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Acute toxicological effect of glyphosate-based herbicide (round up) with cadmium as a co-contaminant on African catfish (*Clarias gariepinus*)

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ABSTRACT: The possible toxicological effect of Round Up (RU) interacting with cadmium on juvenile African catfish was investigated. Juvenile catfish of mean weight 30 ± 0.3 g and mean standard length of 15±0.1 cm were exposed to constant sublethal concentration of 10 mg/L RU glyphosate herbicide and varying sublethal concentrations of cadmium (4.0 and 4.5 mg/L) for 96 hours with physicochemical parameters analyzed concurrently. Thereafter, biochemical parameters were assayed in the liver and kidney of exposed catfish using standard conventional methods. The physicochemical parameters analyzed on the artificial pond were within the standard acceptable range for the culture and survival of catfish. A significant increase in the level of thiobarbituric acid reactive substances (TBARS) was observed in catfish exposed to RU + Cd^{2+} as the concentration of cadmium increased when compared with RU alone and control. Also, there was a significant reduction in the level of inherent antioxidants possessed by the exposed catfish tissues which were determined via DPPH, FRAP and GSH estimation. The deleterious effect of RU with cadmium was accompanied by a marked decrease in the activities of Na^+/K^+-ATPase and ð- ALA-D and an increase in the activities of purinergic enzymes in a concentration-dependent manner when compared with RU alone and control in the tissues examined. Finally, the toxicity of RU with cadmium was established via the harmful effect exerted on the liver and kidney tissue of exposed catfish which was examined through the estimation of liver and kidney function indices. Hence, the present study suggests that RU and cadmium leaching together into an aquatic environment can be considered to have an additive deleterious effect and significantly perturbs the physiology and morphology of aquatic organisms especially the catfish when compared with exposure to individual glyphosate and cadmium.

Keywords: Catfish, Roundup, cadmium, TBARS, ð- ALA D, Na^+/ K^+ ATPase.

Abbreviations: RU: Round Up, **TBARS:** Thiobarbituric acid reactive substances, **ð-ALA-D:** Delta aminolevulinic acid dehydratase, **GSH:** Reduced glutathione, **AST:** Aspartate aminotransferase, **ALT:** Alanine aminotransferase, **Na^+/K^+ATPase:** Sodium potassium adenosine triphosphatase, **NTPDase:** Nucleoside Triphosphate diphosphohydrolase; **ALP:** Alanine phosphatase.

INTRODUCTION

The excessive contamination of aquatic ecosystems which seriously affects both fresh and marine habitats has become a major environmental and health concern worldwide over the past decades (Vutukuru, 2005; Yousafzai and Shakoori, 2006; Vinodhini and Narayanan, 2008; McNeil and Fredberg, 2011; Muhammad *et al*., 2011; Yu *et al*., 2011; El Nemr *et al*., 2012, Abdel-Khalek, 2015a). These water pollutants range from pesticides, industrial wastes, and plastics to other chemicals that are washed into aquatic bodies. Herbicides are commonly used pesticides that are considered to be pollutants and are chemical substances used to suppress or kill unwanted weeds which may reach water bodies directly by overhead spray of aquatic weeds, or indirectly through processes such as agricultural runoff, spray drift and leaching.

Furthermore, glyphosate with its broad-spectrum activity and low cost has become an important and popular herbicide among others in ecological restoration programs (Wilkin *et al*., 2003). Glyphosate has the IUPAC name, N-(phosphonomethyl) -glycine and is a non-selective, broad-spectrum, post-emergent systemic herbicide in which its herbicidal activity is expressed through direct contact with the leaves and subsequent translocation throughout the plant. Also, it is used to control weeds in compounds, gardens, fish ponds, etc. Moreover, due to its benefits and effectiveness in farm practices, glyphosate usage is currently growing at an increasing rate (Bureau of National Affairs, 1998; Erhunmwunse *et al*., 2018) and this has brought about the emergence of the product in different commercial formulations by different manufacturers. Glyphosate alone (analytical grade) or with its formulation products was previously considered to be harmless in normal usage and at chronic exposure in previous testing approaches (Williams *et al.,* 2000). However, its indiscriminate use makes it a major concern for toxicologists due to its effect on non-target organisms especially in the aquatic environment.

