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Ameliorating performance of dimercaptosuccinic acid on heavy metal lead acetate induced protein and amino acid contents alteration in gill and kidney of freshwater fish, *Channa striatus* (Bloch)

Vijaya Francis[1], M Muthulingam[2*]
[1] Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu, India
[2] Department of Zoology, Faculty of Science, Annamalai University, Annamalainagar, Tamil Nadu, India

Abstract
Heavy metal, Lead is a common environmental pollutant released into the surrounding atmosphere as a byproduct of industrial and anthropogenic activities. Lead sources include mining and smelting of ore, manufacture of lead containing products, burning of coal and oil, and waste incineration. Due to the environmental persistence of lead, historic sources also contribute heavily to present contamination. These incorporated leaded gasolines, paints, solder in food cans, pesticides and lead shot and sinkers. Through food chain heavy metal lead can enter fish body subsequently affect human beings. Fish is generally appreciated as one of the healthiest and cheapest source of protein and it has amino acid compositions that are higher in cysteine than most other sources of protein. The aim of the present study was to assess the protein and amino acid levels in gill and kidney of *Channa striatus* was exposed to sublethal concentration of lead acetate further the fish treated with *meso 2,3-* dimercapto succinic acid (DMSA) 2.5 ppm and 5 ppm in group 3 and group 4 respectively for the period of 10, 20 and 30 days. The fish exposed to lead acetate showed a decrease the protein and increase the amino acid levels further the fish were treated with dimercaptosuccinic acid showed gradually the protein contents increased and amino acid levels were decreased for the periods of 10, 20 and 30 days in gill and kidney. The objective of the present investigation was to observe the ameliorative performance of dimercaptosuccinic acid reduced the lead acetate induced alterations in protein and amino acid in the gill and kidney of freshwater fish, *Channa striatus.*

Keywords: protein, amino acid, lead acetate, dimercaptosuccinic acid, *Channa striatus*

Introduction

Environmental pollution is threats widely create disturbances in the life of plants, animals, human being and aquatic animals like fishes and prawns. The heavy metals are the most significant pollutants. The advancement of industries has led to increased discharge of pollutants into ecosystems [1]. Fish and water body contamination remain dispute despite public concern about the relationship between health and the environment. Fishing is an important economic and cultural activity in some ethnic communities. As a result, these groups consume large amounts of fish and have higher exposure to chemicals [2]. Untreated industrial, technological wastes and agricultural practices consist of different heavy metals often pollute natural water bodies. Bio-accumulative and non-biodegradable properties of heavy metal constitute a major group of aquatic contaminants. These heavy metals particulates enter the aquatic medium through effluents discharged from tanneries, textiles, metal finishing, mining, dyeing and printing industries, ceramic and pharmaceutical industries etc. [3].

Human activities are major responsible for water pollution. Aquatic medium get dirty due to pollution and are looked upon with contempt. Water pollution affects the fish rigorously and proves lethal to them. Water pollution imposes this adverse effect on all kinds of aquatic plants and animals including fish. Fishes are mainly affected from the human nuisances. So, it is the need of time to pay adequate attention to this issue and implement necessary remedial measures [4]. Fishes die due to pollution of water from pesticides adjoining the cultivation fields. Agriculture practices leads to the pesticides flow off into the nearby water proving fatal to the aquatic life of non target flora and fauna. Industries, discharged large quantity of wastes in natural water bodies directly or indirectly through open drains either without any treatment or with inappropriate and inadequate treatment processes causing water pollution and leading to serious public health hazard to aquatic organism through food chain it will directly affect the human beings [5].

Heavy metals in the environment have long biological half-life and therefore a major threat to aquatic organisms, especially fishes [6]. In high concentrations of heavy metals will destroy aquatic organisms; in sub acute concentrations heavy metals are gradually intensified in diverse aquatic organisms as they reach upper tropic levels of the food chain [7]. Health hazards produced by heavy metals have become a great concern only when they affected humans via the food chain as in minamata diseases in Japan [8].

