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EFFECT OF LEAD AND CHROMIUM ON THE TOXICITY OF GLYPHOSATE HERBICIDES TO AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL 1822)

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ABSTRACT: *There is a growing increase in pollution of the aquatic ecosystems with a wide range of chemicals including herbicides and heavy metals input through anthropogenic activities, leading to disruption of ecological balance. This study used a static toxicity bioassay to examine the interaction between heavy metals (Pb and Cr) and organophosphorus herbicides (analytical grade glyphosate, commercially formulated - Roundup and Forceup) on African catfish (Clarias gariepinus BURCHELL 1822). Lethal concentrations (LC_{50}) for roundup (RU), forceup (FU), analytical grade glyphosate (AGG), chromium (Cr), and lead (Pb) were 17.23 mg/l, 4877 mg/l, 131.12 mg/l, 21.16 mg/l, and 74.82 mg/l respectively. In addition, fish exposed to varying concentration of chromium (5.0 mg/l and 7.5 mg/l) and lead (20 mg/l and 25 mg/l) with glyphosate (RU: 10 mg/l; FU: 4300 mg/l; and AGG: 100 mg/l) for 96 hours were examined for biochemical parameters. Blood plasma examined for liver and kidney profile indices showed that kidney functions' parameters (protein, albumin, AST-aspartate aminotransferase, ALT-alanine aminotransferase and Bilirubin) and liver functions parameters (urea and creatinine) increased significantly ($p<0.05$) when glyphosates combined with lead and chromium treated groups compared with the control group. This study therefore, showed that low concentrations of combined toxicants of glyphosates and heavy metals (Cr and Pb) will synergistically induce deleterious effects on the liver and kidney of Clarias gariepinus.*

KEYWORDS: Herbicides, Clarias gariepinus, Toxicity, Glyphosates, Heavy metals.

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INTRODUCTION

Herbicide use has increased over time, mostly as a result of increased agricultural activity brought on by higher food demand owing to population growth (Radosevich *et al*., 2007). Herbicides are chemicals that have industrial and agricultural uses; they have the power to kill unwanted plants. Because of their toxicity, they may impact negatively on the health of human, plants, and aquatic lives like fish (Adedeji & Okocha, 2012; Fishel *et al.*, 2013). Herbicide residues have been reported to be present in biotic habitats of the environment, including the soil and water body (Aiyesanmi & Idowu, 2012; Ibigbami *et al*., 2015; Adegbite *et al*., 2020). Herbicides in agricultural wastes are washed and carried away by rain and flood to a closeby aquatic environment, where they would cause damage to aquatic organisms, especially fish. In very small concentrations, several herbicides are harmful to fish. It is known that a number of herbicides can lead to liver and kidney diseases in fish, hence herbicides along with other pesticides are to be blamed for the widespread fish extinction (Adedeji & Okocha, 2012; Fishel *et al.*, 2013, Hogan 2014; Ibigbami *et al*., 2016).

Glyphosate (N-(phosphonomethyl) glycine) is the most commonly used herbicide worldwide (Piccolo *et al*., 1992); it is a broad-spectrum, non-selective, organophosphorus herbicide which contains carboxyl, amino, and phosphonate functional groups (de Jonge *et al*., 2001). Glyphosate is one of the herbicides used to suppress trees, shrubs, broad-leaved weeds, annual and perennial grasses, and other species (Okayi *et al*., 2010). Due to its limited persistence, it is additionally arguably the most significant herbicide ever developed. Nevertheless, some of the surfactants used for the formulation of glyphosate are harmful to aquatic organisms and should not be found used in aquatic environments (Tu *et al*., 2001).

Fish are directly exposed to chemicals from agricultural production through surface runoff or indirectly through the ecosystem food chain, making them excellent candidates to act as bioindicators of environmental pollution and to assess the potential risk associated with contamination in the aquatic environment (Lakra & Nagpure, 2009). In the conduct of experimental investigations like toxicological and some pharmacological studies, fish is regarded as a model organism due to the potential for applicability of the findings from these studies to human health and other environmental health issues (Govind, 2011).

