Original Article

Biochemical Alteration Induced by Cadmium and Lead in Common Carp via an Experimental Food Chain

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ABSTRACT

Background: Evaluation on the toxicity of two mainly contaminant heavy metals, cadmium (Cd) and lead (Pb) through the food chain was the aim of this study.

Methods: A total number of 270 healthy common carp (4±1.14 g) in April, 2015 transported to the Khatam Alanbia University of Technology, Behbahan, Iran. Fishes were divided into three groups and transferred to the 20 L aquaria each containing 30 juveniles. The first group (control) fed by metal-free *Artemia fransiscan anauplii* throughout the experiment. The second and third groups were feeding by Cd and Pb (1.5 mg/L free ion) contaminated nauplia, respectively. The experimental study was carried out for three weeks and sampling was done in 4th, 7th, 14th and 21st days. Finally, the alterations in plasma biochemical responses were determined.

Results: Alanine aminotransferase and aspartate aminotransferase activities increased in response to feeding Pb-contaminated nauplia. Creatine phosphokinase activity showed significant increase in fourth day about both Cd and Pb and at the end of experiment only in Cd treatment (P<0.05). Cholesterol and triglyceride were increased significantly only for Pb (P<0.05). Plasma glucose and creatinine levels increased by both heavy metals compared to the control but glucose just remained high only for Pb at the end of the experiment. Total protein, albumin and globulin were significantly declined in both metal contaminated groups (P<0.05).

Conclusion: It seems Pb had a greater toxicity than Cd through the food chain and it may be due to its more trophic transfer than Cd.

Keywords: Biochemical Alteration, Common Carp, Food Chain, Heavy Metal.

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INTRODUCTION

Environmental concentrations of heavy metals have been increasing globally because of human activities. Various metals have the capability to induce harmful effects on living organisms at ecological relevant concentrations. They are considered important environmental contaminants [1]. The natural aquatic systems have been extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities [2].

Cadmium (Cd) is a toxic heavy metal, a contaminant mainly of aquatic ecosystems, which can enter the aquatic food chain through direct consumption of water or biota and through non-dietary routes such as absorption over epithelia [3]. For fish, the gills, skin, and digestive tract are potential sites for absorption of Cd in water. Fish are often at the top of the aquatic food chain and may concentrate large amounts of Cd from the water. Toxic responses in fish exposed to elevated waterborne Cd are well-documented [4]. However, studies on oral exposures are sparse, even though food is a significant route of Cd contamination in fish under natural conditions [5]. Cd via the alimentary canal occurs with an initial transfer from food to the gut tissue, followed by movement into the blood and subsequent internal distribution via the circulation. The specific internal organs and tissues in fish that accumulate

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Cd appear to vary with the route of uptake and species [6]. For example, in rainbow trout (*Oncorhynchus mykiss*) during waterborne exposure, the organs with the highest Cd accumulation are the gills and kidney [7], whereas dietary Cd accumulates mostly in the gastrointestinal tract and kidney [8].

Lead (Pb) belongs to a group of toxic metals, which have no function in the physiological processes of living organisms [9]. This metal easily accumulates in fish tissues such as bones, gills, kidneys, liver, and scales [10]. Because Pb crosses the blood brain barrier, it can cause various detrimental effects to the body condition, health, and life of fish [11-13]. Waters generally have low Pb levels, even when high concentrations are found on the bottom, so, food is a significant source of these elements for fish [14].

Blood chemistry indices including enzymes, nutrients, metabolites, waste products, and inorganic ions have been used to detect cellular damage and measure the responses to metals [12]. Various responses were recorded in the plasma of fish chemistry due to metal species, metal concentration, and exposure duration [15]. Plasma enzymes such as alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST) are considered important plasma markers to investigate the health of animal species in concern. Likewise, other plasma biomarkers such as glucose, triglyceride, total protein, and urea commonly are used to detect health of animals. Environmental stressors, such as metal exposures, may change any of the above-mentioned parameters [16, 17]. Therefore, measurement of plasma biochemical parameters can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicants [17, 18].

Heavy metals may affect organisms directly by accumulating in the body or indirectly by transferring to the next trophic level of the food chain [5, 19, 20]. Uptake and toxicity of foodborne metals remain a complex and unrevealed puzzle. The facts that dietary metal exposure can be of significant importance and that it has to be included in regulatory guidelines have become generally accepted statements, the need for a more profound mechanistic understanding of the underlying processes increases exponentially. Moreover, the dual uptake pathways associated with the continuous exposure to a polluted medium and the subsequent reliance on contaminated food items remain an eco-toxicological challenge unique to the aquatic environment [8].

