

## Comparative evaluation of antioxidant enzymes, lipid peroxidation, nitric oxide and non – microsomal oxidases in *Galatea paradoxa* exposed to varying concentrations of ‘uproot’ a glyphosate – based herbicide

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### ABSTRACT

This study comparatively evaluated antioxidant enzymes, lipid peroxidation, nitric oxide and non – microsomal oxidases in *Galateaparadoxa* (GP) exposed to varying concentrations of glyphosate-based herbicide uproot. Twenty GP were equally divided into 4 groups (n = 5). Group A was control (no glyphosate exposure) while groups B, C, and D were exposed to 10 mg/L, 20 mg/L, and 30 mg/L of uproot a glyphosate-based herbicide, respectively, for 21 days. At the end of the exposure, the soft tissue of GP was harvested from the shell, homogenized in ice-cold phosphate buffer (0.02 M; pH 7.2), and the supernatant was used to analyse the biochemical parameters employing standard laboratory methods. Results showed elevated (p < 0.05) activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase with respect to increasing concentration of glyphosate exposure. The concentration of nitric acid and activities of superoxide dismutase, catalase, glutathione s – transferase and lactate dehydrogenase were elevated (p < 0.05) in exposed GP as compared with the control proportionate to the concentration of glyphosate exposure. The results also indicated that the reduced glutathione content and glutathione peroxidase activity of exposed GP increased (p < 0.05) at 10 mg/L and 30 mg/L glyphosate exposure as compared with the control group. Again, glyphosate exposure did not inhibit acetylcholinesterase activity and also did not cause changes in the activities of monoamine and sulphite oxidases. Taken together, these results support the possibility of uproot (the glyphosate herbicide) altering the antioxidant defense system in GP and may provide insights into monitoring the ecological consequences which may result from the indiscriminate use of glyphosate in our farmlands and these antioxidant enzymes employed as biomarkers of environmental contamination.

### INTRODUCTION

Herbicides are employed in farmlands to increase agricultural yields by controlling the growth of unwanted weeds. Glyphosate, (N- (phosphonomethyl) glycine (an isopropylamine salt of glycine), is a non – selective herbicide currently among the most widely used agricultural chemicals globally for the control of weeds in cultivars. It was

identified to have herbicidal action in 1970 (Anneth *et al.*, 2014) and released into the agricultural market in 1974 (Duke and Powels, 2008).

The herbicidal action of glyphosate is attributed to its ability to inhibit the shikimate acid pathway through the inhibition of the enzyme 5 – enolpyruyl shikimic acid -3-phospahte synthase (EPSPS), which is responsible for the production of chorismate and consequently interferes with the biosynthesis of aromatic amino acids, phenylalanine, tyrosine, tryptophan and a range of other substances (Amrhein *et al.*, 1980; Mertens *et al.*, 2018). Glyphosate – based herbicide may enter aquatic system through accidental offsite movement in herbicide spray drift, runoff and leaching processes and also by direct applications to control noxious aquatic weeds, thus contaminating the water (Perez *et al.*, 2011; Mishra, 2021). Glyphosate can act directly or indirectly on non – target species in the aquatic environment such as aquatic invertebrates with consequences for the ecosystem functioning (Vick, 2010; Vera *et al.*, 2012; Schaaf. 2017; Sobjak *et al.*, 2017) and these adverse effects of pollutants on aquatic ecosystem is of high concern (Banaee and Taheri, 2019).

Herbicides according to Lau *et al.* (2004), could exert their effects on biological systems through radicals or high – energy molecules which are ultimately reflected as oxidative stress on organisms. An increase in the production of reactive oxygen species (ROS) in organisms inhabiting the aquatic environment has been reported as the main effect of herbicide or other chemical pollutants (Bagchi *et al.*, 1995; Cogo *et al.*, 2009; Rondon – Barragan *et al.*, 2012). These ROS can initiate oxidative damage to nucleic acids, lipid and proteins, eventually leading to organelle damage and finally death. Fanton *et al.* (2020) reported that glyphosate imposed a stress in freshwater copepod, *Notodiaptomus carteri*, while alteration of antioxidant defense system by glyphosate in fish (Langiano and Martinez, 2008; Nwani *et al.*, 2013; Sinhorin *et al.*, 2014), Mussels (Iummato *et al.*, 2013) and Shrimps (Hong *et al.*, 2018) has been documented. The damaging effect of ROS as reported by Sies (1997) is counteracted by the production of antioxidants.

