Biochemistry & Analytical Biochemistry
ISSN: 2161-1009

El-Shafei, Biochem Anal Biochem 2016, 5:2
DOI: 10.4172/2161-1009.1000272

Research Article Open Access

Bioaccumulation of Hexavalent Chromium in Tissues of a Freshwater Fish

Hussien M El-Shafei*
Central Laboratory for Fish Resources Research, Abbassa, Egypt

Abstract

This study aims to investigate the bioaccumulation Tissues. The experiment was carried on for 28 days, meanwhile sampling fish weekly. With continued exposure, the accumulations were increased and fish progressively lost their ability to respond to this increase in exposure period. The control group displayed normal behavior during the test period. The lowest concentrations (10 and 15 mg/L Cr^{6+}) had similar behavior to that of the control group. From the dose 20 mg/ L Cr^{6+}, the fish started to show behavioral disorders. 25 mg/L Cr^{6+} and at the highest concentration (30 mg/L Cr^{6+}) onwards, there was shivering, rather high respiratory disorder and swimming in capsized position in the following order the concentrations of Cr in gills were almost twice, three and four times as high as in the muscle at 20, 25 and 30 ug/L Cr^{6+} mediums, respectively. The Cr accumulation on the skin of Tilapia for all experimental concentrations was nearly twice as much as in the muscle of samples at the end of experiment.

Keywords: Cr (VI); Acute and chronic effects; Aquatic pollutants; Risk assessment

Introduction

The overall toxic impact on organs like gill, kidney and liver may seriously affect the metabolic, physiologic activities and could impair the growth and behavior of fish [1]. Acute poisoning by chromium compounds causes excess mucous secretion, damage in the gill respiratory epithelium and the fish may die with symptoms of suffocation [2]. On chronic exposures, hexavalent chromium severely affected the renal tubules causing hypertrophy of epithelial cells, reduction of tubular lumen [3], Chromium compounds also cause renal failure leading to the loss of osmoregulatory ability and respiration in fish [4]. Sub lethal effects of chromium in fish were directly related to the inhibition of various metabolic processes [5]. The hexavalent chromium induced depletion in the profiles of liver glycogen, total protein and total lipid has been reported [6,7] studied African catfish (*Clarias gariepinus*). Concentrations used varied from 11 to 114 mg/L ($K_2Cr_2O_7$). They found that the main deformity reported was abnormal body axis [8]. After 45 days of exposure significant decline in the protein and carbohydrate content of gills was observed [9]. The objective of our study was to investigate the impact of different chromium (10, 15, 20,25 and 30 ug/L Cr^{6+}) pollutions on the survival of juvenile *Oreochromis aureus* (Tilapia) and to compare the chromium concentrations in fish tissue (muscle, skin and gill).

Material and Methods

Fish

O. aureus (Tilapia), a tropical freshwater and important culture fish, is a species commonly found in brackish water in estuaries all over the world [10-12]. Sixty Juvenile specimens of Tilapia were captured from ponds at Manzalla Lake and transferred to the laboratory where the experiments were conducted during April 2015. The samples were placed in aquarium for one week to allow for adaptation of the fish to the new conditions. Tap water used for the experiment had a pH value of 7.5 ± 0.3 and total hardness of 131 ± 5.3 mg $CaCO_3$ /L. The aquariums were aerated with air stones for proper oxygen saturation (8.5 mg O_2 /L). Fish were hand-fed once a day with artificial feed meal with commercial dry pellets. The feed was comprised of 32% crude protein, 22% crude lipid, 25% ash and 12% moisture. Fish were fed at a feeding rate of 3% of body weight per day for 28 days. Any fish that showed abnormal behavior were removed immediately from the tanks. Acclimatized fish were moved at random into six aquaria volume (50 L), five aquaria for each concentration and one aquarium for control, each containing ten fish.

Chemicals

All reagents were analytical grade. Required is (Merck). A Cr (VI) stock solution was delivered to five test aquariums via automatic pipettes. (Cr^{6+} as 10, 15, 20, 25 and 30 ug/L)

Experimental exposure

Treatment water was monitored every day for dissolved oxygen, pH and Cr concentrations. Exposure period was 28 days, during which samples were taken on day 7, 14, 21 and 28. Experimental units were checked daily for mortality and behavioral changes and any dead fish was immediately removed from the aquariums. For measurement purpose, 3 fishes were taken from each aquarium and their lengths were measured (cm) and weighed (g) before dissection with cleansed tools.