Heavy metals and other types of pollutants build up in aquatic ecosystems as a result of the continuous inflow of agricultural waste. Metal contamination in the aquatic environment slowly poses a possible harm to aquatic creatures, due to their bioaccumulative and non-biodegradable characteristics (Aiyesanmi, 2006; Oyakhilome *et al*., 2013). Fish metabolic, physiological, and biochemical systems are being adversely affected by a variety of sources of heavy metals, including industrial, agricultural, and anthropogenic activities (Heath, 1987; Dethloff *et al*., 1999; Atli & Canli, 2007). Chromium is among other deleterious heavy metals which is considered as potent toxicant to fish as well as other aquatic life even at low concentration, showing its effects at physiological, histological, biochemical, enzymatic and genetic levels (Heath, 1987). Numerous researchers have also observed that Cr has negative harmful effects on biochemical toxicity, immunological response and nonspecific immunity, and genotoxicity in numerous fish species (Sastry & Sunita, 1983; Lemos *et al*., 2001; Maples & Bain, 2004; Prabakaran *et al*., 2007).

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A number of researchers have previously reported the toxic effects of herbicides in fish. Ayoola (2008) studied the acute toxicity, behavioural changes and histopathological effects of glyphosate in *Clarias gariepinus* and *Oreochromis niloticus*. Similarly, Glusczak *et al*. (2011) also reported that acute exposure of *Leporinus obtusidens* to glyphosate affected its oxidative parameters. Similar studies have reported different toxic endpoints such as genotoxicity and cytotoxicity of glyphosate in fish blood (Ayanda *et al.*, 2015; Moreno *et al.*, 2014). Ogamba *et al*. (2011) studied the effects of paraquat dichloride on metabolic parameters including creatinine, albumin, total bilirubin, total urea and total protein in muscle and gill tissues of *C. gariepinus* and reported a decrease in the values of these metabolic parameters, suggesting herbicide-induced stress in the fish. Therefore in this current study, an attempt has been made to investigate the synergistic effects or otherwise of chromium and lead on the toxicity of glyphosate herbicides to the African Catfish, *Clarias gariepinus*

MATERIALS AND METHODS

Test Chemicals

Test chemicals used in this study include analytical grade glyphosate (AGG) obtained from CanSpec Chemical Company (China), while two different brands of commercially formulated grade glyphosate, Roundup (RU) and Forceup (FU) herbicide were obtained at Oba's market, Akure, Ondo State, Nigeria. Aspartate aminotransferase (AST) kit, Alanine aminotransferase (ALT) kit, ALP kit, Bilirubin kit, Albumin kit, total protein kit, Urea kit and Creatinine kit were sourced from Randox Laboratories, Crumlin, Co. Antrim, UK. Lead chloride, potassium dichromate and all other chemicals of analytical grade were obtained from a chemical store in Akure, Nigeria.

Fish Sample

Clarias gariepinus juveniles used in this study averaged 20 ±0.5g of body weight and of a standard body length of 15 ± 2 cm. which was purchased from a reputable fish farm in Akure, Ondo State, Nigeria. Live fish samples were transferred into buckets containing water from the fish farm into glass aquarium tanks in the laboratory. The fishes were held in a 1000 L tank for a fourteen day acclimatisation period in de-chlorinated water and fed twice daily.

Preparation of Test Solutions and Exposure of Fish

After acclimation, 10 fish per tank were randomly distributed into experimental tanks for range finding test. Each group was exposed to logarithmic concentrations (1, 10, 100 and 1000 mg/L to determine suitable concentration range for the definitive experiment. The range finding test was carried out separately and individually using analytical grade glyphosate, roundup, forceup, chromium and lead (Ayoola, 2008). The concentrations obtained through the range test were used for the definitive experiment to determine the 96 hours' acute toxicity value expressed as LC_{50}.

Acute Toxicity Study

Acute toxicity of glyphosate herbicides to *C. gariepinus* was conducted by exposing the fish to lethal concentrations of the herbicides over a 96-hour period at room temperature of 25ºC (De

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Lorenzo *et al.*, 2001). Varying concentrations of toxicants used were AGG (100 mg/l, 125 mg/l, 150 mg/l, 175 mg/l 200 mg/l), RU (10 mg/l, 11.5mg/l, 13 mg/l, 14.5mg/l 16 mg/l), FU (4600 mg/l, 4800mg/l, 5000mg/l, 5200 mg/l,5400 mg/l), Pb^{2+} (70 mg/l, 75 mg/l, 80 mg/l, 85 mg/l, 90 mg/l), and Cr^{6+}(25 mg/l, 30 mg/l, 35 mg/l, 40 mg/l, 45 mg/l, 50 mg/l). Fishes were monitored for 96 hours and the total number of dead fishes were recorded daily. A control group containing fish samples that were not exposed to any toxicant was simultaneously monitored. During the period of the test, the fish were not fed, and test solutions were not changed but adequately aerated. Also, physicochemical parameters like temperature, pH, dissolved oxygen, conductivity and turbidity were observed daily. The fish behaviour and mortality rate were observed and recorded daily for four days and dead fishes were instantly evacuated from the aquarium. Thereafter, the 96 hours LC_{50} of each toxicant were calculated from the mortality rate following the probit analysis method after Finney 1980 and cited by Veeraiah *et al*. (2015).

