Research Article

Ionic Balance in Tissues of Hybrid African Catfish after Exposure to Chronic Levels of *Lepidagathis alopecuroides* Leaves Extracts

F. G. Obomanu, U. U. Gabriel and O. S. Edori*

[1]Department of Chemistry, Rivers State University of Science & Technology, P.M.B 5080, Port Harcourt 5000001, Nigeria
[2]Department of Fisheries and Aquatic Environment, Rivers State University of Science & Technology, P.M.B 5080, Port Harcourt, 5000001, Nigeria
[3]Department of Chemistry, Ignatius Ajuru University of Education, P.M.B. 5047, Rumuolumeni, Port Harcourt Rivers State, 5000001, Nigeria

Abstract

The effect of aqueous extract of *Lepidagathis alopecuroides* leaves (0.25, 0.50, 0.75, 1.00 and 1.25 mg/L) and a control (0.00 mg/L) was examined on the hybrid African catfish, *Heterobranchus longifilus* x *Clarias gariepinus* (mean total length, 29.96 ±2.23cm SD; mean weight, 207.83 ± 12.63g SD) under laboratory conditions to determine its effect on ionic balance in the fish. The test was conducted on a bio-renewal assay for twenty one days. The ions (sodium, potassium and calcium) were examined. The result showed that the plant extract caused changes in the ionic balance in the fish when compared to the control. There was a general increase in the levels of the ions in the different test media. The general trend was sodium> potassium> calcium in all the tissues examined. The most elevated ion was sodium in the muscle (797.50±52.35 mmol/L) at 1.25 mg/L concentration representing 108.68% elevation. Potassium was most elevated in the muscle (66.13±2.67 mmol/L) at the 0.75mg/L which represents 236.48% elevation. Calcium value was highest in the muscle (9.88±3.45 mmol/L) at the control. The trend for sodium showed that muscle > gill > liver > kidney > plasma, while that of potassium showed muscle > liver > kidney > gill > plasma and Calcium trend was muscle > gill > liver > kidney > plasma. The result showed that the plant extract is toxic to the fish and therefore, local fishermen should take precaution while applying it for fishing purposes.

Keywords: *Lepidagathis alopecuroides*, ionic balance, hybrid catfish, aqueous extracts, exposure

***Correspondence**
Author: O. S. Edori
Email: onisogen.edori@yahoo.com

Introduction

Man has resorted to the use of plants and plant products as replacement for the treatment and management of various ailments and diseases [1, 2] and as piscicides [3] without considering their toxic effects and other implications. Plants offer an inexhaustible source of structurally diverse biologically active substances [4] and are preferred to synthetic pesticides for safe and environmentally friendly application as piscicides for cleaning of ponds and catching fish [5, 6]. The preference for plants poison is due to the fact that they are less expensive, highly degradable, readily available, easy to handle and safe for mankind due to the fact that they do not leave any residues after application [7].

Piscicidal substances have been isolated from different parts of plants and the phytochemical screening of most of these plants have been found to possess divers toxic substances and chemical components such as rotenones, saponins, flavanoids, alkaloids, glycosides, tannins, oxalics [8]. Piscicidal plants such as *Tetrapleura tetraptera, Raphia vinifera, Parkia bioglobosa, Parinari polyandra and Khaya senegalenses* are commonly used by fishfolks to catch fish because they are highly potent against fishes [9].

The plant *Lepidagathis alopecuroides* is a tropical shrub belonging to the *Acanthaceae* family. It is predominantly found in the coastal countries of West Africa [10] and some areas in Brazil. The ground leaves of the plant when spread on mudflats at low tide immobilize mudskippers (*Periophthalmus papillio*) and other small aquatic animal occupying the sediments. *Lepidagathis alopecuroides* was found to possess antimicrobial activity against *M. luteus, P. aeruginosa, S. aureus, E. coli and Shigella spp* [10].

Different studies on *Lepidagathis alopecuroides* have shown that the plant caused dose dependent mortality and histopathological changes in the gills and intestine of *Periophthalmus papillio* after exposure for some hours [11], mortality and behavioural changes in *Clarias gariepinu*s and *Heterobranchus bidorsalis* [6], alterations in enzymatic

activities in the organs/ tissues of *Clarias gariepinus* and its hybrid [12, 13], and also altered the haematology, organ indices and plasma enzymes of*Clarias gariepinus* after intramuscular injection of aqueous leaf extracts into the muscles of the fish [14]. It also had negative effects on the sperm quality, fertility and hatchability in gravid *Clarias gariepinus* [15].

