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Aberration of Metals Competing for Iron on Exposure to Lambda Cyhalothrin and Aflatoxins in Dietary Fish from Selected Aquatic Sources in Kenya

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Abstract

Excess or deficiency of minerals may seriously disturb biochemical processes and upset internal homeostasis, leading to various diseases and disorders in fish species due to deficiency or excess of micro and macro elements caused by improper nutrition, avitaminosis or poisoning. The specific objectives of the study were to determine the iron levels and aberration of metals competing on exposure to lambda-cyhalothrin and aflatoxins in dietary fish from selected aquatic sources in Kenya. The concentration of elements cadmium, zinc, and iron in Oreochromis niloticus and Clarias gariepinus bred in Kenya Marine and Fisheries Research Institute at Sagana and obtained from River Nyando was measured using atomic absorption spectrophotometer. Iron availability was lower on treatment with Aflatoxin compared to Lambda-Cyhalothrin with a mean of 3.66 ± 0.84 mg/kg, but on subjection to zinc, competition was 3.82 mg/kg on consideration of zinc competition. The naturally occurring toxins cause micronutrient deprival and therefore relevant stakeholders be keen to prevent contamination from farm to fork.

Keywords

dietary, fish, aberration, metals, competing, aflatoxins, Lambda-cyhalothrin

1. Introduction

Fish is a vital source of food for people and a good source of heme iron (FAO, 2005). Iron, a mineral required by the body to produce red blood cells, occurs in two different forms in the diet heme and non-heme. Heme iron derives from the hemoglobin in animal tissue whereas nonheme iron derives primarily from plant tissues, although a small amount of the iron in animal foods is nonheme iron. The body absorbs heme iron more efficiently than non-heme iron. Eating a wide variety of iron-rich foods, and combining foods that contain heme and non-heme iron will help prevent deficiencies. Sorption and co-precipitation of metals by Fe oxides decreased the bioavailability and toxicity of waterborne metals may increase the dietary supply of metals and lead to toxic effects along the food chain (Vuori, 1995).

Heme iron, found in meat and fish, is more easily absorbed at approximately 15-40% as compared to 1-15% nonheme and is only significantly inhibited by large quantities of dairy products, while fish and meat proteins enhance nonheme iron absorption (Onyangore et al.). The Food and Agricultural Organization (FAO) estimates that about one billion people worldwide rely on fish as their primary source of animal protein (FAO, 2000). It is man’s most important single source of high-quality protein, providing 16% of the animal protein consumed by the world’s population according to the Food and Agriculture Organization (FAO) of the United Nations (2000). It is a particularly important protein source in regions where livestock is relatively scarce fish supplies <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (FAO, 2000). In Kenya, annual fish consumption levels in the country are at 3.7 kilograms per person (Omondi, 2012).

Lambda-cyhalothrin is a synthetic pyrethroid insecticide widely used for pest management and public health applications to control insects. Lambda-cyhalothrin is categorized as a restricted use pesticide in Extension Toxicology Network for its toxicity to fish (Mound, 1998). But its usage for the control of major pest in agriculture is being continued in developing countries. The pesticide exposure causes severe alterations in the tissue biochemistry and histology of fishes (Saravanan et al., 2010; Susan et al., 2010; Velisek et al., 2009).

Aflatoxin is metabolic byproduct of molds *Aspergillus flavus* and *Aspergillus parasiticus*. It is a toxic compound and the cause of high mortality in livestock, poultry, fish and in some cases of human beings (Fotsis et al., 1995; Reed et al., 1987). Toxicogenic *A. Flavus* produces Aflatoxin B_1 and B_2 whereas *A. Parasiticus* produces Aflatoxin G_1 and G_2. Aflatoxin B_1 is classified as a group I carcinogen by international agency for research on cancer. Effect of aflatoxin on fishes and other animals have been reported by many workers. Nunez et al. (1991) reported hepatocellular adenoma and hepatocellular carcinoma in Rainbow trout when exposed to aflatoxin B_1. Caguan et al. (2004) reported a loss of appetite, low survival percent and decreased mean total biomass in tilapia when fed with aflatoxin-contaminated feed.

