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Effect of Pb and Cd on the Iron Solute in Blood (*Chalcalburnus chalcoides*)

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Abstract: This study was carried out within inland Aquaculture Research Center Bandar Anzali (Guilan Province) through Southwestern part of the Caspian Sea; to examine the effect of two heavy metals (lead, cadmium) on (Fe) amount in blood of *Chalcalburnus chalcoides*. This fish is widespread and lives through Southern and Western part of the Caspian. The sampling was randomly done in two stages. The size of fish was 15-16 cm. After transferring fish to the pool, in order to adaptation, has been provided 12 aquariums in which 9 fish were released to each one. Regarding to the lowest capacity (LC50) for these fish, chose the selective densities to pollute aquariums' environs. In such a way that for each aquarium cell with a constant density, we account 0.05, 0.15 and 0.03 ppm for lead and 0.15, 0.25 and 0.75 ppm for cadmium, respectively; while a stereotype aquarium was considered as a non- polluted environ. In each stage, we left 3 fish out of them to take blood from their heart and tail-stem, then we transferred the frozen samples to the lab. This experiment was repeated for the second metal as the same. Acid nitric digested the blood to provide a transparent and colorless solution for the atomic absorption device. With respect to the obtained absorptions by Pb, Cd and Fe in lab, the variance analysis (ANOVA) was carried out in (SPSS) and (Excel) systems. Based on statistical results, cadmium with ratio $p<0.05$ replaced with ferritin (Fe) over the time, but metal (Pb) couldn't so. The results indicated that by increase in lead density within various times, this metal was absorbed by other fish's tissues.

Key words: Cadmium, lead, toxicity, *Chalcalburnus chalcoides*

INTRODUCTION

Caspian Sea is one of the most important of world ecosystems that has three areas of Northern, Middle and Southern. The industrial sewage is entered to this sea and they carry, a heavy metals such as copper, lead, zinc and cadmium that are among the most important of contaminant materials. Among these metals, a lead and cadmium that originate from the industrial material are a little toxic. The water flow of Caspian Sea is such that most of contaminants are conducted from different parts to it's south beach. The intensity of these contaminants is more at the western part of South shore where Gilan Province is there (Anzali pond).

Chalculburnus chalcoides is from the family of cyprinidae such as immigrant species to Anzali pond and Western and Southern parts of Caspian Sea. This fish has a high economic value after a caviar and white fish. *Chalcalburnus chalcoides* live at water of sea Middle and South area as a dispersed. But they are rarely found at North part of sea (Vossoghi and Mostajir, 1371).

When iron is attracted from a small intestine physiologically, it combines with one Beta globulin named apotransferin at a blood plasma immediately and forms a transferin and it is transmitted at a

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cell A iron combines with protein named Apoferitin at a cell A iron combines with protein named apoferitin at a cell cytoplasm and forms a feritin. Therefore, a feritin has an iron. A lead and cadmium are among a heavy metals that cause a extensive contamination. The acceptable concentration of lead at fish and a sea foods is 0.5 ppm.

A cadmium element becomes a free from melting kinds of ore, industrial sewage, waste water and industries in the environment. A cadmium is a element that is accumulated at a kidney and a liver at high amount (Clements, 1991). Therefore, a main aim of this research is that whether can the elements like a lead and cadmium displace with iron and whether do this displacement play a role at a trend of the iron efficiency? And whether can we use from amount of these metals at a blood for approximating the environmental contamination?

MATERIALS AND METHODS

This research was performed at acquatics research center of internal waters at Ghazian Region, Anzali Town at Gilan Province. This region consists the West-South parts of Caspian Sea. The sampling was made within two stages. The fish fishing was performed by Runaround gill net and the method of a sample collection was made based on an accidental sampling.

First, we prepared 12 numbers of aquariums and filled inside them with water and allowed to remain water inside an aquarium for 24 h. After trapping a fish, they were left inside a small pool and allowed to the adaptation of fish with a new environment for 3 days. The required light of sun and an usual lamp and the used water pH (7-7.5) was used.

After remaining water for 24 h at aquarium, fish were inside an aquarium. At each aquarium, 9 fish were left accidentally. The fish size was 15-16 cm had obtained the selected concentrations for contaminating an aquariums using LC50. The LC50 means concentration of one contaminant at one population that half of them is lost (Shah and Altindag, 2005). At about this experiment, a minimum 0.25 concentration of LC50 will be equal to the most concentration for a relative metal. Therefore, the selected concentrations was selected 0.05, 0.15 and 0.3 mg L^{-1} for a lead and 0.15, 0.25 and 0.75 mg L^{-1} for a cadmium. Three of an aquariums were selected with one fix concentration and other 3 numbers with one concentration and other 3 numbers with other concentration. (For a reason of repeat three-times), one aquarium was pat as an evidence (non-pollution environment) for each three aquarium with one fix concentration. We allowed to the adaptation of fish at an aquarium environment for 24 h.

