soil pH

The effect of glyphosate impact on soil pH is presented in Table 3.1. Generally the impacted portions had lower pH values post impact when compared to the control.

Table 3.1: Effect of Glyphosate Impact on Soil pH

Group Designation / Soil type	A Control (-Water -Glyphosate)		B Vehicle (H_2O)		C (1% Glyphosate in H_2O)		D (2% Glyphosate in H_2O)		E (3% Glyphosate in H_2O)	
	pH (Mean±SD)(n)		pH (Mean±SD)(n)		pH (Mean±SD)(n)		pH (Mean±SD)(n)		pH (Mean±SD)(n)	
	PIP	POIP+	PIP	POIP	PIP	POIP	PIP	POIP	PIP	POIP
Top Soil in H_2O	7.01± 0.16(5)	6.75±0.09(4)	7.14± 0.11(5)	6.45± 0.05(4)*	7.83± 0.05(4)	6.88± 0.03(4)*	7.73± 0.06(4)	6.77± 0.11(4)*	7.51± 0.01(4)	6.43± 0.11(4)*
Sub Soil in H_2O	7.27± 0.24(5)	6.79±0.10(5)	6.64± 0.02(5)	6.25± 0.04(4)*	7.46± 0.07(4)	6.50±0.07(4)*	7.52± 0.09(4)	6.59± 0.09(4)*	7.81± 0.07(4)	6.98± 0.06(4)*
Top Soil in KCl	6.82± 0.08(5)	6.40±0.09*(4)	6.57± 0.03(5)	6.41± 0.04(4)*	7.66± 0.09(4)	6.38±0.05(4)*	7.62± 0.08(4)	6.20± 0.04(4)*	7.31± 0.07(4)	6.95± 0.06(4)*
Sub Soil in KCl	6.58± 0.19(5)	6.36±0.10(5)	6.39± 0.08(5)	6.12± 0.07(5)*	7.10± 0.13(4)	6.20± 0.03(4)*	6.96± 0.08(4)	6.28± 0.04(4)*	7.05± 0.13(4)	6.51± 0.11(4)*

*** Value statistically significantly different from the corresponding pre- impact value (P≤ 0.05).**
+ PIP = pre impact; POIP = post impact

Qualitative evaluation of microbial content of the soil

The types of Bacteria and Fungi identified in the soil pre- and post-impact are presented in Table 3.2.The community of microorganisms within the soil were found to be the same pre- and post-impact except for the sudden appearance of ***Arthrobacter sp., Streptococcus sp.*** and ***Aspergillusfumigatus***,and concomitant disappearance of ***Proteus mirabilis*** and ***Saccharomyces sp.***

Table 3.2: Total Heterotrophic Microbial Isolates from Soil Samples

S/N	Bacterial isolates		Fungal isolates	
	Pre-impact (7)	**Post-impact (8)**	**Pre-impact (7)**	**Post-impact (7)**
1	**___**	***Arthrobacter sp.***	*Aspergillusniger*	*Aspergillusniger*
2	*Bacillus licheniformis*	*Bacillus licheniformis*	*Fusarium sp.*	*Fusarium sp.*
3	*Bacillus substilis*	*Bacillus substilis*	*Mucor sp.*	*Mucor sp.*
4	*Escherichia coli*	*Escherichia coli*	*Penicillium sp.*	*Penicillium sp.*
5	*Klebsiellaaerogenes*	*Klebsiellaaerogenes*	*Rhizopus sp.*	*Rhizopus sp.*
6	*Micrococcus luteus*	*Micrococcus luteus*	***Saccharomyces sp.***	**___**
7	*Pseudomonas fluorescens*	*Pseudomonas fluorescens*	*Tichodermasp*	*Tichodermasp*
8	***Proteus mirabilis***	***Streptococcus sp.***	**___**	***Aspergillusfumigatus***

Quantitative evaluation of microbial content of the soil

The level of microorganisms (Bacteria and Fungi) in the soil is presented in Table 3.3. There is no significant difference between the amount of Bacteria and Fungi in the post- impact soil relative to the pre- impact soil.