Since fishes are important sources of proteins, minerals and lipids for humans and domestic animals, so the health of fishes is very important for human beings. Excess or deficiency of minerals may seriously disturb biochemical processes and upset internal homeostasis, leading inconsequence to various diseases. Tacon (1992) reported that disorders occurred in organisms of various fish species due to deficiency or excess of micro and macro elements which were caused by improper nutrition, avitaminosis or poisoning. It is therefore important to monitor levels of macro and micro elements in fish organisms (Brucka-Jastrzębska et al., 2009). The poisoning is majorly caused by toxicants emanating from either feed or water pollution. Previous research work in Kenya has conducted studies on the effects of toxicants on the other aspects of health, but not taken a step further to conduct research on the influence of essential nutrients like iron. The natural and synthetic toxicants in fish may be causing significant changes in the iron levels due to metabolic disruption, and yet no research has specifically checked for the availability of iron. The knowledge gap on nutrient toxin interaction was addressed as findings from this study, therefore, linked toxicants and availability of iron in fish as a heme food source. Results from this study generalized and shared with food scientists, food toxicologists, medical personnel, agriculturalists and the community at large on the magnitude on iron availability in fish on exposure to natural and synthetic toxicants in fish a heme food sources. Previous studies have considered the implications of nutrient toxicant interactions but findings from this study encouraged nutritionists to consider the importance of environmental exposures to their study populations and their research questions. Furthermore, it encouraged the involvement of nutritionists in the design of high quality, rigorous studies of nutritional assessment and interventions in populations exposed to environmental chemicals. As a growing field, the intersection between nutritional science and toxicology benefited from the expertise of nutritionists.

2. Materials and Methods

2.1 Study Design, Species, Sample Size, and Setting

For fish bred at Sagana Experimental study design was adopted where manipulation of independent variables to determine their effect on a dependent variable. A completely randomized block design was adopted. For Fish from River Nyando were collected at three different points (Appendix 31). A Cross-Sectional study design was also adopted where fish samples were collected from River Nyando for laboratory analysis. The independent variables were the treatments and the dependent variable being iron levels (Kothari, 2004). For fish bred at Sagana Identification of *Oreochromis niloticus* and *Clarias gariepinus* species was done by the Kenya Marine and Fisheries Research Institute (KMFRI) staff. Nine Hundred (900) fish, 450 of each species were bred in the lab between January-August 2015.

Sample size calculation for fish bred at Sagana

$2\ SD^2 (Z^t + Z^x)^2 / d^2$

SD = Standard Deviation from Pilot Study

Z^t = Z value from the Z table

Z^x = type 1 error of 5% at 80% interval

D = effect size, the difference in means (iron in mg) (Jaykaran & Kantharia, 2013).

$= 2(1)^2 (1.96 + 0.842)^2 / 3.5$

= 4.48

= 5 fish per tank

Fish from River Nyando

$2\ SD^2\ (Z^t + Z^x)^2 / d^2$

$= 2(1)^2\ (1.96 + 0.842)^2 / 3.5$

$= 2(1)^2\ (1.96 + 0.842)^2 / 0.145$

= 108.29

= 108 fishes

2.2 Sampling Techniques

For fish bred at Sagana, there are about thirty-five species of fish commonly consumed in Kenya were written down, and the two fish species were selected randomly. For the fish bred in an aquarium in the lab, all the fish sampled were used for the experiment. The fish species used were *Oreochromis niloticus* and *Clarias gariepinus* and were cultured in the lab for twelve weeks after demineralization of water. The samples were extracted immediately and analyzed for iron levels cadmium and zinc as metals competing with iron, This was after treatment with aflatoxins in feed prepared and Lambda-Cyhalothrin introduced in water and parameters measured when fish were at various ages. For fish from River Nyando, the sampling procedure for fish species collection was the same as in fish bred in Sagana. The fish were selected at three different sites along River Nyando. For each site fish were collected by means of gill net mesh size. The nets were deployed from early morning and checked two hourly until the required fish quotas were reached. This minimized the amount of time fish spent in the net in order to reduce an imposed stress. Only living fish size 10-60 g were selected, kept in a 100 L plastic tank and immediately transported to the laboratory for sample collection and necropsy analysis. A total of 105 fish specimens were collected from River Nyando. The samples were extracted immediately and analyzed for iron levels and cadmium and zinc as metals competing with iron.

