International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 3, p. 251-257, 2019

INNSPUB

RESEARCH PAPER OPEN ACCESS

Determination of 96-hr LC_{50} and lethal responses of *Wallago attu* to water-borne chromium

Sumra Naz*, Muhammad Javed

Department of Zoology, Wildlife and Fisheries University, of Agriculture, Faisalabad, Pakistan

Key words: Acute toxicity, Wallago attu, Heavy metal, chromium.

http://dx.doi.org/10.12692/ijb/14.3.251-257 Article published on March 27, 2019

Abstract

(Importance)Water pollution by heavy metals, especially chromium pollution from industrial source can affect aquatic life, all ecosystems and human health directly or indirectly through food chain. The present research work was conducted under laboratory conditions to determine 96-hr LC_{50} and lethal responses of a fish, *Wallago attu*, to water-borne chromium. (Brief Method) The fingerlings of fish were kept in glass aquaria having 35-L water capacity. The toxicity tests were conducted at constant total hardness (250mgL^{-1}), water temperature (28 °C) and pH (8) with three replications for each test concentration, separately. Physico-chemical parameters of water viz. total hardness, temperature, pH, carbon dioxide, dissolved oxygen, total ammonia, sodium and potassium were monitored twice a day during each 96-hr trial. The 96 hour LC_{50} and lethal concentrations of chromium, with 95% confidence interval, were computed by using the Probit analysis method. Regression/correlation analyses were also performed to find-out relationships among different variables under study. (Results/key findings) This investigation revealed significant differences between 96-hr LC_{50} and lethal concentrations of chromium for *Wallago attu*. The mean 96-hr LC_{50} and lethal concentrations of chromium for this fish were computed as 59.17±1.45 and 90.65±1.09mgL-1, respectively. (Conclusions)The present investigation also revealed that metallic ion concentrations had significant impacts on the physico-chemical parameters of the test media as total ammonia and carbon dioxide contents showed significant increase with concomitant increase in chromium concentration while dissolved oxygen showed significant decrease. The dissolved oxygen revealed negatively significant relationship with total ammonia. Calcium exhibited statistically highly significant but negative relation with magnesium contents of the test media.

* **Corresponding Author:** Sumra Naz ✉ Sumr002@yahoo.com/03346611046

Introduction

Aquatic pollution has its own significance regarding human life, as water is the main part of living organisms and has great concern with daily life events such as drinking, cleaning and agriculture (Singh *et al.*, 2010). Sewage disposal, industrial wastes, rainfall and soil leaching are considered as important factors that can disturb the stability of natural aquatic ecosystems (Adefemi *et al.*, 2008). The aquatic pollution due to metallic ions is a severe worldwide issue now-a-days (Khare and Singh, 2002). Due to increased use of metal based fertilizers in agricultural revolution, there occurs a continuous increase in metallic ions in the freshwater (Adefemi *et al.*, 2008; Prasath and Arivoli, 2008).

Unlike other contaminants, metals are non-biodegradable and can only be transformed from highly toxic to less toxic forms (Shaukat, 2013). Among the metallic toxins, heavy metals are predominantly severe in action due to their tendency of bio-magnify in the food chain. Toxic impacts of heavy metals are an important risk to the organisms living in the aquatic ecosystems (Selvam *et al.*, 2014).

The harmful impacts of heavy metals on the aquatic environment range from mortality of individuals to complete loss of the biota (Malik *et al.*, 2010; Dhanalakshmi, 2013). The heavy metals may also cause alterations in the structural and physiological aspects of the aquatic inhabitants (Wepener *et al.*, 2001; Singh *et al.*, 2010).

Chromium is extensively distributed in both aquatic and terrestrial environments, occurring mainly in two valences states i.e., hexavalent chromium (Cr^{6+}) and trivalent chromium (Cr^{3+}). Its compounds are used as micronutrients and food supplements. Chromium is discharged into aquatic ecosystems mainly by electroplating, textile and tannery productions (Bagchi *et al.*, 2002; Steinhagen *et al.*, 2004). It is an essential micronutrient necessary to stimulate the action of insulin in tissues so that the body can use fats, proteins and sugars (Piotrowska *et al.*, 2008). However, it is toxic at high concentrations and for long exposure durations (Valko *et al.*, 2005; Lushchak, 2008). The acute exposure of chromium significantly affects the histology of tissues and ultimately results into mortality of fish (Farag *et al.*, 2006).