Chronic Toxicity and Biochemical Assay

Chronic toxicity study was conducted to investigate the effect of long-term exposure of fish to sub-lethal concentrations of glyphosate in the presence of lead and chromium each as co-toxicants. Twenty fish were independently exposed to toxicant solution for 21 days in a plastic aquarium. The experiment was set up in duplicate and grouped accordingly. The treatment solutions in the aquaria were not changed till the end of the study. After the stipulated period of exposure, fish samples were prepared for bioassay. Blood samples were collected through the dorsal vein using a 5 mm syringe into EDTA bottles. The blood samples were centrifuged at 4000 rmp for 10 min to be removed and transferred into a clean dry eppendorf tube. The serum samples were then stored in the refrigerator and kept frozen at -20$^{\circ C}$ prior to the bioassay.

Evaluating the possible liver injury induced by the exposure to toxicant, the activities of AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alanine phosphatase), total protein, albumin and total bilirubin as liver biomarkers for hepatotoxicity in plasma, were determined using commercial kit sourced from Randox Laboratories. Likewise, evaluating the possible renal injury induced by the exposure of the fish to the toxicants, the activities of plasma creatinine and urea were determined using commercial kits. Blood samples used were collected and prepared as described above

Statistical Analysis

Data collected from the study was analysed using Statistical Package for Social Sciences (SPSS version 16) and level of significance was set up at $p \leq 0.05$ using descriptive statistics (mean and standard error of means) subjected to analysis of variance (ANOVA) .

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RESULTS AND DISCUSSION

Table 1: Physicochemical Parameters of the Experimental Water

Parameter	Value
Temperature	25.7^{Oc}
Dissolved Oxygen	6.5± 0.1
Conductivity	210 µs/cm
pH	6.63

Acute Toxicity Study

Table 2 presents the acute toxicity at 96 h (LC_{50}) of glyphosate herbicides (analytical and commercially formulated grade) and heavy metals (chromium and lead) to *C. gariepinus.*

Table2: Acute Toxicity (LC_{50}) of Glyphosate Herbicides, Chromium and Lead to *C. gariepinus*

Toxicants	LC_{50}(mg/l)
RU	**17.23**
F	**4977**
AGG	**131.12**
Cr^{6+}	**21.16**
pb^{2+}	**74.82**

The results for analytical grade glyphosate (AGG) revealed that *C. gariepinus* is sensitive to the herbicides, as concentration leading to 50% mortality of the test organism (LC_{50}) was estimated to be 131.12 mg/L. On the other hand, *C. gariepinus* is highly sensitive to Roundup (RU) with estimated LC_{50} of 17.23 mg/l, while less sensitive to Forceup with LC_{50} of 49.77 mg/l. This observation shows that the surfactant used in the formulation of Roundup was a key factor in its high toxicity level as reported by Kennedy (2017). It could also be inferred that the surfactant used for Forceup formulation has a very significant reduction in the toxicity of glyphosate. It has opined that the level of toxicity of any pesticide depends on its bioaccumulation, the chemistry of the compound forming the pesticide and the reactions of the organisms receiving the toxicant (Neighbor & Richardson, 1980). The LC_{50} value for AGG, though similar, was lower than 160 mg/L reported by Uchida *et al*. (2011). In a comprehensive study by Wan *et al*. (1989) comparing glyphosates, the toxicity of analytical grade glyphosate could be said to be relatively mild as a pesticide with mild toxicity and environmentally friendly.