Water and fish tissue analysis

Samples of 100 ml of water from each treatment were filtered through a 0.45 µm micropore membrane filter and stored at -20°C prior to analysis the chromium concentration in water (Cr^{6+} mg/L). Each filtered sample bottle acidified to 1% HNO_3 and analyzed with Inductively Coupled Plasma Emission Spectrometry (ICP-ES). The absorption wavelength and instrument detection limit were 283.553 nm and 0.007 µg/L. For the analyses, the gill, a part of skin and approximately 5 g of each fish stored at -20°C prior to analysis. The digestion of the fish tissues was in accordance with methods described by [13] with concentrated HNO_3/HCl (1:3 V/V). Metal concentrations were determined with ICP-ES. The concentrations were expressed as micrograms per gram weight (µg/ g. wt.) of tissue in organisms.

***Corresponding author:** Hussien M El-Shafei, Central Laboratory for Fish Resources Research, Abbassa, Egypt, Tel: 002-012-10965203; E-mail: hussienelshafei50@yahoo.com

Received: March 08, 2016; **Accepted:** May 05, 2016; **Published** May 09, 2016

Citation: El-Shafei HM (2016) Bioaccumulation of Hexavalent Chromium in Tissues of a Freshwater Fish. Biochem Anal Biochem 5: 272. doi:10.4172/2161-1009.1000272

Copyright: © 2016 El-Shafei HM. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Chromium conc. (Cr^{6+} ug/L) in water	Mean of measured Chromium con. in water (Cr^{6+} ug/L) (SD)	Initial number of Tilapia per aquarium	Percent survival in exposure days (d)			
			7 days	14 days	21 days	28 days
Control (0)	1 (0.1)	10	100[a]	100[a]	96.4[a]	95.8[a]
10	9.4 (0.6)	10	96.4[a]	96.2[a]	96.3[a]	92.3[a]
15	12.8 (0.5)	10	96.4[a]	95.1[a]	85.4[a]	81.6[b]
20	18.7 (0.6)	10	93.6[a]	94.2[a]	88.7[b]	82.3[b]
25	23.4 (0.8)	10	88.5[a]	88.1[a]	81.4[b]	62.4[b]
30	27.8 (1.1)	10	86.3[b]	75.9[b]	72.1[b]	52.7[c]
Different superscript letters (a, b and c) designates difference at p < 0.05 within a sample day.						

Table 1: Mean of measured chromium concentration in water (Cr^{6+} mg/L), standard deviation (SD) and mean percent survival of juvenile Tilapia (*O. aureus*) exposed to Cr^{6+} as $K_2Cr_2O_7$ during 28 days.

Chromium Conc. (Cr^{6+} mg/L) in water	Maximum level Chromium in different Tissues in exposure days (d)											
	7th day			14th day			21th day			28th day		
	G	S	M	G	S	M	G	S	M	G	S	M
Control (0)	0.001	0.002	0.001	0.004	0.003	0.002	0.004	0.003	0.002	0.007	0.006	0.005
10	1.15	0.91	0. 4	3.20	1.38	0.75	4.20	2.25	0.95	3.06	2.72	1.25
15	3.71	1.6	0.9	6.50	2.85	1.45	6.54	3.25	1.75	7.95	4.91	2.43
20	4.77	2.1	1.2	9.35	4.45	2.20	33.45	18.20	8.64	37.41	19.1	9.03
25	9.85	4.2	2.5	15.95	9.10	4.31	37.85	18.75	9.55	40.61	22.5	10.81
30	11.25	6.4	3.29	23.75	13.25	6.45	41.56	22.10	10.31	44.83	25.3	12.25
G = gill, S = skin and M = muscle												

Table 2: Mean of measured chromium concentration in different Tissues of juvenile Tilapia (*O. aureus*) exposed to Cr^{6+} as $K_2Cr_2O_7$ during 28 days.