2.3 Laboratory Procedures for Heavy Metal Analysis

For both fish samples collected from fish bred at Kenya Marine and Fisheries Industries (KMFRI) Sagana and from River Nyando, the laboratory sites and procedures were similar. At the laboratory, fish were kept in large holding tanks filled with water from each site to minimize stress.

The heavy metal analysis was conducted at KIRDI (Kenya Industrial and Research Development Institute). The sampling containers, preparation and handling of fish samples for analysis, the procedure for the dissection of fish3, sample collection from different parts of the fish, sample collection from different parts of the fish was adopted as per previously published work (ICP Waters report, 105/2010). All glassware was washed in nitric acid solution and rinsed with distilled water. All reagents used during analysis were of analytical grade. This was digested using the procedure recommended by (Chen et al., 2008) as described. An acid mixture of two parts concentrated nitric acid and one part perchloric acid (2:1) was prepared. One gram (1.0 g) dried liver material was weighed and put in a 100 ml digestion tube. 5 ml of the acid mixture was added and the sample was placed on a hot plate. The samples were heated at 60^0C for 15 minutes. The heat was then increased to 120^0C and digested for 75 minutes or until the sample cleared. The tube was removed from the hot plate when the sample was clear. The sample was then cooled and sufficient distilled water added to bring the solution to 100ml and preserved in the cold room at 4^0C awaiting analysis by AAS. A blank comprising of the reagents in the proportion as for the samples but containing no sample material was prepared likewise.

The concentration of elements (Fe Cd and Zn) in muscle and fish liver was measured using atomic absorption spectrophotometer (AAS) AAnalyst 800 (Parkin Elmer Instrument, 2USA) with an acetylene flame (Fe and Zn) and argon non-flame (Cd), after preparation of calibration standards. The overall recovery rates (Mean ± SD) for Cd, Zn were 103 ± 8.3 and 2.3 and 90 ± 3.5 respectively. The detection limit for Fe, Cd, and Zn was 0.04, 0.02 and 0.10 μg/g respectively.

Detection of Lambda-Cyhalothrin was conducted in water from River Nyando. Sample analysis was done using Varian CP 3800 Gas Chromatograph equipped with an Electron Capture Detector. Separation was done using BPX 5 capillary column of dimensions 30 m x 0.25 mm x 0.25 μm film thickness. Confirmatory analysis was done using a BPX35 capillary column of dimensions 50 m x 0.25 mm x 0.25 μm film thickness. A temperature program was used starting from 90^0C (withhold time of 3 minutes), increased to 215^0C at 8^0C/min (withhold time of 25 min), then increased to 270^0C at 5^0C/min (withhold time of 5.37 min), and finally ramped to 275^0C at 5^0C/min (withhold time of 18.63 min). The carrier gas was high purity helium (99.9995%) with white spot nitrogen as the makeup gas. Quantification followed external calibration method using high purity pesticide reference standards mixture obtained from Ultra Scientific USA. Quality control and Quality assurance were ensured by all sampling, extraction, and analysis being done in triplicate to allow verification detected PoPs residues. The samples were spiked with the insecticide during extraction and analysis to minimize errors due to detector fluctuations. Recovery tests were also carried out using the reference pesticide standards to determine the performance of the methodology. Quantification of LCH was carried out using high purity organic pollutants. Heavy metal assessments for fish both from bred in Sagana and River Nyando, the iron cutoffs were 20-80 mg/kg, the zinc ranges 20-55 mg/kg, the cadmium ranges 0.05-5 mg/kg were as per the European Standards**.**

3. Data Management and Analysis

3.1 Data Reporting

For both fish obtained from Sagana and from River Nyando, data included iron levels, zinc levels, cadmium levels. For fish from River Nyando, Lambda Cyhalothrin and aflatoxin were recorded from the water samples (Kroglund et al., 2007; Rognerud et al., 2002).