For the tested heavy metals, the LC_{50} value of chromium to C. *gariepinus* was calculated to be 21.16 mg/l, which was lower to 35.50 mg/l reported by Johnson *et al.* (2015) for Catfish *Clarias batrachus* (Linn.) and 30.90 mg/l reported by Kole *et al*. (2014) for freshwater Catfish *Anabas testudineus* (Bloch). This indicates that the toxicity effect of chromium is a function of fish species, which is higher in C. *gariepinus* than other studied fish. The LC_{50} value for Lead in this study was calculated to be 74.82 mg/l of Pb^{2+} in $PbCl_2$, higher than 50.12 mg/ L reported by Babatunde and Idris (2017) for *C. gariepinus* using $Pb(NO_3)_2$. This also implies that

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toxicants' moiety also plays a significant role in their toxicity level. Okareh and Akande (2015) also reported an LC_{50} of 80.5 mg/l closer to what was reported in this study using lead chloride. Lethal concentration (LC_{50}) obtained for chromium was significantly lower ($p<0.05$) than that of lead suggesting that chromium is more toxic to *C. gariepinus* than lead. Higher LC_{50} connotes lesser toxicity since higher concentration is required to achieve a 50% mortality of test organisms (Hedayati *et al.*, 2010).

Chronic Toxicity Study

The results of biochemical parameters obtained after sublethal exposure of the African catfish *Clarias gariepinus* to the varying concentrations of lead and chromium with glyphosates (both commercial and analytical grade) for 96 hours are presented in **Tables 2** and **3**. The biochemical assay includes the liver and kidney profile test which were conducted on the serum samples from the exposed fish. The results demonstrate that there are significant differences ($p<0.05$) in toxicity effects with varying concentrations of lead and chromium in combination with fixed concentrations of the individual glyphosate herbicides (RU, FU and AGG), as revealed by the biochemical changes in the liver profile indices of the juvenile catfish. The exposure of the catfish to concentrations of 20 mg/l and 25 mg/l of $Pb^{2+}$with the herbicides, as well as Cr^{6+}(5.0 mg/l and 7.5mg/l) resulted in the significant increase ($p<0.05$) in ALT (Alanine transaminase) and AST (Aspartate transaminase) levels when compared with the control. The liver is a crucial detoxification organ, and changes brought on by toxins are frequently linked to a degenerative necrotic disease (Ayanda *et al.*, 2015).

Table 3: Liver Profile in Serum of Catfish Exposed to Chronic Combination of Lead with Glyphosates

Toxicant	Concentration (mg/l)	ALT (u/l)	AST (mMol/l)	Bilirubin (mMol/l)	Albumin (u/l)	Total protein(g/l)
Control	0.0	8.0±0.0^{f}	8.5±0.0^{a}	5.0±0.0^{g}	17.5±0.7^{g}	22.5 ±0.0^{a}
	RU(10.0) alone	9.0± 0.0^{a}	10.0±0.0^{abc}	7.5± 0.7^{b}	15.5 ±0.7^{f}	41.5± 0.7^{fg}
RU+Pb^{2+}	RU +Pb^{2+} (20)	11.5 ± 0.7^{b}	12.5± 0.7^{ac}	10.5 ±0.7^{c}	11.0 ±0.0^{d}	46.0± 0.0^{j}
	RU + Pb^{2+} (25)	12.8± 0.3^{d}	14.5± 0.7^{e}	13.5 ±0.7^{d}	6.5 ±0.7^{b}	49.0± 1.4^{j}
Control	0.0	8.0 ±0.0^{f}	8.5 ±0.0^{a}	5.0 ±0.0^{g}	17.5 ±0.7^{g}	41.5 ±0.7^{a}
FU+Pb^{2+}	FU(4300) alone	13.5± 0.7^{de}	12.5± 0.7^{cd}	6.0± 0.0^{ab}	12.5 ±0.7^{e}	48.5 ±0.7^{j}
	FU+Pb^{2+} (20)	17.8 ±1.1^{g}	14.5 ±0.7^{e}	15.0±1.4^{defg}	9.9 ±0.1^{cd}	52.0± 1.4^{k}
	FU +Pb^{2+} (25)	20.5± 0.7	16.5 ±0.7^{g}	19.0 ±1.4^{i}	5.0± 0.0^{a}	54.5± 0.7^{i}
Control	0.0	8.0 ±0.0^{f}	8.5± 0.0^{a}	5.0± 0.0^{g}	17.5± 0.7^{g}	41.5± 0.7^{a}