Additionally, heavy metals are another class of pollutants that pose environmental concern, due to their persistent characteristic and ability to bioaccumulate in the environment. Freshwater habitats are mostly affected by heavy metals through the discharge of untreated wastes from industrial facilities, some agricultural processes and anthropogenic activities. Problems arising from toxic heavy metals are of great concern as it adversely affects and devastates both aquatic biota and human (Mahurpawar, 2015) due to the dispersal performance and bio-magnification of metals into aquatic food chains in addition to their toxicity and accumulative behavior in the biological tissues (Islam and Tanaka, 2004, Yu *et al*., 2011). However, Cd^{2+} is one of the most devastating heavy metals of global concern presently due to the threats posed on aquatic mediums. It is presently at dump sites close to aquatic banks which can easily leach into the aquatic environment. Also, cadmium is well known as strong toxic heavy metal which is present in paints, mining extracts and liquid wastes (Perez and Wallace, 2004) and its toxicity usually targets the kidney and liver (Ryan *et al*., 2000) of an organism.

Previous studies have revealed that exposure to glyphosate herbicides caused metabolic and oxidative damage in fish (da Fonseca *et al*., 2008; Salbego *et al*., 2010; Glusczak *et al*., 2011). On the other hand, exposure to cadmium induces biochemical changes in tissues and oxidative stress by interfering with the activity of the enzyme (Szebedinszky *et al*., 2001; Flora *et al*., 2012; Jhamtani *et al.*, 2017) and increasing lipid peroxidation in many organs such as brain, liver, kidney and gills (Asagba *et al*., 2008; Lee and Freeman, 2014; Hosseini *et al*., 2018; Nimmy and Joseph, 2018).

However, since glyphosate from farm practices may leach into the aquatic environment as well as heavy metals from the domestic refuse dumped at the aquatic bank and both constitute major environmental concerns, it is worthwhile to investigate the potential influence of both on the fish in aquatic medium and there is a dearth of information about this in literature. It is instead of this that this study was carried out to examine the acute toxicological effect of glyphosate-based herbicide - round-up with cadmium as a co-contaminant on African catfish (*Clarias gariepinus*) in aquatic medium.

MATERIALS AND METHODS

Toxicity tests

Juveniles of *Clarias gariepinus* with a mean weight of 30 ± 0.3 g and mean standard length of 15±0.1 cm (Mean ± SEM) were used for the experiment and were purchased from a reputable farm in Ondo State, Nigeria. Prior to the toxicity experiment, fish were acclimatized under laboratory conditions in 600 L plastic tanks for 7 days with dechlorinated water (temperature 26.0 ± 0.8°C, pH 7.0 and dissolved oxygen 6.3 ± 0.1 mg/L). Also, fish were fed with commercial floating pellets at 10% of their body weight regularly before the experiment.

Short–term static toxicity tests were performed to evaluate the toxicity of Round Up and cadmium to *C. gariepinus.* Preliminary tests were carried out to determine the appropriate concentration range for testing the chemical. The commercial formulation of glyphosate, Roundup (360 g glyphosate L^{-1} or 41% of glyphosate, Monsanto do Brasil LTDA) was used. Experiments were performed in 100 L glass aquaria containing 8 fish each, with continuously aerated dechlorinated water, with the same characteristics described for the acclimation period. The tests consisted of ten groups of fish exposed to five different concentrations of RU and cadmium (10, 11.5, 13, 14.5 and 16 mg/L; and 10, 12, 13, 14, 18,22 and 26 mg/L respectively) and a control group exposed only to water, without the herbicide or heavy metals. The number of dead fish was recorded every 6, 24 and 96 h, and the values of the median lethal concentration (LC_{50}) were estimated by the probit method (USEPA, 1999; Erhunmwunse *et al*., 2018).