The study of the different pieces of the dietary exposure of heavy metals has therefore become an important research topic. Therefore, the objective of this study was to evaluate the toxicity of two mainly environmentally contaminant heavy metals, Cd and Pb through the experimental food chain.

MATERIALS AND METHODS

Acclimation Conditions

At Aprill 2016, Common carp (*Cyprinus carpio*) fingerlings (with average body weight 4±1.14 g) were obtained from the fish hatchery located at the Reproduction and Rearing Center of Shoush (Khuzestan), southern Iran. They were transferred to the Khatam Alanbia University of Technology (Behbahan) and were maintained in 300 L tanks filled with dechlorinated fresh water (25±2ºC, pH=7.3). Two weeks before the start of experiments, the carp were divided into the three groups (with triplicates) and were transferred to the separate 20-L aquaria each containing 30 juveniles and individually equipped with air stone and heater. Initially, all fish were fed 3% body weight on a control diet (*Artemia nauplii*) without Cd or Pb contamination to acclimate them to experimental conditions.

Artemia Hatching, Rearing and Contamination

Artemia fransiscana cysts were hatched following standard procedures [21]. Newly hatched nauplia were divided into three batches and transferred into the 5 L transparent plastic containers separately. Nauplia for experimental groups were exposed to a sub-lethal concentration of each metal ion for a period of 24 h as static non-renewal condition. The concentration of each toxicant was selected as nominal sub-lethal concentration and based on available literature data for common carp [5, 22, 23]. Analytical grade cadmium chloride and lead nitrate supplied by Merck (Germany) were used as metal toxicant throughout the experiments. Same concentration

of Cd and Pb based on 1.5 mg/L free metal ion were then added to the two containers and last one was remained intact (for control group). Nauplia in all groups were fed with baker's yeast (*Saccharomyces cerevisiae*) twice a day.

Feeding Trial, Sampling and Plasma Preparation

After 24 h, contaminated nauplia were harvested, rinsed and used for feeding of carps in experimental groups. Intact nauplia were also considered for feeding of control group accordingly. The feeding trial was continued 21 days according above procedure for all mentioned group. Sampling was done 4, 7, 14 and 21 days after experiment initiation. Five fish were sampled from each aquarium to determine biochemical analysis. After removal from the aquarium, fish were anesthetized and blood was drawn by cutting the tail aseptically and then drained into a sterile heparinized Eppendorf tube. The pooled up blood of five fishes was taken as a single sample for all three groups. The blood was apportioned and treated for enzymatic investigations. The blood was then centrifuged for 10 min at 3,000 rpm and the plasma samples were then stored at -30˚C until the analysis.

Plasmaenzyme Activity and Metabolite Level

The activities of ALT, AST, ALP and LDH and the concentrations of glucose, total protein, albumin, globulin, triglyceride (TG), cholesterol, and creatinine (Cr) in plasma were measured using a Spectrophotometer Biochrom (England). Reactants for all the measurements were supplied from Pars Azmun Company (Iran) for the analyses.

ALT and AST activities were determined using UV test technique [24]. ALP activity was determined using the colorimetric assay [25]. LDH activity was assayed using UV test technique [26]. Creatine phosphokinase (CPK) assay was carried out [27] for the reverse reaction (hydrolysis of phosphocreatine).

The cholesterol [28], TG [29], Cr [30] and glucose [25] level were determined by enzymatic colorimetric test. The total protein was measured using colorimetric test. The operation of the kit was based on a previous study [31]. Albumin was measured following the method of Wotton and Freeman [32]. Globulin (G) concentration was calculated as the difference between total protein and albumin [33].

Statistical Analysis

Study was done as experimental assay and carried out based on simple completely randomized design. All values of the enzyme and metabolite assay were analyzed statistically by analysis of variance (ANOVA) using SPSS version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Duncan's multiple range tests was used to evaluate the mean difference among individual groups at the 0.05 significance level. Data are presented as mean ± standard error.

RESULTS

Results from enzyme activity are illustrated in Figure 1. Plasma AST activity was increased at 21 days in juveniles that fed by Pb contaminated nauplia ($P<0.05$). Similar results was obtained for ALT reharding Pb. ALP and LDH activity seems were not affected in both treatments ($P>0.05$). CPK activity displayed significant increase in fourth day as for both Cd and Pb and at the end of experiment (day 21) only in Cd treatment.