Antioxidants cellular systems which include enzymatic [superoxide dismutase (SOD); catalase (CAT); glutathione peroxidase (GPx), glutathione- s-transferase (GST)] and non – enzymatic [reduced glutathione (GSH)] help to mitigate the effect of ROS. These together with malondialdehyde (indices for lipid peroxidation) can be used as biomarkers (biochemical and physiological responses) of oxidative stress in the assessment of the quality of the coastal environment (Adams *et al.*, 2000; Narbonne *et al.*, 2005; Bonifacia *et al.*, 2016). Since aquatic organism are constantly exposed to various contaminants (Banaee *et al.*, 2016) including glyphosate – based herbicides, the effects of on aquatic system could be described by assessing the changes in selected biomarkers in animals inhabiting the aquatic environment such as *Galatea paradoxa*.

*Galatea paradoxa* (Born 1778) is a freshwater snail, a bivalve mollusk of the order:- *Veneroidea*, superfamily:- *Tellinoidea* and family:- *Donocidae* (Purchon, 1963). *Galateaparadoxa* is well spread and commonly found in freshwaters of Bayelsa state, Niger Delta, Nigeria. It constitutes a source of cheap animal protein and also provides and important income earning and livelihood for our local fishermen. Bivalve mollusks which include clam are now commonly employed as standard bioindicators of aquatic pollution due to their sedentary life history, abundance, large tissue size for analysis, good adaptation to laboratory conditions and ability to bioaccumulate certain elements over natural background (Tessier *et al.*, 1993; Naimo, 1995; Dutta, 2016).

Studies on the elemental status of *Galatea paradoxa* (Ekut *et al.*, 2000), bioaccumulation of heavy metals (Amisah *et al.*, 2011), mercury in whole tissues of clam (Obirikorang *et al.*, 2010), and tissue toxicity of chromium, copper and zinc to freshwater clam (*Galatea paradoxa*) (Ugwu *et al.*, 2005) have been reported. The hygienic status of processed fresh water clam in Yenagoa metropolis, Niger Delta, Nigeria have been carried out by Kingdom *et al.* (2018). However, studies on the effect of ‘uproot’ a glyphosate – based herbicide formulation on *Galatea paradoxa* have not been reported. This research seems to fill in the gap and thus comparatively evaluate antioxidant enzymes, lipid peroxidation, nitric oxide, acetylcholinesterase, lactate dehydrogenase and non- microsomal oxidases in *Galatea paradoxa* exposed to varying concentrations of ‘uproot’ a glyphosate – based herbicide.

## MATERIALS AND METHODS

### Collection of *Galatea paradoxa*

*Galatea paradoxa* samples were bought from a local market in Otuoke, Ogbia Local Government Area, Bayelsa State. The clams were placed in a sterile container for four weeks in order to allow the clams acclimatize. The temperature was kept constant during the entire experiment. The clams were fed regularly and their water changed daily.

### Experimental Design

Treatment with glyphosate based herbicide commenced on the 5<sup>th</sup> week after acclimatization. *Galatea paradoxa* samples were divided into four groups (A, B, C, and D) of five clam per group (n =5) in 10L of water in a plastic bowl (60 cm x 38 cm x 30 cm). Group A was considered as control (no glyphosate exposure). Groups B, C, and D were exposed to 10 mg/L, 20 mg/L and 30 mg/L of ‘uproot a glyphosate – based herbicide, respectively. The water was changed after every 24 hours and the exposure was repeated which lasted for 21days. The clams were sacrificed afterwards facilitating the harvesting of its tissue from the shell. The samples were collected and kept in clean and appropriate sample containers and biochemical analysis commenced immediately in the laboratory of the Department of Biochemistry, Federal University Otuoke.