Interaction of round-up glyphosate and cadmium

To evaluate the influence of cadmium on Round-Up, catfish were exposed to constant sublethal concentration of Roundup (10 mg/ L) and varying sublethal concentrations of cadmium (4.0 and 4.5 mg/L) in an experimental artificial plastic pond for 96 hours (4 days) and a control group in 100 L glass aquaria containing 10 fish each. Also, the experimental water was constantly monitored by analyzing the physicochemical parameters of water in each artificial plastic pond. After the exposure, the kidney, liver tissues and blood of the surviving exposed fish samples were analyzed for biochemical parameters.

Immediately after removal from the aquaria, the fish were decapitated under mild ether anesthesia and blood samples were taken from the caudal vein into heparinized plastic syringes. Subsequently, animals were killed by cervical section and the kidney as well as liver were immediately removed.

Biochemical analyses

Tissue preparation

Tissue samples (liver and kidney) of the surviving fish from the acute toxicity study were quickly removed, placed on ice and homogenized in cold 50 mM Tris–HCl pH 7.4 (1/10, w/v). The homogenate was centrifuged at 4000×*g* for 10 min to yield the low-speed supernatant (S1) fraction that was used for all biochemical assays. Protein was measured using a modification method of Lowry *et al.* (1951) by Kade and Rocha (2010) using bovine serum albumin as a standard.

Lipid peroxidation Assay

Production of TBARS was determined as described by Ohkawa *et al.* (1979) and modified by Kade *et al.* (2009b). The color reaction was developed by adding 300 µL 8.1% SDS to S1, followed by the sequential addition of 500 µL acetic acid/HCl (pH 3.4) and 500 µL 0.8% TBA. This mixture was incubated at 95°C for 1 h. TBARS produced were measured at 532nm, and the absorbance was compared to that of a standard curve obtained using malondialdehyde (MDA).

Redox systems

GSH Level Estimation: GSH levels of liver and kidney tissues of fish exposed to glyphosate were determined was estimated using Ellman's reagent after deproteinization with TCA (5% in 1 mmol/EDTA) following the method modified by Kade *et al*. (2009b). In all cases, the yellow color formed in the reaction systems was measured at 412 nm.

DPPH - Free Radical Scavenging Ability: The free radical scavenging ability of the kidney and liver tissues against DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radicals were evaluated according to Kade and Rocha (2010). Briefly, tissue homogenates were mixed with 600 µL, 0.3 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance of the resulting golden-yellow products was measured at 516 nm.

Ferric Reducing Antioxidant Properties (FRAP): Ferric reducing antioxidant properties of the compounds against TPTZ (2, 4, 6-Tri(2-pyridyl)-s-triazine). Briefly, 300 µL of the protein-free tissue homogenates were mixed with 300 µL and 900 µL of TPTZ solution as cited by Kade *et al*. (2009a). The mixture was left in the dark for 10 minutes, and absorbance values were measured at 543 nm.

Enzymes activity

ð-Aminolevulinate dehydratase (ð-ALAD) activity: Kidney and liver ð- ALAD activities were assayed according to the method cited by Kade *et al*. (2009a). by measuring the rate of product porphobilinogen (PBG) formation except that 1M potassium phosphate buffer, pH 6.8 and 2.4mM ALA were used. Incubations were carried out for 2 hr at 37°C. The reaction product was determined using modified Ehrlich's reagent at 555 nm for the Ehrlich–PBG salt.

Purinergic enzymes

Nucleotidase triphosphate dehydratase (NTPDase) activity: NTPDase-like activity was determined in a reaction medium as cited by Kade and Rocha (2010). A 100 µL of protein of the enzyme preparation was added to the reaction mixture and preincubated for 10 min at 37°C. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Released inorganic phosphorous (Pi) was measured by the method modified by Kade *et al.* (2008). Protein was measured by the method of Lowry *et al.* **(**1951) using bovine serum albumin as standard.

