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Comparing Effect of Lead Nitrate on Proximate Composition of *Clarias gariepinus* and *Oreochromis niloticus*

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Abstract: This study investigated the effects of lead nitrate exposure on the proximate composition of African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*). The research was conducted in the research farm of the Department of Agriculture, Alvan Ikoku Federal University of Education, Owerri Nigeria. Five treatment levels were considered: 0, 1.55, 3.10, 4.65, and 6.20 mg/L of lead, using Lead nitrate as the lead source. The proximate parameters evaluated were moisture, fibre, ash, fat, protein, and carbohydrate contents. The results indicated significant differences in all measured parameters in both fish species, with reported p-values of 0.032 or lower. In *C. gariepinus*, moisture content increased from 6.39 in the control to 22.03 at 3.10 mg/L before declining to 10.10 at 6.20 mg/L. Fibre content increased from 5.57 to 13.74 at 4.65 mg/L, while ash content declined from 1.81 to 0.37 at the highest exposure level. Fat content decreased from 18.84 to 15.10, whereas protein content increased from 35.36 to 44.80. Carbohydrate content decreased from 33.64 to 4.48 at 3.10 mg/L before increasing to 22.31 at 6.20 mg/L. In *O. niloticus*, moisture and fibre generally increased, while fat and protein showed slight declines relative to the control. Ash content also declined, whereas carbohydrate content varied only slightly. These findings suggest that lead exposure altered the proximate composition of both species, with more pronounced numerical changes in *C. gariepinus*. The study highlights the potential nutritional consequences of lead contamination in aquatic environments.

Keywords: *Lead Nitrate, Proximate Composition, Heavy Metals, African Catfish, Nile Tilapia, Aquatic Pollution*

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I. INTRODUCTION

Toxic heavy metals have become a major worry for researchers and scientists globally. Among these, lead is particularly concerning because it offers no biological benefit to plants, animals, or humans. Like other heavy metals such as cadmium, arsenic, and mercury, lead can be dangerous even in tiny amounts. It's often seen as the most critical toxic heavy metal in our environment (Offor et al 2024a; Offor et al 2025). Since lead doesn't break down and is continuously released into the environment, its levels keep climbing, which increases the risks we face (Offor et al 2024b). Research has shown that heavy metals can accumulate in fish due to polluted waters (Offor et al 2024b). Moreover, the degree of heavy metal bioaccumulation can vary among different species, and their growth responses can differ as well. Factors such as the nature and chemical behavior of the toxicant, its solubility, and its breakdown rate all influence how likely fish are to accumulate these metals and the overall impact on their health. While lead poisoning from consuming fish is considered rare, lead can still accumulate in fish from contaminated water, posing health risks to humans. In nature, lead typically enters water bodies—like oceans, rivers, and lakes—mainly through industrial waste. Fish can absorb lead through their gills, skin, and diet, storing it in their soft tissues and organs like the liver and kidneys.

Lead pollution poses a threat to aquatic ecosystems and their inhabitants when concentrations exceed safe levels. Its non-biodegradable nature and persistent presence in the environment lead to toxicity in fish (Taslima et al 2022). Different aquatic species may respond differently to the same level of toxicant pollution. The African catfish, known for its resilience, serves as an excellent bioindicator of toxicants, as shown in various studies. It's also a widely farmed fish species throughout Africa (Anyanwu et al 2015a and Anyanwu et al 2015b). Nile tilapia, another popular freshwater fish, is also extensively cultivated in the region.

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Proximate composition is a key indicator of how nutritious fish are. It typically includes moisture, crude protein, crude fat, ash, crude fiber, and carbohydrate levels. These elements not only shed light on the nutritional value of fish but can also indicate changes in tissue composition due to environmental stress. Protein is vital as it provides essential amino acids necessary for growth and tissue repair, while fats act as energy reserves and support crucial physiological functions. The moisture content plays a significant role in the makeup of fish tissues and is important for processing and preservation. Ash content gives a rough estimate of total inorganic residue, though it doesn't pinpoint specific minerals. When carbohydrate content can be reliably measured, it helps paint a fuller picture of the sample's overall composition. In Nigeria, African catfish (Clarias gariepinus) and Nile tilapia (Oreochromis niloticus) are vital freshwater fish species, contributing significantly to food security, livelihoods, aquaculture development, and the provision of animal protein. However, their nutritional profiles can be influenced by environmental factors and biological differences between the species. Salim et al. (2024) highlighted variations in the protein, fat, and ash contents of C. gariepinus and O. niloticus collected from Zobe Dam, showing that proximate composition can differ among commercially significant freshwater fish species.