### **Preparation of tissue supernatant of *Galatea paradoxa***

The whole tissue of the *Galatea paradoxa* was homogenized in ice cold 4.5 mL phosphate buffer (0.02M, pH 7.2). The homogenates were centrifuged at 4000 xg for 10 mins and supernatant decanted into a 5 mL sterilized plain container and stored frozen at --30°C until.

### **Biochemical Analysis**

Total protein concentration in the obtained tissue supernatant was determined using the method of Doumas *et al.* (1981). Superoxide dismutase activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 as described by Misra and Fridovich, (1972). Catalase activity was estimated from the rate of consumption of hydrogen peroxide levels (Kaplan and Groves, 1972). Lipid peroxidation level was determined in terms of thiobarbituric acid reacting substances (TBARS) using Malondiadehyde (MDA) as standard (Buege and Aust, 1978). Tissue reduced glutathione (GSH) was determined by the method of Ellman, (1959) while, the activity of glutathion S-transferase was determined by Habig *et al.*, (1974) using 1 – chloro – 2, 4-dinitrobenzene (CDNB) as substrate. The activities of glutathione peroxidase and acetylcholinesterase were determined by the methods of Moin, (1986) and Ellman, (1961), respectively. The activities of sulphite oxidase and monoamine oxidase were estimated using the methods of Macleod *et al.* (1961) and Tabor *et al.* (1954), respectively. The methods of Reitman and Franklin (1957) and Roy (1970) were used to analyse the activities of aminotransferases and alkaline phosphatase respectively, while the lactate dehydrogenase activity was assayed by the method of Amador *et al.* (1963), and the concentration of nitric oxide was measured with the method of Green *et al.* (1982).

### **Statistical Analysis**

Mean  $\pm$  standard deviation of replicate experiments with triplicate sampling were taken for each analysis. Significant differences of results were established by one way Analysis of Variance (ANOVA) and differences between / within groups at  $p < 0.05$  were carried out by Duncan Multiple Range Test. All statistical analysis was carried out using SPSS version 21.0.

## RESULTS

**Table 1: Activities of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in *Galatea paradoxa* Exposed to ‘uproot’ a glyphosate herbicide**

Parameters	A	B	C	D
Alanine aminotransferase (U/L)	30.20 ± 2.17 <sup>a</sup>	35.20 ± 1.15 <sup>b</sup>	41.00 ± 1.58 <sup>c</sup>	36.60 ± 2.51 <sup>b</sup>
Aspartate aminotransferase (U/L)	58.60 ± 3.21 <sup>a</sup>	64.80 ± 3.77 <sup>bc</sup>	62.00 ± 3.54 <sup>b</sup>	68.20 ± 3.27 <sup>c</sup>
Alkaline phosphatase (U/L)	4.063 ± 0.55 <sup>a</sup>	4.902 ± 0.80 <sup>b</sup>	5.139 ± 0.91 <sup>b</sup>	5.384 ± 0.26 <sup>b</sup>

Value are expressed as Mean ± SD; with n = 5. Mean not sharing the same superscript alphabet on a given row differ significantly at p < 0.05. A = Control; B = 10 mg/L glyphosate; C = 20 mg/L glyphosate; D = 30 mg/L glyphosate

Table 1 indicated an elevation (p < 0.05) in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in *Galatea paradoxa* exposed to 10 mg/L, 20 mg/L and 30 mg/L of glyphosate herbicide as compared with the control clams. Although, higher activity of ALP observed in exposed clams were comparable (p > 0.05). The highest AST activity (68.20 ± 3.27 U/L) was recorded for *Galatea paradoxa* exposed to 30 mg/L glyphosate while that of ALT (41.00 ± 1.58 U/L) according to the results was observed in *Galatea paradoxa* exposed to 20 mg/L glyphosate.