This study was carried out to investigate the effects of the plant *Lepidagathis alopecuroides* the ion (electrolytes) regulatory properties in the organs/tissues of a hybrid catfish after chronic exposure.

Materials and Methods

Tank raised hybrid catfish, *Heterobrachus longifilus* x *Clarias gariepinus* (mean total length, 29.96 ±2.23cm SD; mean weight, 207.83 ± 12.63g SD) were purchased from a private farm at Abuloma, Port Harcourt, Rivers State and transported to the Research Laboratory, Chemistry Department, Rivers State University of Science and Technology in aerated aquaria. The fish were acclimated individually in plastic aquaria with 25L volume of borehole water. The fish were fed 35% crude protein diet at one percent biomass once daily during the period of the acclimation and experiment. Fresh leaves of the plant *Lepidagathis alopecuroides* obtained from the wild were air-dried to constant weight in the laboratory and were subsequently powdered with an electric blender and stored in dry air tight containers.

Five graded concentrations (0.25, 0.50, 0.75, 1.00 and 1.25mg/L) and a control 0.00ml/L of the aqueous extract were prepared in quadruplicates after range finding test was conducted. Experimental fish were added singly in each of the aquaria and covered with netted material which has a slit at the middle to prevent escape of fish. The aquaria were washed daily to remove uneaten food and feacal matters. Water in the control and the test solutions were renewed daily.

At the end of the exposure (21days) blood was collected from the fish by inserting 21G size needle behind the anal fin. When the needle pierced the kidney, blood flowed freely into the syringe. The blood samples were transferred into heparinized bottles for electrolyte analysis. The fish were then killed with a blow on the head and dissected to remove the liver, kidney, gills and muscle tissues. A sample, 0.5g of each of the tissues was homogenized in 5ml de-ionised water. The homogenate was centrifuged at 3000rpm for 5min. The supernatant was decanted into a 5ml plain bottle and stored at -2^{o}C for electrolytes (ions) analysis. The electrolytes, sodium (Na^{+}), potassium (K^{+}) and calcium (Ca^{2+}) content were determined colorimetrically by the method of [16]. The data obtained were subjected to analysis of variance (ANOVA) and differences among means were separated by Duncan Multiple Range Test (DMRT) [17].

Results

In the plasma, sodium ion (Na^{+}) increased slightly in all the exposure concentrations, but the increase was not significantly different (P>0.05) from that of the control value. Levels of potassium ion (K^{+}) were either increased or decreased in the various test concentrations. Calcium ion (Ca^{2+}) showed non-significant (P>0.05) increase in all the exposure concentrations as against that of the control value (**Table 1**).

Table 1 Cations in the plasma of catfish hybrid exposed to *L. alopecuroides* for 21days (Mean±SD)

Conc. of L. alopecuroides (mg/L)	Na^{+} (mmol/L)	% of control	K^{+} (mmol/L)	% of control	Ca^{2+} (mmol/L)	% of control
0.00	135.50±5.57^{a}	100	2.20±1.16^{a}	100	2.80± 1.01^{a}	100
0.25	137.50 ±5.57^{a}	101.48	2.20.±1.16^{a}	100.00	2.35± 0.06^{a}	83.93
0.50	140.25±1.89^{a}	103.51	1.05.±0.66^{a}	47.73	2.35 ± 0.25^{a}	83.93
0.75	135.75 ± 3.95^{a}	100.18	3.50±1.55a	159.10	2.21± 0.46^{a}	79.02
1.00	138.00 ± 2.83^{a}	101.85	1.95± 1.17^{a}	88.64	2.23 ±0.33^{d}	79.46
1.25	139.25±3.30^{a}	102.77	3.50±2.34^{a}	159.10	2.25±0.13^{a}	80.36

Means with the same alphabet in the same column are not significantly different (P>0.05)

The levels of sodium ion in the liver were significantly (P>0.05) higher or lower than the control value. Potassium ion levels only decreased at 1.00mg/L concentration of the toxicant but increased in all the other test concentrations. The value of calcium ion increased slightly above the control value only at 1.00mg/L concentration. The decreased values observed in the other concentrations were significantly different (**Table 2**).

