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The effects of cadmium chloride on the hematological and biochemical parameters in giant Sturgeon fish (*Huso huso*)

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Abstract

Cadmium, heavy metal regularly is being reported in flesh of one of the most importantcaviar,Sturgeon fish(Huso huso)in Caspian Sea.This level of heavy metal were detected by atomic absorption spectrophotometer device in the albumin content of this fish.The goal is, to study the effect of heavy metal cadmium on sturgeon fish (Huso huso) by using certain hematology and bio-chemical variations.The sub-lethal concentrations (LC50) of cadmium chloride on *Huso huso* was found out for 96 h (28 mg/L), and 1/15th, 1/10th, and 1/5th of the LC50 were 1.93, 3.11 and 5.78 mg/L respectively. The results indicated that the values of the leucocytes and the RBC were in the range of 13.74±0.42 to 40.64 ± 2.01 (×103cell /mm3) and 4.31±0.35 to 2.28±0.35 ($p<0.05$) respectively. Concentrations of M.C.H (pg), M.C.H.C (g/LD), hematocrit and hemoglobin were significantly decreased ($p<0.05$).

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INTRODUCTION

Today it's been well discovered that environmental problems have accelerated mainly because of a rapid increase in human population. However, industries often cause damages to aquatic life when their toxic effluents discharged into water.

Among the aquatic animals, fishes are mainly utilized as food supplies. “Dominant portion of inorganic contaminants are heavy metals. They have some different problems than organic contaminants” (Dreibach and Robertson, 1987; Gad and Saad, 2008). “Toxic metals can penetrate the human body via aspiration, ingestion, and skin contact.

The superlative prevalent effects of toxic metals are hemotoxic nephrotoxic, effects on respiratory and reproduction system” (Dreibach and Robertson, 1987). “The existence of metals differs between different fishes; is related to age, developing stage, and additional physiological factors. Pollutants usually supply partly rapid changes in blood specificities of fish” (Ezzat et al., 1998).

Therefore, hematological and biochemical data can supply helpful information in assessing the health of fish and in monitoring stress responses. The hematological parameters like hemoglobin, hematocrit, blood cell counts, and ion concentrations can be used to find the physiological response of the contaminated environment. The environmental condition factors affect the toxicity of heavy metals as well as cadmium. The accumulation of cadmium, copper and zinc and the induction of metallothioneins (MT) in liver of three freshwater fish species (*Gobio gobio*, *Rutilus rutilus*, and *Perca fluviatilis* was studied by Bervoets L, et a. 2013 at 6 sampling sites along a cadmium and zinc gradient and one reference site in a tributary of the Scheldt River in Flanders (Belgium). They conclude that different fish species exposed tothe same pollution gradient differentially accumulate metals and differentially induce metal binding proteins. (Bervoets L et al., 2013).

Toxicity of Heavy metals is due to the accumulation of them in soft tissues because body does not metabolize them. Several investigations indicate that bioaccumulation of heavy metal occurred in various parts of fish body such as skin, gill, brain, liver, muscle, kidney, intestine, gonads, and blood cells. (Witeska, 1998; Vinodini and Narayanan, 2008). Cadmium accumulation in these organs seems to be depended on the presence of cadmium-binding molecules called metallothioneins (Carpene E. et al., 1987).

Cadmium is one of the highly toxic heavy metals which can interfere with the metabolism of carbohydrates, proteins, and lipids by inhibiting the enzymes involved in the processes (Surya Kumari Vadlamani et al., 2017).

The effects of exposure depend on the dose, exposure time, the mode of exposure, personal habits, traits, and presence of other chemicals(Bujjamma P, Padmavathi P., 2018).

The lethal and sub-lethal concentration, cadmium has accumulative poisoning impact and can cause severe disruptions in fish metabolism such as locomotor anomalies. (Cicik and Engin, 2005). Most cadmium contamination comes from metal foundries,
paint industry , production of plastics, and accumulators. This contact results in pathological changes in water ecosystems mainly, indicated in fishes, which affected by heavy metals through the respiratory, digestive systems, and the skin. *Forexample, researches by Baker and Montgomery(2001)* indicate that cadmium ions are responsible for the decreased olfactory function and changed rheotaxis behavior associated with damage to the lateral line system in freshwater fish. Daei et al., (2009), *research* on (*Chalcalburnus chalcoides*) revealed that cadmium with a ratio ($P<0.05$) replaced with ferritin (Fe) within the exposure time in the fish blood, but metal Pb could not do so. Those results showed that by adding lead, in fishes, this metal was absorbed by other tissues (Daei et al., 2009).