Statistical evaluation

For the survival tests, Statistical Analysis of data was carried out with SPSS statistical package program. A value of $p < 0.05$ was considered to be significant for the accumulation tests.

Results

The concentrations of Cr measured in the water aquariums throughout the experiment were ± 10% of the nominal concentrations (Table 1). Therefore, nominal concentrations from now on will take this value as the basis. The general agreement of Cr^{6+} with the Cr remained in the Cr^{6+} form throughout the experiment [14]. Tables 1 and 2 summarize the measured Chromium concentration in water (Cr^{6+} ug/L) and the survival of the fish during a 4- week-exposure period for juvenile specimens. Survival of control fish was nearly 100% throughout the experiment. Chromium concentrations (25 and 30 mg/L Cr^{6+}) reduced fish survival beginning from the first week of experiment, where high mortality rate of Cr-exposed fish occurred within 14 to 21 days. Survival rate decreased to 52.7c % in fish exposed to 30 mg/L Cr^{6+} dose from days 21 to 28 (Table 2). The behavioral changes of the control group and Tilapia exposed to various doses of K_2CrO_4 were compared with each other during the experiments. The control group displayed normal behavior during the test period. The lowest concentrations (10 and 15 mg/L Cr^{6+}) had similar behavior to that of the control group. From the dose 20 mg/L $Cr^{6+,}$ the fish started to show behavioral disorders such as loss of equilibrium, sudden startling and respiratory difficulties. 25 mg/L Cr^{6+} and at the highest concentration (30 mg/L Cr^{6+}) onwards, there was shivering, rather high respiratory disorder and swimming in capsized position. The accumulations of chromium (ug/g .wt) in the gill, skin and muscle tissues of Tilapia during the exposure period are shown in Figures 1a-1e. The initial Cr^{6+} concentrations were, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L, 30 mg/L. The chromium muscle at 20, 25 and 30 mg/L Cr^{6+} mediums, respectively. The Cr accumulation on the skin of Tilapia for all experimental concentrations was nearly twice as much as in the muscle of samples at the end of experiment. Concentrations in the tissues of control fish (<0.007ug/L Cr^{6+}) throughout the experiments. The concentrations (μg/ g. wt.) of Cr in the organs fish increased when they were exposed to Cr (Figures 1a-1e). Chromium 1.25 to 12.25 μg/g. wt., while concentrations in the gill were within the range of 3.06 - 44.83 μg/ g. wt. (Figures 1a-1e). As can be seen in Figure 1a, maximum level of Cr^{6+} was 12.25 μg /g.wt. on fish muscle having lived 28 days at 10 ug/L Cr^{6+} initial concentrations. On the other hand, higher concentration of Cr^{6+} (12.25 μg/g. wt.) was observed on fish muscle after 28 days at 30 ug/L Cr^{6+} (Figure 1e). Maximum levels of Cr^{6+} concentrations on the muscle tissues at 15 (after 28 days) in Figure 1b, 20 (after 28 days) in Figure 1c and 25 (after 28 days) ug/L Cr^{6+} treatments in Figure 1d were 2.43, 9.03 and 10.81 μg /g. wt., respectively. The concentrations of Cr in gills were almost twice, three and four times as high as in the muscle at 20, 25 and 30 ug/L Cr^{6+} mediums, respectively. The Cr accumulation on the skin of Tilapia for all experimental concentrations was nearly twice as much as in the muscle of samples at the end of experiment.

Discussion

It is widely known that metal toxicity is more accurately measured in fresh water than in sea water, because metal appear to a great extent as complex compounds in sea water and this reduces the toxicity of metal ions. In the present study, the mortality increased with an increase in Cr^{6+} concentration and also the duration of the exposure to Cr^{6+}. This may be rather significant, because smaller fish being generally more active than larger ones, metal uptake and elimination of metal could also occur in higher rates in these smaller ones [15,16]. A comparison of toxicity values for 10 μg/L Cr^{6+} to 30 μg/L Cr^{6+} experiments demonstrated a decline in survival rate following longer exposure periods. Even though the magnitude of this decline varied between the different chromium concentrations, lower (10 to 20 μg/L Cr^{6+}) mediums were about 10 to 20% mortality while higher (25 and 30 μg/L Cr^{6+}) mediums were 38 to 45% mortality at the end of experiments. Nearly no mortality was recorded in the experiment controls. The Cr concentrations [10 to 30 μg/L (ppb) Cr (VI)] used in the present study has been proved not to be sub lethal for juvenile Tilapia and have been considered as representatives of environmental exposure, fish showed externally abdominal distention and hemorhagic (reddening) and-

