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Accumulation and Depuration of Cadmium and Chromium in the Muscles of Tilapia Fingerlings After Sub-Lethal Exposure

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Abstract
Consumption of fish contaminated with heavy metals over time can lead to an adverse effect on humans who consume them. This study determined the depuration level of cadmium (Cd) and chromium (Cr) accumulation and the histopathology of tilapia (*Oreochromis niloticus*) fingerlings following sub-lethal exposure and depuration. A total of 150 tilapia fingerlings were placed into 5 groups (A-E) with triplicates of 6 fingerlings each and subjected to different metal concentrations. After 14 days of exposure, fish muscle tissues were harvested from each triplicate to assess the bioaccumulation rate and histopathology. The remaining fingerlings were depurated in clean water. The result showed the mean bioaccumulation of each group and their depuration percentage. All groups experienced reduction in fish weight except Cd/Cr 1.5 mg/L, 10 mg/L group which had increase in weight all through the experiment. The histological result showed alterations and lesions, the severity of the alterations increased with dosage. The histology examination after depuration revealed reduced alteration; recovery of muscle tissues from heavy metal contamination was not complete. The studies suggest that the depuration of accumulated cadmium and chromium in the muscles is dependent on the concentration, weight of fish and its uptake rate.

Keywords: Accumulation, Depuration, Cadmium, Chromium, Bioaccumulation, Histopathology

Introduction

Heavy metal contamination is a major source of pollution in aquatic ecosystems and is becoming a serious global environmental problem because of their toxic nature even at low trace levels (Isangedighi & David, 2019). Studies of different tilapia species from different rivers in the Niger Delta have shown high toxicity of heavy metals released into waterways as a result of increased household activities, urbanization, and continuous industrial and agricultural growth (Abiaobo et al., 2020). Locals in these areas frequently rely on wild fishing, notably for the commonly consumed and very cheap tilapia fish. The Nile tilapia, *Oreochromis niloticus*, has worldwide distribution and is frequently used as a target biological model in toxicological research to examine the toxicity of different pollutants. The risk of consuming fish that may have been exposed to high amounts of cadmium and chromium is high since aquatic life frequently accumulates these metals. Over exposures are possible despite the presence of trace amounts due to its low permissible exposure limit. Cadmium can enter freshwater and saltwater systems directly by atmospheric deposition, runoff, and direct discharges into water bodies or watersheds (Al Naggar et al., 2018). Though its metal form is insoluble in water; its salts in chloride and sulphate are freely soluble (Elinder, 2019). It basically has no biological function to aquatic life (Vajargah, 2021), hence, when ingested by aquatic animals like fish, the gills and digestive system are the two significant sites for its absorption. From the observations of Zhai et al. (2017), Abdel-Tawwab and Wafeek (2017) and Soegianto et al. (2022), Cd levels in Nile tilapia increase significantly in the blood and tissue with the kidney, gills and liver having the highest level of concentration. However, cadmium accumulation by the body muscles is always reported as comparatively low (Guo et al., 2019; Pavlaki et al., 2021), though it can also be found in other tissues like bone and the placenta. Chromium, on the other hand, released into the environment from anthropogenic activity occurs mainly in the hexavalent form, Cr (VI), which is more toxic and highly soluble in water than the divalent form (Chen et al., 2018). The trivalent form, Cr (III), which is an essential trace element, can easily be oxidized to Cr (VI) in a natural environment (DesMarias & Costa, 2019). In an aquatic environment, the toxicity of Cr in fish is dependent on the fish species, its age/developmental stage, body weight, temperature, salinity, pH and hardness of the water they inhabit (Bakshi & Panigrahi., 2018; Tumolo et al., 2020) while its level of accumulation differs depending on the type of tissues. In fish, for instance, its concentration is highest in the gills, kidney and liver, and is low in

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the muscles (Yin et al., 2020). The degree to which aquatic organisms are susceptible to cadmium and chromium varies widely in aquatic habitats.

Many techniques have been employed to remove heavy metals from fish before consumption; some of them have been successful, while others have not. One of this method, which is also a typical fishing technique is depuration. Whether complete elimination of heavy metals is achieved during depuration is still a subject of debate, despite this, other scientists believe that depurating contaminated fish is not feasible because of the high expenses involved and the lengthy retention periods of toxins present in many species. The histological condition of the organisms after this time is also essentially unknown, and nothing is known about how various species react to depuration. The state of the fish's soft tissue must be assessed by histopathological analysis in order to diagnose the degree of contamination based on structural alterations in the organs that are the primary targets of contaminants. Various histopathological experiments have been conducted on different parts of fish: digestive tract (Breccia et al., 2022), gills (Nursanti et al., 2017), liver (Hamed et al., 2017; Jabeen et al., 2018), heart (Islam et al., 2019), muscles (Yacoub et al., 2021), tongue (Abbate et al., 2020) kidney (Ratn et al., 2018), skin (Hamed et al., 2017) and spleen (Zhang et al., 2018). Thus, the aim of the research is to determine the depuration level of Cadmium (Cd) and Chromium (Cr) accumulation and the histopathology of Nile tilapia (*O. niloticus*) fingerlings following sub-lethal exposure and depuration. It also highlights the importance of depuration as an effective practice for detoxification of fish contaminated with Cd and Cr. The findings of the research shall aid in providing rich practical information on the effectiveness of short-term depuration as compared to several other studies on long term depuration.