Lead is a non-essential heavy metal with no biological function and can be toxic to aquatic animals when given in access amounts. Previous studies have shown that Pb^{2+} causes disruption of Na^{+}, Cl and Ca^{2+} regulation and disruption in hemoglobin synthesis [9]. Pb^{2+} interacts with other elements synergistically or antagonistically. There are evidences on the antagonism between Pb^{2+} and Ca^{2+}, by which this metal directly competes with Ca^{2+} for uptake at calcium binding sites and can enter the cells through similar transport pathways

[10, 11]. Lead has a combination of physical and chemical properties that make it extremely useful in industries. Nowadays, the major use of lead in battery production since a large drop has occurred in the demand for gasoline additives containing lead. In the past, lead use in the chemical industry for preparing paints, pigments and colored inks was widespread, but many countries, now restricted to use of lead [12]. The natural concentration of lead in surface water has been estimated at 0.02 µg.L–1 and it rarely exceeds a few micrograms/L. However, high levels of lead are associated with areas in the vicinity of lead mines, smelters and battery-producing industries [11].

Chelating agents have been used clinically as antidotes for both acute and chronic metal poisoning [23]. Chelators not only enhance excretion but also decrease the clinical signs of toxicity by preventing metals from binding to cellular target molecules. This can be achieved when the chemical affinity of complexing agent for the metal is higher than the affinity for the bioligands. Effective chelation therapy depends on the lipophilic character of the chelating agent and its effectiveness in successful removal of the metal from intracellular spaces where metal is firmly bound. Meso-2, 3-dimercaptosuccinic acid (DMSA) is a water soluble, safe and effective chelator and also recommended for clinical use to reduce metal burdens [14, 15]. Fish are susceptible to aquatic pollutants as fishes are in direct contact with the surrounding water through their gills [16]. Gills surface comprises over half of the body surface area of fish and only a few microns of gill epithelium separate the internal environment of the fish from external environment [17]. Proteins are macromolecules considered as the architecture of cell. Proteins are involved in physical and chemical activities to maintain the homeostasis of the cell. Amino acids are regarded as building blocks of proteins. Amino acids are essential intermediates in protein synthesis and its degradation products appear in the form of different nitrogenous substances. Therefore, the assessment of the total protein content can be considered as a diagnostic tool to determine the physiological process of the cell [18]. Fish protein is an essential source of nutrients for many people, especially in developing countries. It is estimated that worldwide, one billion people depend on producing, processing and trading fish for their livelihood [19, 20].

Materials and Methods

The fish *Channa striatus* having mean weight 17 - 21 g and length 11 – 13 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1% KMNO4 solution and then kept in cement tank for acclimatization for a period of two weeks. They were fed twice daily i.e. morning and evening on boiled chicken eggs approximately 4% of fish body weight divided into two equal meals daily. The lead acetate was used in this study and stock solutions were prepared. Lead acetate, LC_{50} was found out for 96 h (32 ppm) [21] and 1/10th (3.2 ppm) taken as sublethal concentration for this study. Forty fish were selected and divided into 4 groups of 10 each. The first group was maintained in free from lead acetate and served as the control. The other 3 groups were exposed to sub lethal concentration of lead acetate for 30 days. The 3rd and 4th groups were reexposed to 2,3 meso dimercapto succinic acid (DMSA) 2.5 ppm and 5 ppm respectively for 10, 20 and 30 days.. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein and amino acid estimation. The protein and amino acid content in gill and kidney of *Channa striatus* were estimated by the method of [22] and [23] respectively. The data obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance [24].

Results

Protein content in gill

The protein contents were observed in the control gill to be 118.34 ± 9.01, 119.53 ± 9.10 and 123.38 ± 9.40 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The protein contents were significantly decreased when the fish *Channa striatus* exposed with sublethal concentration of lead acetate for the periods of 10, 20 and 30 days showed 97.14 ± 7.40, 89.28 ± 6.80 and 75.65 ± 5.76 respectively. Sublethal concentration of lead acetate exposed fish after 30 days treated with DMSA (2.5 and 5 ppm) showed increased the protein content (101.67 ± 7.74, 103.30 ± 7.87 and 108.23 ± 8.24) and (105.24 ± 8.01, 111.24 ± 8.47 and 117.64 ± 8.96) for 10, 20 and 30 days respectively (Fig 1). However, more gill protein contents were observed in DMSA (5 ppm) treated fish, *Channa striatus.* (Fig 1)