Table 3.3: Total Heterotrophic Microbial Counts of Selected Soil Samples

	Bacterial population		**Fungal population**	
	Mean ± SEM (n=5) x10^6 (Cfu/g)		**Mean ± SEM (n=5) x10^6 (Cfu/g)**	
Soil type	**Pre-impact**	**Post-impact**	**Pre-impact**	**Post-impact**
Top soil	**3.42 ± 0.51**	**4.30 ± 0.51**	**0.12 ± 0.03**	**0.18 ± 0.09**
Sub soil	**2.94 ± 0.46**	**4.02 ± 0.79**	**0.13 ± 0.04**	**0.15 ± 0.52**

Effect of glyphosate impact on soil *A. longa* GSH status.

The effect of glyphosate impact on soil *A.longa*GSH status as well as the distribution of GSH within the different regions of the earthworm is presented in Table 3.4. No significant difference was observed post impact relative to pre impact except a decrease which was significant at 2% and 3% respectively. The distribution of GSH was more abundant in the Head region when compared to other regions with the Tail being the least.

Table 3.4: Effect of glyphosate Impact on soil *A. longa* reduced Glutathione (GSH) status

Group Designation / *A.longa* Region	A Control (-Water -Glyphosate)		B Vehicle (H_2O)		C (1% Glyphosate in H_2O)		D (2% Glyphosate in H_2O)		E (3% Glyphosate in H_2O)	
	GSH (Mean±SEM; n=4) mmol/lHomogenate		GSH (Mean± SEM; n=4) mmol/l Homogenate		GSH (Mean± SEM; n=4) mmol/lHomogenate		GSH (Mean± SEM; n=4) mmol/lHomogenate		GSH (Mean± SEM; n=4) mmol/lHomogenate	
	PIP	POIP+	PIP	POIP	PIP	POIP	PIP	POIP	PIP	POIP
Head Region	1.12±0.28[m]	19.78±1.06[m]	21.70±0.98[m,a]	19.66±3.09	18.00±0.35[l,a]	13.18±3.36[l]	19.08±1.00	11.79±2.09*[m]	20.49±1.37	11.50±2.73*
Mid Region	20.99±0.91	19.53±0.48	18.25±0.15[l,a]	19.42±2.34[l]	18.68±0.49	17.24±2.11[m]	17.76±0.82[l]	10.13±2.52*[l]	20.52±0.86[m]	10.38±2.95*
Tail Region	20.07±0.36[l]	15.36±2.58[l]	19.98±0.64	21.21±3.62[m]	20.27±0.81[m,a]	14.37±4.92	19.84±0.96[m]	10.40±3.29*	19.67±1.09[l]	8.05±1.60*

*** Value statistically significantly different from the corresponding pre- impact value (P≤ 0.05).**

+ PIP = pre impact; POIP = post impact
m = most abundant region
l = least abundant region
a = pre impact is significant
b = post impact is significant

Effect of Glyphosate Impact on Soil *A. longa* Acid Phosphatase (ACP) Activity.

The effect of glyphosate impact on soil *A.longa* Acid phosphatase activity is presented in Table 3.5. There was a general increase in the ACP activity of soil *A.longa* when impacted with glyphosate. On comparing the post impact to the pre impact, the increase was not significant, but the distribution of the enzyme was even in the Head and Tail regions of the earthworm.