Materials and Methods

Sample Collection and Acclimation

Freshwater Tilapia fingerlings (*Oreochromis niloticus*) of both sexes were purchased from African Regional Aquaculture Centre (Port Harcourt, Nigeria). A total of one hundred and fifty (150) tilapia fingerlings were purchased and transported using plastic aquaria to C.B. Powel Biodiversity & Toxicity Laboratory in the Department of Animal and Environmental Biology, University of Port Harcourt. Mean total length (5.7±0.7) and mean total weight (3.76±0.38) were taken and recorded. Purchased fingerlings were randomly distributed into 4 plastic aquaria and kept to acclimatize for 5 days. Fingerlings were fed twice a day with 1.5mm fish feed.

Range Finding

After 3 days of acclimation, 32 fingerlings were separated into different plastic aquaria for range finding of cadmium (0.5mg, 1.5mg, 2.5mg, and 3.5mg/L) and chromium (10mg, 30mg, 50mg and 70mg/L) with each concentration containing 4 fingerlings. Cadmium chloride ($CdCl_2$) and Chromium (III) nitrate, ($Cr(NO_3)_3$ were used.

Exposure to Heavy Metals Salt

After range finding, the fingerlings were randomly distributed in 15 plastic aquaria of 2 litres (L). The plastic aquaria were separated into 5 groups comprising 3 plastic aquaria (triplicates) per group with 6 fingerlings in each. Group A served as the control while other groups were used for exposure, labelled groups B to E. Four groups of tilapia were exposed to different concentrations of cadmium (1.5 mg/L and 2.5 mg/L), chromium (20 mg/L) and a combination of the two metal salts (Cd/Cr 1.5 mg/L, 10 mg/L). The groupings are group A (control), group B (Cd/Cr 1.5 mg/L, 10 mg/L), group C (Cd 1.5 mg/L), group D (Cr 20 mg/L), and group E (Cd 2.5 mg/L). Each exposure was performed in triplicate. Metal salts were introduced initially after every two days but was changed after day 4 to after every three days. Fingerlings were fed once daily as their feeding rate was observed to have reduced except for those in the control group. Exposure period lasted for 2 weeks (14 days). Fingerlings were kept in a well-ventilated room in the laboratory.

Depuration

After 14 days of exposure period, the fish samples were transferred to clean water for 4 days to study recovery responses. Water was changed and the plastic aquaria were cleaned every two days to avoid refiltration of depurate contaminants (fingerlings were fed once a day).

Organ Harvesting

The first organ harvesting was carried out after the 2 weeks (14 days) exposure period; the second was done after 4 days depuration experiment. One fish was collected from each triplicate of the different groups and was sent to the Environmental Biology Laboratory of the Department of Animal and Environmental Biology where the muscle of each fingerling was harvested. Muscle sample from different concentration for heavy metal analysis were put in universal bottles and transported in a container of ice to the lab, while those for histological

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examination were transported in 5% formaldehyde solution to Anatomy Laboratory, Department of Pharmacy, University of Port Harcourt.

Metal Analysis

Muscle samples were dried in the oven at 105ºC for 24 hours. The samples were then grinded to powder form using laboratory mortar and pestle. After which, 0.5g of each homogenized samples were weighed and then digested using $10cm^3$ of concentrated hydrogen trioxonitrate (V), HNO_3, and $5cm^3$ of concentrated hydrogen tetraoxosulphate (VI), H_2SO_4, until a colourless solution was produced. The digested samples were then filtered and diluted in a 100ml volumetric flask of distilled water. Finally, the samples were taken to the atomic absorption spectrometer (AA spectrometer) for readings. Sens AA model from GBC Scientific Equipment was used.

Tissue Preparation and Staining of Sections

Harvested muscle was fixed in buffered formalin (5% formaldehyde) and removed after 24 hours to reduce brittleness from acidity of the fixative. Water was removed from the fixed muscle specimen by passing it through 70% to 100% ethanol concentrations. To clear from alcohol (i.e. ethanol), the specimen was passed through xylene, used as a clearing agent. The muscle specimen was impregnated with the clearing agent xylene, then placed in a warm mold filled with melted paraffin, which cooled and solidified after being removed from the heat source. The paraffin block was then cut to the specimen's size and placed on a microtome, where the thin paraffin sections were collected and floated in a warm water bath before being placed on a glass slide covered with a thin layer of albumen, which acted as an adhesive medium for the specimen. Paraffin was first dissolved from the specimen using xylene to stain the tissue in the sections. The sections were rehydrated with decreasing ethanol (alcohol) concentrations and then stained with H&E stains (Haematoxylin Solution (Harris Solution) and Eosin Y). Following staining, the sample was once more dried out and submerged in xylene. It was then mounted and covered with a glass coverslip for protection on the slide. Mounted tissue sections were examined with a scanning electron microscope (SEM) and viewed on the monitor.