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AGG+ Pb^{2+}	AGG (100) alone	10.5± 0.7^{g}	15.5 ±0.7^{ef}	11.0± 1.4^{c}	14.3 ±0.2^{f}	28.5 ±$0.^{b}$
	AGG +Pb^{2+} (20)	15.5± 0.7	18.5± 0.7^{g}	15.7 ±0.3^{defg}	10.8± 0.2^{d}	35.0 ±0.0^{c}
	AGG +Pb^{2+} (25)	18.5± 0.7^{gh}	21.0 ±0.7^{h}	17.0 ±1.4^{gi}	9.8± 1.3^{cd}	38.0 ±1.4^{d}

Different alphabets show significant difference ($p<0.05$)

Table 4: Liver Profile in Serum of Catfish Exposed to Chronic Combination of Chromium with Glyphosates

Toxicant	Concentration (mg/l)	ALT (u/l)	AST (mMol/l)	Bilirubin (mMol/l)	Albumin (u/l)	Total protein (g/l)
Control	**0.0**	**8.0± 0.0^{f}**	**8.5± 0.0^{a}**	**5.0± 0.0^{g}**	**17.5± 0.7^{g}**	**22.5 ±2.1^{a}**
RU+Cr^{6+}	**RU(10.0) alone**	**9.0 ±0.0^{a}**	**10.0 ±0.0^{abc}**	**7.5± 0.7^{b}**	**11.0± 0.0^{d}**	**41.5± 0.5^{fg}**
	RU+Cr^{+6} (5.0)	**14.5± 0.7^{ef}**	**11.2± 0.6^{b}**	**19.5 ±0.7^{j}**	**11.0 ±0.0^{d}**	**44.5 ±0.0^{k}**
	RU+Cr^{6+}(7.5)	**17.7± 0.4^{g}**	**12.9± 0.4^{cd}**	**21.5 ±0.7^{g}**	**8.5± 0.7^{c}**	**49.0 ±0.1^{k}**
Control	**0.0**	**8.0 ±0.0^{f}**	**8.5 ±0.0^{a}**	**5.0± 0.0^{g}**	**17.5± 0.7^{g}**	**22.5± 2.1^{a}**
FU +Cr^{6+}	**FU(4300) alone**	**13.5± 0.7^{d}**	**12.5 ±0.7^{cd}**	**6.0 ±0.0^{ab}**	**12.5± 0.7^{e}**	**48.5± 0.7^{j}**
	FU+Cr^{6+}(5.0)	**20.5 ±0.7^{j}**	**18.5± 0.1^{g}**	**16.1 ± 1.1^{g}**	**9.0 ± 0.1^{c}**	**49.0 ±0.1^{k}**
	FU+Cr^{6+}(7.5)	**23.5± 0.7^{g}**	**20.5 ±0.7^{h}**	**17.7 ±0.4^{deg}**	**8.5± 0.2^{c}**	**53.5 ±0.2^{k}**
Control	**0.0**	**8.0 ±0.0^{f}**	**8.5± 0.0^{a}**	**5.0 ±0.0^{g}**	**17.5± 0.7^{g}**	**22.5 ±2.1^{a}**
AGG+Cr^{6+}	**AGG(100) alone**	**10.5 ±0.7^{g}**	**15.5 ±0.7**	**11.0 ±1.4^{c}**	**14.30 ±0.2^{e}**	**28.5± 0.0l**
	AGG+Cr^{+6}(5.0)	**17.5± 0.7**	**15.5 ±0.7^{ef}**	**14.0 ±0.0^{d}**	**13.0 ±0.0^{e}**	**39.5 ±0.7^{def}**
	AGG+Cr^{+6}(7.5)	**19.5 ±0.7^{ih}**	**20.0 ± 1.4^{h}**	**17.5± 0^{gj}**	**11.0 ± 1.4^{d}**	**42.5 ±0.7^{h}**

The most prevalent serum biomarkers of liver damage are Aspartate transaminase (AST) and Alanine transaminase (ALT), with levels significantly rising as metal concentrations and herbicide exposure increased. This was seen in fish tissues after exposure to herbicides and

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chromium in admixture, as reported by Kole *et al.* (2014), thus suggesting a synergistic effect between the various toxicants.

An impaired liver function is known to affect metabolism, which also affects the concentrations of waste products. Exposure of fish to different contaminants have been known to present substantial variability in most physiological and biochemical variables (Sadauskas-Henrique *et al.*, 2011; Nwani *et al.*, 2013). AST and ALT have been used extensively in a lot of ecotoxicological studies to assess stress induced by various contaminants in the environment (El Sayeed, 2007). Their increase means enhanced transamination, a sensitive indicator of stress imposed by the herbicide and may provide an overview of dysfunction and structural damage to such vital organs as the spleen, kidney and liver (Bhattacharya *et al*., 2008; Banaee *et al*., 2011).