Table 2 Cations in the liver of catfish hybrid exposed to *L. alopecuroides* for 21days (Mean±SD)

Conc. of L. alopecuroides (mg/L)	K^+ (mmol/L)	% of control	Ca^{2+} (mmol/L)	% of control	Na^+ (mmol/L)	% of control
0.00	18.50±2.93^a	100	6.70±0.56^c	100	585.00± 29.64^{ab}	100
0.25	30.63 ±18.34^a	165.64	4.53.±1.86^b	67.54	591.50±140.71^{ab}	101.11
0.50	25.00±10.69^a	135.14	3.60.±2.38^{ab}	53.73	326.75±280.63^a	55.85
0.75	19.55±7.29^a	105.68	1.38±0.52^a	20.52	566.50±129.53^{ab}	96.84
1.00	16.53 ± 2.57^a	89.32	6.88± 1.03^c	102.61	667.75±89.00^b	114.15
1.25	27.03±12.23^a	146.08	3.00±1.41^{ab}	44.78	669.00±77.12^b	114.34

Means with the same alphabet in the same column are not significantly different (P>0.05)

In the kidney, the levels of sodium ion were significantly (P>0.05) altered, with lower values recorded at 0.50 and 0.75mg/L test concentrations. However, levels that were higher than control values were noted in other concentration. Potassium ion increased significantly (P>0.05) in all the exposure concentrations as against that of the control value. Calcium ion values increased in the lower concentrations (0.25 and 0.50mg/L) and at 1.0mg/L, but decreased below the control value at 0.75 and 1.25mg/L (**Table 3**).

Table 3 Cations in the kidney of catfish hybrid exposed to *L. alopecuroides* for 21days (Mean±SD)

Conc. of L. alopecuroides (mg/L)	K^+ (mmol/L)	% of control	Ca^{2+} (mmol/L)	% of control	Na^{2+} (mmol/L)	% of control
0.00	9.33±8.00^a	100	3.50±2.66^{ab}	100	315.00± 240.03^{ab}	100
0.25	29.15 ±16.54^b	312.60	4.30.±0.82^{ab}	122.86	424.25±66.61^{ab}	134.68
0.50	14.48±6.33^{ab}	155.23	3.57.±2.70^{ab}	102.14	210.75±143.50^a	66.90
0.75	10.33±7.73^a	110.72	1.95±2.11^a	55.71	264.50±186.69^{ab}	83.97
1.00	16.20 ± 4.37^a	173.73	6.28± 2.43^b	179.29	330.75±228.92^{ab}	105.00
1.25	19.95±7.17^{ab}	214.94	2.48±0.57^a	70.71	560.00±113.63^b	177.78

Means with the same alphabet in the same column are not significantly different (P>0.05)

In the gills, sodium ion decreased progressively in the lower concentrations below the control value and increased above that of the control value in the higher concentrations. The value of potassium ion and calcium ion in the gills were lower than that of the control value in all the exposure concentrations except at the highest concentration where an increase was observed. However, the decrease in both potassium ion and calcium ion were not concentration dependent (**Table 4**).

Table 4 Cations in the gill of catfish hybrid exposed to *L. alopecuroides* for 21days (Mean±SD)

Conc. of L. alopecuroides (mg/L)	K^+ (mmol/L)	% of control	Ca^{2+} (mmol/L)	% of control	Na^{2+} (mmol/L)	% of control
0.00	19.15±7.36^{ab}	100	6.90±1.10^{bc}	100	602.00± 69.01^{ab}	100
0.25	12.53 ±3.53^a	65.43	4.50.±2.02^{ab}	65.22	552.00±11.11^{ab}	91.69
0.50	15.50±9.05^{ab}	80.94	5.13.±3.07^{ab}	74.28	355.00±110.51^a	58.97
0.75	10.80±3.59^a	96.40	1.65±0.0.24^a	23.91	602.75±95.35^{ab}	100.08
1.00	11.89 ± 6.94^a	62.08	6.03± 1.83^{abc}	87.32	618.75±143.20^{ab}	102.78
1.25	23.63±5.38^b	123.37	9.63±5.15^c	139.49	718.75±17.50^b	119.39

Means with the same alphabet in the same column are not significantly different (P>0.05)

In the muscle sodium levels increased slightly at 0.25, 1.00 and 1.25mg/L test concentrations. Decreased levels were observed at 0.50 and 0.75mg/L test concentrations. The value of potassium ion was not significantly (P>0.05) higher than that of the control value in all the exposure concentrations except at 0.75mg/L concentration. The value of calcium ion in the gills was significantly (P>0.05) lower than that of the control value in all the exposure concentrations (**Table 5**).