3.2 Data Analysis

The independent variables were the treatments aflatoxins and lambda-cyhalothrin. The dependent variables were iron levels and metal levels competing for iron availability, the data was entered into a computer and analyzed using the excel spreadsheets as database and GENSTAT version 12 analysis for means, frequencies and cross-tabulations. For fish obtained from river Nyando, SPSS version 21 was used for data analysis. One way ANOVA was carried out to determine differences in means of iron levels, zinc levels, and cadmium levels lambda-cyhalothrin, aflatoxins. Post hoc HSD (turkey) (was used for Post-hoc discrimination between means. The effects of aflatoxins and lambda-cyhalothrin were analyzed using linear regression analysis after performing an outlier analysis.

The interaction between lambda-cyhalothrin, aflatoxins, iron levels, zinc and cadmium competing for iron was analyzed by ANCOVA. In all statistical tests, 5% significance level was applied.

3.3 Ethical Considerations

All experiments with fish were conducted in accordance with national and institutional guidelines for protection of animal welfare (Prevention of Cruelty to Animals Act, Cap 360 of the Laws of Kenya). Authority to conduct research was sought from the Fisheries department of Kenya and from the various sites, KEMFRI Sagana, and KIRDI Nairobi.

4. Results

4.1 Determine Iron Levels

4.1.1 Iron Levels in *Oreochromis Niloticus* and *Clarias Gariepinus*

The mean Fe in both species was 5.0369 ± 1.443 mg/kg with a maximum level of 7.767 mg/kg. The level of iron of 5.22 mg/kg in *Oreochromis niloticus* was higher than that in *Clarias gorgeous* which was 4.86 mg/kg.

Table 1. Comparison of Means of Iron on Treatment with Lambda-Cyhalothrin versus Aflatoxin in *Clarias Gariepinus* and *Oreochromis Niloticus* in Fish from Sagana River Nyando, Metal Concentrations in mg/g DW and n=54

Environment	Species1	SEM	Species2	SEM	P value
1 Sagana	4.86 ± 1.49	0.29	5.22 ± 1.40	0.27	0.367
2 River Nyando	17.11 ± 4.47	0.66	25.68 ± 3.52	0.44	0.000

Results from Table 1 indicate that there were significant differences of treatments on iron levels between species *Clarias gariepinus* and *Oreochromis niloticus* $P > 0.05$ in fish bred in River Nyando and no significant differences in iron levels in species in fish bred in Sagana. Fish in River Nyando had higher iron levels with a mean of 17.11 ± 7.81 mg/kg and 25.68 mg/kg in *Clarias gariepinus* and *Oreochromis niloticus* respectively than in fish bred in River Nyando.

Comparing means on iron levels on analysis of variance in Clarias *gariepinus* and *Oreochromis niloticus* on exposure to Aflatoxin and Lambda-Cyhalothrin in selected environments.

Table 2. Comparing Means on Iron Levels after Analysis of Variance in Clarias *Gariepinus* and *Oreochromis Niloticus* on Exposure to Aflatoxin versus Lambda-Cyhalothrin in Sagana and River Nyando

Condition	Lambda Cyhalothrin	S.E.M	Aflatoxin	S.E.M	P value
Sagana	4.78 ±0.72	0.17	3.66 ±0.84	0.200	0.01
River Nyando	22.09 ±7.81	0.59	ND	ND	0.97

Results from this study (Table 2) show that the type of treatment had a significant effect ($p = 0.01$) on iron availability ($p < 0.05$). However, treatment had no significant effect on iron availability from fish in River Nyando. Fish from River Nyando had higher iron levels than those bred in Sagana.

4.2 The Significance of the Type of Treatment

Table 3. Tukey Means Separation to Show the Significance of Types of Treatment on Aflatoxin and Lambda-Cyhalothrin on *Clarias Gariepinus* and *Oreochromis Niloticus* in Cultured Fish

Treatment	Mean(Sagana)	S.E.M	P value
Aflatoxin	3.66^a	0.20	**0.01**
Control	6.68^c	0.13	
Lambda Cyhalothrin	4.78^b	0.17	

Both of the treatments had lower iron levels as compared to the control and Lambda-Cyhalothrin treatment had higher iron levels as compared to aflatoxin treatment at 3.66 mg/kg (Table 3). The treatments influenced the tissues differently, i.e., the treatments were significantly different.