The toxicity of Cd is imputed to its capability to generate reactive oxygen species that may act as inducing molecules in the stimulation of gene expression and apoptosis (Waisberg et al., 2003), reduce endogenous radical scavengers, and harm a variety of transport proteins such as the Na^+ / K^+ - ATPase.

At low levels, Cadmium is toxic to plants, fish, birds, humans, and microorganism's life (Eisler, 1985; ATSDR 2008). The toxic impacts of cadmium on fish are multidirectional and manifested by many alterations in the physiological and chemical processes of their body systems (Dimitrova et al., 1994). Cadmium is a metal with no known advantageous specificities that support life - there is no document that it is either biologically needful or helpful (Eisler, 1985).

The present research designed to study the hematological and biochemical effect resulting from the exposure of the Caspian Sea sturgeon (*Huso huso*) exposed to sub-lethal concentrations of cadmium chloride.

MATERIAL AND METHOD

Experimental design

The *Huso huso* larvae gained from the Research Institute of Shahid Rajaee, Sari, Mazandaran province (Iran). Total of 60 fishes (70 - 100 g) was adapted to laboratory environments for two weeks before use. Fishes were allocated randomly into four groups of 15 each. The first group maintained in free from cadmium chloride and served as the controls.

The other three groups were exposed to sub-lethal concentration of cadmium chloride in the 100-liter capacity tank. During the experiment, pH and dissolved oxygens were 7.1 ± 0.5 and 8 mg/l, respectively. The tank temperature maintained at 27±1°C using thermostats. Throughout the feeding trial, all tanks maintained under normal photoperiod (11 h light, 13 h dark).

During the experiment (60 days), the fishes were fed per day (twice a day) equal of 3% of their body weight. Siphoning three-quarters tanks was done every day. The waste removed and replaced it by an equal volume of water having the same concentration of Cd. Dead fish removed and recorded daily. Ingredients and structure of experimental diets are shown in Table 1.

Table 1: Experimental diet formulation and proximate composition

Ingredients (%)	Diet
Fish meal	60
Wheat meal	20
Fish oil	6
Soybean oil	6.5
Molasses	2.3
Vitamin mixture[1]	2
Mineral mixture[2]	3
Anti oxidane	0.2
Proximate composition	
Dry mater (%)	89.86
Protein (%DM)	42.72
Lipid (%DM)	15.67
Ash (%DM)	12.79
Digestible energy (kj g^{-1} diet)	20.18
Gross energy (kj g^{-1} diet)	22.13

[1]**Vitamin mixture** (mg kg-1 diet): retinyl acetate, 40; cholecalciferol, 0.1; a-tocopheryl acetate, 80; niacin, 168; riboflavin, 22; pyridoxine HCl, 40; thiamin mononitrate, 45; *D*-Capantothenate, 102, biotin, 0.4; folic acid, 10; vitamin B12, 0.04; ascorbic acid, 1000 and inositol, 450.

[2] **Mineral mixture** (g kg-1of premix): $NaH_2PO_4 \cdot H_2O$, 200; KH_2PO_4, 200; $MgSO_4 \cdot 7H_2O$, 10; $MnSO_4 \cdot H_2O$, 2; $CuCl_2 \cdot 2H_2O$, 1; $ZnSO_4 \cdot 7H_2O$, 2; $FeSO_4 \cdot 7H_2O$, 2; NaCl, 12; KI, 0.1; $CoCl_2 \cdot 6H_2O$, 0.1.

Blood and hematological factor

Blood was collected by venipuncture using syringes coated with heparin (Sigma-Aldrich®) and transferred directly into 9 ml capacity lithium heparin vacuities (Greiner®) on the ice, and the serum was separated by centrifugation. Hematocrit (PCV) value (% red blood cell) was analyzed in micro-hematocrit- heparinized capillaries within 40 min after blood sampling, using a micro-hematocrit centrifuge _13,000 rpm for 3 min.

The total leucocytes counted by standard hematological procedures and Hemoglobin (Hb) level was determined calorimetrically by determining the formation of cyanmethemoglobin using a commercial kit. The mean corpuscular hemoglobin (MCH, pg = (Haemoglobin [g/dL] × 10 / (RBC count [in millions/lL])) and mean corpuscular hemoglobin concentration (MCHC,g/dl= Hemoglobin [g/dL] / Hematocrit [%]) were calculated from hematological data. 2.3.

Serum, The concentration of serum total protein, globulin, glucose, was determined by the methods described by (Shimeno et al., 1990), while the albumin content was determined spectrophotometrically using a standard kit (Glaxo, India). Total Serum cholesterol was measured by the method of Allain (1974).

