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Environmental Contamination and Toxicology

Effects of Lead and Cadmium Interactions on the Metal Accumulation in Tissue and Organs of the Nile Tilapia (*Oreochromis niloticus*)

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Due to industrialization, the entrance of toxic metals into aquatic systems is on the increase and this creates an important environmental problem ecologically. Lead and cadmium are highly toxic non-essential heavy metals and they do not have a role in biological processes in living organisms. Thus even in low concentrations, both lead and cadmium could be harmful to living organisms (Burden et al., 1998). The main effect of the increase in concentration of lead and cadmium in aquatic systems to teleost (Tao et al., 1999) and aquatic invertebrates (Gundacker, 1999) is the accumulation of lead and cadmium in tissues and organs of these organisms. Specifically, it has been demonstrated that Cd might cause pathological changes in liver (Friedman and Gesek, 1994), testicle (Shen and Sangiah, 1995), brain and nervous system (Novelli et al., 1999), gill (Voyer et al., 1975) and skeletal (Muramoto, 1981) systems. cadmium also changes osmoregulatory behaviour by affecting enzyme activities and membrane integrity, the ionic composition of blood plasma (Sjöbeck et al., 1984) and the secretion of neurotransmitter compounds (Gill and Epple, 1992). Lead, on the other hand , was found to inhibit the impulse conductivity by inhibiting the activities of monoamine oxidase and acetylcholine esterase (Katti and Sathyanesan, 1986), to cause pathological changes in tissue and organs (Rubio et al., 1991) and to impair the embryonic and larval development of fish species (Dave and Xiu, 1991).

Metals do not exist alone in natural environments. There are numerous investigations on the effects of metal mixtures to the metal accumulation in teleost tissues and organs (Allen, 1995; Pelgrom et al., 1995). However, there is little known about the effects of lead and cadmium interaction on metal accumulation in tissues and organs of Nile tilapia (*Oreochromis niloticus*). It is important to investigate the effects of heavy metal mixtures on the accumulation of metals in fish tissues and organs in relation to biotransformation of these through the body, their excretion and evaluation of contamination caused by heavy metals in aquatic systems (Wicklund et al., 1988). Since heavy metals do exist together in contaminated areas and their accumulation and toxic effects change by the type of metal and exposure time, it is neccessary to demonstrate the effect of metal mixtures on fish. This investigation was aimed at demonstrating the effects of the mixture of certain concentrations of lead and cadmium on the accumulation of metals in structurally different and metabolically active tissues like liver, gill, kidney, spleen and muscle in *Oreochromis niloticus* for 7 and 15 day periods.

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MATERIALS AND METHODS

Fish used in the experiment were randomly selected from stock ponds and brought into a laboratory where they have been acclimatised to the controlled environment in 40 H x 100 L x 40 W cm glass stock aquaria for one month. Fish were measured for weight and length at the end of this period.The average length and weight of fish to be used in the experiment were measured as 14.44 ± 0.41 cm and 59 ± 0.68 g respectively. The temperature in the laboratory was kept at 25 ± 1 °C. The photoperiod was 12 L ; 12 D throughout the experimentation. Experiments were conducted in two series to determine the accumulation levels in tissue and organs of *O. niloticus* when exposed to Pb, Cd and the mixture of Pb and Cd for both 7 and 15-day exposure time. In each series, 6 aquaria similar in size to stock aquaria were used and arranged as three groups. The first aquarium in each group was filled with 120 L tap water having no measureable amount of lead and cadmium. Following the fill up, the concentrations of cadmium, lead and Cd – Pb mixture in these aquaria were adjusted to 1.0 mg/l. The second aquarium in each group, on the other hand, was filled again with same tap water and designated as control. Several water quality parameters were measured as follows; pH; 7.86 ± 0.17; Dissolved oxygen; 7.47 ± 0.23 mg/l; Total hardness; 195 ± 6.02 $CaCO_3$ mg/l; Total alkalinity; 240 ± 3.44 $CaCO_3$ mg/l. Oxygen was supplied to the system through a central aeration unit. Six fish were randomly allocated to each tank for this experiment. Due to adsorption, precipitation and evaporation, the concentrations of lead and cadmium in aquaria were maintained using freshly made stock solutions in every two days throughout the experimentation.