**Table 2: Concentrations of total protein, malondialdehyde and nitric oxide and activities of superoxide dismutase and catalase in *Galatea paradoxa* exposed to glyphosate**

Parameters	A	B	C	D
Total protein (g/dL)	1.305 ± 0.29 <sup>a</sup>	1.399 ± 0.05 <sup>a</sup>	1.263 ± 0.13 <sup>a</sup>	1.214 ± 0.14 <sup>a</sup>
Superoxide dismutase (Unit/g tissue)	26.36 ± 2.80 <sup>a</sup>	29.49 ± 0.72 <sup>b</sup>	29.14 ± 1.02 <sup>b</sup>	28.81 ± 0.38 <sup>b</sup>
Catalase (Unit/g tissue)	39.48 ± 3.78 <sup>a</sup>	45.90 ± 4.24 <sup>b</sup>	47.26 ± 3.07 <sup>b</sup>	48.09 ± 1.14 <sup>b</sup>
Nitric Oxide (%)	39.63 ± 1.47 <sup>a</sup>	46.02 ± 2.65 <sup>b</sup>	55.25 ± 2.70 <sup>c</sup>	41.27 ± 3.58 <sup>a</sup>
Malondialdehyde (Unit/mg of wet tissue)	1.152 ± 0.45 <sup>a</sup>	1.440 ± 0.16 <sup>a</sup>	1.413 ± 0.13 <sup>a</sup>	1.202 ± 0.25 <sup>a</sup>

Value are expressed as Mean ± SD; with n = 5. Mean not sharing the same superscript alphabet on a given row differ significantly at p < 0.05. A = Control; B = 10 mg/L glyphosate; C = 20 mg/L glyphosate; D = 30 mg/L glyphosate

Results in Table “2” showed that exposure of clam to glyphosate herbicide statistically increased ( $p < 0.05$ ) the activities of superoxide dismutase and catalase as compared with the control animals. However, the observed increase in enzyme activities was not dose – dependent. The results in Table “2” also indicated that the concentrations of total protein were comparable ( $p > 0.05$ ). However, *Galatea paradoxa* exposed to 20 mg/L and 30 mg/L of glyphosate had a lower ( $1.263 \pm 0.13$  g/dL and  $1.214 \pm 0.14$  g/dL) total protein concentrations.

Table “2” also revealed that the lipid peroxidation level reported as the concentration of malondialdehyde (MDA) in clam’s tissues exposed to vary concentrations of glyphosate increased but was not statistically ( $p > 0.05$ ) different from the MDA concentration of the control clam. It was also observed that the nitric acid concentration (NO) increased in *Galatea paradoxa* exposed to 10 mg/L and 20 mg/L of glyphosate as compared to the control clams. *Galatea paradoxa* exposed to 30 mg/L of glyphosate had comparable ( $p > 0.05$ ) nitric oxide concentration with the control animals.

**Table 3: Levels of reduced glutathione and the activities of glutathione s- transferase and glutathione peroxidase in *Galatea paradoxa* exposed to glyphosate**

Parameters	A	B	C	D
Reduced glutathione (GSH) (Unit/mg protein of wet tissue)	$33.02 \pm 3.12^a$	$38.09 \pm 4.14^b$	$33.11 \pm 2.43^a$	$39.66 \pm 3.68^b$
Glutathione s-transferase (GST) (nmol/mg protein of wet tissue)	$17.60 \pm 1.75^a$	$26.47 \pm 2.47^b$	$28.64 \pm 2.82^b$	$27.34 \pm 2.77^b$
Glutathione peroxidase (GPx) ( $\mu$ mol/mg tissue/min)	$42.88 \pm 2.22^a$	$47.65 \pm 2.03^b$	$40.59 \pm 2.11^a$	$36.87 \pm 3.47^c$

