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BIOACCUMULATION OF LEAD IN *CIRRHINUS MRIGALA* (HAMILTON,1822) AND *CTENOPHARYNGODON IDELLA* (STEINDACHNER, 1866)

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ABSTRACT

Continuous exposure of organisms to low concentrations of metals such as lead may result in their bioaccumulation and subsequent transfer to humans through the food chain. Few acute toxicity studies with non-essential trace metal lead have been carried out in carps, but sublethal toxicity studies are lacking in most endemic and exotic carps. In the present study, static bioassays were conducted on fingerlings of *Cirrhinus mrigala* (8±0.5 g) and *Ctenopharyngodon idella* (8.5±1 g), to study bioaccumulation of lead in six tissues, both edible (skin and muscle) and non-edible (gill, brain, liver and kidney), over a period of four weeks under sublethal conditions. Lead nitrate was used as the lead agent. Fish were exposed to 1/5 of LC 50 of lead calculated for *C. mrigala* (0.06 mg l^{-1} Pb) and that calculated for *C.idella* (0.04 mg l^{-1} Pb).The concentrations in the edible parts of both the species after 28 days of sublethal exposure, were well below the provisional tolerable daily intake of 7 ug/kg body wt. of lead per person, established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA),1982.

Key Words: *Cirrhinus mrigala, Ctenopharyngodon idella,* lead, sublethal toxicity, bioaccumulation

While literature shows many field studies on bioaccumulation of heavy metals by fishes, few studies are carried out on experimental basis under acute toxicity condition. While acute toxicity generally deals with the adverse effects of single doses, delayed effects may occur due to accumulation of the chemical in tissues or other mechanisms. Keeping this aspect in view, an attempt has been made to study the bioaccumulation of the non-essential metal lead, under sublethal conditions. Lead is held to be the most dangerous, since the continuous exposure of organisms to low concentrations may result in its bioaccumulation and subsequent transfer to humans through the food chain.

Metal accumulation is affected by some of the same parameters that affect toxicity (water chemistry and particulate matter), as they are absorbed either from solution or from food or particles [1,2]. There are two main routes of metal acquisition, one is directly from the water and the other is from the diet [3]. After reviewing available literature, Bryan [1] came to the conclusion that, in the majority of cases, food is a much more important source of metals than the water. However, which route is more important depends on environmental circumstances, and has not always been properly documented [4]. Aquatic environment is complex and exhibits a changeable structure. These changing conditions affect the chemical reactions of substances and pollutants [5,6].

Study of metal accumulation is one of the most valuable tools for identifying and quantifying the impact of metals in aquatic environments [7,8,9,10]. Fishes being one of the main aquatic organisms in the

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food chain, may often accumulate large amounts of certain metals [11,12]. Fishes assimilate these heavy metals through ingestion of suspended particulates, food materials, and / or by constant ion-exchange process of dissolved metals across lipophilic membranes like the gills or adsorption of dissolved metals on tissue and membrane surfaces .Various metal ions get biologically magnified when taken up from the surrounding water in their various tissues as they grow. This uptake and bioaccumulation is well documented in skin, gills,stomach, muscles, intestine, liver, brain, kidney and gonads, but their main target organs are liver, kidney and muscles depending on the exposure concentration and time [13,14,15,16,17,18].

Lead is mainly soluble in soft and slightly acidic water [19]. Lead that leaks from fishery boats, or which enters through erosion and leaching from the soil, or through domestic and industrial waste discharges into the aquaculture ponds, will easily dissolve in the water. Culturing fish in soft and slightly acidic pond waters over a period of 3-5 months. Once the trace elements are absorbed, they are transferred from the gills and intestine to the blood and distributed to other parts of the body [20], implying a need for monitoring lead accumulation in fish organs. Study on metal concentration in fish muscle is a means to estimate the amount of metals transported to human body through food.

In the present investigation, bioaccumulation of lead in six tissues, both edible (skin and muscle) and non-edible (gill, brain, liver and kidney), has been estimated over a period of 28 days under sublethal conditions.