H_o There is no significant aberration of iron on exposure to Lambda-cyhalothrin versus Aflatoxins in dietary fish in selected environments in Kenya.The null hypothesis was rejected because the treatments influenced the iron levels differently.

4.3 Assess Metals Competing for Iron Availability

4.3.1 Zinc and Cadmium Availability

Table 4. Summary Statistics of Zinc in Livers of Clarias Gariepinus and Oreochromis Niloticus on Treatment with Lambda-Cyhalothrin and Aflatoxin in Fish Bred in Sagana

Size (n)	Mean (mg/kg)	Variance	SD	SEM
54	19.85	11.58	3.403	0.4631

The mean zinc levels were 19.85 ±3.403 mg/kg (Table 4).

4.3.2 Mean Comparisons of Zinc and Cadmium in Levels in the Selected Environments

The zinc levels in fish from River Nyando were higher at 34.585mg/kg as opposed to the fish bred in

Sagana. Results from this study further showed that the type of treatment had a significant difference on zinc levels ($p < 0.05$) in fish bred in Sagana as opposed to from River Nyando. There was no significant effect of treatment on the cadmium levels in River Nyando (Table 5).

Table 5. Comparison of Means on Analysis of Variance in *Clarias Gariepinus* and *Oreochromis Niloticus* on Zinc Levels on Treatment with Lambda-Cyhalothrin and Aflatoxin in Fish Bred in Sagana and River Nyando

Condition	Lambda-Cyhalothrin	S.E.M	Aflatoxin	S.E.M	P value
Sagana	16.60 ±0.09	0.22	18.72 ±0.14	0.33	0.01
River Nyando	34.585 ±14.66	1.411	ND	ND	0.575
Sagana(Cd)	ND	ND	ND	ND	ND
River Nyando	5.82 ±1.42	0.136	ND	ND	0.429

4.3.3 Comparison of Means of Zinc and Cadmium on Exposure to Lambda-Cyhalothrin versus Aflatoxins

Table 6. Comparison of Means on Exposure with Lambda-Cyhalothrin versus Aflatoxin in *Clarias Gariepinus* and *Oreochromis Niloticus* Bred in Sagana and from River Nyando

Environment	Species1	S.E.M	Species2	SEM	P value
Sagana Zn	19.78 ±3.54	0.68	19.91	0.94 ±3.32	0.64
River Nyando	32.76 ±14.62	2.18	35.88 ±14.67	1.85	0.278
Sagana Cd	ND		ND	ND	ND
River Nyando	5.79 ±1.36	0.20	5.84 ±1.47	0.18	0.863

There was no significant effect ($p > 0.05$) in the type of treatment among the species from both environments as presented in Table 6. Cadmium was not detected in fish that was bred in Sagana.

Table 7. Tukey's Comparison of Means of Zinc on Treatment with Lambda-Cyhalothrin and Aflatoxin in Clarias Gariepinus and Oreochromis Niloticus in Fish Bred at Sagana

Treatment	Means	S.E.M	P value
L-cyhalothrin	16.60^{a}	0.22	0.01
Aflatoxin	18.72^{b}	0.33	
Control	24.22^{c}	0.18	

Results from this study show that the treatments affected the zinc levels differently. The control had the highest zinc level of 24.22 mg/kg. Lambda-Cyhalothrin had lower zinc levels compared to Aflatoxin of

18.72 mg/kg (Table 7).

H₀ There is no significant aberration of metals competing for iron bioavailability in Lambda-Cyhalothrin versus Aflatoxins compared in dietary fish in selected environments in Kenya

The hypothesis was rejected because the treatments influenced the metals competing with iron levels significantly.

4.3.4 Significance of Iron Availability on Coexistence with Zinc on Exposure to Aflatoxins and Lambda-Cyhalothrin

On conducting an Analysis of Covariance (ANCOVA), the type of treatment on the zinc and iron interaction was significant ($p > 0.05$).

