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Evaluating Short-Term Effect of Cadmium on Proximate Composition of *Clarias gariepinus* and *Oreochromis niloticus*

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Abstract: Cadmium is a harmful heavy metal that can negatively impact the physiology, metabolism, and nutritional quality of fish. This study looked into how cadmium nitrate exposure affects the proximate composition of two fish species: African catfish (Clarias gariepinus) and Nile tilapia (Oreochromis niloticus). The experiment was conducted in research farm at Alvan Ikoku Federal University of Education Owerri Nigeria. Completely randomized design was adopted which involved five treatments and three replicates. Several parameters were evaluated, including moisture, fiber, ash, fat, protein, and carbohydrate levels at cadmium concentrations of 0, 0.9, 1.8, 2.7, and 3.6 mg/L. The results revealed that cadmium exposure changed the proximate composition for both species, though the effects varied between them. For C. gariepinus, moisture rose from 6.39 in the control group to 15.87 at 1.8 mg/L, while protein saw a slight increase at 0.9 mg/L before dropping at higher concentrations. The fat content peaked at 0.9 mg/L and then decreased with higher levels of cadmium. Ash content fell from 1.81 in the control to 0.10 at 3.6 mg/L, but the statistical analysis didn't show a significant effect on ash. In O. niloticus, protein decreased from 33.41 in the control to 27.28 at 1.8 mg/L, and fat dropped from 10.48 to 8.44 at the higher concentrations. Moisture and fiber generally increased at 1.8 and 3.6 mg/L, while ash levels varied across treatments. The statistical results indicated significant treatment effects on all measured parameters in O. niloticus. Overall, these findings suggest that exposure to cadmium nitrate can alter the nutritional makeup of both fish species, which could have important implications for fish health, aquaculture productivity, and food quality.

Keywords: *Cadmium Nitrate, Proximate Composition, Clarias Gariepinus, Oreochromis Niloticus, Heavy Metal Toxicity, Nutritional Quality.*

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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 heavy metals do not break down and is continuously released into the environment, its levels keep climbing, which increases the risks we face (Babu et al 2021, Offor et al 2024b). Research has shown that heavy metals can accumulate in fish due to polluted waters (Offor et al 2024b).

Cadmium (Cd) is a heavy metal that isn't essential for life, yet it has garnered a lot of attention due to its toxicity and its tendency to build up in aquatic organisms. Fish can come into contact with cadmium through polluted water, sediments, and their food sources. Once absorbed, cadmium can disrupt normal cellular functions, throw mineral regulation out of balance, increase oxidative stress, and cause tissue damage. Liu et al. (2022) pointed out that cadmium toxicity in fish involves a range of physiological and biochemical processes, including oxidative damage and disruptions to regular biological functions. In a similar vein, Lee et al. (2023) highlighted oxidative stress and altered immune responses as significant effects of cadmium exposure in fish.

Two key freshwater fish species that are commercially important are the African catfish (Clarias gariepinus) and Nile tilapia (Oreochromis niloticus). Both are widely farmed and eaten, but they have distinct biological traits, feeding habits, and physiological reactions to their environments. By

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comparing how they respond to cadmium nitrate exposure, we can uncover differences in how their nutritional makeup shifts under metal stress.

Recent reviews have stressed the need to assess cadmium toxicity using a variety of biological indicators instead of just survival rates (Das et al., 2023; Lee et al., 2023). Looking at proximate composition adds another layer of understanding, as it can highlight changes in the nutritional profiles of the affected fish. However, it's crucial to interpret these changes alongside other indicators, such as growth, feed efficiency, tissue cadmium accumulation, oxidative stress levels, and histopathological changes.

Despite the significance of these factors, understanding how cadmium nitrate affects the proximate composition of C. gariepinus and O. niloticus requires a species-specific approach under clearly defined exposure conditions. This study aims to compare the reported levels of moisture, fiber, ash, fat, protein, and carbohydrates in these two species.

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*

Cadmium nitrate [Cd (NO3)2]. 4H2O 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 Cadmium 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 Cadmium (0mg/l; 0.9mg/l; 1.8mg/l; 2.7mg/l; 3.6mg/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 analysed.

➢ *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

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

➢ *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

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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 Cadmium on Proximate composition of Clarias Gariepinus

Treatment	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
0.9mg/l	14.39±..052	5.18±..011	.28±..005	22.07±..010	36.40±..015	21.83±..099
1.8mg/l	15.87±..050	8.25±..135	.16±..019	17.80±..011	33.73±..152	24.36±..070
2.7mg/l	11.77±..091	8.37±..024	.23±..020	18.29±..105	31.18±..030	30.26±..149
3.6mg/l	11.88±..018	7.69±..055	.10±..027	16.80±..165	32.86±..060	30.53±..184±
Sig(p-value)	0.00	0.00	0.27	0.00	0.00	0.00

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Table 3 Effects of Cadmium on Proximate Composition of Nile Tilapia