Mortality Ratio

To evaluate mortality, visual and tactile analysis was carried out on each group on a daily basis.

Method of Data Presentation and Analysis

The data collected were presented in table, charts and graphs for clarity purposes. The results are presented as mean ± SD values using Excel 2010.

Results

Fish Mortality

The mortality rate observed during range finding for chromium treated group (10, 30, 50 and 70 mg/L) is indicated in Table 1. Cr at 10 mg/L and 30 mg/L had 25% mortality, 50 mg/L had 50% mortality and 70 mg/L had 75% mortality. Cadmium at 0.5 mg/L had 0% mortality, 1.5 mg/L had 25%, 2.5 mg/L had 50% while 3.5 mg/L had 75% mortality. Cadmium mortality on exposed fingerlings is reported on Table 2.

Table 1. Mortality of fingerlings exposed to chromium during range finding.

Chromium Concentration (mg/L)	No. of fingerlings exposed	Survival	Mortality	Mortality Percentage (%)
10	4	3	1	25
30	4	3	1	25
50	4	2	2	50
70	4	1	3	75

Table 2. Mortality of fingerlings exposed to cadmium during range finding.

Cadmium Concentration (mg/L)	No. of fingerlings exposed	Survival	Death	Mortality Percentage (%)
0.5	4	4	0	0
1.5	4	3	1	25
2.5	4	2	2	50
3.5	4	1	3	75

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Effect of Bioaccumulation on Fish Weight

Figure 1 indicates the differences in weight (kg) in the exposed groups during 14 days exposure. The highest weight at day 1 of exposure was observed in the group in Cd 2.5 mg/L while the lowest was the combination group (Cd/Cr 1.5; 10 mg/L). At day 9, all the groups increased in weight except the Cd 2.5 mg/L which had steady decline. The Cr 20 mg/L group had very insignificant increase in weight at day 9. At day 14, all the groups experienced decrease in weight with exception to the Cd/Cr 1.5; 10 mg/L group.

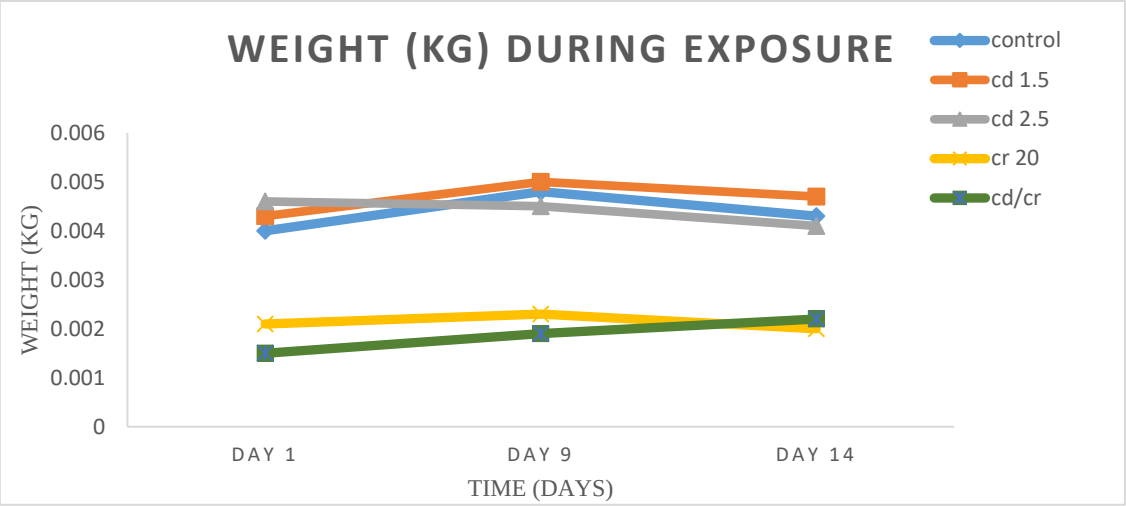

Fig 1. Weight of fingerlings during exposure to heavy metals

Metal Bioaccumulation

Figure 2 showed that cadmium exposure (Cd (exp)) and Cadmium depuration (Cd (dep)) was significant in all the groups except the Cd 1.5 mg/L group. Chromium exposure (Cr (exp)) and Chromium depuration (Cr (dep)) was significant in all the groups. Cr accumulation during exposure was higher singly than in combination with cadmium. In the control group, no accumulation was observed for cadmium and chromium.

Depuration Percentage

Table 3 showed the control had no heavy metal hence there was no depuration percentage, the depuration of Cd 1.5 mg/L was insignificant, the Cd 2.5 mg/L group had 57.14% depuration percentage but in the combination group (Cd/Cr 1.5; 10 mg/L) had 40%. For chromium, the combination group (Cd/Cr 1.5; 10 mg/L) eliminated (depuration) more chromium than the Cr 20 mg/L group.