This study aimed to compare how different concentrations of lead nitrate affect the proximate composition of C. gariepinus and O. niloticus. It specifically looked at changes in moisture, fiber, ash, fat, protein, and carbohydrate levels at exposure concentrations of 0, 1.55, 3.10, 4.65, and 6.20 mg/L. By comparing these two species, we can gain insights into how they respond compositionally to lead exposure, which may help us better understand the nutritional implications of heavy metal pollution in freshwater ecosystems.

II. MATERIALS AND METHODS

➢ *Area of Study*

The experiment was set up in the research farm of the department of Agricultural Science, Alvan Ikoku Federal University of Education, Owerri Imo State Nigeria. Owerri is situated at 5.48° North latitude, 7.03° East longitude and 159 meters elevation above the sea level. The annual rainfall averages between 1,500mm to 2,200mm (60 to 80 inches). An average annual temperature above 20 °C (68.0 °F) creates an annual relative humidity of 75%, with humidity reaching 90% in the rainy season (Offor et al., 2020).

➢ *Research Design*

The research is pure experimental research which involved manipulating one variable (independent variable) and observing the effect on the other variable (dependent variable). The experiment is a factorial experiment and completely randomized design (CRD) was adopted in assigning treatments and replicates.

➢ *Procurement of Materials*

Lead (II) nitrate [Pb $(NO_3)_2$] was procured from ChemiSciences LTD Owerri Imo State. Fingerlings for the experiment were collected from the research farm of the School of Agriculture and Technology, Federal University of Technology Owerri.

➢ *Experimental Set-Up*

Two fish species were used for the experiment (*Clarias gariepinus* and *Oreochromis niloiticus*). Five treatments of lead (ii) nitrate salt (0g/40l ; 0.1g/40l; 0.2g/40l; 0.3g/40l; 0.4g/40l) designated A, B, C, D, and E for each fish representing the five concentrations of lead (0mg/l; 1.55mg/l; 3.1mg/l; 4.65mg/l; 6.20mg/l) respectively were prepared. The experiment comprised a total of 10 treatments and 30 experimental units/ replicates.

Thirty plastic aquaria (30) of fifty litres capacity were used for the experiment. Forty litres of analysed tap water of known physico-chemical properties were poured into each of the aquaria. Lead salt were added at the pre-determined concentrations. The solutions were properly stirred and left for 2hours to allow for proper water-heavy metal mixture.

➢ *Experimental Period*

Total of five (500) fingerlings, 250 for each specie were procured and kept in two plastic ponds each tank. Fish were acclimatized to laboratory conditions using dechlorinated tap water for seven (7) days. During acclimatization, fish were fed on commercial diet of 40% crude protein twice daily.

➢ *Water quality Parameters*

Water parameters (pH, Temperature, total alkalinity) of the water used for the experiment were anaysed.

➢ *Proximate Analysis Procedures:*

Proximate analysis was by AOAC methods as reported by Anyanwu et al (2015a) and Anyanwu et al (2015b).

➢ *Determination of Moisture Content*

A crucible was washed and dried in the oven

- 2g of the beans sample was weighed into crucible
- The weight of the crucible and sample was noted before drying
- The crucible and sample were put in the oven for 2hrs at temperature of 105^{o}C.The drying procedure was continued until a constant weight was obtained

$$\% \text{ moisture content} = \frac{\text{weight lost}}{\text{Weight of sample}} \times 100$$

➢ *Ash Content*

Empty platinum crucible was washed, dried and the weight was noted. 2g of the beans sample was weighed into the platinum crucible and placed in a muffle furnace at 500^{0}C for 3 hours. The sample was cooled in a dessicator after burning and weighed.

Calculations

$$\% \text{ Ash content} = \frac{\text{weight lost}}{\text{Weight of sample}} \times 100$$

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➢ *Determination of Crude Proteins*

1g of beans samples each was weighed into a 300ml kjehdal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stoppered and shaken. Then 0.5g of the kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack on electric hot plate until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling about 100ml of distilled water was added to avoid caking and then 5ml of the filtrate and 5ml of 40% Na0H was transferred to the kjedahl distillation apparatus. A 250ml receiver beaker containing 10ml of 10% boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methylene blue was placed under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution. Then 5ml of 40% sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until 50 drops gets into the receiver beaker , after which it was titrated to pink colour using 0.01N hydrochloric acid.