Citation: El-Shafei HM (2016) Bioaccumulation of Hexavalent Chromium in Tissues of a Freshwater Fish. Biochem Anal Biochem 5: 272. doi:10.4172/2161-1009.1000272

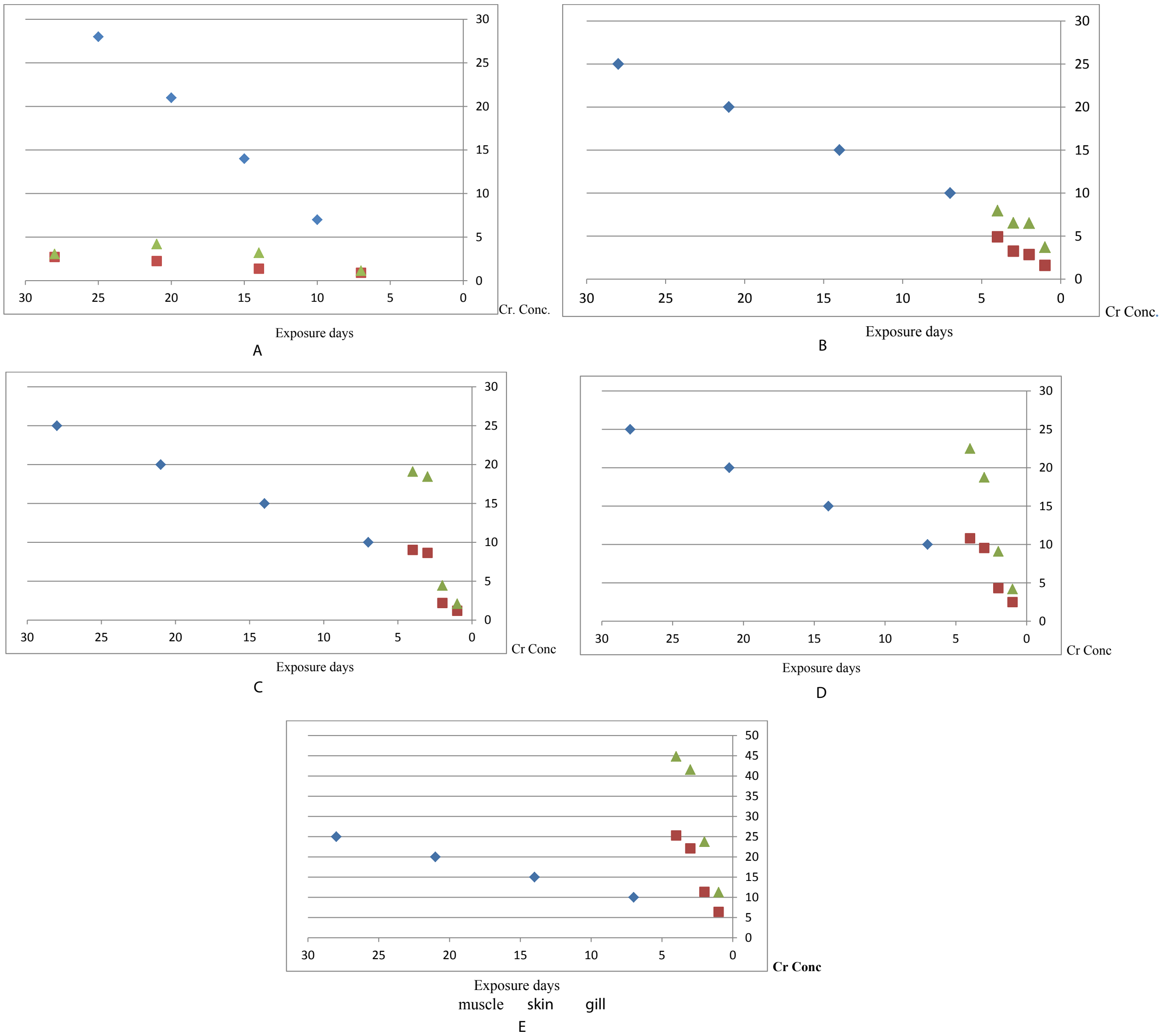

Figure 1(a-e): Time-dependent bio-concentration levels of chromium µg per gram weight (µg/g. wt.) in muscle(♦), skin(■) and gill (▲) tissues of Tilapia. The initial Cr6+ concentrations: 10 mg/L (a), 15 mg/L (b), 20 mg/L (c), 25 mg/L (d) and 30 mg/L (e).

uregenital pours. Erosion and fusion were also detected in lamellar epithelium of gills. Several studies showed the effects on aquatic living things have been evaluated; some data exist on salmon [14].

Other researcher studied heavy metal accumulation in tissues of Tilapia, a freshwater fish [17-20]. It was noted that Cr accumulation in the tissues showed the following sequence: gills > skin > muscle tissues (least). Other study found that the lowest concentration of Cr was detected in the muscle tissue and skin and the gills showed similar levels ($p > 0.05$) [21]; chromium in the spleen, muscle and gills; copper in the kidney; zinc in the gills [9]. Another study indicated that concentrations of heavy metals on wild fish were higher in all of the skin samples than in the muscles [22]. The reason for high metal skin complexion with the mucus that is impossible to remove completely from the tissue before analysis. In general, higher metal concentrations in gills reflect metal concentrations of the water where the fish live. Some studies showed that the gills which are very soft part of the body involved in the respiratory process start damaging expose at lowest concentration of Cr (25 mg/L) [23].