Wallago attu is one of the large inland catfishes present in southern Punjab. It is commonly distributed in the reservoirs, rivers and in connected tributaries of Pakistan, Bangladesh, India, Sri Lanka, Nepal, Vietnam, Thailand, Burma, Cambodia and Indonesia (Mirza, 2003). Due to over-exploitation, demolition of brood fishes from freshwater territories, environmental degradation, contamination and absence of appropriate management practices, the population of *Wallago attu* is declining day-by-day (Mijkherjee *et al.*, 2002; Patra *et al.*, 2005). In freshwater ecosystems, *Wallago attu* is considered as a chemoreceptor and plays an important role in detecting the occurrence of odoriferous matters in aquatic environments, therefore, it would naturally be meaningful to determine the acute toxic effects of heavy metals for this fish (Chakrabarti and Ghosh, 2008).

Keeping in view the importance of the survival of aquatic animals especially fish, the current research was conducted to study the adverse effects of heavy metals to the water bodies.

Materials and Methods

This research work was conducted to determine the acute toxicity of chromium in terms of 96-hr LC_{50} and lethal concentrations for *Wallago attu* under controlled laboratory conditions.

The fingerlings of *Wallago attu* were obtained from their natural breeding grounds having an average weight and total length of 11.50±0.71g and 155.00±4.20mm, respectively. They were kept in cemented tanks for two weeks prior to the start of experiment for acclimatization and fed with pelleted diet (2.90 $Kcalg^{-1}$ digestible energy and 35% digestible protein). However, fish was not fed during the last

24-hr of acclimatization and throughout the acute toxicity experiments. Before start of these experiments, all glass aquaria were washed thoroughly with tap water and stocked with fish for each test concentration of metal. Three replications were used for each test concentration for the determination of 96-hr LC_{50} and lethal concentration. Stock solution of chromium was prepared on molar basis by dissolving the pure chloride compound of chromium in deionized water.

In order to minimize stress to the fish, the concentration of the metal in each aquarium was increased gradually as 50% test concentration was maintained within 3 hours and full toxicant concentration in 6 hours. The physico-chemical parameters of test media (water) *viz*. total hardness, temperature, pH, carbon dioxide, dissolved oxygen, total ammonia, calcium, magnesium, sodium and potassium were determined at 12-hr intervals (A.P.H.A. 1998). All static bioassay tests for *Wallago attu* were performed at constant total hardness (250 mgL^{-1}), water temperature (28 °C) and pH (8). During whole experiment, fresh air was supplied to each test media with an air pump. Each test was performed under 12-hr light and dark illumination cycle and fish mortality was recorded on hourly basis. The dead fish obtained during 96-hr acute toxicity tests were removed from the media and their mortality data was compiled for analyses.

Results and discussion

Acute toxicity of chromium

Table 1 shows the mortality of *Wallago attu* during 96-hr exposure of different chromium concentrations for three replications.

(Agreed)The 96-hr LC_{50} and lethal concentration values of chromium for the fish were obtained by performing *Probit analysis* on fish mortality data. Normal distribution of data was estimated at 95% confidence interval revealing LC_{50} value of 60.56±0.27mgL^{-1} with confidence interval range of 55.12-65.00mgL^{-1}.

However, the lethal concentration of chromium was estimated as 89.40±0.17mgL^{-1} having confidence interval range of 82.24-102.26mgL^{-1}.

Table 1. Calculated 96-hr LC_{50} and lethal concentrations of chromium for the fish.