Table 3.5: Effect of Glyphosate Impact on Soil *A. longa* Acid Phosphatase (ACP) Activity

Group Designation / *A. longa* Region	A Control (-Water -Glyphosate)		B Vehicle (H_2O)		C (1% Glyphosate in H_2O)		D (2% Glyphosate in H_2O)		E (3% Glyphosate in H_2O)	
	ACP IU/L homogenate(Mean±SEM; n=4)		ACP IU/L homogenate (Mean± SEM; n=4)		ACP IU/L homogenate (Mean± SEM; n=4)		ACP IU/L homogenate (Mean± SEM; n=4)		ACP IU/L homogenate (Mean± SEM; n=4)	
	PIP	POIP+	PIP	POIP	PIP	POIP	PIP	POIP	PIP	POIP
Head Region	5.59 ±0.56	6.88±1.72	4.73±1.34	11.18±2.69 m	6.02±1.22	7.53±3.36 l	5.59±0.82 m	8.17±2.59	10.48±5.26m,a	14.84±5.2m,b
Mid Region	7.74±0.79m,a	8.39±1.18 m	7.96±2.52 m	7.31±0.43l	6.24±0.41 m	9.89±2.12 m	5.38±0.73	6.67±2.68	4.73±1.08l,a	4.73±1.08l,b
Tail Region	4.73±1.14l, a	5.81±1.24l	5.16±0.93l	10.97±1.87*	5.81±0.41l	8.34±0.73*	5.16±2.43l	2.79±0.41l	6.02±0.79	6.02±0.79

*** Value statistically significantly different from the corresponding pre- impact value (P≤ 0.05).**

+ PIP = pre impact; POIP = post impact
m = most abundant region; l = least abundant region; a = pre impact is significant; b= post impact is significant

Effect of Glyphosate Impact on Soil *A. longa* Alkaline Phosphatase (ALP) Activity

The effect of glyphosate impact on soil *A.longa* Alkaline phosphatase activity is presented in Table 3.6. There was a general decrease at 1% and 2% and increase at 3% in the ALP activity of soil *A.longa* when impacted with glyphosate. On comparing the post-impact to the pre-impact, the decrease was significant in the tail region at 1%; also, the distribution of the enzyme was even at the Head and Tail regions of the earthworm.

Table 3.6: Effect of Glyphosate Impact on Soil *A. longa* Alkaline Phosphatase (ALP) Activity

Group Designation on *A.longa* Region	A Control (-Water -Glyphosate)		B Vehicle (+water, H_2O)		C (1% Glyphosate in H_2O)		D (2% Glyphosate in H_2O)		E (3% Glyphosate in H_2O)	
	ALP UI/L homogenate (Mean±SEM; n=4) PIP	POIP+	ALP UI/L homogenate (Mean±SEM; n=4) PIP	POIP	ALP UI/L homogenate (Mean± SEM; n=4) PIP	POIP	ALP UI/L homogenate (Mean± SEM; n=4) PIP	POIP	ALP UI/L Homogenate (Mean± SEM; n=4) PIP	POIP
Head Region	15.58±2.79^{l,a}	32.19±2.18*,^{b}	33.23±6.98	37.46±2.59	39.78±4.25^{l}	32.59±3.19	38.53±9.42^{m}	38.89±2.39^{m}	30.83±8.89^{m}	41.21±3.10^{m}
Mid Region	36.54±4.62^{m,a,n}	40.09±5.51^{m,b,n}	40.97±8.31^{m,a}	48.08±2.36^{m,b}	45.93±5.72^{m}	28.51±6.50	35.18±6.37	35.78±3.13	27.52±5.75	40.34±2.42
Tail Region	33.95±7.89^{a,n}	22.44±2.12^{l,b,n}	20.49±2.12^{l,a}	16.85±2.47^{l,b}	41.85±6.44	21.25±1.86*,^{l}	34.19±6.58^{l}	24.12±2.71^{l}	24.12±5.91^{l}	24.28±4.8 2^{l}

*** Value statistically significantly different from the corresponding pre- impact value (P≤ 0.05).**

+ PIP = pre impact; POIP = post impact
m = most abundant region
n = mutually insignificant
l = least abundant region
a = pre impact is significant; b = post impact is significant