The levels of sodium in the tissues of the fish after exposure to *L. alopercuroides* showed that the ion was most elevated in the kidney, which was followed by the values observed in the gill and then the values observed in the liver, which was followed by the values observed in the kidney. The least value was observed in the Plasma (**Figure 1**).

Table 5 Cations in the muscle of catfish hybrid exposed to *L. alopecuroides* for 21days (Mean±SD)

Conc. of L. alopecuroides (mg/L)	K^+ (mmol/L)	% of control	Ca^{2+} (mmol/L)	% of control	Na^{2+} (mmol/L)	% of control
0.00	27.95±16.52[a]	100	9.88±3.45[c]	100	733.75± 27.80[b]	100
0.25	34.75 ±10.30[b]	142.33	7.13.±3.47[bc]	72.15	736.25±27.80[b]	100.34
0.50	30.13±17.21[a]	107.78	5.00.±3.76[ab]	50.63	350.00±105.46[a]	47.70
0.75	66.13±2.69[b]	236.48	1.88±0.48[a]	18.99	663.75±17.02[b]	90.46
1.00	39.50 ± 11.62[a]	141.32	8.00± 1.35[bc]	81.01	797.50±52.36[b]	108.69
1.25	28.38±6.86[a]	101.52	6.00±2.31[abc]	60.76	747.50±23.63[b]	101.87

Means with the same alphabet in the same column are not significantly different (P>0.05)

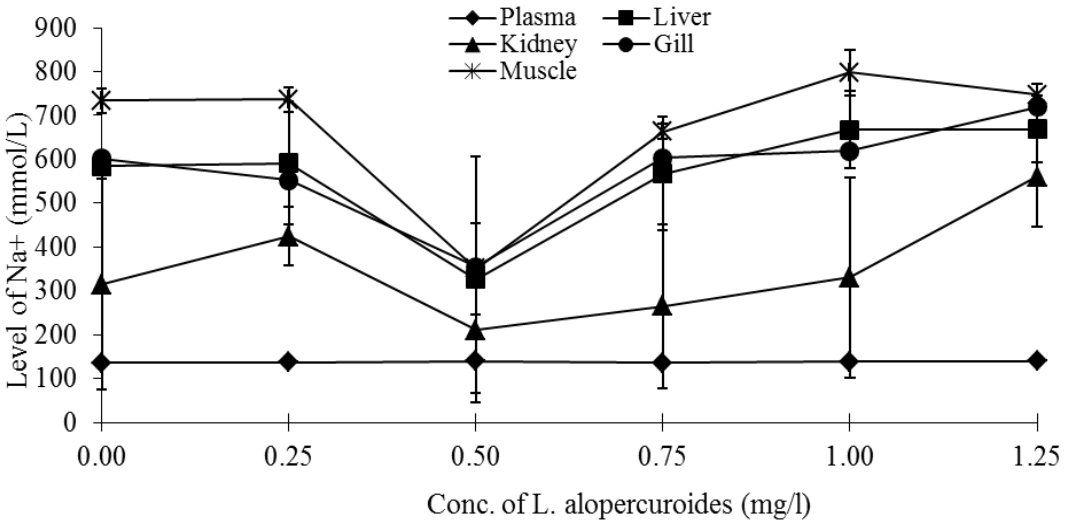

Figure 1 Comparative levels of sodium ion in the various tissues of the hybrid catfish

The levels of potassium in the tissues of the fish showed that the muscle tissue had the highest level of potassium ion, which peaked at 0.75 mg/L of the toxicant *L. alopercuroides*, which was followed by the values observed in the liver, which peaked at 1.25 mg/L. this was followed by the values observed in the kidney and then the gills. The least value was observed in the plasma with the highest value observed at 1.25 mg/L (**Figure 2**).