At the end of 7 and 15 day exposure time, six fish from each aquarium were taken out and anaesthetised using MS 222 (tricane methanesulphonate, 75 mg/l). Liver, gill, kidney, spleen and muscle tissues of these fish were used to determine the level of metal accumulation. There was no statistical difference between the length and weight measurements of fish in each aquarium before and after the experimentation ($p > 0.05$). Throughout the experiment fish were fed with commercial fish feed containing no measureable amount of lead and cadmium at 2 % BodyWeight once per day. In order to determine the dry weights, all the tissues taken from each experimental fish were oven dried at 120 °C for 48 hours. Dry tissue samples were put into digestion flasks and 2 ml nitric and 1 ml percholoric acid 2:1 v/v (Merck) were added to them. The digestion flasks were then put on a hot plate set to 130 °C (gradually increased) until all the materials were dissolved. After digestion the digests were diluted with deionised water appropriately in the range of standards that were prepared from stock standard solution of the metals (Merck). Metal concentrations were measured using a Perkin Elmer AS 3100 flame Atomic Absorption Spectrophotometer. The blanks were also included in each run of the instrument.Data were analysed statistically using Student Newman Keul's test.

RESULTS AND DISCUSSION

The means and standard errors of metal accumulation levels found in liver, gill, kidney, spleen and muscle tissues of *O. niloticus* after 7 and 15 - day exposure to Cd, Pb and mixture of Cd and Pb concentrations are shown in Table 1 - 4.

Table 1. Cadmium levels (μg Cd/g d.w.) in the tissues of *O. niloticus* exposed to cadmium alone and cadmium – lead mixture for 7 days exposure periods.

Groups	N	Tissues				
		Liver	Gill	Kidney	Spleen	Muscle
		X ± sx *	X ± sx *	X ± sx *	X ± sx *	X ± sx *
Cont	6	ND **a**	ND **a**	ND **a**	ND **a**	ND **a**
Cd	6	16.3 ± 1.2 **bs**	18.7 ± 2.0 **bs**	23.7 ± 2.1 **bt**	15.5 ± 1.5 **bs**	3.0 ± 0.5 **bx**
Cd-Pb	6	17.7 ± 1.3 **bs**	19.4 ± 1.6 **bs**	25.4 ± 1.9 **bt**	15.7 ± 1.9 **bs**	3.3 ± 0.3 **bx**

* SNK= Letters a and b show differences among Control, Cd and Cd + Pb mixture; and s, t and x among tissues. Data shown with different letters are significantly different at the P<0.05 level., ND= Not detectable. X ± sx : Mean ± standard error.

Table 2. Cadmium levels (μg Cd/g d.w.) in the tissues of *O. niloticus* exposed to cadmium alone and cadmium – lead mixture for 15 days exposure periods.

Groups	N	Tissues				
		Liver	Gill	Kidney	Spleen	Muscle
		X ± sx *	X ± sx *	X ± sx *	X ± sx *	X ± sx *
Cont	6	ND **a**	ND **a**	ND **a**	ND **a**	ND **a**
Cd	6	61.9 ± 3.0 **bs**	31.2 ± 1.7 **bt**	113.6 ± 2.6 **bx**	36.0 ± 1.1 **by**	3.6 ± 0.6 **bz**
Cd-Pb	6	69.8 ± 1.9 **cs**	26.9 ± 1.7 **ct**	123.5 ± 2.3 **cx**	33.7 ± 1.4 **by**	3.5 ± 0.2 **bz**

* SNK= Letters a, b c show differences among Control, Cd and Cd + Pb mixture; and s, t, x,y and z among tissues. Data shown with different letters are significantly different at the P<0.05 level. ND= Not detectable. X ± sx : Mean ± standard error.

Compared with the control, the accumulation of Pb and Cd in liver, gill, kidney, spleen and muscle tissues of fish exposed to Pb, Cd and the mixture of Cd and Pb was significantly increased after 7 and 15 – day experimental period ($p<0.05$). After 15 day of exposure, the highest amount of Pb and Cd was found in kidney tissues of fish exposed to Pb, Cd and the mixture of Pb and Cd. However, the lowest accumulation levels were found in muscle tissues whilst there was no statistical difference between tissue and organs regarding the Cd accumulation levels except in kidney and muscle tissues of fish exposed to only Cd and the mixture of Cd and Pb after a 7 - day experimental period ($p>0.05$), there was a significant difference between the tissue and organs investigated regarding the Cd accumulation levels after 15 - day exposure time ($p<0.05$). The following relationship was found regarding the Cd accumulations among tissues and organs;

Kidney > Liver > Spleen > Gill > Muscle

After 7 - day of exposure to the mixture of Cd and Pb, the accumulation of Cd in liver, gill, kidney, spleen and muscle was not different from levels of fish exposed to Cd only for the same duration. The accumulation of Cd in kidney and liver tissues was increased by the mixture of Cadmium and lead, decreased in gill tissue and was unchanged in spleen and muscle after 15 - day of exposure.