Protein content in kidney

The protein contents were observed in the control kidney to be 91.36 ± 6.96, 92.63 ± 7.05 and 93.74 ± 7.14 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The protein contents were significantly decreased when the fish *Channa striatus* exposed with sublethal concentration of lead acetate for the periods of 10, 20 and 30 days showed 83.52 ± 6.36, 77.46 ± 5.90 and 64.59 ± 4.92 respectively. Sublethal concentration of lead acetate exposed fish after 30 days treated with DMSA (2.5 and 5 ppm) showed increased the protein content (84.39 ± 6.43, 86.44 ± 6.58 and 89.86 ± 6.84) and (86.72 ± 6.60, 88.33 ± 6.73 and 92.66 ± 7.06) for 10, 20 and 30 days respectively (Fig 1). However, more kidney protein contents were observed in DMSA (5 ppm) treated fish, *Channa striatus.* (Fig 2)

Amino acid content in gill

The amino acid contents were observed in the control gill to be 3.86 ± 0.29, 3.83 ± 0.29 and 3.81 ± 0.29 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The amino acid contents were significantly enhanced when the fish *Channa striatus* exposed with sublethal concentration of lead acetate for the periods of 10, 20 and 30 days showed 5.04 ± 0.86, 7.55 ± 0.57 and 11.34 ± 0.86 respectively. Sublethal concentration of lead acetate exposed fish after 30 days treated with DMSA (2.5 and 5 ppm) showed declined the amino acid contents (4.97 ± 0.38, 5.24 ± 0.40 and 7.18 ± 0.55) and (4.10 ± 0.31, 4.68 ± 0.36 and 4.79 ± 0.39) for 10, 20 and 30 days respectively (Fig 1). However, more declined gill amino acid contents were observed in DMSA (5 ppm) treated fish, *Channa striatus.* (Fig 3)

Amino acid content in kidney

The amino acid contents were observed in the control kidney to be 3.25 ± 0.25, 3.33 ± 0.25 and 3.38 ± 0.26 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The amino acid contents were significantly enhanced when the fish *Channa striatus* exposed with sublethal concentration of lead acetate for the periods of 10, 20 and 30 days showed 5.14 ± 0.39, 7.58 ± 0.58 and 8.49 ± 0.65 respectively. Sublethal concentration of lead acetate exposed fish after 30 days treated with DMSA (2.5 and 5 ppm) showed declined the amino acid contents (4.67 ± 0.36, 5.42 ± 0.41 and 5.53 ± 0.42) and (3.85 ± 0.29, 3.91 ± 0.30 and 4.08 ± 0.31) for 10, 20 and 30 days respectively (Fig 1). However, more declined kidney amino acid contents were observed in DMSA (5 ppm) treated fish, *Channa striatus.* (Fig 4)

Discussion

Aquatic ecosystems are considered as suitable places for disposal of toxic and domestic wastes. However, constantly increasing pollution load and over exploitation of the water for potable supplies, irrigation, industries and thermal power plants to meet the requirements of the increasing population, significantly minimizes their assimilative capacity. Thus, the dual stress exerted on the watercourses is ultimately faced by the biological communities inhabiting them. The fish is one of the most important aquatic communities concerning human beings [25]. Aquatic organisms are generally exposed to chronic metal contamination, though they may also suffer acute exposures in areas where industrial effluents are discharged. Metal exposure can lead to several toxic effects, such as disruption of ion homeostasis, neurotoxicological abnormalities, alterations in biochemical and physiological mechanisms and ultimately cause mortality of aquatic organisms [26, 27]. Proteins are the mainly central and plentiful biochemical ingredient present in the animal body, predominantly in fish. Protein normally contains a series of amino acid residues of the polypeptide chains. Fish, a familiar resource of protein contains a greater quantity of protein than any other living animals, contributing around in relation to 75% of the weight of fish [28]. Gills represent a thin and extensive surface in intimate contact with water. They carry out three main functions, gas exchange, ion regulation and excretion of metabolic waste products. Due to the constant contact with the external environment, gills are the first targets of waterborne pollutants [29]. The kidney, which is an important organ of excretion and osmoregulation, is indirectly affected by pollutants through blood circulation [30]. The impact of contaminants on aquatic ecosystem can be assessed by measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination [31]. Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms [32].