5'-Nucleotidase activity: The 5'-Nucleotidase activity was determined in a reaction medium essentially as described (Heymann *et al*., 1984; Kade and Rocha*,* 2010) with 100 µL of protein added. The reaction was mixed and preincubated for 10 min at 37^{0}C and initiated by the addition of AMP to a final concentration of 2.0 mM and incubated for 20 min.

Kidney and liver tests

The activities of creatinine, urea, ALT, AST, ALP, bilirubin and albumin were determined according to the method of

Table 1. Determination of redox status in tissues of catfish exposed to RU + cadmium.

Parameters	Control	RU (10 mg/L)	RU+Cd^{2+}(4.0 mg/L)	RU+Cd^{2+}(4.5 mg/L)
A) TBARS				
Kidney	229.05±11.5	278.26±13.9	309.36±15.5*	334.25±16.7*
Liver	189.62± 8.8	250.270±11.7	299.79±13.8*	327.88±15.8*
B) DPPH				
Kidney	0.616±0.001	0.720±0.04	0.808±0.04*	0.895±0.05*
Liver	0.716±0.04	0.731±0.04	0.789±0.04	0.828±0.04*
C) FRAP				
Kidney	0.263±0.01	0.181±0.01	0.139±0.01*	0.106±0.01*
Liver	0.171±0.01	0.169±0.01	0.165±0.01	0.120±0.01*
D) GSH				
Kidney	0.098±0.01	0.078±0.004	0.074±0.004*	0.052±0.003*
Liver	0.124±0.01	0.107±0.005	0.063±0.003*	0.054±0.003*

[A]Unit of TBARS is µM malondialdehyde (MDA)h^{-1}g $tissues^{-1}$, [B]Unit of DPPH is % free radicals unscavenged properties, [C]Unit of FRAP is % ferric reducing antioxidant properties, [D, E]Reduced glutathione (GSH) levels are presented as µmol/g tissue, *Significantly different from the control group (ANOVA/Duncan, p<0.05).

Kade and Rocha, (2010) as described by the manufacturer's manual (Randox Laboratories Ltd).

Statistical analysis

Results were analyzed by appropriate analysis of variance (ANOVA) and this is indicated in the text of the results. Differences between groups were considered to be significant when p<0.05.

RESULTS

Physiochemical analysis of test water

The physicochemical characteristics of the water during sub-lethal tests of the interaction of RU and cadmium concentrations, in all the exposure periods, remained stable. The mean values (±SE) for control and experimental groups were Temperature: 25.3±1.3°C and 25.8±1.3°C; pH: 7.13± 0.36 and 5.81±0.29; Dissolved oxygen: 5.69±0.28 and 4.90±0.24 mg/ L; Conductivity: 195.7±9.75 and 246.5±12.3 µS/cm respectively.

Lipid peroxidation

Experimental catfish exposed to RU at 10 mg/L increased level of TBARS produced in comparison with control and became highly significant when RU combined with different concentrations of cadmium in a concentration-dependent manner (p<0.05) in kidney and liver homogenates examined as shown in Table 1A.

Redox system

DPPH free radical scavenging properties

The DPPH free radicals scavenging ability of kidney and liver tissue homogenate of catfish exposed to 10 mg/L of RU as presented in Table 1B showed an elevated level of DPPH free radicals when compared with the 10 mg/L RU alone as well as with control and this effect became more pronounced when RU combined with cadmium in a concentration-dependent manner.

Ferric reducing antioxidant power

The effects of RU alone at 10 mg/L and its combination with varying concentrations (4.0 and 4.5 mg/L) of cadmium on the ferric reducing antioxidant properties of the brain, kidney and liver of an exposed catfish is presented in Table 1C and a reduction in the ferric reducing antioxidant properties was observed in catfish exposed to 10 mg/L RU alone and marked reduction (p<0.05) was observed as the concentration of cadmium increases in the catfish exposed to RU with cadmium when compared with the control group.