DISCUSSION

The intensive use of herbicides is an environmental problem, partially because of the potential hazardous effects of these chemicals on soil biological processes and non-target organisms. Biological and biochemically mediated processes in soils are of the utmost importance to ecosystem function. Soil microbes are the driving force behind many soil processes including transformation of organic matter, nutrient release and degradation of xenobiotics (Zabaloy*et al*., 2006). Several biological parameters have been used to assess soil quality and health as affected by agricultural practices (Beneditti and Dilly, 2006; Filip, 2002; Anderson, 2003). Among them, microbial activity is expected to be more efficient indicators than physical and chemical parameters as they are able to respond immediately to environmental change (Nannipierietal, 2002; Avidano*et al*., 2005).

Glyphosate is the systemic herbicide that is commonly used to control a broad spectrum of weed in crops and pastures worldwide (Zabaloy*et al*., 2006).

The significantly decreased soil pH of the impacted portions post impact when compared to the pre impact (Table 3.1) was a likely indication of the rate of application of the herbicide (Glyphosate) and its active metabolite Aminomethylphosphonic acid (AMPA). Glyphosate with high solubility and relatively non-selective can adsorb to soil when sprayed. It has a half life of 3 to 141 and AMPA is between 119 and 958days. The decrease could be attributed to the active metabolite. In the soil, glyphosate form complexes with ions such as ca^{2+} and mg^{2+} (Vijver*et al*., 2010).

Soil is an important component of the ecosystem, serves as a medium for plant growth through the activity of microbial communities. The microbial communities (like bacteria, fungi and actinomycetes) play critical role in litter decomposition and nutrient cycling, which in turn, affect soil fertility and plant growth (chauhan*et al*., 2006; Tripathi*et al*., 2006; Pandey*et al*., 2007). Soil microorganisms participate in the processes that are crucial for long-term sustainability of agricultural systems

(Nannipieri*et al*., 2003). In organic systems, plant production depends primarily on nutrient cycling in soils that are controlled by microbes and soil enzymes (Monokrousos*et al*., 2006, Karaca*et al*., 2011).

The communities of microorganism within the soil pre- and post-impact were found to be the same except for the sudden appearance of *Athrobacter sp., Streptococcus sp. And Aspergillusfumigatus*and concomitant disappearance of*Proteus mirabilis* and*Saccharomyces sp.*Available reports indicate that glyphosate application stimulates populations of fungi with general increases in overall microbial biomass (Araujo*et al*., 2003).

Report from the quantitative evaluation of the total heterotrophic microbial counts of selected soil samples indicate that there was an increase in the microbial biomass which is not significant. This is in agreement with the result obtained by Sebiomo*et al*. (2011). This can be traced to the use of the Herbicide which disadvantageously affects the soil microorganisms (Jarvan*et al*., 2014). As a weed killer, glyphosate targets a single enzyme called 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Franz *et al*., 1997) which plays important role in the shikimic acid pathway responsible for biosynthesis of aromatic amino acids, and this enzyme is widely present in plants and microorganisms, including bacteria and fungi (Ca Jacob *et al*., 2004). The presence of EPSPS proteins in bacteria and fungi, therefore, made the microorganisms vulnerable to glyphosate.

Glyphosate (GP) treatment led to increasing bacterial population in soil because bacteria were able to adapt to the toxic effects of GP and utilize it as nutrient source (Partoazar*et al*., 2011). The bacterial growth in the presence of GP as sole C source indicates that the GP was a source of energy for microbial activity (Haney *et al*., 2000). This conclusion agrees with Lancaster *et al*. (2010), who found that after repeated application of GP, microorganisms were better able to utilize it. According toPartoazar*et al*. (2011), bacterial population in the presence of GP as P source was significantly higher than those of other sources. Glyphosate as an organophosphonate can be used as a source of P, C or N by either Gram-positive or Gram negative bacteria (Zabaloy*et al*., 2008). It is evident that the effects of GP on bacterial populations are dose-dependent and highly temporal and could be explained by a rapid enrichment of opportunistic bacteria that use the compound as a nutrient and/or C source

(Ratcliff*et al*., 2006).