The aeration was made by the air pump correctly. After this time, the relative metal concentration was added as a solution to the water of the aquarium. The needed biometry was accounted from all of fish. After time three periods, we took a blood from a fish. As we took out three fish at each 24 h and took a blood from a heat and a gill part (without the anesthesia of fish). A special dishes was transferred to a freezer, immediately after taking a blood. We performed an experiment for a second metal the same as it.

The Frozen Dishes Were Transferred to a Laboratory

We used from Nitric acid for digesting blood, so that we can prepare a colorless solution for an atomic absorption device. We added 10 cc acid to the dishes and then transferred it to a test tube and those tubes were put at hot water bath with 60° for 1 h. After taking out those tubes, the samples were purred inside volumetric flask 10 mL, it was approach to a special volume with the distilled water. From each fish, about 1-1.5 cc blood was taken. The samples were provided to an atomic absorption device and the related absorptions to a lead and cadmium were obtained. Using these absorptions, we obtained the existing concentrations in the blood for each three metals.

Finally, for determining being or not being meaningful of the difference, we used from ANOVA variance analysis at SPSS system and the related charts were drawn and set using Excel program.

RESULTS

A different living beings show a different sensitive to a toxic elements. This study was reviewed in order to effect a lead and a cadmium on an iron amount variation at chalculburnus chalcoides blood. The results are as follows: about a lead metal given three concentrations of the aquarium contaminant namely 0.05, 0.15 and 0.3 ppm, the performance of the analysis and ANOVA variance between a different concentrations showed that there is no a meaningful difference between them ($p>0.05$).

Meanwhile, there is no a meaningful difference between average iron concentration at the contaminated environment to 0.05, 0.15 and 0.3 ppm given that each one was measured to a time ($p>0.05$).

Also, between three concentrations 0.05, 0.15 and 0.3 ppm of a lead, the fix concentration lead 0.3 mg L^{-1} showed a meaningful level at a different times ($p<0.05$).

Also, we didn't obtain a meaningful difference between a fix times and a different concentrations about a lead metal. About a cadmium metal, the results are as follows: The performance of analysis and ANOVA variance between a different concentrations of a cadmium showed that there statistically a meaningful difference between an average cadmium concentration at the blood at the contaminated environment to 0.15 ppm of cadmium during 24 h with an average cadmium concentration at the blood at the contaminated environment to 0.75 concentration ppm during 24 h and 0.15, 0.25 and 0.75 ppm during 48 h and 0.15, 0.25 and 0.75 ppm during 72 h of cadmium ($p<0.05$). There is a meaningful difference between a fix concentrations 0.15, 0.25 and 0.75 ppm of a cadmium at environment at a different times ($p<0.05$).

Also there was a correlation between a different concentrations of the environment contaminating to cadmium and a cadmium concentration at the blood and the measured iron concentration at a fix times with a different concentration, 0.15, 0.25 and 0.75 ppm of the environment cadmium and it is a meaningful difference statistically.

The studies showed that there was a correlation between a cadmium concentration at the blood and an iron concentration at the blood and it is a meaningful difference. Therefore, according to the statistic results, the cadmium with $p<0.05$ possibility, is displaced with a solution iron at the blood, with passing a time. The lake of a meaningful relationship between an iron concentration at the blood and a lead concentration at the blood shows that with increasing a lead concentration at a different times, a lead is absorbed by other fish textures (Fig. 1).

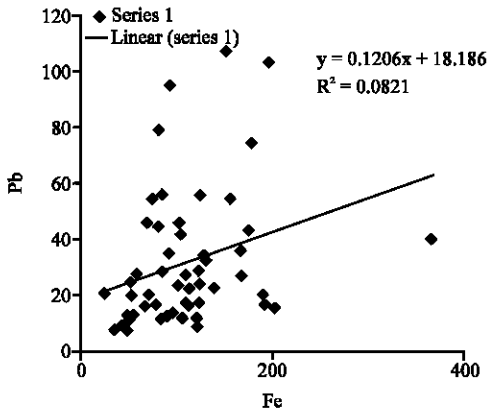

Fig. 1: Correlation between a lead concentration at the blood and an iron at the blood