Fish species		Mean 96-hr LC_{50} (mgL^{-1})	95% confidence interval (mgL^{-1})	Mean lethal concentration (mgL^{-1})	95% confidence interval (mgL^{-1})
Labeo rohita	Replication I	61.43±2.72 a	55.28 - 66.57	98.09±5.69 a	89.27 - 113.74
	Replication II	58.54±2.92 a	51.94 - 64.02	99.54±6.18 a	89.94 - 116.41
	Replication III	56.37±2.88 a	49.60 - 61.58	94.39±5.40 a	85.95 - 109.04
	Mean±SD	58.78±2.54 b		97.34±2.66 a	

Means with similar letters in a single column and row are statistically non-significant at $p<0.05$.

This model shows the *Deviance Chi-Square* value of 0.98 and goodness of fit test, "p" value of 1.00 revealing high precision of this regression model. From the *Probit* curve computed for second replication, the 96-hr LC_{50} value of chromium was estimated as 57.67±0.11mgL^{-1} (55.22-62.59mgL^{-1} confidence interval). The lethal concentration of chromium for *W. attu* was determined as 91.28±0.29mgL^{-1} with confidence interval range of 83.02-106.80mgL^{-1}. For third replication, the calculated 96-hr LC_{50} value of chromium (59.28±0.19mgL^{-1}) was obtained with 95% confidence interval range of 53.63-64.02mgL^{-1}. However, the lethal concentration for the fish species was determined as 91.28±0.31mgL^{-1} with confidence interval range of 83.37-105.40mgL^{-1}.Analysis of variance revealed non-significant variations among the three replicates for both 96-hr LC_{50} and lethal concentration of chromium for the fish. (Contrast)However, significant differences were found between LC_{50} and lethal concentrations of chromium for *Wallago attu*. Murti (2005) were performed the trials for the short-term toxicity of potassium dichromate to *Channa punctatus*.

Table 2. Means (±SD) for physico-chemical characteristics of the test media recorded during acute toxicity trials for *Wallgao attu*.

Concentrations (mgL^{-1})	Total hardness (mgL^{-1})	Temperature (°C)	pH	Carbon dioxide (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Total ammonia (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Sodium (mgL^{-1})	Potassium (mgL^{-1})
5.00	250.01±0.00	28.00±0.00	8.00±0.00	0.49±0.03	5.81±0.52	0.28±0.23	20.56±4.67	49.65±13.45	210.45±9.39	11.21±4.51
10.00	250.00±0.00	28.00±0.00	8.01±0.01	0.33±0.01	5.76±0.49	0.31±0.27	22.78±5.93	48.26±10.99	201.32±10.01	10.99±4.48
15.00	250.00±0.00	28.01±0.01	8.00±0.00	0.52±0.04	5.85±0.57	0.37±0.31	21.89±5.31	48.82±12.01	232.71±11.63	12.64±4.78
20.00	250.02±0.01	28.00±0.00	8.00±0.00	0.57±0.05	5.73±0.38	0.49±0.43	22.91±6.01	48.19±10.67	221.41±11.23	13.11±4.85
25.00	250.00±0.00	28.02±0.01	8.00±0.00	0.61±0.06	5.71±0.34	0.55±0.46	23.42±6.88	47.86±9.56	301.13±11.87	11.76±4.59
30.00	250.01±0.00	28.01±0.01	8.02±0.01	0.55±0.04	5.69±0.31	0.58±0.49	23.92±7.93	47.55±9.09	247.13±12.11	13.83±5.02
35.00	250.00±0.00	28.00±0.00	8.00±0.00	0.64±0.06	5.74±0.43	0.45±0.37	22.09±5.42	48.70±11.31	261.98±13.32	14.09±5.11
40.00	250.00±0.00	28.00±0.00	8.00±0.00	0.69±0.07	5.65±0.25	0.63±0.55	24.38±8.87	47.26±8.65	309.23±14.88	12.53±4.68
45.00	250.00±0.00	28.00±0.00	8.01±0.00	0.56±0.05	5.55±0.17	0.71±0.63	24.79±9.09	47.01±8.41	267.28±15.09	15.76±5.67
50.00	250.01±0.00	28.01±0.01	8.00±0.00	0.77±0.08	5.49±0.15	0.77±0.67	23.61±7.32	47.75±9.33	273.56±15.71	14.49±5.19
55.00	250.00±0.00	28.00±0.00	8.00±0.00	0.79±0.09	5.41±0.11	0.66±0.59	25.55±9.91	46.53±8.55	323.89±16.47	13.23±4.87
60.00	250.03±0.01	28.01±0.00	8.00±0.00	0.81±0.10	5.38±0.07	0.81±0.71	24.08±8.11	47.46±8.91	295.39±17.42	16.19±5.87
65.00	250.00±0.00	28.00±0.00	8.02±0.01	0.83±0.12	5.35±0.07	0.88±0.76	23.11±6.54	48.06±10.11	239.53±18.78	14.78±5.21
70.00	250.00±0.00	28.00±0.00	8.00±0.00	0.86±0.13	4.96±0.02	0.92±0.82	24.87±9.45	46.96±8.23	315.31±19.87	15.32±5.32
75.00	250.01±0.00	28.03±0.01	8.00±0.00	0.89±0.14	5.23±0.05	1.03±0.87	26.31±10.23	46.06±7.33	256.89±19.11	13.81±4.99
80.00	250.00±0.00	28.00±0.00	8.01±0.01	0.95±0.16	5.19±0.05	1.21±0.91	25.83±10.01	46.36±7.89	320.29±20.61	12.91±4.81
85.00	250.00±0.00	28.00±0.00	8.00±0.00	0.98±0.17	5.08±0.04	1.37±0.97	26.95±10.67	45.65±7.11	289.73±21.23	14.88±5.29
Means±SD	250.01±0.01	28.01±0.01	8.00±0.01	0.70±0.18	5.50±0.27	0.71±0.31	23.94±1.68	47.54±1.05	268.66±39.31	13.62±1.53