Table 3. Lead levels (μg Pb/g d.w.) in the tissues of *O. niloticus* exposed to lead alone and lead – cadmium mixture for 7 days exposure periods.

Groups	N	Tissues				
		Liver	Gill	Kidney	Spleen	Muscle
		X ± sx *	X ± sx *	X ± sx *	X ± sx *	X ± sx *
Cont	6	ND **a**	ND **a**	ND **a**	ND **a**	ND **a**
Pb	6	15.1 ± 0.3 **bs**	36.0 ± 1.5 **bt**	66.1 ± 1.9 **bx**	9.6 ± 1.1 **by**	1.2 ± 0.2 **bz**
Pb-Cd	6	14.5 ± 0.3 **bs**	52.2 ± 2.5 **ct**	60.6 ± 1.4 **cx**	10.0 ± 0.9 **by**	1.0 ± 0.1 **bz**

* SNK= Letters a, b and c show differences among Control, Pb and Pb + Cd mixture; and s, t, x, y and z among tissues. Data shown with different letters are significantly different at the P<0.05 level.
ND= Not detectable. X ± sx: Mean ± standard error.

Table 4. Lead levels (μg Pb/g d.w.) in the tissues of *O. niloticus* exposed to lead alone and lead – cadmium mixture for 15 days exposure periods.

Groups	N	Tissues				
		Liver	Gill	Kidney	Spleen	Muscle
		X ± sx *	X ± sx *	X ± sx *	X ± sx *	X ± sx *
Cont	6	ND **a**	ND **a**	ND **a**	ND **a**	ND **a**
Pb	6	32.5 ± 1.9 **bs**	45.6 ± 1.7 **bt**	225.2 ± 7.6 **bx**	16.7 ± 1.3 **by**	2.0 ± 0.1 **bz**
Pb-Cd	6	42.8 ± 1.1 **cs**	55.3 ± 1.2 **ct**	184.6 ± 3.3 **cx**	21.7 ± 1.7 **cy**	2.7 ± 0.1 **cz**

* SNK= Letters a, b and c show differences among Control, Pb and Pb + Cd mixture; and s, t, x, y and z among tissues. Data shown with different letters are significantly different at the P<0.05 level.
ND= Not detectable. X ± sx : Mean ± standard error.

However, there were significant differences in Pb accumulation levels in tissues of fish after exposed to Pb and Pb - Cd mixtures for 7 and 15 - day experimental periods and the relationship found between the tissues and organs regarding Pb accumulations is as follows:

Kidney > gill > liver > spleen > muscle

Following 7 - day exposure, the mixture of Cd and Pb increased the accumulation of Pb in gill tissues and decreased the levels in kidneys. There was no significant effect on Pb accumulations in other tissues and organs. However, following 15 - day exposure, the mixture of Pb and Cd increased Pb accumulation levels in liver, gill, spleen and muscle tissues compared with the accumulation levels found when fish were only exposed to Pb. Levels were decreased in kidney tissue.

Metal accumulation in fish tissues and organs not only depends on the type of metal and its concentrations, time of exposure and the physical and chemical parameters of Water like temperature, salinity and hardness (Allen, 1995) but also to species and their age and metabolic activities (Pelgrom et al., 1995). Organs like liver, kidney