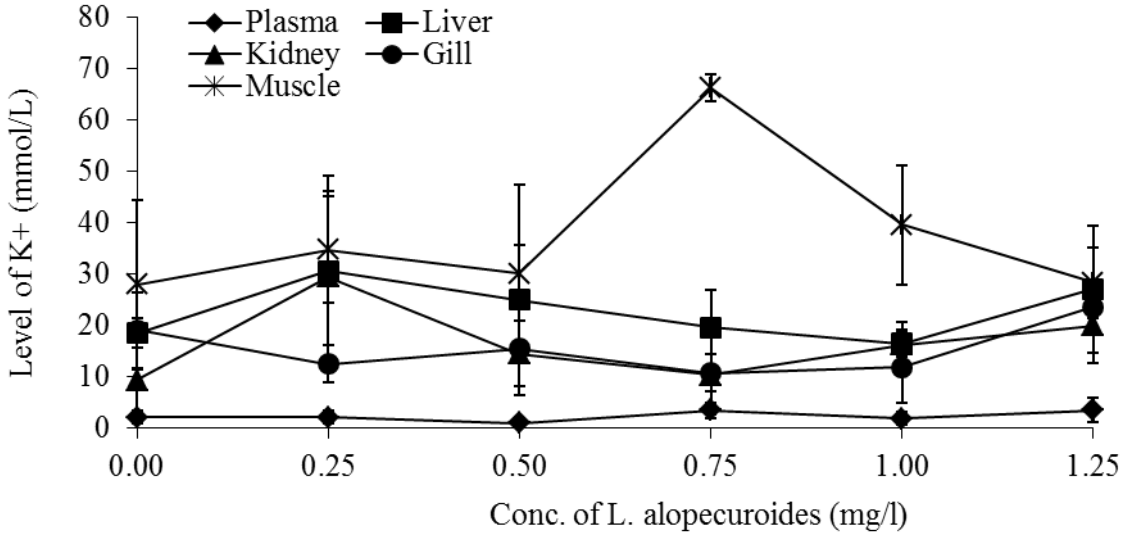

Figure 2 Comparative levels of potassium ion in the various tissues of the hybrid catfish

The levels of calcium ion in the tissues showed that the muscle calcium was the highest, though it decreased progressively at 0.25 – 0.75 mg/L concentrations and then increased in the higher concentrations. In the gill, there were fluctuations in the value which was finally elevated at the highest toxicant concentration. This was followed by the value observed in the liver and then kidney and the lowest values being those observed in the plasma.

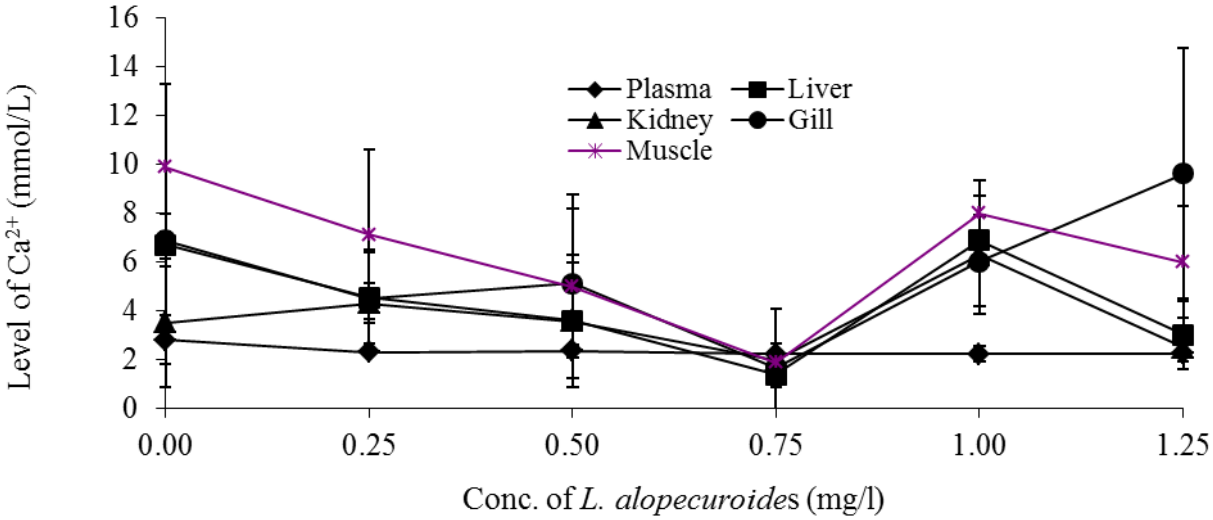

Figure 3 Comparative levels of calcium ion in the various tissues of the hybrid catfish

Discussion

Electrolytes are ions which conduct electricity and transport electrical signals in the body fluid, tissue and blood of living organisms. Impairment of organ functions manifests in a variety of different clinical forms which may sometimes be asymptomatic and therefore can only be detected by routine examinations in the laboratory [18]. In the presence of environmental assault, these ions are altered in content in the plasma and the various organs and tissues, thus indicating impairment of organ functions in the organism [19].