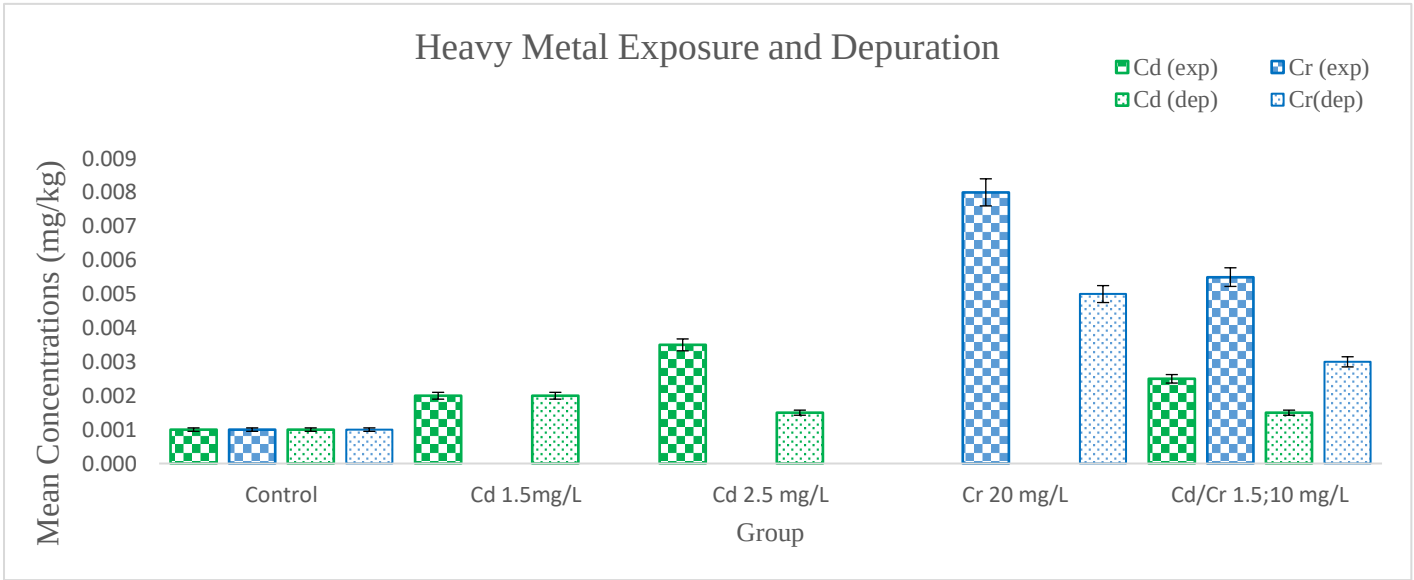

Fig 2. Heavy metals exposure and depuration at different concentrations

Table 3. Depuration Percentage

Groups	Cd(%)	Cr(%)
Control	0	0
Cd 1.5mg/L	0	
Cd 2.5 mg/L	57.14	
Cr 20 mg/L		37.50
Cd/Cr 1.5;10 mg/L	40.00	45.45

Histological Examination

Histological examination of muscles of fingerlings exposed to heavy metals and after depuration (recovery response) as represented in Plates 1 – 10. Plate 1 showed enlargement of muscle fibre and hypertrophy although cartilage and muscle fibres were intact. Mild muscle tissue distortion. Plate 2 indicated inflammatory cells infiltration within the muscle fibres and hypertrophy. Muscle fibres were unaffected. Plate 3 showed severe muscular distortion, general degeneration, necrosis and muscular hypertrophy. Moderate tissue distortion. Plate 4 showed some degenerating muscle tissues, broken muscle tissue and regenerating muscle tissues. Plate 5 revealed degeneration, necrotic fibres, muscular hypertrophy and severe distortion of muscle tissues. Plate 6 showed degenerating muscle tissue, broken muscle fibres and necrosis in some muscle tissues. Faint staining of muscle fibres was also observed. Plate 7 indicated general degeneration, necrotic fibres, inflammatory cells infiltration, muscular hypertrophy of muscle fibres and chronic distortion of muscle tissues. Plate 8 indicated mild hypertrophied muscle tissue and degeneration. Some muscle fibres showed necrosis, inflammatory cell infiltration and distorted muscle tissue with high recovery of muscle tissues. Plate 9 showed degeneration, necrotic fibres and inflammatory cells infiltration. Muscular hypertrophy of muscle fibres and severe muscle distortion are observed. Plate 10 revealed hypertrophied muscle tissue, some broken muscle fibres, necrosis in some muscle fibres and inflammatory cell infiltration. Distorted muscle tissue was also observed.