and gills are metabolically very active in fish and metals in these organs tend to accumulate in large concentrations compared with organs like muscle and gonads, which control locomotion and reproductory processes, respectively (Amiard et al., 1987). The highest metal accumulation was found in kidney and liver under different concentrations of Cd in *Oncorhynchus mykiss* (Melgar et al., 1997) and Pb in *Oreochromis aureus* (Allen 1995) whereas the lowest levels were recorded in muscle tissue. Although Cd in *Cyprinus carpio* accumulates in liver and kidney, the accumulation levels in kidney tissue were 50 times higher than the levels measured in muscle tissue. The accumulation of Cd in muscle tissue, however, has been demonstrated to reach significant levels only after a 106 day exposure time (DeConto-Cinier et al., 1999). Compared with liver, the highest level of Pb accumulation was found in spleen and gills in *Gillichthys mirabilis*, whereas the lowest accumulation was in muscle tissue of this species (Somero et al., 1977). In this experiment with *O. niloticus*, it was shown that the highest metal accumulation was found in kidney and liver under the effects of Cd and Pb separately, and the lowest accumulation was in muscle tissue. The differences between tissues and organs in accumulation levels could be explained by the differences in metabolic processes of these tissues and organs and changing in accumulation levels with species.

Although the Cd accumulation in liver of *C. carpio* exposed to Cd only was higher than that of gill tissues (DeSmet et al., 2001), the Pb accumulation in gill tissues of *Anabas testudineus* exposed to different concentrations of Pb was higher than liver tissues (Tulasi et al., 1992). Another investigation with *Tilapia zilli* has demonstrated that gill tissues could accumulate more Pb than liver tissues (Karataş and Kalay, 2002). Except kidney, the highest Pb accumulation was also found in gill tissues compared with other tissues and organs in *O. niloticus*. It is known that metal binding molecules like metallothionein, which has low molecular weight and is rich in cysteine, could increase under heavy metal presence and detoxify metals by binding them (Cousins, 1985). Liver and kidney are the main MT synthesizing organs in fish (Thomas et al., 1983) and the higher accumulation levels of cadmium in these tissues and organs could be justified by this. Gill tissues in fish are involved with respiration rather than detoxification (Pelgrom et al., 1995) and there is no information available on the synthesis of metal binding proteins like MT in fish gills (Roesijadi, 1992). It was found that mucus excretion from gills was markedly increased in *Oncorhynchus kisutch* when exposed to lead, resulting in increased lead concentrations in gill tissue (Varanasi and Markey, 1978). The higher accumulation of lead in gill tissue of *O. niloticus* could be also explained by mucus excretion and structural damages to tissues.

The metal accumulation in fish depends both the structure of tissues and organs and the interactions of heavy metals in the environment (Pagenkopf, 1983). It was demonstrated that under natural concentrations, the interaction of Cu and Cd affected the metal accumulations increasing the levels above those of fish exposed only to Cd in liver, kidney, gill and gut tissues. However, there was no significant effect on muscle tissue from the Cu and Cd interaction (Pelgrom et al., 1995). Whilst it was shown that Pb and Hg did not affect Cd accumulation in tissue and organs of *O. aureus*, the highest accumulation of cadmium was measured in kidney, liver and

spleen whereas the lowest levels were in muscle and brain (Allen, 1995). In experiments with *Clarias lazera* (Hilmy et al., 1987) and *Tilapia nilotica* (Kargın and Erdem, 1992), it was found that whilst Zn prevented the uptake of Cu, copper did not affect the uptake of Zn in these species. In this experiment with *O. niloticus* it was also demonstrated that the mixture of Pb and Cd increased the accumulation of Cd in kidney and liver and decreased the levels in gills. The combination also increased Pb accumulation in liver, gill, spleen and muscle tissues and decreased Pb levels in kidney. The accumulation levels and toxic effects of metals to organisms change both with the interactions between metals and the type of organism. Although it was found that while Pb and Cd mixture in mammals (Skoczynska et al., 1994) and Cu and Cd mixture in *Mytilus edulis planulatus* (Eliot et al., 1986) decreased Cd accumulation in tissues and organs of these organisms, they increased the Cd accumulation in *O. mossambicus* (Pelgrom et al., 1995).

Metal accumulation, compared with controls, was found to increase in liver, gill, kidney, spleen and muscle tissues of *O. niloticus* exposed to Pb, Cd and the mixture of these metals in this investigation. The mixture of Pb and Cd increased the Cd accumulation levels in kidney and liver, compared to fish only exposed to Cd and increased Pb accumulation in liver, gill, spleen and muscle tissues. The combination decreased Cd levels in gills and Pb accumulation in kidney. These results may be explained by the increase in metal binding proteins like MT in liver, kidney and spleen tissues and organs and the competition between Pb and Cd for the binding sites in the structure of MT and uptake regions on the gill surface.

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