Statistical analysis:

Data analyzed by one-way analysis of variance (ANOVA) and Duncan's comparison of means. Percentage of data transformed to square-root arcsine values to homogenize variance. All statistical tests were done using SPSS software (SPSS, Ver. 14.0, SPSS, Chicago, IL.). Variances were considered as statistically significant when P

< 0.05.

RESULTS

Cadmium chloride LC50 was found out for 96 h (28 mg/L), and 1/15th, 1/10th, and 1/5th of the LC50 values were 1.93, 3.11 and 5.78 mg/L respectively taken as sub-lethal concentrations for this study. Summary of hematological and biochemical parameters indices were presented in Tables 2 and 3.

Table 2. Effects of cadmium exposure on hematological parameters of *Huso huso*.

parameter	Control	T1	T2	T3
Haemoglobin(gl−1)	9.98± 1.54	9.94± 0.52	9.11± 0.17	8.46± 1.42*
Hct(%)	31.00± 0.13	29.54± 1.46	28.71± 0.78	27.16±2.11*
Leucocytes(×10³cell/mm³)	13.74±0.42	28.43± 1.04*	32.10±2.06	40.64 ± 2.01*
M.C.H (pg)	66.01± 2.04	55.07±0.23*	49.24±2.31*	38.58± 2.19*
M.C.H.C (g/ld)	35.25 ± 0.06	35.34 ±1.84	35.14± 3.61	30.77 ±2.17*
RBC(×10³cell/mm⁴)	4.31±0.35	4.11±0.17	3.17±0.04*	2.28±0.35*

*Significant difference with control (P<0.05). Values are mean ± standard error.

Table 3. Effects of cadmium exposure on biochemical parameters of *Huso huso*.

parameter	control	T1	T2	T3
Total protein(g/L)	45.19± 2.35	40.62±0.28	33.25± 1.08*	28.71± 2.06*
Albumin(g/L)	5.18± 1.47	4.27± 1.48	3.35± 3.46*	3.10±1.69*
Globulin(g/L)	36.18±0.05	31.19± 1.28*	31.14±2.41*	25.34 ± 0.01*
glucose (mg/dl)	56.31± 0.02	64.02±0.20	71.14±1.01*	75.05± 0.19*
Cholesterol (mg/dl)	150.12 ± 0.41	158.45 ±0.25	178.10± 0.36*	186.24 ±1.43*

*Significant difference with control (P<0.05). Values are mean ± standard error.

Regarding hematological parameters, cadmium exposure for 60 days significantly dwindled RBC count, HCT, MCHC, MCH, and hemoglobin concentration in *H. huso* in comparison with control. In the present study, the mean value of PCV was 31.00± 0.13 in the control group, which diminished progressively (29.54, 28.71, 27.16) in trial groups. A decline in the percent of hematocrit demonstrates the worsening of an organism state and anemia expansion.

DISCUSSIONS

The decrease of hematological factors in fishes at sub-lethal levels of cadmium might be from the demolition of mature RBCs and the suppression of erythrocyte generation due to the mitigation of haem synthesis that may be induced by pollutants (Wintrobe, 1978, 2018).

Also, the RBCs count detraction may be attributed to haematopathology that results in severe anemia in most animals, including fishes exposed to different pollutants (Khangarot & Tripathi, 1992). According to Pamila et al. (1991), the reduction in hemoglobin content in fish exposed to toxicant could also be from the inhibitory influence of the toxic substance on the enzyme system responsible for the synthesis of hemoglobin.

Also, Gill & Epple (1993) found a significant reduction in the RBCs, Hb, and HCT in American eel (*Anguilla* *rostrata*) after exposure to 150 ug Cd L-1. Karuppasamy et al. observed an important reduction in total erythrocyte count, hemoglobin content, hematocrit value and mean corpuscular hemoglobin concentration in air-breathing fish, *Channa punctatus* after exposure to the sub-lethal dose of Cd (29 mg Cd L-1)(Karuppasamy et al., 2005).

Leucocytes counts were found increased following cadmium exposure, as presented in Table 1. Similar findings were also recognized meaningfully higher in fish exposed to increasing of cadmium concentration (Bujjamma P, Padmavathi P., 2018). Mishra and Srivastava (1980) also stated an increase in leucocytes count when they exposed fishes to heavy metals.

Respecting the serum protein, cadmium exposure for 45 days meaningfully reduced the amount of total protein, albumin, and globulin ratio comparing with control. The decrease in serum protein may indicate some liver dysfunction. When exposed to the stressor, the gills become permeable to water and ions, regularly causing in osmoregulatory imbalances.

Therefore, the decline in serum total protein, albumin, and globulin may also be due to a degree of have modulation under stress from contaminations. Heavy metals increment the glucose content in serum, due to intensive glycogenolysis and the synthesis of glucose from extrahepatic tissue proteins and amino acids (Almeida et al., 2001). Many other workers reported hypoglycemic condition in fishes due to contaminants. (Sastry, 1984).