Treatment	Moisture	Fibre	Ash	Fat	Protein	CHO
0mg/l	20.89± .024	6.61±.083	2.39±.006	10.48±.057	33.41±.175	26.12±.030
0.9mg/l	19.94±.061	5.90±.035	1.64±.057	13.45±.248	31.09±.060	27.93±.053
1.8mg/l	26.17±.106	11.2±.035	3.37±.075	8.44±.010	27.28±.225	23.38±.021
2.7mg/l	19.90±.101	5.89±.023	1.63±.036	13.38±.015	31.21±.076	27.94±.012
3.6mg/l	26.18±.038	11.3±.151	3.36±.040	8.44±.055	27.37±.213	23.38±.019
Sig	0.00	0.00	0.00	0.00	0.00	0.00

The findings reveal that exposure to cadmium nitrate led to noticeable changes in the proximate composition of both C. gariepinus and O. niloticus. However, the extent and nature of these changes varied between the two species and across different parameters. In both species, protein content dropped at higher treatment concentrations. For C. gariepinus, the lowest protein level recorded was 31.18 at 2.7 mg/L, compared to a control value of 35.36. Meanwhile, O. niloticus showed a minimum protein value of 27.28 at 1.8 mg/L, with a slightly higher value of 27.37 at 3.6 mg/L, against a control value of 33.41. These results suggest that the reduction in protein content was more pronounced in tilapia than in catfish at the highest concentration tested. Protein plays a crucial role in tissue maintenance, growth, enzyme production, and various physiological functions. The oxidative stress and biochemical disturbances caused by cadmium may hinder normal protein metabolism or increase the demands for physiological upkeep (Lee et al., 2023; Liu et al., 2022). Fat content also showed variability in both species. In catfish, the peak fat value was observed at 0.9 mg/L, followed by generally lower values at higher concentrations. In tilapia, the lowest fat values were noted at 1.8 and 3.6 mg/L, while the value at 2.7 mg/L remained relatively high. This indicates that the relationship between exposure concentration and fat content varied between the species. Moisture content saw a significant increase at certain concentrations in both fish, although the patterns were somewhat erratic. Cadmium can interfere with ionic regulation, cellular function, and normal physiological processes in fish (Lee et al., 2023; Liu et al., 2022). These disruptions could explain the changes in tissue water content. However, the inconsistent fluctuations in the results suggest that moisture content doesn't respond in a straightforward manner to concentration levels.

The rise in moisture at certain concentrations might also impact the apparent levels of protein, fat, and other components if these were measured on a wet-weight basis. Heavy-metal toxicity can trigger oxidative stress and throw normal cellular functions off balance, which could interfere with how lipids are utilized and maintained (Lee et al., 2023; Shahjahan et al., 2022). The ash results showed some interesting differences: in catfish, the numerical values dropped across treatments, while tilapia exhibited a pattern of alternating low and high values. The statistical analysis further revealed that ash levels in catfish weren't significantly affected ($p = .27$), but tilapia showed a significant change (reported $p = .00$). Toxic stress might influence how energy substrates are used and stored as fish adapt to environmental challenges (Shahjahan et al., 2022). A decrease in carbohydrates could indicate shifts in carbohydrate availability or metabolism. These variations might point to species-specific physiological responses, differences in tissue composition, or how each fish species reacted to the exposure conditions. Recent reviews on cadmium toxicity highlight that responses can differ based on exposure concentration, duration, fish species, and the biological endpoint being measured (Das et al., 2023; Lee et al., 2023; Liu et al., 2022). The findings align with the broader understanding that cadmium exposure can lead to changes in fish physiology and biochemical makeup.

IV. CONCLUSION AND RECOMMENDATION

The study compared the effects of different concentrations of cadmium on the proximate composition of Clarias gariepinus and Oreochromis niloticus. The results showed that the measured moisture, fibre, fat, protein, and carbohydrate values varied across treatment concentrations in both species. The findings align with existing research showing that cadmium can disrupt fish physiology and biochemical processes, primarily through oxidative stress, cellular function disturbances, and metabolic disruptions (Das et al., 2023; Lee et al., 2023; Liu et al., 2022). However, the current results don't pinpoint the exact physiological mechanisms behind the observed changes in composition. To wrap it up, exposure to cadmium nitrate was linked to alterations in the proximate composition of both fish species. Based on these findings, here are some recommendations:

- *Regular Monitoring of Cadmium Contamination:*

Aquaculture facilities and environmental authorities should keep an eye on cadmium levels in fish-farming water and other potential contamination sources. It's crucial to implement measures that prevent untreated industrial waste and other cadmium-laden materials from entering aquatic environments.

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- *Protection of Aquaculture Water Quality:*

Fish farmers should source water from locations with verified quality and adopt effective water management practices to minimize the risk of heavy metal exposure.

- *Further Investigation of Nutritional Composition:*

More studies are needed to explore how cadmium nitrate affects proximate composition, using well-defined sample types, validated analytical methods, and clearly reported units. Special attention should be paid to confirming the fiber measurements noted in this study.

- *Assessment of Cadmium Accumulation in Fish Tissues:*

Future research should focus on measuring cadmium levels in the muscle, gills, liver, and kidneys of both species. This would help clarify the connection between environmental exposure, tissue accumulation, and changes in proximate composition.

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