Value are expressed as Mean $\pm$ SD; with  $n = 5$ . Mean not sharing the same superscript alphabet on a given row differ significantly at  $p < 0.05$ . A = Control; B = 10 mg/L glyphosate; C = 20 mg/L glyphosate; D = 30 mg/L glyphosate

Reduced glutathione (GSH) concentration and the activities of glutathione s- transferase (GST) and glutathione peroxidase (GPx) are expressed in Table “3”. The results indicated significant increase in GSH ( $p < 0.05$ ) in clam exposed to 10 mg/L and 30 mg/L glyphosate as compared with the control. Elevated ( $p < 0.05$ ) activities of GST were observed in exposed clams (groups B, C and D) as compared with the control clams. The activities of GPx statistically ( $p < 0.05$ ) increased ( $47.65 \pm 2.03$   $\mu$ mol/mg tissue/min) in *Galatea paradoxa* exposed to 10 mg/L of glyphosate and thereafter decreased ( $36.87 \pm 3.47$   $\mu$ mol/mg tissue/min) ( $p < 0.05$ ) in clam exposed to 30 mg/L of glyphosate as compared with the control clams.

**Table 4: Activities of lactate dehydrogenase, acetylcholinesterase, monoamine and sulphide oxidases in *Galatea paradoxa* exposed to vary concentrations of glyphosate**

Parameters	A	B	C	D
Lactate dehydrogenase (U/L)	8.60 ± 0.96 <sup>a</sup>	11.53 ± 1.07 <sup>b</sup>	13.38 ± 1.91 <sup>bc</sup>	13.78 ± 1.43 <sup>c</sup>
Acetylcholinesterase (AChE) (nmol/min/mg protein)	0.054 ± 0.001 <sup>a</sup>	0.049 ± 0.003 <sup>a</sup>	0.077 ± 0.001 <sup>b</sup>	0.055 ± 0.001 <sup>a</sup>
Monoamine oxidase (MO) (Unit/mg protein)	0.898 ± 0.24 <sup>a</sup>	1.262 ± 0.22 <sup>a</sup>	1.412 ± 0.13 <sup>a</sup>	1.123 ± 0.14 <sup>a</sup>
Sulphide oxidase (SO) (Unit/mg protein)	1.875 ± 0.04 <sup>a</sup>	1.852 ± 0.03 <sup>a</sup>	1.866 ± 0.05 <sup>a</sup>	1.892 ± 0.02 <sup>a</sup>

Value are expressed as Mean ± SD; with n = 5. Mean not sharing the same superscript alphabet on a given row differ significantly at p < 0.05. A = Control; B = 10 mg/L glyphosate; C = 20 mg/L glyphosate; D = 30 mg/L glyphosate

Table “4” indicated a dose – dependent increased (p < 0.05) in the activities of lactate dehydrogenase in Clams exposed to 10 mg/L, 20 mg/L and 30 mg/L of glyphosate ( i.e., 11.53 ± 1.07 U/L, 13.38 ± 1.91 U/L and 13.78 ± 1.43 U/L) as compared with the control clam (8.60 ± 0.96 U/L). Results in Table “4” also showed that except for calms exposed 20 mg/L of glyphosate, which had an elevated (p < 0.05) actylcholinesterase activity, *Galatea paradoxa* exposed to 10 mg/L and 30 mg/L of glyphosate had comparable (p > 0.05) actylcholinesterase activity with the control clam. The activities of monoamine and sulphide oxidases were comparable (p > 0.05) with that of the control calms.

## DISCUSSION

In recent times, most problems associated with the aquatic environment are attributed to the production and release of toxic chemicals into the aquatic environment and the aquatic organisms are constantly exposed to these toxic chemicals. The contaminants produce stress on the aquatic organisms like clams or fishes that cannot avoid being exposed to these chemicals that are usually suspended or dissolved in water (Nahed, 2009). The impact of environmental pollutants such as ‘uproot’ a glyphosate – based herbicide on *Galatea paradoxa* could be evaluated by changes in the biochemical parameters such as activities of alkaline phosphates (ALP), alanine aminotransferase (ALT), aspartate Aminotransferase (AST), lactate dehydrogenase (LDH), acetylcholinesterase (AChE), total protein concentration (TP), antioxidant defense

system [superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), glutathione-s-transferase (GST) and lipid peroxidation level]

In the present study, *Galatea paradoxa* was exposed to varying concentrations (10 mg/L; 20 mg/L and 30 mg/L) of 'uproot' a glyphosate – based herbicide.