The metabolite changes in all experimental groups are shown in Figure 2. Cholesterol and TG were indicated same changing pattern and were increased only about Pb ($P<0.05$). Except of 14th day, Cr was increased in carp fingerlings fed by nauplia contaminated by Cd. Pb group just showed apparent increment compared to the control group in 21st days feeding trial ($P<0.05$). Plasma glucose increased concerning both experimental heavy metals compared to the control during the experiment but just remained high only for Pb contaminated group ($P<0.05$). Total protein and albumin were decreased in all sampling periods for Cd and Pb. Decrease in total protein in both experimental metals showed a similar level approximately. Globulin was also decreased as results of feeding from Cd and Pb contaminated nauplia, but significant decrease was observed in days 14 and 21 for cd and 7, 14 and 21 for Pb groups.

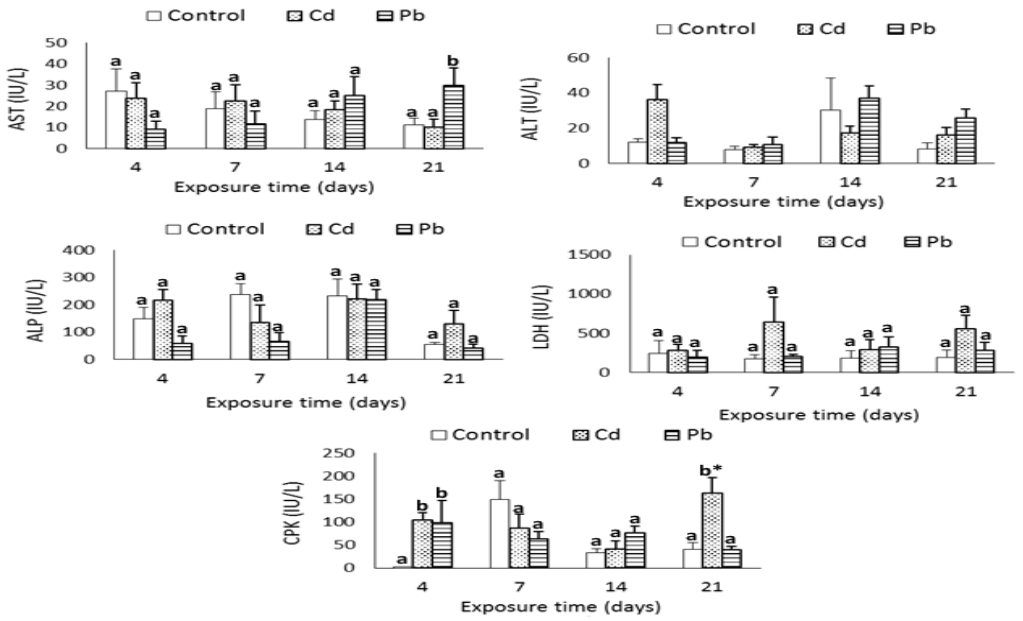

Figure 1. Mean+SE of the activities of plasma enzymes in common carp juveniles during different time feeding by Cd and Pb contaminated nauplia. Different letters indicate significant differences among groups at the same time (P <0.05). Asterisk shows the mean difference at P<0.01.