Table 8. Comparing the Significance of Iron Availability on Competition and without Competition with Zinc on Exposure to Aflatoxins and Lambda-Cyhalothrin Treatments in *Clarias Gariepinus* and *Oreochromis Niloticus* Cultured Fish

Treatment	Means (iron/zinc)	Df	P value	Means (Iron)
L-cyhalothrin	3.816^{a}	2	**0.140**	3.659^{a}
Aflatoxin	5.222^{b}			4.769^{b}
Control	6.070^{b}			6.681^{c}

The means did not differ significantly on exposure when zinc and iron interacted on exposure to the two treatments. The means of iron without competition were (3.659 and 4.769) and got higher on exposure to treatments and on the competition with zinc (3.816, 5.222). However, in the control, the iron levels were higher on interaction with zinc 6.681 mg/g (Table 8). The treatment was not significant $p < 0.05$.

5. Discussion

Fish is a major dietary heme source in our diets and hence should be safe and contain uncompromised iron levels when consumed. Exposure of fish to contaminants through feed and environment is likely to cause compromised safety to humans and altered nutritional levels. Most insecticides have been known to be highly toxic to nontarget organisms that inhabit natural environments close to agricultural fields. Several studies reported that some surface waters and surrounding environments were contaminated with different insecticides (Arjmandi et al., 2010; Bagheri, 2007; Ghassempour et al., 2002; Rahiminejad et al., 2009; Tarahi Tabrizi, 2001). Findings from this study conform to the studies that insecticides like lambda-cyhalothrin were detected in river Nyando.

Results from this study show that iron levels were higher in *Oreochromis spp* than in *Clarias gariepinus* when tests were conducted in the cultured fish. The same result was obtained from the fish collected from River Nyando showed that the *Clarias gariepinus* had lower levels of iron than

Oreochromis niloticus. Fish obtained fromRiver Nyando had higher levels of iron of 22.09 mg as compared to 5.04 mg in from river Nyando. Fish found in water bodies with high levels of metals accumulate higher amounts of metals than those found in uncontaminated water bodies because they absorb these metals from gills, skin oral consumption of water, food and non-food particles. It is expected that iron levels in *C. gariepinus* fish caught in the River Nyando should have higher values as fish absorbs metals from the sediment, polluted water, and food and thus leads to contamination of the food chain. The low iron levels contrary to other studies could be attributed to toxicity from pesticides in water. Metals are not easily biodegradable and consequently can be accumulated in human vital organs. This situation causes varying degrees of illness based on acute and chronic exposure (Demirezen et al., 2006; MATHENGE, 2013). The introduction of these elements into the food chain may affect human health as the excess of iron can cause toxicity (Coulate, 1992). Further, the iron levels measured by (Oyoo-Okoth et al., 2010) in *Rastrineobola argentea* are more or less similar to the *C.gariepinus* of this study, but *R.argentea* is herbivorous and thus lower on the food chain than *C.gariepinus*. It can be expected that *C.gariepinus* from Lake Victoria investigated by (Oyoo-Okoth et al., 2010), would have had greater levels, due to bio-magnification, as *R.argentea* are prey to *C.gariepinus*.

The results presented suggest a greater effect of aflatoxin on iron levels in cultured fish livers as compared to lambda-cyhalothrin. Since fish is a vital source of proteins and lipids for humans and domestic animals, the health of fish is very important to human beings. Fish like other aquatic organisms may be exposed to a great range of insecticides during the course of their life cycle. In fish, different insecticides can be absorbed through gills, skin or alimentary ducts (Mahdi Banaee, 2012; M Banaee et al., 2011). Fishes are particularly sensitive to environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (M Banaee et al., 2011). The differences in iron levels could be attributed to a greater physiological damage the aflatoxin causes as compared to the pyrethroid and hence the effects of aflatoxins on fishes are of great concern at its mode of action is hepatotoxicity.

The concern of iron levels is of vital importance more so because fish might look apparently healthy despite accumulating metals to a concentration which substantially exceed maximum values considered safe for human consumption. Aflatoxicosis is a major problem related to aquaculture that leads to economic losses and health complications in fish. Aflatoxins are well recognized as a cause of liver cancer, but they have additional important toxic effects. In farm and laboratory animals, chronic exposure to aflatoxins compromises immunity and interferes with protein metabolism and multiple micronutrients that are critical to health. These effects have not been widely studied in humans, but the available information indicates that at least some of the effects observed in animals also occur in humans (Jonathans et al., 2004). Although aflatoxin is known to affect nutritional concentrations of iron in animals (Dhanasekaran, 2011) its potential effect on human iron concentrations is not known.