GSH levels

Hepatic and renal GSH levels reduced in catfish exposed to RU 10 mg/L only and significantly diminished in catfish exposed to RU with cadmium at increasing concentration in comparison with control (Table 1D).

Table 2. Activity of ð ALA D, NTPDase and 5' Nucleotidase in tissues of catfish exposed to RU and cadmium. Data are expressed as means ± SEM of ten catfish.

Parameters	Control	RU (10mg/L)	RU+Cd^{2+}(4.0mg/L)	RU+Cd^{2+}(4.5mg/L)
A) ð ALA D[b]				
Kidney	220.05±11.0	173.36±8.7	136.89±6.8*	119.44±6.0*
Liver	218.32±10.9	187.92±9.4	178.87±8.9	144.72±7.2*
B) NTPDase[a]				
Kidney	108.47±5.4	141.32±7.1	184.04±9.2*	228.90±11.4*
Liver	107.47±5.4	168.32±8.4	204.04±10.2*	218.90±10.9*
C) 5' Nucleotidase[a]				
Kidney	24.17±1.2	44.89±2.2	60.60±3.0*	91.89±4.6*
Liver	26.72±1.3	45.15±3.3	68.02±3.4*	79.73±4.0*

Data are expressed as means ± SEM of ten catfish. [a] NTPDase and [a]5' Nucleotidase are presented as nmolPi/mgProtein/minute, [b] ð ALA D activities are presented as nmol of PBG/µg protein/hr, *Significantly different from the control group (ANOVA/Duncan, p<0.05).

Table 3. Biochemical parameters of catfish exposed to RU + cadmium. ALT, AST and ALP. Data are expressed as means ± SEM of ten catfish.

Parameters	Control	RU (10mg/L) only	RU+Cd^{2+}(4.0mg/L)	RU+Cd^{2+} (4.5mg/L)
A) Hepatic markers				
ALT[a]	7.0±0.4	8±0.4	9.8±0.5*	10±0.5*
AST[a]	8±0.5	10±0.6	11±0.6*	13±0.7*
ALP[a]	14±0.7	18±0.9	22±1.1*	23±1.2*
Bilirubin[b]	4.0±0.2	6±0.3	10±0.5*	14±0.7*
Albumin[b]	16.1±0.81	14.2±0.76	12.6±0.73*	10.4+0.67*
B) Renal markers				
Urea[b]	2.3±0.1	3±0.2	3.4±0.2*	3.7±0.2*
Creatinine[b]	14±0.7	16±0.8	17±0.9*	19±1.0*

Data are expressed as means ± SEM. ten catfish. [b]Data of renal markers and hepatic levels are presented as mmol/L. [a]Data of enzyme activities are presented as U/L. *Significantly different from the control group (ANOVA/Duncan, p<0.05).

Enzyme activity

δ-ALA D

The activity of δ-ALAD was observed to reduce in the kidney and liver of catfish ($p<0.05$) exposed to RU (10 mg/L) and became highly pronounced in the kidney and liver of catfish exposed to RU with cadmium as the concentration increases when compared with control as presented in Table 2A.

Purinergic enzymes

Activities of NTPDase and 5' Nucleotidase were elevated in the kidney and liver of catfish exposed to RU at 10 mg/L and this increase was significant ($p<0.05$) in catfish exposed to RU + cadmium as the concentration of cadmium increased when compared with the catfish exposed to control as presented in Table 2C and 2D respectively.