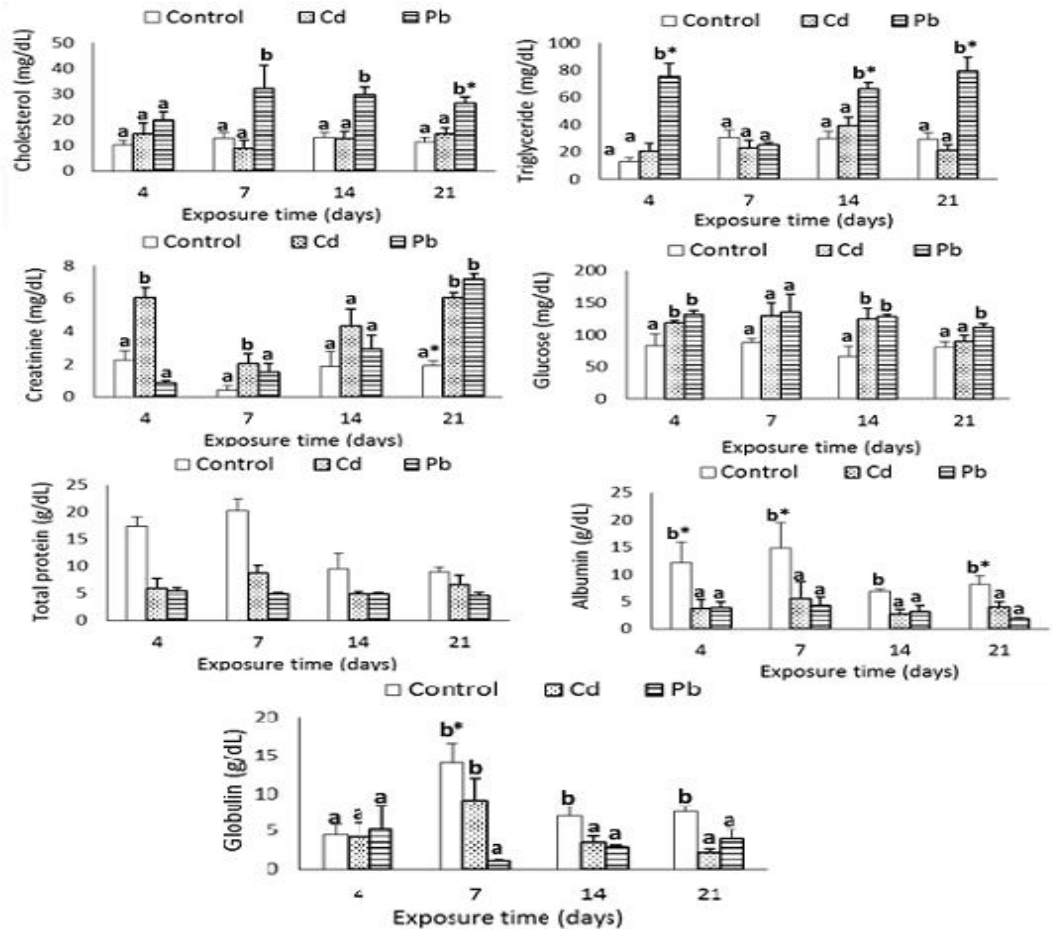

Figure 2. Mean+SE of plasma metabolite change in common carp juveniles during different time feeding by Cd and Pb contaminated nauplia. Different letters indicate significant differences among groups at the same time (P<0.05). Asterisk shows the mean difference at P<0.01.

DISCUSSION

The role of blood enzymes in monitoring and detecting stress or disease has led to a growing concern in using them as biochemical indicators to trace environmental pollutants [34, 35]. ALT and AST were increased only for juveniles fed by Pb contaminated nauplia. Increased in plasma ALT and AST activity because of metal exposure has been widely reported in several investigations [36-39]. Generally, increased activity of these enzymes in extracellular fluid or plasma is a sensitive indicator of cellular damage [39]. Blood levels of ALT and AST may increase because of cellular damage in the liver and high levels of these enzymes in plasma are usually indicative of disease and necrosis in the liver of animals [40]. Therefore, increased ALT and AST activity in the plasma of *C. carpio* juveniles as a results of Pb contamination is probably caused by leakage of these enzymes from liver cytosol into the bloodstream because of liver damage caused by metal exposure [39].

CPK activity showed significant increase in 4^{th} for both Cd and Pb but it seems be modulated especially for Pb, although remained high at the end of experiment about Cd treatment. Cr synthesis begins in the kidney and completed in the liver. It travels through the bloodstream to other tissues, particularly muscle where it reacts with ATP to form the high-energy compound creatine phosphate through CPK action [41]. CPK is used to diagnose and monitor liver, kidney, and heart disease, although it can be elevated during stress [42]. The significantly elevated CPK in blood plasma, particularly concerning Cd treated fish could be consequent to damaged liver and/or to myocardial infarction (necrosis of the heart tissue) [35, 43].

Cholesterol and TG have been used for demonstrating the nutritional status in animals. TG is used to evaluate lipid metabolism; high concentrations may occur with nephritic syndrome or glycogen storage disease [44]. Oral exposure of metals especially lead in the present research induced a significant rise in blood TG level in the carp juveniles. The levels of blood TG were significantly increased in the animals treated with different metals [37, 45, 46]. There are a number of reports indicating that pollutants (e.g. Cd, Pb and Mercury) influence thyroid function [47, 48]. Since decreased thyroid secretion (hypothyroidism) greatly increases TG level in the blood, the observed hypertriglyceridemia may be due to hypothyroidism induced by lead and/or liver dysfunction because the liver is the principle center of lipid metabolism [49].