Materials and methods

Male and female fingerlings of an endemic carp *Cirrhinus mrigala* (mrigal), and an exotic carp *Ctenopharyngodon idella* (grass carp) ranging in length from 3 ½ to 4'' and weight 8 ± 0.5g and 8.5 ± 1g respectively, were procured from a private fish farmer in Kaikaluru, Andhra Pradesh. They were acclimated at a temperature of 28 ± 2 ^{0}C and fed with rice bran and oil-cake. The lead in water and feed used for the experiments was below detectable level.

The sub-lethal toxicity experiment was conducted with lead in both male and female fingerlings of *C. mrigala* (measuring 31/2 - 4" and weighing 8 ± 0.5 g) and *C. idella* (measuring 31/ 2 - 4" and weighing 8.5 ± 1 g) after acclimation for one week. Feeding was terminated 24 hrs. prior to the experiment .

Groups of 25 fish were exposed to 1/5 of LC 50 of lead calculated for *C. mrigala* (0.06 mg l^{-1} Pb) and that calculated for *C.idella* (0.04 mg l^{-1} Pb) in 500 litre fibre-glass tanks, not exceeding 1 g fish/l, using static test method. Aeration was avoided, as it might alter the results of the tests. Fish were maintained at 29 ± 2 0 C and fed on a weekly basis, once in the morning and once in the evening, for four weeks during the 28-day long experiment. Control fish were maintained under the same conditions, in water devoid of lead detectable.

Prior to exposing the fish to sublethal concentration, skin, muscle, gill, brain, liver and kidneys of control groups of both species were sampled. Every 7th, 14th, 21st and 28th day, 25 fish (belonging to both species) were sampled from the lead exposure groups, dissected, and skin, muscle, gill, brain, liver and kidneys neatly separated. 1 gm. weight of each tissue was weighed into 25 ml. conical flasks, and digested overnight with 7 ml. of pure nitric acid (AR grade, specific gravity : 1.42, Qualigens, India) and 3 ml. of hydrogen peroxide.

The tissues were analysed for lead concentration following the method of AOAC official method 999.10 (AOAC 2000) [21].Samples were digested in Teflon containers using a microwave digester (LEM MARS 240/50 Niulab, Hyderabad, India). Tissues were homogenized, 3.0 g. of wet tissue was weighed into 100 ml. Teflon vials and digested overnight with 7 ml. of pure nitric acid (AR grade, specific gravity:1.42, Qualigens, India) and 3.0 ml. of hydrogen peroxide. The microwave parameters were 700 W power for 1 hr., with 40 minute heating time and 20 minute ventilation time. The digested contents were transferred to acid washed polypropylene bottles and made up to 25 ml. with double distilled water and subjected to lead content analysis by Atomic Absorption Spectrophotometer (Spectra AA 220, Varian, Australia). Statistical analysis was

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performed by two way ANOVA procedure of MINITAB, to determine any significant difference in lead accumulation level in the chosen tissues of *C.mrigala* and *C.idella*.

Results & Discussion

The amount of lead in the skin, muscle, gill, brain, liver and kidney of the control group of *C.mrigala* are 0.011, 0.000, 0.016, 0.000, 0.016 and 0.015 ug/g wet wt. respectively (Fig.1).

On exposure to sublethal concentration (1/5 of LC 50) of lead (0.06 ppm.) for 7, 14, 21 and 28 days, the amount of lead in skin of *C. mrigala* recorded values 0.014, 0.020, 0.027 and 0.036 ug/g wet wt., in muscle, 0.003, 0.006, 0.007 and 0.013 ug/g wet wt., in gill, 0.019, 0.022, 0.026 and 0.029 ug/g wet wt., in brain, 0.004, 0.006, 0.007 and 0.010 ug/g wet wt., in liver, 0.037, 0.038, 0.041 and 0.047 ug/g wet wt., and in kidney, 0.035, 0.037, 0.039 and 0.043 ug/g wet wt. respectively.