This may be to cope with high-energy demand in stress situations. Cholesterol is the substantial sterol occurring in animal fats that equally distributed between plasma and red blood cells, but in the adrenal cortex, it appears in the esterified form — the cholesterol increase as white (or) faintly yellow, nearly odorless granules. In the current study, the serum cholesterol amounts were meaningfully ($p<0.05$) increased in heavy metal exposed experimental groups (Table 2).

CONCLUSION

The cadmium chloride causes deleterious effects on fishes and much altars the biochemical characterization of blood and serum. Pollution of water by heavy metals because of acute or chronic actions constitutes the extra source of stress for organisms lives in an aquatic environment. Sub-lethal concentrations of toxicants in the environment will not essentially infer in absolute mortality of organisms lives in water.

Cadmium chloride LC50 had been found out commonly in fish for 96 h (28 mg/L) by Sprague, 1971(Sprague, J.B., 1971). In this study, this dosage as LC50 for 96 h, has been confirmed for applying it to specific fish, *Huso hoso*. The sub-lethal concentrations of this heavy metal were found out for 1.93, 3.11, and 5.78 mg/L respectively.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

Valiallahi Jalal conceived the experiments, analyzed the data, prepared figures, and tables, authored or revised drafts of the paper, approved the final draft.

Pourabbasali Mohsen conceived and designed the experiments, analyzed the data, contributed reagents /materials/analysis tools, prepared figures, and tables.

Data Availability

All the data are shown in the tables of this article.

Ethics Statement

The study was conducted by national and international guidelines (Directive 2007/526/EC of the European Commission) for the protection of animal welfare. Also approved by Scientific Association of Environmental Education and Sustainable Development(EESD) http://www.ac.ir/environment

Supplemental Information

Supplemental information for this article can be found online at ???

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APPENDICES 1

Sturgeon fish (*Huso huso*), is one of the largest and the most important caviar fish in the world, and one of the vulnerable species of Caspian Sea fish. The grownups of this fish live in Caspian Sea with the salinity of 10-13 ppt, but spawning happens in freshwater Rivers, fry lives in rivers and after two months, returns back to Caspian Sea.

Caspian Sea is an inlands lake that is charged by several freshwater rivers, numerous of pollutants, like heavy metals, etc. discharged to Caspian sea cause *H. huso*, to be recorded as threatened and endangered species (Moghim et al. 2002). In this context,

Sturgeon farming has been practiced in Iran for about half a century. At the past, the belly of these fish in captive was been cut off for taking out the eggs, but at a recent decade, the Iranians fish propagation specialist are using modern methods for taking out eggs from females, such as cesarean section. After taking caviar (female Strugeon fish egg) from female for propagation, fish will send to cesarean section for surgery and recovery (figure. 1).

Figure 1. After taking caviar (female Strugeon fish egg) from female for propagation, fish will send to cesarean section for surgery and recovery. Shahid Rajaee Strugeon fish propagation center for stock enhancement. Gorgan, Golestan, Iran

There is several main farming complex in the north of Iran, have been specialized for sturgeon farming. One of them is Shahid Rajaee Sturgeon fish propagation center (figure 1, 2, and 3 Appendices 1). This fish farming is mainly used for fingerling rearing in order to restocking inland waters to compensate decreasing of sturgeon populations.

In addition, during the past 30 years, profitable culture of sturgeons has been established for meat and caviar production. Iranian Fisheries Organization (IFO) yields up to 20 million fingerlings annually for discharge into the Caspian Sea(IFO, 2009). The IFO 2016 reports, indicates that 2870000-sturgeon fingerling has been propagated and released to rivers.

Figure 2. Taking sperm (male Strugeon fish sexual product) from male fish for propagation. Shahid Rajaee Strugeon fish propagation center for stock enhancement. Gorgan, Golestan, Iran 1990.

Annual production of caviar fish for the year 2016 has been 650 tons of all 5 commercial sturgeon fish (IFO 2016). The beluga (*H. huso*) is one of the largest freshwater species of the world and the most important Caviar fish that are produced in Iran. Five commercially important sturgeon fish including beluga (*H. huso*) are progressively significant aquaculture fishes in Iran due to the diminishing natural sources for their caviar and meat. These fishes are commercially important and meat are sold both fresh and as frozen fillets. So, the cultures of sturgeon should increase the supply of caviar and decrease pressure on the natural stocks.

Figure 3. live food culture pond, for propagation. Shahid Rajaee Strugeon fish propagation center for stock enhancement. Gorgan, Golestan, Iran 1990.