Proteins are the most abundant organic molecules in living system and play an essential role in the physiology of living organism by providing information on the general energy mobilization of an animal (Adams *et al.*, 1990). As the basis of structure and function of life, proteins are broken down under stress condition by organisms to amino acid to meet their metabolic need. There was no significant difference ( $p < 0.05$ ) in the total protein concentration between the exposed and control clams, however, *Galatea paradoxa* exposed to 20 mg/L and 30 mg/L glyphosate showed lower total protein concentration. Bhattacharya *et al.* (2006) reported decrease in total protein concentration in *Pila globosa* exposed to an untreated tannery effluent.

The activities of ALP, AST, and ALT increased ( $p < 0.05$ ) in clam exposed to glyphosate as compared with the control. ALP according to Chen *et al.* (2012), catalyse the hydrolysis of phosphate monoesters and is essential to protein synthesis. The enzyme (ALP) has also been reported as an important biomarker because it is involved in adaptive cellular response to the potential and genotoxicity of pollutants and could serve as a good indicator of intoxication (Boge *et al.*, 1992; Leohner *et al.*, 2001). The marked increase in ALP activity recorded in clam maybe related to the degree of stress the animals were experiencing as a result of exposure to glyphosate. ALT and AST activities are biomarker of acute hepatic damage and are enzymes associated with the conversion of amino acid to keto acids, thus allowing interplay between carbohydrate and protein metabolism during fluctuating energy demands of the organisms in various adaptive situation. Such increase in the transaminases (ALT and AST) activities in clam exposed to glyphosate as observed in this study, may suggest dysfunction resulting from glyphosate exposure. Again, disruption process of energy supply through amino acid metabolism might be responsible for the elevation of these transaminases.

Exposure of glyphosate – based herbicides to organisms has been previously reported to be implicated in the initiation of oxidative stress (Romero *et al.*, 2011; Glusczak *et al.*, 2011; Menezes *et al.*, 2011). The activities of SOD and CAT were increased ( $p < 0.05$ ) in exposed clam (Table 2). This elevation may not be unconnected with the effect of pollutants on antioxidant defense system. The enzymes SOD and CAT constitute an indispensable part of first line defense system against the radicals generated by ambient oxidative pollutants. SOD converts superoxide radical to  $H_2O_2$  in all aerobic organisms, while CAT decompose the  $H_2O_2$  into water and oxygen in the peroxisomes and GPx in the cytosol and mitochondria (Baumard *et al.*, 1999; Banaee *et al.*, 2013). An increase in nitric oxide (NO) concentration in *Galatea paradoxa* exposed to glyphosate (Table 2) could be attributed to change in the gene expression of nitric oxide in response to increased production of superoxide radical ion as a result of clam's contact with



glyphosate. Nitric oxide reacts with superoxide radical to form peroxynitrite ( $\text{ONOO}^-$ ) (Sawyer and Valentine, 1981), which also scavenges lipid peroxyl radical ( $\text{LOO}^*$ ); thus is viewed as an antioxidant (Goss *et al.*, 1997; Radi, 2018).