Previous studies show that increased transamination under pesticide challenge is related to fish needing more energy (Vani *et al*., 2012). Increased activities were also observed in common carp, *Cyprinus carpio* exposed to a related herbicide and in other fishes exposed to different environmental contaminants (Prusty *et al*., 2011; Saravanan *et al*., 2012). The accumulation and binding of the herbicide in the tissues of the exposed fish may cause stressful situations, which would then cause the transamination pathway to be elevated to combat the ensuing energy crisis. Albumin, bilirubin, and total protein are other indicators that indicate liver injury. When different lead concentrations of 20 mg/l and 25 mg/l interacted with various herbicides, the bilirubin level (Tables 2 and 3) showed significant elevation ($p<0.05$) compared to the control. Additionally, significant increase ($p<0.05$) was observed with increase in lead concentrations. Similarly, varying chromium concentrations (5.0 mg/l and 7.5 mg/l) in admixture with the glyphosate herbicides showed significant increase ($p<0.05$) in bilirubin level compared with the control, as well as increase in metal concentrations. Diseases of the liver and/or biliary tract are the cause of elevated serum total and conjugated bilirubin concentrations (Iyanagi & Accoucheur, 1998; Okonkwo & Ejike, 2011). This may be due to disruption of the hepatic architecture such that the conjugation of bilirubin and excretion of bilirubin is altered. On the other hand,the albumin level significantly decreases ($p<0.05$) with increase in concentrations of lead and chromium in admixture with the different glyphosate herbicides when compared with the control. Similar decrease was observed with increase in the metal concentrations, which was more pronounced with Pb than Cr. This decrease in albumin level may lead to impairment of vascular transport for nutrients and reduced antioxidant capacity.

There was a significant increase ($p<0.05$) in concentration of serum total protein level compared with the control when the organism was exposed to varying concentrations of lead and chromium in admixture with the herbicides. The increase in serum total protein level may be attributed to liver dysfunctions and disturbance in protein biosynthesis (Abdel-Tawab *et al*., 2011). However, some previous studies reported protein reduction in tissues of fish exposed to various environmental contaminants (Crestani *et al*., 2007; Dogan & Can, 2011). Borges (2007) and Fırat *et al*. (2011) reported reduced total protein in catfish on exposure to pesticide and it was linked to adverse effects of herbicide which caused loss of protein by either reduced protein synthesis or increased proteolytic activity or degradation. This change may indicate systematic and gradual dysfunction of the liver. Changes in protein levels have been generally used as sensitive indicators of environmental stress in aquatic organisms (Dogan & Can, 2011).

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The levels of urea and creatinine in serum in each combination of the different herbicides with lead and chromium at different concentrations were significantly different ($p< 0.05$) compared with the control (**Tables 5 and 6**). Similarly, increased metal concentration from 20 mg/l to 25 mg/l for lead and 5.0 mg/l to 7.5 mg/l for chromium resulted in significant increase ($p<0.05$) in the urea and creatinine levels in the serum, especially with FU and AGG. The observed high levels of urea and creatinine may be caused by increased tissue breakdown, diet, impaired excretion, and increased synthesis, decreased kidney clearance of these chemicals from the urine, or decreased degradation of these substances (Adham *et al*., 2002). The marked increase in blood urea nitrogen could be attributed to impaired kidney excretion of urea, since ammonia is urea in fish, the end product of protein degradation. This explanation is supported by rising blood creatinine levels, which are a more sensitive and specific indicator of impaired kidney function (Cameron, 1996). Mahmoud *et al*. (2013) recorded an increase in the serum creatinine level in *C. gariepinus* after exposure to lead. The current study revealed an increase in serum creatinine level when the metals are combined with the herbicides, in contrast to the studies by Okonkwo and Ejike (2011), which found that the recorded values were not significantly different ($p>0.05$) from the control. It is important to note that all toxicants have several biological effects that might harm an organism's structure and function and eventually jeopardise its existence.