The LC_{50} (median lethal concentration) value computed for potassium dichromate, after 96 hour exposure period, was 1.84mgL^{-1}. He concluded that the mortality rate of fish was both concentration and time dependent. Sanjay et al. (2006) exposed the *Channa marulius* (air breathing teleost fish) to chromium for 96 hours. They concluded that with an increase in concentration of metal and exposure time, the mortality rate was also increased. The 96 hours acute exposure of chromium revealed that LC_{50} (50% lethal concentration) for *Channa marulius* was 121.79mgL^{-1}. Domingues *et al.* (2010) also observed a significant increase in fish mortality with the increase in chromium concentrations. Their results showed that the acute toxicity value of chromium in terms of 96 hour LC_{50} for zebra fish was 39.4mgL^{-1}. Dural *et al.* (2002) investigated the lethal concentration of chromium for the African catfish (*Clarias gariepinus*). Their results revealed that the 96-hr LC_{50} value for *C. gariepinus* was computed as 288mgL^{-1}. Joshi and Patil (2002) evaluated the impact of acute toxicity of chromium to *Clarias batrachus*. The median lethal concentration of chromium for the fish, *C. batrachus*, was found as 105mgL^{-1}. Singh and Singh (2002) exposed the fish (*Channa punctatus*) over 96 hours to 40 and 80% of chromium concentrations. After acute exposure of chromium, the LC_{50} value for *C. chanos* was calculated as 25.6mgL^{-1} with 95% confidence limit of 22.4-29.2mgL^{-1}.

Physico-chemistry of chromium test media

The physico-chemical parameters of the test media influenced the toxicity mechanisms in the fish. During present experiment, it was observed that the level of carbon dioxide and total ammonia increased gradually while the dissolved oxygen decreased as a result of increasing chromium concentration in the test media. These results indicated that increased concentration of chromium lead to a significant stress on the fish and ultimately carbon dioxide, ammonia excretion increased along with enhanced consumption of dissolved oxygen.

Total hardness, water temperature, pH, carbon dioxide, dissolved oxygen, total ammonia, calcium, magnesium, sodium and potassium of the test media were analyzed daily on 12 hour basis. During acute toxicity trials of chromium, mean total hardness (250mgL^{-1}), water temperature (28 ^{o}C) and pH (8) were kept constant. The data regarding physico-chemical parameters of the test medium used for acute toxicity of chromium to *Wallago attu* are presented in the Table 2.