DISCUSSION

The iron at a blood plasma is combined with one beta globulin known as Apotransferin and is carried as a transferin. The extra iron at the blood is deposited at all of cells especially, liver cells and with less amount at a bone marrow. The iron at a cell cytoplasm is combined with a protein named apoferitin and it forms a feritin. This reserved iron as a feritin is known as a reserve iron. The absorption of iron is made from all of parts of small intestine and commonly with aiding apotransferin. The apotransferin is connected to a free iron inside small intestine and it causes a transferin and it is relived as a plasma transferin inside a blood. The regulation of iron amount is applied with a variation at iron absorption amount through an intestine canal. It means that with the saturation of a reserve apoferitin with iron, the iron absorption amount decline rather high. The proposed mechanism is such that the relief of iron from a plasma transferin decline to a textures, when all of body apoferitin is saturated from iron. As a result a transferin can not accept a new iron from intestine mucus cells (Houston *et al*., 1996).

Of 90 existing elements at a nature, some of them are a poisonous. A lead, mercury and cadmium metals are named a heavy metals that they are a toxic with a concentrations more than an allowed limit. More than 90% lead at the blood is connected to a red globules. Among a poisoning effects with a lead, it's effect on biosynthesis also is very important (Brumbaugh *et al*., 2005). These variations consist the increase of synthetaz (ALA) delta-aminoloo linic acid and co-oxygenaz and dehidrataz ALA restraint. The deficiency at a poisoning with a lead is seen for this reason that a ferric iron is not regenerated to a ferrous and this process also is an important for a synthesis (Fig. 3).

The Bone is a Largest Reserve of a Lead at a Body

A lead may be change a vitamin D 2-1-5- dihydroxy blood levels This problem is a remarkable, because vitamin D and paratiroid is a responsible for getting a calcium from a bone (Rhona and Sandhu, 2003). A lead is absorped to a protein at a body textures and displaced with a calcium at cells and it distrupts an activity of body elements that a calcium play a basic role at it (Fig. 2).

The interaction of a lead and calcium was reviewed by Witeska (2005). These findings were added to a previous findings based on the interaction of a lead with some mechanism related to a calcium such as calmodiolin, kinaz protein C, potassium canals related to a calcium at a plasma membranes and norotransmiters free.

Simons introduced a lead as strong restrainer of calcium entrance inside norons through a calcium canals related to voltage. It weakened a nervious cells relationship together and distrupted at muscles contraction and a blood blockage time and kidneys activity. A lead with restraining two main enzymes

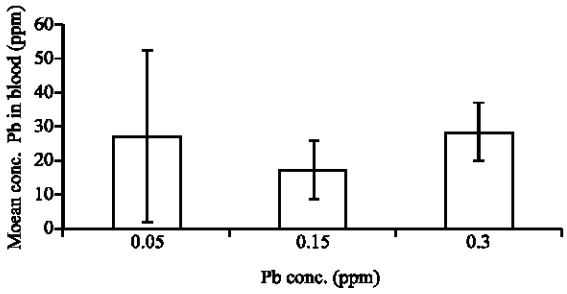

Fig. 2: Average lead concentration at the blood at the contaminated environment to a lead at 72 h

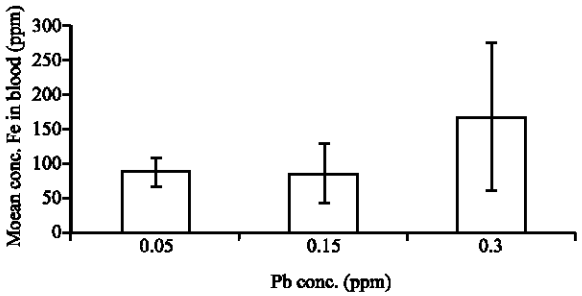

Fig. 3: Average iron concentration at the blood at the contaminated environment to a lead at 72 h

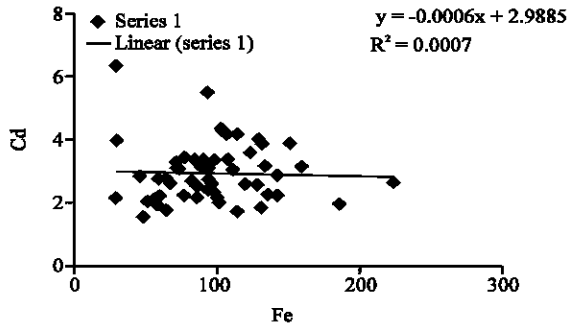

Fig. 4: Correlation between a cadmium concentration at the blood and iron at the blood