Analysis of variance for bioconcentration of lead by control (Group A) and lead treated (Group B) of *C.mrigala* is shown in Table 1. There is a significant difference between the A Group (Control group) and C Group (Lead exposure group) at 5% level of significance. Further, there is a significant mean difference in micrograms among the tissues at 5% level as per the significant p-values of the F-test mentioned above

Table 1. Analysis of variance for bioconcentration of lead by Groups A(control group) & C(lead exposure group) of *C. mrigala*

Source	DF	SS	MS	F	P
Group	1	0.0036	0.0036	8.104	0.017
Tissues	10	0.0044	0.0004	242.291	0.000
Error	24	0.0000	0.0000		
Total	35	0.0081			

The amount of lead in the skin, muscle, gill, brain, liver and kidney of the control group of *C.idella* are 0.011, 0.000, 0.016, 0.000, 0.014 and 0.013 ug/g wet wt., respectively.

On exposure to sublethal concentration (1/5 of LC 50) of lead (0.04 ppm.) for 7, 14, 21 and 28 days, the amount of lead in skin of *C.idella* recorded values 0.015, 0.019, 0.028 and 0.035 ug/g wet wt., in muscle, 0.001, 0.003, 0.004 and 0.007 ug/g wet wt., in gill, 0.020, 0.022, 0.024 and 0.028 ug/g wet wt., in brain, 0.001, 0.002, 0.004 and 0.008 ug/g wet wt., in liver, 0.016, 0.018, 0.020 and 0.024 ug/g wet wt., and in kidney, 0.021, 0.023, 0.025 and 0.027 ug/g wet wt. respectively.

Analysis of variance for bioconcentration of lead by control (Group A) and lead treated (Group B) of *C.idella* is shown in Table 2. The results show a significant difference between the A Group (Lead) and C Group at 5% level of significance. Further, there is a significant mean difference in micrograms among the tissues at 5% level as per the significant p-values of the F-test mentioned above.

Table 2. Analysis of variance for bioconcentration of lead by Groups A(control group) & C(lead exposure group) of *C. idella*

Source	DF	SS	MS	F	P
Group	1	0.0014	0.0014	5.142	0.047
Tissues	10	0.0027	0.0003	177.433	0.000
Error	24	0.0000	0.0000		
Total	35	0.0041			

Among the tissues, muscle and brain recorded the lowest level of lead in the control groups of both the species. While both gill and liver recorded higher level in the control group of *C. mrigala,* the highest level of lead was recorded by gill alone in the control group of *C. idella.* Muscle recording a lower level of lead may

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be explained by the physico-chemical nature of the concerned heavy metals, which dictate their penetration across the blood-brain barrier and other barriers. Analysis of variance for bioaccumulation of lead by the control and exposure groups is shown in Table 1 and comparison of bioaccumulation of lead by different tissues of control group (A Group) and lead exposure group (B Group) is shown in the interval plot (Fig.1)

Comparison of bioconcentration of lead by different tissues of control (A Group) and lead treated (B Group) *C.mrigala* and *C.idella* is shown in Figures 1 and 2.

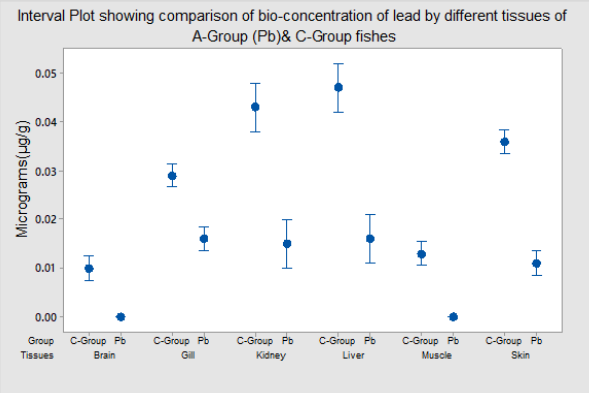

Figure 1. Comparison of bioconcentration of lead by different tissues of the control (A Group) and lead treated (B Group) *C.mrigala*