Abedi *et al.* (2012) examined the reduced oxygen consumption and increased ammonia exceretion by *Pangasius hypophthalmus* (scale less freshwater fish) during 96-hr acute exposure of chromium. It was observed that this fish was sensitive to physico-chemical parameters like hardness, temperature, pH and dissolved oxygen of the test media. Kawade and Khillare (2012) concluded an inverse relationship between the metallic ion concentration of chromium and pH of the test media during the acute exposure of chromium to *Channa gachua*.

Carbon dioxide

An increase in the metallic ions concentration in the test media caused a gradual rise in the carbon dioxide concentration. The mean minimum and maximum carbon dioxide concentrations in the test media of *Wallago attu* were estimated as 0.33±0.15 and 0.98±0.05 at 10.00 and 85.00mgL^{-1} chromium concentrations, respectively.

Dissolved oxygen

The dissolved oxygen contents showed significant decrease with an increase in chromium concentration of the test media revealing an inverse relationship between dissolved oxygen and metal concentrations during this investigation. The minimum and maximum dissolved oxygen concentrations of the test media, used for chromium toxicity trials, varied between the mean values of 5.08±0.11 and 5.85±0.07mgL^{-1} at 85.00 and 15.00mgL^{-1} chromium concentrations, respectively. . Guedenon *et al.* (2012) reported that chromium toxicity may decrease the oxygen consumption by *Clarias gariepinus*.

Total ammonia

The total ammonia contents of the test media during 96-hr LC_{50} and lethal concentration trials for *Wallago attu* were ranged from 0.28±0.07 to 1.37±0.05mgL^{-1} at 5.00 and 85.00mgL^{-1} chromium concentrations, respectively.

Calcium

The maximum mean value of calcium was observed as 26.95±0.08mgL^{-1}, while it was minimum as 20.56±0.55mgL^{-1} chromium concentration.

Magnesium

During the 96-hr acute toxicity trials, the magnesium contents of the test media were ranged from 45.65±0.05 to 49.65±0.34mgL^{-1} at 85.00 and 5.00mgL^{-1} concentrations of chromium.

Sodium

The sodium contents of test media ranged from 201.32±5.97 to 323.89±2.21mgL^{-1} at 10.00 and 55.00mgL^{-1} concentrations, respectively.

Potassium

The maximum and minimum potassium contents of the test media were determined as 16.19±0.31 and 10.99±0.68mgL^{-1} at 60 and 10mgL^{-1} concentrations of chromium.

Conclusion

There existed significant differences between 96-hr LC_{50} and lethal concentrations of chromium for *Wallago attu*. The mean 96-hr LC_{50} and lethal concentrations of chromium for the fish were computed as 59.17±1.45 and 90.65±1.09mgL^{-1}, respectively. The oxygen consumption by the fish increased significantly with the increase in chromium concentration in the test media while carbon dioxide and ammonia concentrations increased concomitantly. The chromium concentration exhibited statistically highly significant positive relation with calcium, sodium and potassium while it showed highly significant but inverse correlation with magnesium contents of the test media. Carbon dioxide showed significantly direct relationship with total ammonia while it exhibited highly significant but inverse correlation with dissolved oxygen. The dissolved oxygen revealed negatively significant relationship with total ammonia. Calcium exhibited statistically highly significant but negative correlation with magnesium contents of the test media.

References

Abedi Z, Khalesi KM, Eskandari KS, Rahmani H. 2012. Comparison of lethal concentrations (LC_{50} 96-hr) of $CdCl_2$, $CrCl_3$ and $Pb(NO_3)_2$ in Common Carp (*Cyprinus carpio*) and Sutchi catfish (*Pangasius hypophthalmus*). Iranian Journal of Toxicology **6,** 672- 680.