Calculations

% Nitrogen = Titre value x 0.01 x 14 x 4

% Protein = % Nitrogen x 6.25

➢ *Determination of Fat Content*

Dry 250ml clean boiling flasks in oven at 105-110^{0}c for about 30 minutes. Transfer into a desiccator and allow to cool. Weigh about 2g of the samples each accurately into labeled thimbles. Weigh correspondingly labeled, cooled boiling flasks. Fill the boiling flasks with about 300ml of petroleum ether (boiling point 40-60^{0}c). Plug the extraction thimble lightly with cotton wool. Assemble the soxhlet apparatus and allow to reflux for about 6 hours. Remove thimble with care and collect petroleum ether in the top container of the set-up and drain into a container for re-use. When flask is almost free of petroleum ether, remove and dry at 105^{0}C - 110^{0}C for 1 hour. Transfer from the oven into a desiccator and allow to cool and then weigh.

Calculation:

$$\% \text{ Fat} = \frac{\text{weight lost o fat}}{\text{weight of sample}} \times 100$$

➢ *Determination of Crude Fibre*

- Weigh 2g of the sample into a conical flask containing 200ml of 1.25g of H_2SO_4 solution per 100ml
- Boil under reflux for 30 minutes.
- Filter the solution through linen or several layers of cheese cloth on a fluted funnel
- Wash with boiling water until the washings are no longer acidic at pH 7.0
- Transfer the residue to a beaker and boil for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml
- Filter the final residue through a thin but close pad of washed and ignited asbestos in a Gooch crucible
- Dry in an electric oven at temperature of 105^{0}C and weigh
- Incinerate in muffle furnace at temperature of 300^{0}C for 2 hours, cool and weigh

The loss in weight after incineration x 100 is the percentage of crude fibre.

$$\% \text{ crude fibre} = \frac{\text{weight lost}}{\text{Weight of sample}} \times 100$$

➢ *Carbohydrate Determination*

% Carbohydrate = 100 – (%Protein + %Moisture + %Ash + %Fat + %Fibre +)

➢ *Data Analysis*

Data generated were subjected to one-way ANOVA using SPSS version 20. Mean separation was by Duncan Multiple Range Text. Test of significance was set at 95% confidence level.

III. RESULTS AND DISCUSSIONS

Table 1 The Physico-Chemical Characteristics of the Water Used:

Parameter	Value
Temperature	24 °C
Dissolved oxygen	7.2mg/l
Hydrogen ion concentration	6.8

Table 2 Effects of Lead on Proximate Composition of *clarias gariepinus*

Treat.	Moisture	Fibre	Ash	Fat	Protein	CHO
0mg/l	6.39±.284	5.57±.256	1.81±2.30	18.84±.130	35.36±.026	33.64±.097
1.55mg/l	8.86±113	7.03±.151	3.60±.200	19.04±.060	39.13±.112	22.29±.132
3.1mg/l	22.03±.115	11.33±.155	2.31±.072	18.80±.155	40.87±.174	4.48±.055
4.65mg/l	18.76±.151	13.74±.150	1.18±.012	16.23±.060	43.40±.065	6.64±.388
6.2mg/l	10.10±.015	7.37±.107	.37±.027	15.10±.095	44.80±.100	22.31±.075
Sig (p-value)	0.00	0.00	0.032	0.00	0.00	**0.00**

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Table 3 Effects of Lead on Proximate composition of Nile Tilapia

Treat.	Moisture	Fibre	Ash	Fat	Protein	CHO
0mg/l	20.89± .043	6.63±.075	2.36±.046	10.58±.105	33.40±.220	26.15±.090
1.55mg/l	23.83±.149	7.36±.030	1.49±.006	8.93±.016	32.89±.017	25.57±.035
3.1mg/l	20.91±.020	6.61±.075	2.37±.014	10.53±.007	33.39±.115	26.33±.037
4.65mg/l	23.73±.027	7.38±.025	1.49±.006	8.92±.030	32.88±.015	25.56±.058
6.2mg/l	23.71±.043	7.36±.005	1.49±.006	8.92±.054	32.88±.081	25.59±.101
Sig	0.00	0.00	0.00	0.00	0.00	0.00