Table 5: Urea and Creatinine in Plasma of Catfish Exposed to Lead–Glyphosate Combination

Experimental Toxicant	Concentration (mg/l)	Urea (mMol/l)	Creatine (mMol/l)
Control	0.0	2.0 ± 0.1^{a}	34.5 ± 0.7^{a}
RU +PB^{2+}	RU (10.0) alone	2.3 ± 0.0^{b}	39.5 ± 0.7^{b}
	RU +Pb^{2+} (20.0)	3.0 ± 0.2^{d}	43.5 ± 0.7^{c}
	RU + Pb^{2+} (25.0)	3.3 ± 0.1^{ef}	47.5 ± 0.7^{d}
Control	0.0	2.0 ± 0.1^{a}	34.5 ± 0.7^{b}
FU +PB^{2+}	FU (4300) Alone	2.7 ± 0.1^{c}	47.01 ± 1.4^{d}
	FU + Pb^{2+} (20.0)	3.7 ± 0.0^{k}	72.5 ± 2.1^{h}
	FU +Pb^{2+} (25.0)	4.7 ± 0.1^{i}	95.0 ± 1.4^{k}

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Control	0.0	2.0± 0.1[a]	34.5 ± 0.7[b]
AGG + PB^{2+}	AGG (100) Alone	2.5± 0.1[c]	48.0 1.4[d]
	AGG +Pb^{2+} (20.0)	3.1 ±0.1[de]	73.0± 1.4
	AGG +Pb^{2+} (25.0)	3.4± 0.1[f]	81.0 ±1.4[i]

Table 6: Urea and Creatinine in Plasma of Catfish Exposed to Chromium–Glyphosates Combination

Experimental Toxicant	Concentration (mg/l)	Urea(mmol/l)	Creatine (mmol/l)
Control	0.0	2.0 ±0.1[a]	44.5 ±0.7[a]
RU +Cr^{6+}	RU (10.0) alone	2.3± 0.0[b]	39.5±0.7[b]
	RU + Cr^{6+} (5.0)	3.4 ±0.0[fg]	60.0± 0.0[cg]
	RU+Cr^{6+} (7.5)	3.6 ±0.1[hi]	60.0 ±0.0[g]
Control	0.0	2.0± 0.1[a]	44.5 ±0.7[a]
FU +Cr^{6+}	FU (4300) alone	2.7± 0.1[c]	47.0 ±1.4[d]
	FU+Cr^{6+} (5.0)	3.3± 0.1[f]	77.0 ±1.4[i]
	FU+Cr^{6+} (7.5)	4.0 ±0.1[k]	108.5 ±0.7[i]
Control	0.0	2.0 ± 0.1[a]	44.5 ±0.7[a]
AGG +Cr^{6+}	AGG(100) Alone	2.5 ±0.1[c]	48.0 ± 1.4[d]
	AGG +Cr^{6+} (5.0)	3.4 ±0.0[f]	51.5± 0.7[f]
	AGG + Cr^{6+} (7.5)	3.6 ±0.1[hj]	64.0 ±0.7[h]

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IMPLICATION TO RESEARCH AND PRACTICE

The implication of this study showed a synergistic influence between herbicides and heavy metals contaminants on African catfish *Clarias gariepinus*. Therefore, due to the nutritional benefits derived from consumption of fish, the design and proper management of earthen ponds to prevent co-contamination should be considered. Field bio-monitoring efforts should be designed on a frequency to detect the bioavailability of the metals and early warning indicators of toxicity addressed. There should also be a standard baseline toxicity formulation range policy for all the commercial glyphosate herbicides available in the markets to cater for their different usage.

CONCLUSION

This study investigated the effect of lead and chromium on the toxicity of glyphosate-based herbicides to African catfish. The in-vivo (toxicological) study was used to determine the toxic effects and the mechanism of toxicity. Exposure of catfish to the different contaminants have been known to present substantial variability in most physiological and biochemical indices, with the current study showing that the toxicant altered the entire biochemical indices. Thus, such changes in biochemical levels under the effect of heavy metals (lead and chromium) toxicity in combination with glyphosate based herbicides resulted in the impairment of energy requiring vital processes and hence, which gives an idea about the health status of the fish. The cumulative effect of these different toxicants is that lead and chromium in combination with different types of glyphosate herbicides could result in synergistic toxic effects even at low concentrations, dangerous to the aquatic ecosystem.

FUTURE RESEARCH

Based on this work, there should be more studies to monitor the composition, degradability and toxicity of the herbicide in water and soil.

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