It is, therefore, becoming a matter of concern to environmentalist since the presence of pollutants, particularly; essential metals apparently accumulate in the sediments. In a study that was conducted in Lake Kanyaboli, metal level in the liver of *C.gariepinus* was significantly associated with heavy metal concentration in lake sediment indicating that fish liver is an effective indicator of fish exposure to heavy metals (Akwiri et al.).

In the fish obtained from River Nyando, the lambda-cyhalothrin levels were not significant to the iron levels both in the liver. The many available pesticides for use and elevated metals concentrations from effluent has caused an indirect association between pesticides use and iron levels. There was no aflatoxin association in the fish from the river. The iron levels generally on exposure to both lambda-cyhalothrin and aflatoxin were lower than iron levels in other studies. In a study conducted in Serbia to ascertain the levels of microelements in freshwater fish, the liver values ranged from 4.6-37.3 mg/kg (Brucka-Jastrzębska et al., 2009). In another study conducted in Bangladesh to determine iron levels in long-whiskered catfish, iron levels were higher than in the present study with ranges 54.93 ± 4.33 mg/kg. This could be attributed to the interference of the toxicants with normal physiological processes involving iron synthesis.

In this study the effects of lambda-cyhalothrin, a synthetic pyrethroid was compared to those of aflatoxin a naturally occurring toxicant on iron levels contained in fish.

5.1 Effects of Aflatoxins and Lambda-Cyhalothrin on Levels of Metals Competing for Iron Bioavailability in Fish as a Food Source in Kenya

Results from this study indicate that there were higher zinc levels in aflatoxin-exposed fish, a metal which competes with iron. In this study, the type of treatment had a significant difference in zinc levels. The treatments affected the zinc levels differently with the treatments having the lower zinc levels compared to the control lambda-cyhalothrin treatment which had a higher level of zinc as compared to aflatoxin treatment. Zinc is known to compete with iron during metabolism.

Further, results from this study indicated that iron levels increased on the association to zinc availability. In the ideal, zinc competition could have seen lower iron levels in fish liver. Ewa in a study to determine micro and macro elements in fresh water reported higher and within normal physiological ranges of zinc than in the present study. This study also found out that zinc levels were affected and it is evident that zinc competition was insignificant in the presence of the treatments; this is probably due to the impairment of receptors by the respective treatments. Studies also revealed that co-administration of zinc with lambda-cyhalothrin to treat animals retained the level of GSH and the activity SOD and GPx at the normal values. Catalase, CAT, GST activity and LOP level were improved, and such alterations were still significant in zinc- LCH-treated rats. The observed normalization trend of GSH, SOD, and GPx following zinc treatment could possibly be due to dismutation of O^{-2} to H_2O which is catalyzed by SOD. Zinc is known to induce the production of metallothionein, which is very rich in cysteine, and is an excellent scavenger of -OH. Also, the NADPH oxidases are a group of plasma membrane-associated enzymes, which catalyze the production of O^{-2} from oxygen by using NADPH as the electron donor. Zinc is an inhibitor of this enzyme. Cytochrome P_{450} enzymes are essential for the metabolism and detoxification of many xenobiotics (e.g., pesticides). It has been reported that many chemicals (e.g., pesticides, drug) interactions are the result of an alteration of CYP_{450} metabolism, LCH decreased cytochrome P_{450} activity in LCH-treated rat. This may be due to the inhibition of heme synthesis and destruction of cytochrome P_{450}. Previous studies showed that many pesticides have been reported to inhibit the activity and alteration in the expression of various cytochrome P_{450} isoforms (e.g., parathion, methomyl). These changes may increase the sensitivity of cells against reactive endogenous metabolites or other xenobiotics. Co-administration of zinc to LCH-treated animals improved the activity of cytochrome P_{450} compared to an LCH-treated rat. This change may due to the antioxidant role of zinc and alter the enzyme activities associated with antioxidant defense mechanisms (Abbasy et al., 2014). The same reaction is likely to occur in fish.