Microbial activity is an important factor in the behavior of GP in the soil. The application of GP to the soil led to a significant increase in phosphatase activities with respect to untreated control soil samples. This might be due to increase in microbial population with the potential of utilizing GP as carbon or other nutrient sources.

Many reports have shown that insecticides and heavy metals can induce an increase in reactive oxygen species (ROS) in earthworms (Saint-Denis *et al*., 2001). ROS include the superoxide anion radical (O_2), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (OH).In earthworms, the mechanism to resist ROS consists of enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST), and small molecules, such as glutathione (GSH), that act as anti-oxidants. SOD is responsible for the transformation of O_2 into H_2O_2, while CAT decomposes H_2O_2 into oxygen and water. GST may combine electrophilic reagents with GSH, and it therefore plays an important role in anti-oxidation. An increase in cellular ROS augments not only the oxidative stress but also cellular lipid peroxidation in the organism. Cellular malondialdehyde (MDA) content can serve as an indicator of the degree of lipid peroxidation because it is the main product of lipid peroxidation. Yu *et al*. (2008) also found that (1-octyl-3- methylimidazolium bromide) ILs caused a change in the activity of anti-oxidant enzymes in mice. All pesticides caused a significant increase in GSH concentration, an efficient scavenger against ROS (Hayes *et al*., 2005); however, no dose response of GSH concentration after exposure to the investigated pesticides was observed, and no differences in GSH responses between the species were recorded.

Contrary to the findings of Velki and Hackenberger (2013) which recorded significant increases in GSH concentration after exposure to all three pesticides: dimethoate, pirimiphos-methyl and deltamethrin , the present study indicated a decrease in GSH status at application rate greater than 1% but this decrease was not significant. The reduction observed may be due to increased degradation or

decreased synthesis of GSH and this can be attributed to the microorganisms present in the gut of the earthworm. It can be concluded that GSH concentration is not a suitable biomarker for determining the antioxidant status of the investigated earthworm species.

Earthworms are physically aerators, crushers and mixers; chemically degraders; and biologically stimulators in the decomposer system. They effectively harness the beneficial soil microflora, destroy soil pathogens and convert organic wastes into vitamins, enzymes, antibiotics, growth hormones and protein rich casts. Earthworm bioreactors have an in-house supply of enzymes such as amylase, cellulose, nitrate reductase and acid and alkaline phosphatases. These enzymes biodegrade the complex biomolecules into simpler compounds. The digestive enzymes of earthworms are responsible for the decomposition and humification of organic matter. These enzymes are active at a very narrow pH range and efficiently maintain the highly non-linear pH parameters (Gajalakshmi and Abbasi, 2004).

Phosphatases play an important role in transforming organic phosphorus into inorganic forms, suitable for plants. They may also be used for biochemical soil characterization. Acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) are non-specific phosphatases that are grouped as acid or alkaline based on their optimum pH below or above pH 7. They are enzymes that catalyse the hydrolysis of phosphate esters to produce inorganic phosphate and their main function is to supply inorganic phosphate that is required for the maintenance of cellular metabolism (Mishra *et al*., 2008).
The increase observed in the activity of both ACP and ALP of soil *A.longa* when impacted with the herbicide (glyphosate) post impact relative to pre impact was not significantly different. Available reports suggest that the increase was due to increased presence of microorganisms in the earthworm and this microbial population as shown in Table 3.5 and 3.6 is more abundant in the head and mid regions and least in the tail region.

In summary this studyrevealed that glyphosate can reduce the level of GSH, increase activities of ALP and ACP in soil *A.longa.*

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