Based on obtained results, cholesterol in blood plasma was significantly higher in fish fed on nauplia contained Cd or Pb compared to the control group. Increased plasma cholesterol concentrations can also be resulted from damage of liver or nephrotic syndrome. The present data are supported by other studies showing increased plasma cholesterol concentrations in metal-exposed fishes [36, 46, 50]. The concentrations of cholesterol, an essential structural component of membranes and the precursor of all steroid hormones, may be increased due to the liver and kidney failure causing the release of cholesterol into the blood. Exposure of fish to heavy metals seems to increase the level of plasma cholesterol probably due to stress they cause. Therefore, increases in cholesterol may be a good indication of environmental stress [18].

Levels of Cr were significantly enhanced in both metal-treated fish in the current study. This refers to kidney failure and/or increased muscular tissue catabolism [43]. Heavy metals accumulate mainly in metabolically active tissues such as the liver and kidney [51]. The increased Cr level indicated that metal toxicity had a marked effect on kidney function, perhaps due to the action of diet-borne Cd and Pb on glomeruli filtration rate and/or pathological changes to the kidney resulting in dysfunction. Similar results were reported earlier [52, 54].

The data showed that plasma glucose concentrations were increased in treated than control fish especially for Pb treated fish. These finding suggest that metal-treated fish were experienced the stress situation [16]. Exposure to water pollutants including pesticides and heavy metals similarly resulted in elevated plasma glucose in fish [43]. Literatures showed similar reports as for *Oreochromis niloticus* [41], *Clarias gariepinus* [43], and *Cryptobranchus alleganiensis* [42]. The enhanced level of glucose coupled with depletion of total protein concentration suggested that metallic stress enhanced the potential of gluconeogenesis because of stressful condition and therefor excess demanding for energy resources [41].

The depletion in plasma total protein of carp fingerlings in the present study, observed for both

experimental metals, may be due to impaired or low rate of protein synthesis under metallic stress or a consequent of their utilization in the formation of mucoproteins, eliminated in the form of mucous. Further, direct and indirect utilization of proteins and lipids for energy needs was also reported [45]. In addition, the utilization of proteins in cell repair and organization as causes of their depletion in the tissues should not be ruled out [33, 54].

Plasma protein mainly contains albumin and globulin. Research on the function and uses of plasma albumin continue to be a great interest not only because of its dominant position in the plasma protein, but also its dramatic alterations in the level of this plasma protein in many diseases and other stress situations [55]. Albumin as well as globulin was significantly tended to decline in both Cd and Pb contaminated fish. The complex interactions of immunocompetent cells, required to produce an immunological response of fish, are susceptible to the many biochemical and physiological disturbances, which can be induced by heavy metals [55]. Such adverse effects may suppress the immune response of fish and make them more vulnerable to the environmental pathogens [56].

The lower toxicity of Cd in this study may be related to the lower accumulation of this metal through food chain. Accumulation of Cd in each trophic level depends on Cd concentration in the lowest trophic level and Cd contamination in the water body [57]. They also concluded that Cd tend to more absorption in primary producers. However, our results showed that the Cd concentration was not increased with trophic level. Assimilation efficiency of Cd is depending to external factors including prey type [8]. Liu et al. [58] reported assimilation efficiency for Cd and Zn was around 6% and 30%, respectively, when feeding zebra fish on contaminated daphnids. Vinodhini and Narayanan [4] on the other hand exposed the common carp to sub-lethal waterborne concentration of some heavy metals including Cd and Pb and consequently reported the higher bioaccumulation of Pb compared to Cd. The same results were reported for common carp [59].

CONCLUSION

Based on our obtained results and previous studies, it can be concluded that Pb may had a higher assimilation and toxicity than Cd in both waterborne and food borne condition.

Our results clearly demonstrate the potential dangers of heavy metal pollution in aquatic environments. Feeding by contaminated *A. nauplii* showed adverse plasma biochemical alteration for both metals, suggest the high potential toxicity of heavy metals through the food chain. Based on plasma biochemical analysis, it seems that Pb had a greater toxicity than Cd through the food chain and it maybe because of its more trophic transfer than Cd.

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