at a hemoglobin cycle declines it's production and causes an anemia (Jezierska and Witeska, 2001). A cadmium is viable two capacitance cation and macromolecules have more than one connection site, have a more tendency to cadmium. When a cadmium is absorpted from digestive tract, it is entered to the blood and can bond with high molecule. Weight proteins (40000-600000), plasma metalotionin (MT) and/or enter directly to erythrocyits. A cadmium inside erythrocits may be bond with hemoglobin, molecule weight proteins more than hemoglobin and/or a low molecule weight proteins. Most of erythrocyts cadmium is bonded to a low molecule weight proteins similar to MT. The MT is a low molecule weight protein that it is rich of cictein. The most capacity of a cadmium is seen at a liver. The being toxic of cadmium is related to exit a zinc from a special enzymes and their inactivation.

When a cadmium enters to a body, it becomes viable and causes a defects like an iron deficiency at the blood. A cadmium first go to a liver by a blood (Fig. 5).

It is connected to proteins at a liver and forms a complex that go to a kidney. A cadmium is accumulated at a kidney and disturbs a filteration process. The cadmium repulsion at a kidney takes a long time.

The iron as two capacitance at intestine mucus cells is converted to an three capacitance iron (Feritin) and when required, it takes out as two capacitance from a mocus cells and is combined with a solution oxygen at the blood and is converted to three capacitance iron and becomes as complex by beta globulin that it is an iron transmission way (Shah, 2006).

The heavy metals increase blood acidity and the body exit calcium from bones in order to protect a suitable pH. The heavy metals cause an inflammation at textures that it causes a move discharge of calcium toward a textures as buffer. If the loosed calcium doesn't compensate, then a permanent uptake of this mineral from bones will cause osteoporosis. Cadmium is absorpted by fish and other animals. A cadmium element causes an iron deficiency at the blood over time (Fig. 6).

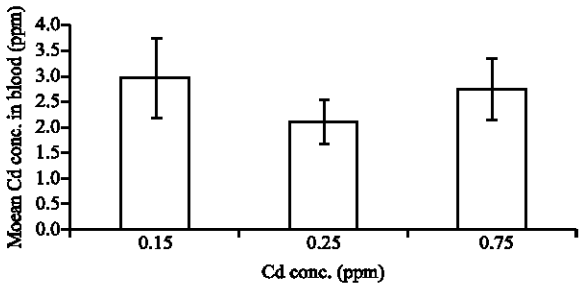

Fig. 5: Average cadmium concentration at the blood at the contaminated environment to a cadmium at 72 h

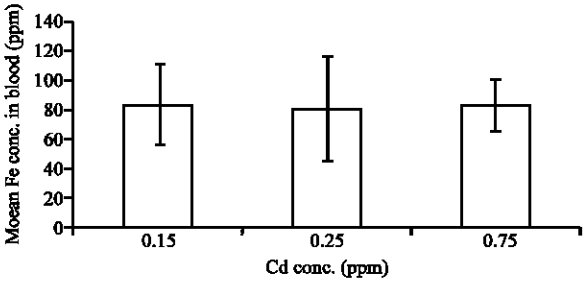

Fig. 6: Average iron concentration at the blood at the contaminated environment to a cadmium at 72 h

Cadmium increases a calcium and phosphorus excretion at urine that causes osteomalacia. The absorption of heavy metals occur from the level of fish organs that consist, gills, skin, fins and intestine, that the portion of gills is more than other two ways and chloride cells play the most important of role at this organ (Vinodhini and Muthuswamy, 2008). The known mechanism for transferring a heavy metals at fish is by chloride cells through gill way (Vosyliene, 1999). The high tendency of cadmium to a calcium ion connection sites at a gill and also connecting a cadmium to a calcium pumps active sites at chloride cells is a main mechanism for entering a cadmium to fish's gill. A cadmium is entered to a blood circulation resulting from ATPAZ calcium after entering to a gill through a calcium ducts at part of cover cells. The heavy metals are distributed at a body organs after entering to a blood circulation finally. The entered metals to a blood, are carried as a free or connected to a protein. These metals with a weak chemical connection with proteins are transferred to environmental blood from around gill.

The studies showed that there was a correlation between a cadmium at the blood iron concentration at the blood and statistically, the difference is a meaningful. Therefore, according to statistic results, the cadmium with $p<0.05$ possibility is displaced with a solution iron at the blood over time (Fig. 4). The lack of meaningful relationship between iron concentration at the blood and a lead concentration at the blood shows that with increasing a lead concentration at a different times, a lead is absorpted by other fish textures (Fig. 1).

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