Effect on liver and kidney functioning

Plasma AST, ALT, ALP, and Bilirubin increased and the level of albumin decreased in catfish subjected to RU but the leakage of these liver indices into the plasma became more pronounced in the catfish exposed to RU + cadmium when compared to the control group as the concentration of cadmium increases (Table 3A). Similarly, levels of plasma urea and creatinine which are used for estimating kidney dysfunction were also elevated in the catfish exposed to RU and significantly increased in the plasma of catfish exposed to RU + cadmium at increased cadmium concentration when compared with control (Table 3B).

DISCUSSION

Although no literature data have given any insight into the possible toxicological potentials of interacting RU + cadmium in aquatic organisms, the sub-lethal concentrations chosen for RU and cadmium administered to some sets of catfish cause obvious signals of toxicity. Since tissue damage associated with toxicity has been related to oxidative stress, enzymic and non-enzymic antioxidant status of the kidney and liver were evaluated in exposed catfish.

Lipid peroxidation is usually measured as a level of thiobarbituric acid reactive substances (TBARS) which is damaging because the formation of TBARS generates a cascade of free-radical reactions that can greatly alter the physicochemical properties of membrane lipid bilayers, resulting in severe cellular dysfunction (Catala, 2006). TBARS production has therefore been frequently used to analyze the effect of pollutants (Livingstone, 2001; Lushchak *et al.,* 2009 Lushchak, 2011; 2014). In this light, an increase in the level of TBARS generated at an increased concentration of RU + cadmium as observed in the present study (Table 1A) suggests the participation of free radical-induced oxidative cell injury and can cause more harm to the cellular system or impair oxidative defenses of the catfish. However, the results gotten here are similar to an earlier report by Modesto and Martinez (2010) that glyphosate exposure caused an increase in lipid peroxidation and also impair the oxidative defense system of *Prochilodus lineatus*. To gain more insight into the effect of RU + cadmium on the exposed catfish, an evaluation of the level of inherent antioxidant properties of the catfish tissues is necessary.

One of the methods to evaluate the antioxidant property of the tissues of catfish is to determine their ability to scavenge free radicals. DPPH (2,2-diphenyl-1-picrylhydrazyl) is described as a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Prior *et al*., 2005; Garcia-Moreno, 2014) since antioxidants can readily donate their hydrogen to DPPH. Apparently, a decrease in the ability of the catfish tissues to scavenge DPPH free radicals as observed (Table 1B) showed a reduction in the inherent antioxidant capacity of the tissues of the exposed catfish to scavenge DPPH free radicals thereby increasing the free radicals produced in the cells of the catfish which consequently induce oxidative stress in the exposed catfish. However, these findings from the DPPH radical scavenging ability of these pollutants call for more antioxidant assays for further investigation.

Hence, ferric reducing antioxidant property is another way to evaluate the antioxidant property of tissues of catfish and is a determination of the ability of catfish tissues to reduce iron (III) due to inherent antioxidant capacity in them. Transition metals such as iron are known to be important in the proper functioning of most biological systems and effective reductive conversion of Fe^{3+} to Fe^{2+} may be considered an antioxidant mechanism. Hence, the reduction in the ferric reducing antioxidant properties (Table 1C) of the catfish tissues in converting Iron III to Iron II after exposure may suggest an onset of oxidative stress. Different studies have reported that there is a direct correlation between antioxidant activities and the reducing powers of some bioactive compounds (Duh *et al.,* 1999; Jemil *et al.,* 2008).

Moreover, Glutathione (γ-glutamylcysteinylglycine, GSH) is a sulfhydryl (-SH) antioxidant, an antitoxin and an enzyme cofactor whose intracellular content is a function of the balance between use and synthesis. The depletion of GSH (Table 1D) which became more pronounced during the interaction of glyphosate with cadmium as observed here may be attributed to the alterations in the intracellular level of GSH as Woods and Ellis (1995) and cited by Kade and Rocha, 2010) reported that the presence of heavy metals was discovered to alter and deplete GSH level in catfish. Consequently, this decreased GSH level diminishes the antioxidant capacity of the catfish which eventually induces oxidative stress thereby exposing the cells to degenerative syndromes (Jones *et al.*, 2000; Nogueira *et al.*, 2004).