The present investigation, protein content had decreased whereas amino acids content had increased in the gill and kidney of freshwater fish, *Channa striatus* was exposed with sublethal concentration of lead acetate for the periods of 10, 20 and 30 days exposure. This trend has been changed when the freshwater fish, *Channa striatus* reexposed to 2.5 and 5 ppm of DMSA showed gradually protein contents were increased and amino acid level declined significantly. Simillarly the protein levels were increased lead with lycopene or vitamin E supplements with diet than lead alone treated fish, *Clarias gariepinus* [33]. The altered mobility and low content of proteins reflects a change in the rate of synthesis and degradation of protein. Proteins are mainly involved in the architecture of the cell. During stress conditions they are a source of energy as fish need more energy to detoxify the toxicant and to overcome stress. The amount of carbohydrates in fish is less so protein is the alternative source of energy to meet the increased energy demand [34]. Protein levels were decreased and amino acid levels increased in liver, kidney and brain of freshwater fish Labeo rohita when treated with sublethal concentrations of nickel chloride [35]. Protein levels were decreased and amino acid contents increased significantly in gill, liver and kidney of *Cyprinus carpio* exposed to sublethal concentration of pharmaceutical effluent [36]. The protein content decreased in the liver, brain and kidney tissues of *Channa punctatus* during lihocin treatment [37]. The protein contents were reduced whereas amino acid contents elevated in gill, liver and kidney of *Hypopthalmichthys molitrix* when exposed to sublethal concentration of cadmium chloride [38].

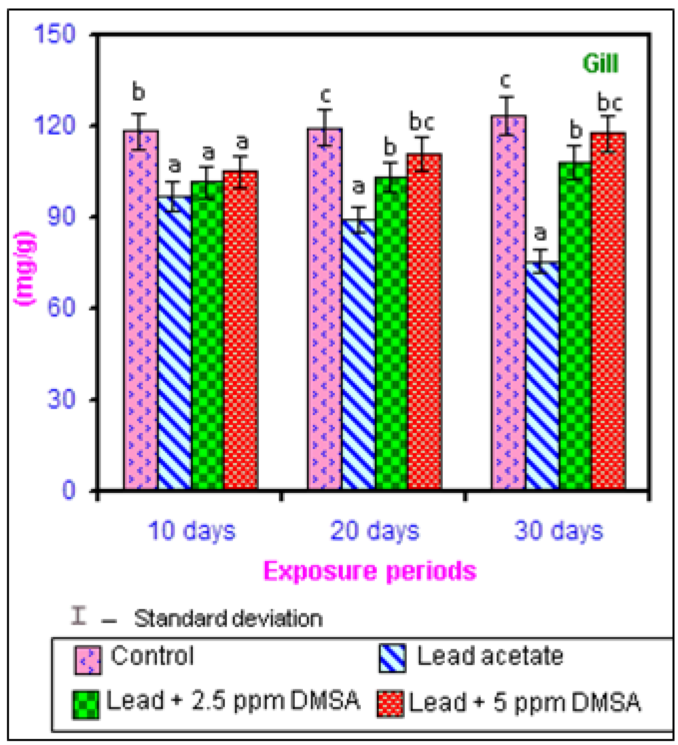

Fig 1: Protein level changes (mg/g) in gill of *Channa striatus* exposed to sublethal concentrations of lead acetate ameliorated by DMSA

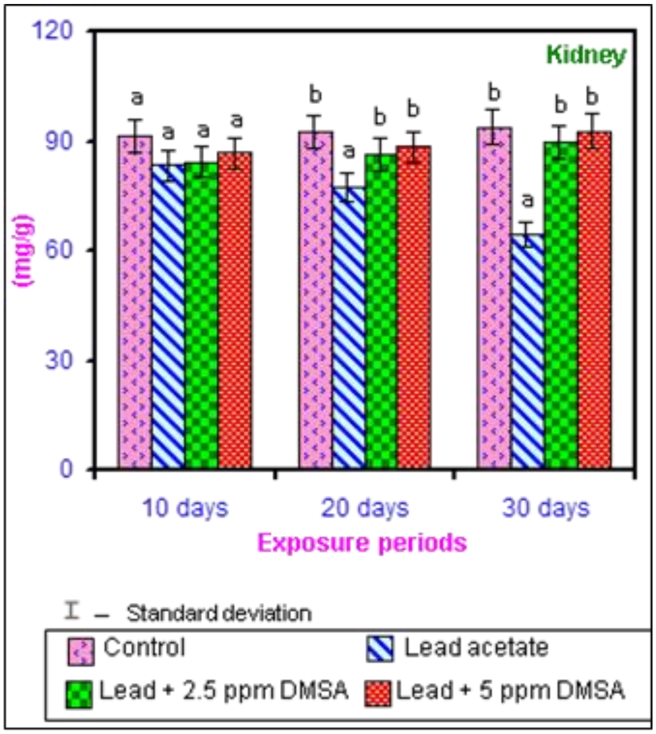

Fig 2: Protein level changes (mg/g) in kidney of *Channa striatus* exposed to sublethal concentrations of lead acetate ameliorated by DMSA