The damage was noted increasing at the exposure of 30 mg/L onwards. In the lowest concentration through the effect was basement membrane and sub mucosa. The gill filaments and its constituents were damaged gradually which reached to the total collapsed level at the highest concentration (40 mg/L). At the higher concentration, the gill rakers start hypertrophy and that was follow by the hypertrophy in gill filaments by their hypertrophy. All these changes caused the fish to avoid taking food and intake of oxygen. These patterns of changes have also been reported by many workers [24,25]. It was also observed that the heavy metals in muscle tissue were at low levels compared with other organs [26]. The results also show that chromium is more accumulated in the samples of gill than in the skin or muscles [23].

References

1. Vinodhini R, Narayanan M (2008) Bioaccumulations of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). Int J Environ Sci Tech 5:179-182.

2. Benoit DA (1976) Toxic effects of hexavalent chromium on brook trout *(Salvelinus fontinalis)* and rainbow trout (*Salmo gairdneri)*. Water Res 10: 497-500.

3. Mishra AK, Mohanty B (2009) Chronic exposure to sublethal hexavalent chromium affects organ histopathology and serum cortisol profile of a teleost, *Channa punctatus* (Bloch) Sci. Total Environ 407: 5031-5038.

4. Arillo A, Melodio F (1988) Effects of hexavalent chromium on trout mitochondria. Toxicol Lett 44: 71-76.

5. Nath K, Kumar N (1987) Effect of hexavalent chromium on the carbohydrate metabolism of a freshwater tropical teleost *Colisa fasciatus* Bull. Inst Zool Acad Sin (Taipei) 26: 245-248.

6. Saxena D, Tripathi M (2007) Hexavalent chromium induces biochemical alterations in air-breathing fish, *Channa punctatus*. J Ecophysiol Occup Health 7: 171-175.

7. Nguyen LTH, Janssen CR (2002) Embryo-larval toxicity tests with the African Catfish *(Clarias gariepinus)*: Comparative sensitivity of endpoints. Arch Environ Contam Toxicol 42: 256-262.

8. Virk S, Sharma A (2003) Changes in the biochemical constituents of gills of *Cirrhinus mrigala* (Ham.) following exposure to metals. Indian J Fish 50: 113-117.