However, since RU + cadmium exhibited pro–oxidant activities in the redox and antioxidant indices earlier investigated, it is reasonable to speculate that redox-sensitive proteins may likely be their primary target. Therefore, it is necessary to evaluate the oxidative effect of these toxicants on redox-sensitive enzymes in the exposed catfish tissues.

δ-ALA-D is a sulfhydryl-containing enzyme that plays a fundamental role in most living aerobic organisms by participating in heme, chlorophyll and corrin biosynthesis (Jaffe, 1995; Rocha *et al*., 1995; Kade *et al.*, 2009c). It catalyzes the condensation of two molecules of ð -aminolevulinic acid (ALA) to the formation of porphobilinogen which is a heme–precursor (Jaffe, 1995). δ -ALA-D inhibition as observed in the kidney and liver of catfish exposed to RU + cadmium (Table 2A) may impair heme biosynthesis (Kade *et al.*, 2008) and can result in the accumulation of ALA, which may consequently affect the aerobic metabolism and may have some prooxidant activity (Emanuelli *et al.,* 2001; Kade *et al.*, 2008). However, the results gotten of this research work were in contrast to what was gotten in previous work by Addison *et al.* (1990) where ALA-D distributions in mitochondria and cytosol of the liver and kidney of rainbow trout were investigated and was reported that lead accumulation in liver and kidney does not inhibit the ALA-D activity.

NTPDase (Nucleoside triphosphate diphosphohydro-lases) are well-characterized ectoenzymes in the central nervous system (Rocha *et al.,* 1990; Kade and Rocha, 2010) and is related closely to the presence of urinoreceptors on the cells in tissues. This enzyme hydrolyzes ATP and ADP resulting in the formation of nucleoside monophosphates thereby playing a crucial role in preventing the activation of platelets and their aggregation (Gendron *et al.,* 2002). Therefore, an increase in the activity of the NTPDase in catfish exposed to glyphosate at increasing cadmium as recorded depicted a massive leakage of nucleotide due to cell lysis and may also subsequently result into cell death through activation of platelets and their aggregation.

5'- nucleotidase is an enzyme that catalyzes the hydrolysis of nucleoside-5'- monophosphates to nucleosides and inorganic phosphate. The enzyme is widely distributed in animal tissues and the activity in serum is caused by its released from the membrane of liver cells by bile salts and has been used as a marker for liver impairment. Hence, an increase in the activity of 5'Nucleotidase level result in intra- or extra-hepatic obstruction which are harmful to the liver and kidney of exposed catfish and culminates into eventual liver/cell damage (Kim *et al.,* 2005). However, since an increase in the activity of 5'-Nucleotidase depicted liver impairment, it is essential to further validate this observation by evaluating liver function parameters in the plasma of the exposed catfish.

Exposure of catfish to RU with cadmium caused evident signs of liver injury as indicated by the activities of plasma AST, ALT, ALP, bilirubin and albumin relative to their normal levels (Table 3). This may be associated with the leakage of these indices from the liver cytosol into the bloodstream (Navarro *et al*., 1993; Kade and Rocha, 2010), which indicates the hepatotoxic effect of interacting RU with cadmium in an aquatic medium.

It is also noteworthy that interacting RU with different concentrations of cadmium in catfish alters the plasma levels of urea and creatinine which are considered significant markers of renal dysfunction (Almdal and Vilstrup, 1988; Kade and Rocha, 2010).

In conclusion, RU with cadmium on catfish displayed a likely additive toxicological effect that depletes the antioxidant capacity of the catfish tissues as well as the activity of crucial but redox-sensitive enzymes. It also has deleterious effects on liver and kidney functions of the catfish, hence a need for concerted efforts to check the use of herbicides as well as electronic waste management to reduce the additive toxicological impact of these agents on aquatic life, fish quality and health of humans, the ultimate consumers.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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