In this study, there was a general increase in the levels of sodium, potassium and calcium ions in the plasma and the various organs or tissues, except calcium in the plasma in all the exposure concentrations. The electrolytes (sodium, chloride, potassium and calcium) serve electrochemical, enzymatic and structural functions in living organisms. The concentrations of monovalent ions and the osmolality of the plasma are found to be relatively constant in most teleost species [20]. The ions or electrolytes sodium, potassium, chloride and calcium function primarily in osmoregulation in fishes either through the exertion of osmotic pressure or by uptake and elimination.

The increased level of sodium in both the plasma and the organs is described as hypernatremia. Increased uptake of sodium implies a reduction in the amount of water (dehydration) in the fish. This situation leads to lethargy and weakness in the fish. Sodium has one electron in its valence shell. It is found in outside of the cell fluids and about 93% of the ions (bases) in blood, is sodium. The major role of sodium in the animal is the regulation of osmotic pressure and acid-base balance regulation. It affects muscle irritability, and the absorption of carbohydrate. Increased sodium also results in muscle twitching and ineffective nerve impulse transmission [21, 22]. Hypernatremia can also be linked to overfunctioning of the organs responsible for its production. Potassium is the major cation found in the internal cell fluid. It regulates osmotic pressure in the internal cell of organisms and the acido-basic balance of animals in stressed conditions. Potassium behaves like sodium in that it stimulates effects on muscle response (irritability or sensitivity) to the environment. Potassium is also required for glycogen and protein synthesis, and the metabolic breakdown of glucose. Potassium is responsible or involved in neuromuscular and cardiac functions. The increased concentration of potassium is a situation described as hyperkalemia. This condition results in fatigue, weakness and abdominal cramps and in severe cases leads to cardiac arrest. This situation in humans leads to hypertension [22].

Sodium and potassium are the major cations in the extracellular and intracellular fluid respectively [23]. The increase in these ions in the fish results from *L. alopecuriodes* toxicosis. The functions of many enzymes is facilitated by these ions and they have been implicated in the transport of ATP and, Na^+ and K^+ATpase located in cell membrane and have been found to be involved in their transport [22, 24].

Calcium is very important in the biological processes of fish. According to [25], when calcium exists in ionic (free) form at relatively high concentrations culture water, it helps to reduce the loss of other salts such as sodium and potassium from fish body fluids (for example blood). Calcium is an activator of several key enzymes, which are pancreatic lipase, acid phosphatase, cholinesterase, ATPases, and succinic dehydrogenase [26].

Calcium activates enzyme, stimulates muscle contraction (ie. promotes muscle tone and normal heart beat) and also regulates nerve impulse transmission from one cell to another by controlling the rate of production of acetylcholine in animal cells. Calcium, in conjunction with phospholipids, plays a key role in the regulation of the

permeability of cell membranes and consequently strengthens the cell walls are which then hold the contents of fruits together [27].

Increased calcium content is referred to as hypercalcemia. Calcium is involved in the transmission of nerve impulse, the contraction of muscle, formation of bones, blood clotting and other metabolic reactions. The need to carry out these functions necessitates the absorption of calcium from water or food. In bony fishes, it combines with phosphorus for the deposition of bones and has also been found to be an important factor in reproduction and mitochondrial functions [20, 28, 29]. Hypercalcemia causes constipation, lethargy, nausea, hypertension and bone pain. When the amount of calcium is increased in fish and other organism, the phosphorus content is decreased which then leads to confusion and non-coordination in the organism and in severe cases may lead to hypertension (in humans) and death of the organism. However, decreased calcium levels in fish can be caused by low level of calcium in water, which cause the element to leak out to the water environment [25]. In general, the regulation of calcium metabolism in fish depends on certain endocrine hormones namely prolactin and somatolactin [30], which may also have been affected by the toxicant.

According to [31], increased levels of these ions in the fish resulted in hyperfunction of the organs/tissues under study. It is also indicative of the fish ability to osmoregulate in the presence of environmental stressors. The increase in these ions is indicative of stress mediated injury in the gills, kidney and the liver of the hybrid catfish. If this condition is allowed to persist, the fish will eventually die of organ damage and dysfunction.

Conclusion

The distortion in ionic content of the fish organs showed that *L. alopecuroides* is toxic to the fish. This imbalance in the level of ions if unchecked can lead to serious damages in the fish. This condition if allowed to continue will eventually lead to other environmental consequences. Therefore, the use of *L. alopecuroides* as fish poison should be controlled or discouraged.

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