9. Begum G, Venkateswara RJ, Srikanth K (2006) Oxidative stress and changes in locomotor behavior and gill morphology of *Gambusia affinis* exposed to chromium. Toxicol. Environ. Chem 88: 355-365

10. Vijayan MM, Morgan JD, Sakamota T, Grau EG, Iwama GK (1996) Food deprivation affects seawater acclimation in tilapia: hormonal and metabolic changes. J Exp Biol 199: 2467-2475.

11. Almeida JA, Diniz YS, Marques SFG, Faine LA, Ribas BO, et al. (2002) The use of oxidative stress responses as biomarkers in Nile Tilapia *(Oreochromis niloticus*) exposed to in vivo cadmium contamination. Environ Int.27: 673-679.

12. Turan F (2006) Improvement of growth performance in Tilapia *(Oreochromis aureus*, Linnaeus) by supplementation of red clover *(Trifolium pratense)* in diets. Bamidgeh, Isr. J Aquac 58: 34-38.

13. Yılmaz AB (2003) Levels of heavy metals (Fe, Cu, Ni, Cr, Pb and Zn) in tissue of *Mugil cephalus* and *Trachurus mediterraneus* from Iskenderun Bay, Turkey. Environ Res 92: 277-281.

14. Farag AM, Mayb T, Marty GD, Easton M, Harper DD, et al. (2006) The effect of chronic chromium exposure on the health of Chinook salmon *(Oncorhynchus tshawytscha)*. Aquat Toxicol 76: 246-257.

15. Canli M, Furness RW (1993) Toxicity of heavy metals dissolved in sea water and influences of sex and size on metal accumulation and tissues distribution in the Norway lobster *Nephrops norvegicus*. Mar Environ Res 36: 217-236.

16. Buhl KJ, Hamilton SJ (1991) Relative sensitivity of early life stages of arctic grayling, coho salmon, and rainbow trout to nine inorganics. Ecotoxicol Environ Saf 22: 184-197.

17. Ay Ö, Kalay L, Tamer M, Canli M (1999) Copper and lead accumulation in tissues of a freshwater fish Tilapia zilli and its effects on the branchial Na, K-ATPase activity. Bull. Environ Contam Toxicol 62:160-168.

18. Wepener W, Vuren van JHJ, Preez du HH (2001) Uptake and distribution of a copper, iron and zinc mixture in gill, live rand plasma of a freshwater teleost, *Tilapia sparrmanii.* Water SA 27: 99-108.

19. Aleya B, Md. Nurul A, Satoshi K, Kiyohisa O (2005) Selected elemental composition of the muscle tissue of three species of fish, *Tilapia nilotica, Cirrhina mrigala* and *Clarius batrachus,* from the fresh water Dhanmondi Lake in Bangladesh. Food Chem 93: 439-443.

20. Zirong X, Shijun B (2007) Effects of waterborne Cd exposure on glutathione metabolism in Nile tilapia *(Oreochromis niloticus)* liver.Ecotoxicol. Environ Saf 67: 89-94.

21. Storelli MM, Barone G, Storelli A, Marcotrigiano GO (2006). Trace metals in tissues of mugilids *(Mugil auratus, Mugil capito, and Mugil labrosus)* from the Mediterranean Sea. Bull Environ Contam Toxicol 77: 43-50.

22. Camusso M, Vigano L, Balestrini R (1995) Bioconcentration of trace metals in rainbow trout: A field study. Ecotoxicol Environ Saf 31: 133-141.

23. Solang FN, Shaimkh SA, Narejo NT (2012) Toxic effect of chromium on gills of cyprinid fish, *Cyprinus Carpio*. Res Jour Sci Ser 44: 445-448.

24. Ahmad I, Maria VL, Oliveira M, Pacheco M, Santos MA (2006) Oxidative stress and genotoxic effects in gill and kidney of Anguilla anguilla L exposed to chromium with or without pre-exposure to β- naphthoflavone. Mut Res 608: 16-28.

25. Ashish KM, Banalata M (2008) Acute toxicity impact of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the fresh water fish Channa puntatus. Environ Toxicol Pharmacol 26: 136-141.

26. Karadede-Akin H, Ünlü E (2007) Heavy metal concentrations in water, sediment, fish and some benthic organisms from Tigris River, Turkey. Environ Monit Assess 131: 323-337.