Fish from the river had higher zinc levels of 34.56 mg as compared to those bred in Sagana. Findings to this study suggested that species had no significant influence on metals competing with iron. Contrary to findings in this study is that zinc /iron competition is due to species composition and not due to the treatment. The study finding in Solomons and Jacob’s found that zinc from Atlantic oysters which are the most zinc-rich natural food items in the occidental diet, was impervious to the inhibitory effects of a 2:1 ratio of iron to zinc, even when 54 mg of zinc (from oysters) and 100 mg of iron as ferrous sulfate (a total of 154 mg of the metals) were given. The absence of an inhibitory interaction may be explained by the chemical form of zinc in the oyster, a physical-chemical protection against the intrusion of iron or accelerated binding of the iron or oxidation of the iron to its ferric form by the oyster (Solomon et al., 2013).

Cadmium (cd) was not detected in fish under controlled conditions but was detected in the fish from the River Nyando. Concentrations of elements such Cd have been previously reported in water and fish (Birungi et al., 2007; Oyoo-Okoth et al., 2013; Wandiga et al., 2002). The findings from this study are consistent with studies conducted by Saeed and Shaker, 2008 who presented a report about concentrations of Fe, Zn, Cu, Mn, Cd, and Pb in *O. niloticus* (Tilapia) fish tissues, water and sediments in northern Delta Lakes. They found that the edible part of *O. niloticus* from Lake Edku and Manzala contained the highest levels of Cd while fish from Manzala Lake contained the highest level of Pb. They reported that Nile tilapia caught from these two Lakes may pose health hazards for consumers. Furthermore, (Olowu et al., 2010) determined the concentrations of Zn, Ni, and Fe in tissues of two fish species, Tilapia and Catfish from two stations in Lagos, Nigeria. They concluded that both fish species may be considered safe for consumption, but the need for continuous monitoring to prevent bioaccumulation is necessary.

Results from this study revealed that *C garipenus* had higher concentrations of zinc and cadmium as compared to zinc. This is consistent with findings from studies conducted in *M. furnieri* which gave the highest concentrations of Pb, Zn, and Cd by 0.552, 20.535 and 0.090 µg/g, respectively, also *M. furnieri* accumulated highest concentrations of Cd and Zn than other species in present study, this may be interpreted by the living and feeding habits of *M. furnieri* (croaker) which is a bottom-dwelling marine species, found in muddy and sandy bottoms in coastal waters. It is considered as a benthic feeder, it feeds on benthic migratory crustaceans and sessile mollusks and occasionally preying on fish. (Romeo et al., 1999), reported that levels of metals found in tissues of benthic fish were always higher than those found in pelagic fish as in *C. garipenus*

High concentration of both Zn and Cd in *M. furnieri* may be attributed to the relation between them which has been studied in terrestrial and marine mammals. The increase in Zn concentration was attributed to compensating the increase in Cd concentration due to pollution processes, and this mechanism probably includes the synthesis of metallothioneins (or metallothionein-like proteins), which would bind both Cd and Zn in a molar ratio of 1:1 (Marcovecho, 2004).

Zinc (Zn) is an essential trace element, is relatively nontoxic and is integral to several key functions in human metabolism Not only has Zn been identified as a component of key enzymes and regulatory proteins, but it was also recently suggested that the preventive effects of zinc may partly be mediated through an increase in cytochrome P_{450} enzymes.

6. Conclusion

Stored iron in the liver is higher on treatment with lambda-cyhalothrin than with aflatoxin. The latter causes liver damage. The iron levels in both treatments are lower than in the control which signifies impaired iron metabolism. Further aflatoxins give a significantly higher level of zinc than lambda-cyhalothrin. On treatment aflatoxins with the facilitation of zinc to compete is better than with lambda-cyhalothrin. This is because aflatoxins disrupt the divalent binding receptors. The fish caught in the wild have the highest levels of toxic metals like cadmium and highest levels of essential elements as compared to cultured fish. Many processes and factors in the water body affect these levels. Lambda-cyhalothrin, in this particular study a synthetic insecticide has less severe effects on metabolism and iron availability than in the naturally occurring toxin aflatoxin.

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