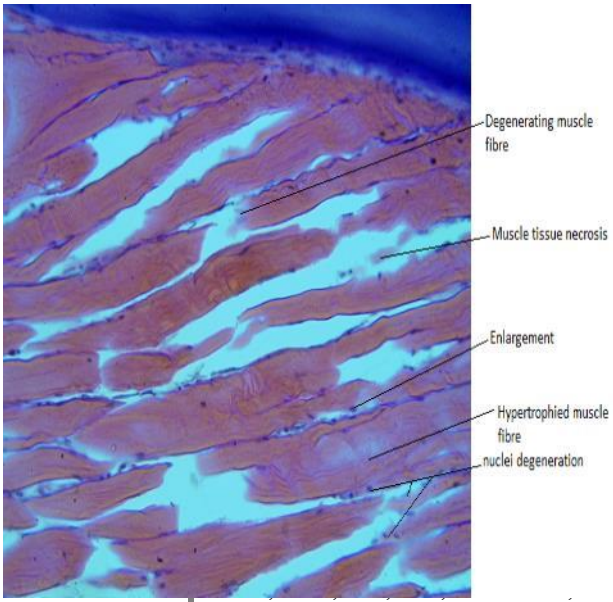

Plate 1. Photomicrograph of the muscle tissue (control) (H & E, X400 – Exposure)

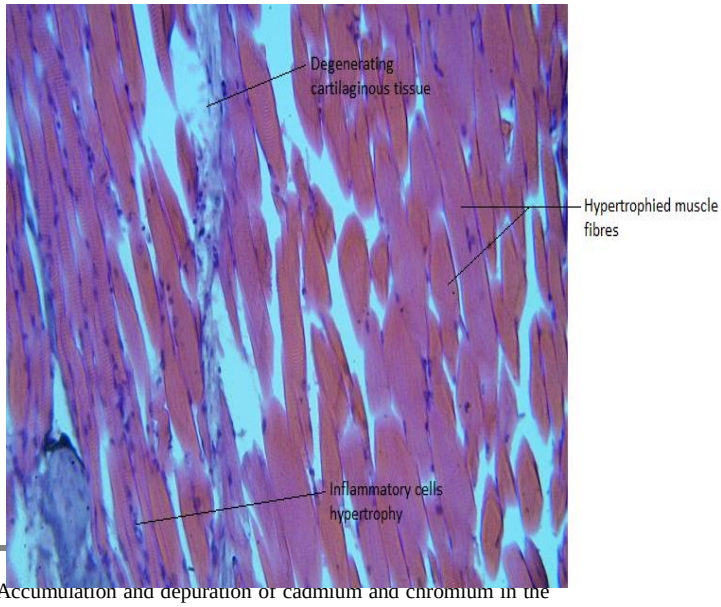

Plate 2. Photomicrograph of muscle tissue (control) H & E, X400 - Recovery

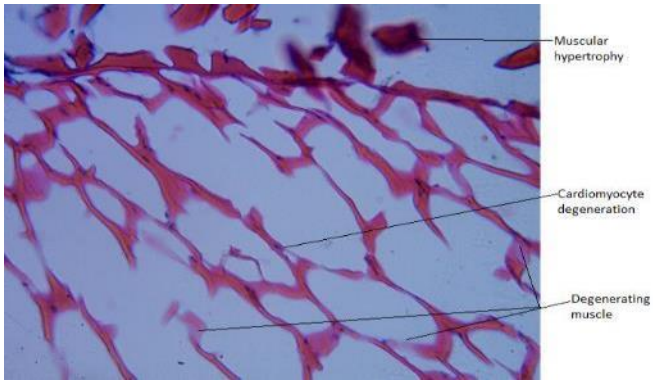

Plate 3. Photomicrograph of the muscle tissue exposed to Cd/Cr1.5, 10 mg/L. H & E, X400 –

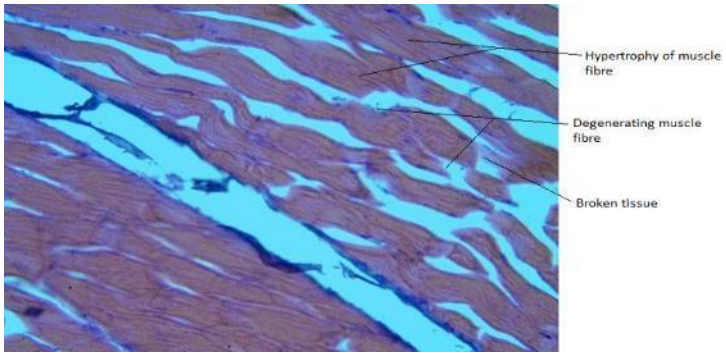

Plate 4. Photomicrograph of muscle tissue exposed to Cd/Cr 1.5, 10 mg/L H & E, X400 -