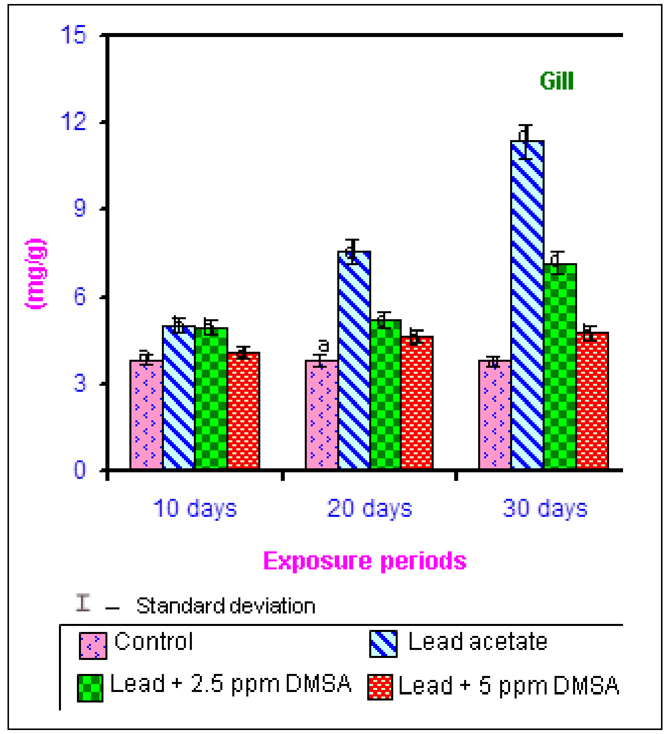

Fig 3: Amino acid level changes (mg/g) in gill of *Channa striatus* exposed to sublethal concentrations of lead acetate ameliorated by DMSA

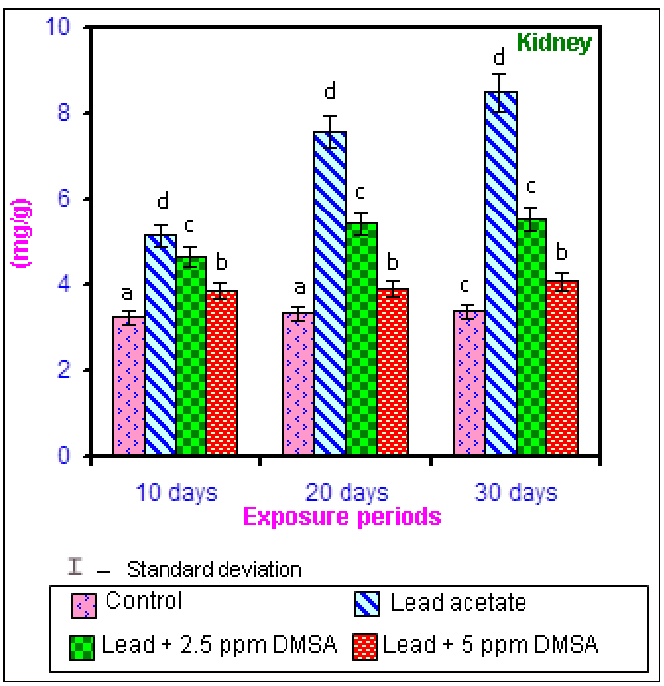

Fig 4: Amino acid level changes (mg/g) in kidney of *Channa striatus* exposed to sublethal concentrations of lead acetate ameliorated by DMSA

Conclusion

Fish is an integral part of the diet of a most of the population rarely some vegetarian people avoid fish and is one of the sources of protein for the people living in coastal areas. The nutritional value of different species of fish depends on their biochemical components such as protein. The alteration in the proximate component could disturb the metabolic system in fish, affecting the food value of fish. The lead acetate treated fish gradually improved the protein metabolism while exposed to antidote DMSA.

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