Looking at Tables 2 and 3, we can see some interesting differences in the proximate composition of C. gariepinus and O. niloticus after being exposed to lead nitrate. The most notable change was in protein content. For C. gariepinus, protein levels jumped from 35.36 in the control group to 44.80 at a concentration of 6.20 mg/L. In contrast, O. niloticus experienced a slight drop in protein, going from 33.40 to 32.88. This stark difference indicates that the two species reacted quite differently when it came to their protein levels.

The findings are broadly compatible with the concern that heavy metal exposure may interfere with normal fish physiology. Uzochukwu et al. (2024) found that lead exposure adversely affected the growth performance of African catfish. Although their findings support the biological importance of lead toxicity, they do not directly establish the mechanism responsible for the lipid changes observed in O. niloticus.

When it comes to fat content, both species showed a decline at the highest concentration of lead exposure. C. gariepinus saw a reduction of about 19.9%, while O. niloticus experienced a 15.7% decrease. This similar trend suggests that lead exposure might be linked to changes in lipid composition for both species, although we should keep in mind the differences in their baseline values and the methods used for analysis.

The ash content also dropped for both species at the 6.20 mg/L level compared to the control. C. gariepinus showed a significant decrease from 1.81 to 0.37, while O. niloticus went from 2.36 to 1.49. This indicates a more substantial reduction in the inorganic residue for C. gariepinus.

Moisture and fiber content varied between the two species, but their responses didn't consistently align with lead concentration levels. C. gariepinus exhibited particularly large fluctuations in carbohydrate content, whereas O. niloticus maintained relatively stable values.

These differences might point to unique physiological traits, tissue compositions, or varying responses to environmental stressors between the species. Still, to draw any firm conclusions about their comparative susceptibility, we would need to conduct a factorial statistical analysis that examines the effects of species, exposure concentration, and their interactions.

Recent researches have shown that C. gariepinus and O. niloticus not only differ in their nutritional compositions but also in how they accumulate heavy metals. Salim et al. (2024) highlighted these differences between these species, while Thomas (2026) reported tissue-specific differences in heavy metal accumulation in both species collected from River Benue. These studies support the importance of considering species and tissue characteristics in aquatic pollution research, although neither establishes the specific cause of the compositional patterns reported in the present experiment.

The overall findings indicate that lead nitrate exposure was associated with changes in the measured proximate components of both fish species. The magnitude and direction of the changes varied by parameter and species, highlighting the need for further investigation of the biochemical mechanisms, tissue metal concentrations, and nutritional implications of lead exposure.

IV. CONCLUSION

The study found that varying levels of lead nitrate exposure led to some noticeable shifts in the proximate composition of Clarias gariepinus and Oreochromis niloticus. However, the degree and type of these changes differed between the two species and across the nutritional parameters evaluated.

In C. gariepinus, lead exposure resulted in an increase in reported protein content, along with significant variations in moisture, fiber, and carbohydrate levels. Interestingly, the fat and ash contents were lower at the highest exposure levels compared to the control group. Conversely, O. niloticus exhibited changes in moisture and fiber content across different treatments, while fat and ash levels tended to be lower at several exposure concentrations. The shifts in protein and carbohydrate contents were relatively minor.

These findings suggest that lead exposure affected the proximate composition of both species, with C. gariepinus showing more pronounced numerical changes in several parameters.

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This research highlights the urgent need to manage lead contamination in aquatic environments to protect fish health, boost aquaculture productivity, and preserve the nutritional quality of fish as a food source.

RECOMMENDATIONS

Based on the study's findings, here are some recommendations:

- Control lead pollution: Environmental authorities should enhance the monitoring and regulation of lead-containing industrial effluents, mining wastes, and other sources of heavy metal contamination entering aquatic ecosystems.
- Investigate biochemical mechanisms: Further studies should measure oxidative stress indicators, liver function, tissue lipid composition, and relevant metabolic enzymes to clarify the mechanisms underlying the observed changes.
- Improve aquaculture management: Fish farmers should regularly assess water quality and avoid using contaminated water sources for fish production. Appropriate treatment and pollution-prevention measures should be implemented where necessary.

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