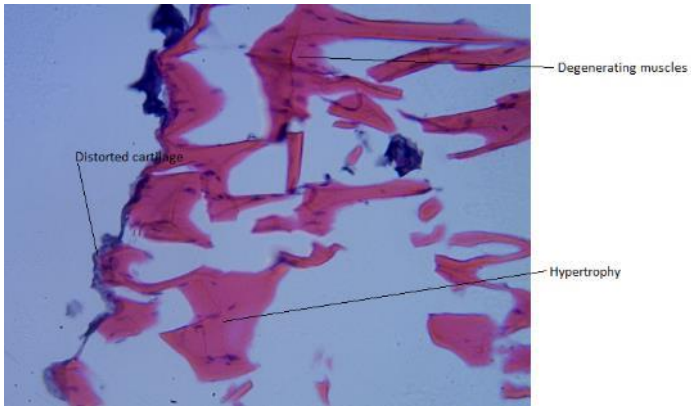

Plate 5. Photomicrograph of the muscle tissue exposed to Cd 1.5 mg/L H & E, X400 - Exposure

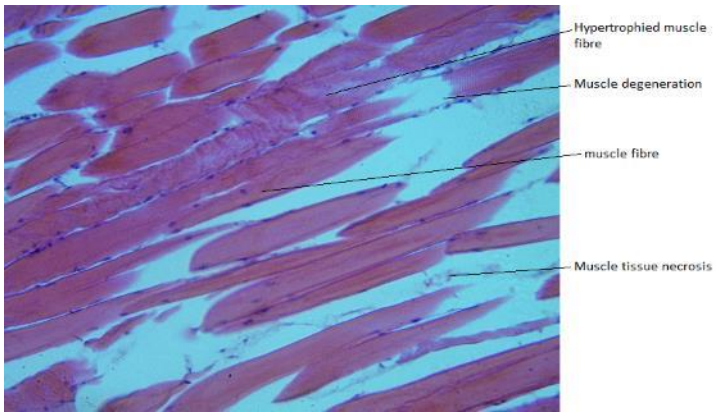

Plate 6. Photomicrograph of muscle tissue exposed to Cd 1.5 mg/L H & E, X400 - Recovery

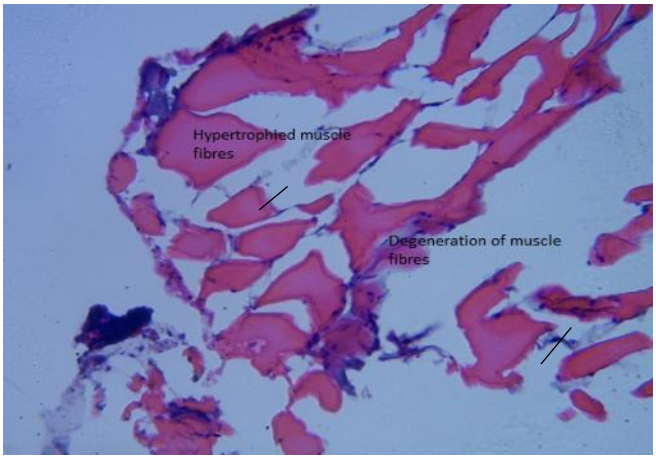

Plate 7. Photomicrograph of the muscle tissue exposed to Cd 2.5 mg/L H & E, X400 - Exposure

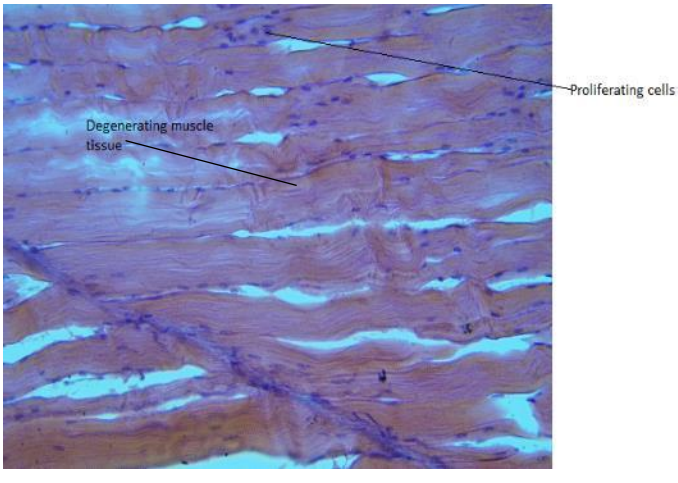

Plate 8. Photomicrograph of muscle tissue exposed to Cd 2.5 mg/L H & E, X400 - Recovery

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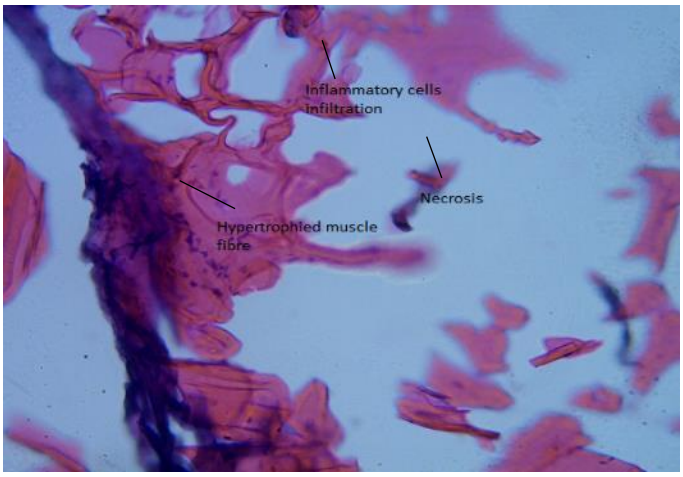