*Galatea paradoxa* exposed to 'uproot' a glyphosate – based herbicide had comparable ( $p > 0.05$ ) malondialdehyde (MDA) concentration with the control clam. MDA is the product of lipid peroxidation and serves as a marker for oxidative stress (Aust, 1985; Viarengo *et al.*, 2007). The observed results of malondialdehyde, in this study, could mean that the induction of antioxidants in clam was able to curtail the free radicals generated by glyphosate at the exposed concentrations or represent adaptive response. Reduced glutathione (GSH) is a sulfhydryl (-SH) antioxidant and the most abundant non-protein thiol in cells (Ueno *et al.*, 2002). GSH acts as primary line of defense to cope with the deleterious effects of ROS (Bradley and Nathan, 1984). It detoxifies  $\text{H}_2\text{O}_2$  and lipid hydroperoxides via reactions catalysed by glutathione peroxidase (Ahmed, 2005) and as a cofactor in biotransformation reactions of glutathione S-transferase (GST) (Jakoby, 1985). Significant ( $p < 0.05$ ) high level of GSH and elevated activities of GST and GPx was observed in *Galatea paradoxa* exposed to 10 mg/L glyphosate. This could be due to enhanced physiological stress that induces protein synthesis and may also indicate a defense response to oxidative stress. Clam exposed to 20 mg/L and 30 mg/L glyphosate also showed higher GST activity indicating increased herbicide biotransformation process by clam and could also be related to changes in gene expression in response to increased  $\text{H}_2\text{O}_2$ . The utilization of cellular GSH content and activation of GST is required to maintain homeostasis of the organism's internal cell environment. Lower activity of GPx observed in clam exposed to 20 mg/L and 30 mg/L glyphosate may not be unconnected to adaptive responses or might be that much of the  $\text{H}_2\text{O}_2$  are produced in the peroxisomes where CAT act on them.

Lactate dehydrogenase (LDH) activity is an important biochemical parameter of tissue damage and hypoxia. The extracellular activity of LDH has been reported to be increased under condition of oxidative stress (Jovanovic *et al.*, 2010). Significant increase ( $p < 0.05$ ) in LDH activity was observed in clam exposed to varying concentrations of glyphosate as compared with the control animals. This increase in LDH activity may be related to response to stressful environment occasioned by the glyphosate exposure. Tunholi *et al.* (2017), explain that snails exposed to physiological stress may act through fermentation or anaerobic routes to provide energy. In a stressful environment LDH converts pyruvate into lactate. Such increase in LDH activity has been previously reported following exposure of snail to heavy metal (Abdel-Halin *et al.*, 2013) and untreated sewage (Banaee and Taheri, 2019). Results from this study did not associate exposure to glyphosate – based herbicide with the inhibition of acetylcholinesterase activity in *Galatea paradoxa*. Acetylcholinesterase activity in clam exposed to 10 mg/L and 30 mg/L were comparable ( $p > 0.05$ ) while animals exposed to 20 mg/L of

glyphosate expressed higher ( $p < 0.05$ ) AChE activity which indicates no inhibition. The non- microsomal oxidases investigated in this study, sulphite and monoamine oxidases showed comparable ( $p > 0.05$ ) enzyme activities with the control clam at all exposed concentrations of glyphosate. This observation could be due to adaptive responses by clam or the concentration of glyphosate employed fail to modulate the activities of these enzymes.

## CONCLUSION

This study showed that exposure of *Galatea paradoxa* to varying concentrations of uproot a glyphosate – based herbicide was able to cause significant changes ( $p < 0.05$ ) in the activities of ALT, AST and ALP in its soft tissues. Alterations in the activities of antioxidant enzymes (SOD and CAT) were observed with increased herbicide biotransformation process as indicated by the elevated GST activity. Lipid peroxidation was not significant as exposed clam showed comparable ( $p > 0.05$ ) MDA levels with the control. Of interest in this study is the increase in LDH activity in experimental clam which could be related to stressful environment caused by glyphosate exposure. Acetylcholinesterase; and monoamine and sulphite oxidases activities were not altered by the concentration of glyphosate in exposed clam. Taken together, these results support the possibility of uproot (the glyphosate herbicide) altering the antioxidant defense system in *Galateaparadoxa* and may provide insights into monitoring the ecological consequences, which may result from the indiscriminate use of glyphosate in our farmlands and these antioxidant enzymes employed as biomarkers of environmental contamination.

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