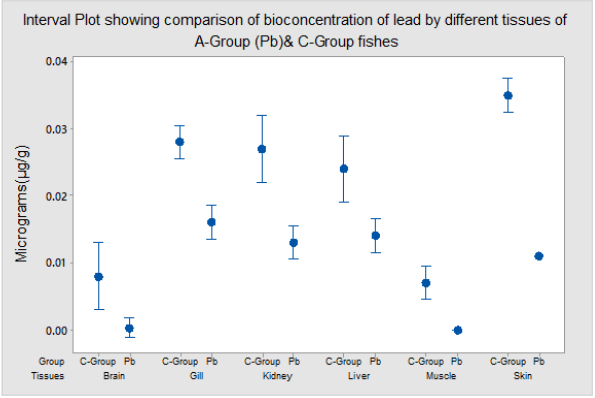

Figure 2. Comparison of bioconcentration of lead by different tissues of the control (A Group) and ;lead treated (B Group) *C.idella*

In the lead treatment groups of both species, the bioaccumulation of lead was high in skin and gills compared to muscle, and increased with increasing exposure period.

The data indicated the following rank order of lead uptake and accumulation (from highest to lowest values of lead at the end of the exposure period) in *C. mrigala* – liver > kidney > skin > gill > muscle > brain.

Similar observation has been made by Ahmed and Bibi [22] in fingerlings of *Catla catla* exposed to waterborne lead. The reason for high concentration of lead in liver might be due to metal-binding proteins. Tissue like liver, which is a major producer of metal-binding proteins, show high concentrations of most heavy metal detoxification [23,24,25,26], which eventually result in clearance of heavy metal ions from the body. Furthermore, the physiological differences and the position of each tissue in the fish can also influence the accumulation of a particular metal [27,28].

The rank order of lead uptake and accumulation (from highest to lowest values of lead at the end of the exposure period) in *C. idellus* is as follows – skin > gill > kidney > liver > brain > muscle.

Rashed [29] observed very high amount of lead accumulation in the skin (scales) of *T. nilotica*Tao et al. [30] described that lead ions from water do bind to the mucous layer present on general body surface, as well as on the gills of the fish, leading to high uptake and absorption of metal ions in the skin and gills. Pouring et. al., [31] documented lead accumulations in edible tissues of five species of sturgeon and it was high in those tissues which were in immediate contact with water carrying heavy metal ions.

The amount of a metal accumulated is influenced by various environmental, biological and genetic factors, leading to differences in metal accumulation between different individuals, species, age, tissues, seasons and sites [32]. It is important to know that fishes from water reservoirs like rivers and lakes receiving industrial effluents containing variable concentration of toxicants including heavy metals from various sources like industries, agricultural run-off or domestic waste water, may have accumulated heavy metals in their tissues as they grow, and these toxicants and metals will be transferred to humans (being at the end of the food chain) when consumed and may impair body metabolism [14,33,31]. *C. mrigala* and *C. idella* are

preferred freshwater fish for human consumption. They are found naturally in all freshwater bodies including rivers and lakes, which are receiving untreated industrial effluents containing various heavy metals, that may lead to their accumulation, including copper and lead, in their tissues, especially muscles.

In literature, heavy metal concentrations in the tissue of freshwater fish varies considerably among different studies possibly due to differences in metal concentrations and chemical characteristics of water from which fish were sampled, ecological needs, metabolism and feeding patterns of fishes and also the season in which studies were carried out [34].

Conclusion

In the present study, the concentrations of lead in skin (0.036 ug / g wwt. and 0.035 ug / g wwt.) and those in muscle (0.013 ug / g wwt. and 0.007 ug / g wwt.) of *C. mrigala* and *C. idella* respectively, after 28 days of sublethal exposure were high, compared to the provisional tolerable daily intake of 7 ug/kg body wt. of lead per person, established by the FAO/WHO Joint Expert Committee on Food Additives [35]. Extrapolating the results of the present study implies a need for monitoring lead pollution in culture ponds and bioaccumulation of lead in the edible parts of fish, keeping in view the exponential increase of the metal in pond waters, over a period of 3-5 months of culture.

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