Plate 9. Photomicrograph of the muscle tissue exposed to Cr 20 mg/L, H & E, X400 - Exposure

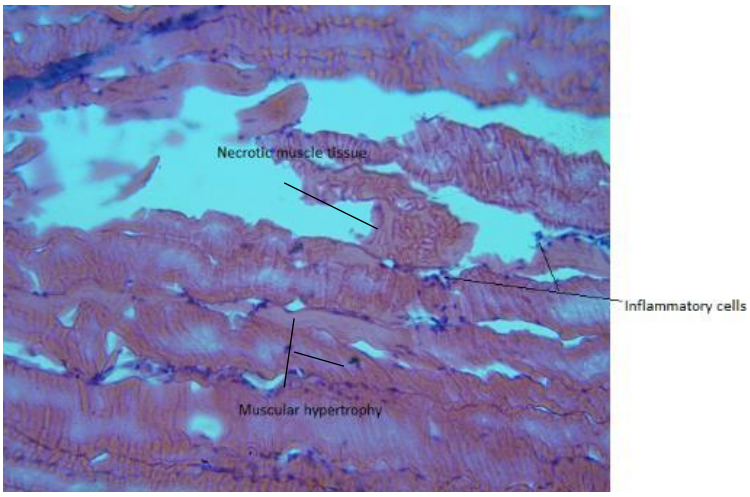

Plate 10. Photomicrograph of muscle tissue exposed to Cr 20 mg/L, H & E, X400 - Recovery

The Semi-quantitative Evaluation of Histopathology

The semi-quantitative evaluation of the histopathology in exposed muscles of Nile tilapia from the pictograph was reported in Table 4 for exposure and Table 5 for recovery. Minus sign (-) represents no alteration while the plus sign (+) represents the presence of alteration, the number of plus signs is used the quantify the severity of alterations.

Table 4. Semi quantitative scoring of the histopathology in the muscles of *Oreochromis niloticus* exposed to cadmium and chromium 14-days exposure.

Histopathological Lesions	Control	Cd/Cr 1.5 mg/L; 10 mg/L	Cd 1.5 mg/L	Cd 2.5 mg/L	Cr 20 mg/L
Degeneration of muscle bundles	-	+++	++	++++	++
Infiltration of inflammatory cells	-	+	++	+++	++
Focal areas of necrosis	+	++	++	+++	++
Splitting of muscle fibres	-	++	+++	+++	++
Distorted Cartilage	-	+	+++	+++	++
Muscle Hypertrophy	+	+++	+++	+++	+++
Loss of interstitial fibres in between the muscle fibres	-	++	+++	++++	++

Score: (-) No alteration, (+) Mild alteration, (++) moderate alteration, (+++) severe alteration (++++) chronic alteration.

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Table 5. Semi quantitative scoring of the histopathology in the muscles of *Oreochromis niloticus* exposed to cadmium and chromium 4-days recovery.

Histopathological Lesions	Control	Cd/Cr 1.5 mg/L; 10 mg/L	Cd 1.5 mg/L	Cd 2.5 mg/L	Cr 20 mg/L
Degeneration of muscle bundles	-	+	++	+	++
Infiltration of inflammatory cells	-	-	-	+	++
Focal areas of necrosis	-	-	+	++	+
Splitting of muscle fibres	-	+	++	++	++
Distorted Cartilage	-	-	++	+	+
Muscle Hypertrophy	-	+	++	+	++
Loss of interstitial fibres in between the muscle fibres	-	+	++	+	+

Score: (-) No alteration, (+) Mild alteration, (++) moderate alteration, (+++) severe alteration (++++) chronic alteration

Discussion

Organisms living in water bodies overtime accumulate these heavy metals and they have deleterious effect on the tissues and organs. Fingerlings exposed to combination of cadmium and chromium (Cd/Cr 1.5; 10 mg/L) increased in weight throughout the 14 days of exposure while those exposed to other concentrations had a steady decline in weight. Combination of cadmium and chromium (Cd/Cr 1.5; 10 mg/L) recorded the highest weight during exposure compared to the control group and Cd 1.5 mg/L group which had a significant increase in weight on Day 9 and declined at the end of day 14. On the other hand, Cd 2.5 mg/L group experienced decline in weight all through the exposure. The decrease in weight is in conformity with Abbas et al. (2019) and Mohammed et al. (2020) who observed a decrease in weight in Nile tilapia exposed to cadmium and Cr^{+6} respectively. Rahman et al. (2018) reported that at varying concentrations, fish growth in weight and length was highest in the control group and lowest in the group with the highest concentration of cadmium, implying that the level of effect of Cd on growth is dependent on the concentration administered. Hu et al. (2021) connected DNA methylation alterations, GH/IGF axis dysregulation, and decreased antioxidant capacity to the weight loss of Nile tilapia exposed to cadmium for 45–90 days. According to the study, chromium and cadmium, respectively, have an adverse influence on Nile tilapia growth performance. Fish exposed to heavy metals typically exhibit behavioral abnormalities. In the experiment, fingerlings exposed to chromium and cadmium respectively was observed to have shade alteration and reduced feeding habit which subsequently led to a decline in their weight. Nisha et al. (2016) observed that trivalent chromium had an effect on the eating habit, swimming pattern and colour of zebra fish.

Muscle is the main edible part of the fish. The trend of cadmium and chromium in the muscles was in the order Cr>Cd. This implies that the muscle absorbs chromium faster than cadmium. In the group exposed to combination of cadmium and chromium (Cd/Cr 1.5; 10 mg/L), uptake and elimination of chromium was higher than for cadmium. The accumulation of cadmium from the result can be deduced to be time and dose dependent, as accumulation of Cd 2.5 mg/L was higher than that of Cd 1.5 mg/L, subsequently, the Cd 2.5 mg/L group eliminated the metal salt faster than the Cd 1.5 mg/L group. The result of this experiment disagrees with the findings of Maurya et al. (2019) and Magna et al. (2021), who observed that chromium in combination with other metal salts, had the least accumulation rate, which is in conformity with the findings of El-Shaer & Alabssawy (2019), and Adorno et al. (2023). Rahman et al. (2018) reported that in different organs of Nile tilapia exposed to Cd, the muscle had the least accumulation, thus establishing that the accumulation tendency of Cd in the muscle is comparatively low, this conforms with the results of Madzingira et al. (2020), were three freshwater fish species exposed to Pb and Cd, only recorded Pb in the muscles with no detection of Cd in the muscles.

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Cadmium in the Cd 1.5 mg/L group had no significant depuration; the Cd 2.5 mg/L group had a 57% depuration percentage, which is the highest for cadmium. The combination group (Cd/Cr 1.5; 10 mg/L) recorded the highest depuration percentage for chromium. Increase in concentration subsequently led to increase in mortality in both metal salts. Comparing weight with depuration rate, it was deduced that depuration aside being concentration dependent is also weight dependent. Fingerlings with higher weight were observed to have depurated faster than those with lesser weight. Chen et al. (2018) examined depuration of Cr (0.5 and 8 mg/L) in Japanese medaka (*Oryzias latipes*) and obtained a 50% and 60% depuration respectively after 14 days. In this experiment, Cr 20 mg/L had a depuration percentage of 37.50%. Babatunde et al. (2020) exposed African catfish juveniles to 5, 10, 15, 20, 25 mg/L Cd and obtained depuration percentage of 18.68, 10.17, 5.95, 1.41 and 8.26 respectively, their result was lower than the result from this experiment were Cd 2.5 mg/L had 57% depuration.

Significant histological changes were observed in the muscles for cadmium and chromium uptake and elimination respectively. Histological examination for the effect of chromium on the muscle tissues was mild compared to cadmium. The severity of cadmium on the muscle tissues increased with increasing concentrations. Cd 1.5 mg/L caused severe damages to the muscle tissues, but 2.5 mg/L Cd caused chronic damage. The faint stains in muscle bundles observed in recovery tissues can be attributed to a reduction in carbohydrate levels (polysaccharide materials) in the muscle. This implies that depuration has no effect on the carbohydrate levels altered during cadmium and chromium exposure (Sayed et al., 2020; Khanh et al., 2022). Group Cd 2.5 mg/L experienced more lesions than other groups. The control group muscles are made up of long muscle fibers called myotomes that are bundled together and split apart by connective tissues called myosepta. Myotomes and Myosepta in exposed tissues could not be seen but were clearly seen in recovery tissues. Abbas et al. (2019) exposed Nile tilapia to 1.5 mg/L Cd for 45 days and observed histological alterations in the muscles such as edema, degenerative and necrotic changes as observed in muscle tissues from this study. Haredi et al. (2020) observed significant changes in the histological examination of exposed tissues and depurated tissues of Nile tilapia from Lake Edku.

Conclusion

The findings from the studies suggest that the accumulation of cadmium and chromium is dose-time dependent, and their elimination is dependent on the uptake. Natural depuration of heavy metals is possible as established in the experiment, but the depuration rate differs from one heavy metal to another. Synergetic effect of combination of cadmium and chromium alters the effect of the individual metal salts on the weight of the fish. This synergy also alters the rate of uptake and elimination of individual metal salts. Depuration rates cannot be used to determine the overall quality of fish before consumption, but it can serve as a complement to other toxicity removal measures. It may be used by farmers and consumers to reduce heavy metal load on fish.

Recommendations

1. Nile tilapia caught in the wild can lose some amount of toxicants when kept in toxicant-free water, hence, fish should be put in clean water to eliminate some percentage of the toxicant.
2. More studies should be conducted to ascertain the synergistic effect of cadmium and chromium on the growth performance of Nile tilapia. Further research is needed to corroborate the relationship between exposure concentrations and depuration rates and patterns.

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