Adefemi SO, Asaolu SS, Olaofe O. 2008. Determination of heavy metals in *Tilapia mossambicuis* fish, associated water and sediment from Ureje Dam in South-Western Nigeria. Research Journal of Environmental Sciences **2,** 151- 155.
http://dx.doi.org/10.3923/rjes.2008.151.155

American Public Health Association (A.P.H.A). 1998. Standard methods for the examination of water and wastewater. 20th Ed. American Public Health Association, New York, p. 1193.

Bagchi D, Stohs SJ, Downs BW, Bagchi M, Preuss HG. 2002. Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology **180,** 5- 22.
http://dx.doi.org/10.1016/S0300-483X(02)00378-5

Chakrabarti P, Ghosh SK. 2008. Ultrastructural organisation and functional aspects of the olfactory epithelium of *Wallago attu* (Bleeker). Folia Morphologica **68,** 40- 44.

Dhanalakshmi B. 2013. Acute and chronic toxicity of chromium on biochemical composition of the fresh water major carp *Cirrhinus mrigala* (hamilton). Asian Journal of Science and Technologies **4,** 21- 26.

Domingues I, Oliveira R, Lourenco J, Grisolia KC, Mendo S, Soares MVMA. 2010. Biomarkers as a tool to assess effects of chromium (VI): Comparison of responses in Zebrafish early life stages and adults. Comparative Biochemistry and Physiology **152,** 338- 345.
http://dx.doi.org/10.1016/j.cbpc.2010.05.010

Farag AM, May T, Marty DG, Easton M, Harper DD, Little EE, Cleveland L. 2006. The effect of chronic Cr exposure on the health of Chinook salmon (*Onchorhynchus tshawytscha*). Aquatic Toxicology **76,** 246- 257.
http://dx.doi.org/10.1016/j.aquatox.2005.09.011

Guedenon P, Edorh PA, Hounkpatin YSA, Alimba GC, Ogunkanmi A, Nwokejiegbe GE, Boko M. 2012. Acute Toxicity of chromium ($CrCl_3$) to African Catfish, *Clarias gariepinus*. Research Journalof Chemical Sciences **2,** 41- 45.

Joshi SN, Patil SH. 2002. Effect of water temperature, pH and hardness on the toxicity of chromium trioxide to fish *Clarias batrachus*. Proc. Indian natnional Science Academy **6,** 347- 350.

Kawade SJ, Khillare KY. 2012. Toxicity of chromium on the biochemical contents of certain tissues of freshwater fish, *Channa gachua* (ham.) International Journal of Applied Biology and Pharmaceutical technology **3,** 242- 251.

Khare S, Singh S. 2002. Histopathological lessons induced by copper sulphate and lead nitrate in the gills of fresh water fish *Nandus*. Journal of Ecotoxicology and Environmental Monitoring **12,** 105- 111.

Lushchak OV, Kubrak IO, Nykorak ZM, Storey BK, Lushchak IV. 2008. The effect of potassium dichromate on free radical processes in goldfish:

Possible protective role of glutathione. Aquatic Toxicology **87,** 108- 114. http://dx.doi.org/10.1016/j.aquatox.2008.01.007.

Malik N, Biswas AK, Qureshi TA, Borana K, Virha R. 2010. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. Environmental Monitoring and Assessment **160,** 267- 276. http://dx.doi.org/10.1007/s10661-008-0693-8.

Mijkherjee M, Praharaj A, Das S. 2002. Conservation of endangered fish stocks through artificial propagation and larval rearing technique in West Bengal, India. Aquaculture Asia **7,** 8- 11.

Mirza MR. 2003. Check list of freshwater fishes of Pakistan. Pakistan Journal of Zoology Supplement series **3,** 1- 30.

Murti R, Omkar, Shukla GS. 2005. Chromium toxicity to a freshwater teleost *Channa punctatus*. Toxicology Letters **18,** 257- 261.

Patra MK, Acharjee SK, Chakraborty SK. 2005. Conservation categories of siluroid fishes in North-East Sundarbans, India. Biodiversity and Conservation **14,** 1863- 1876.

Piotrowska A, Mlyni K, Siwek A, Dybala M, Opoka W, Poleszak E, Nowak G. 2008. Antidepressant like effect of chromium chloride in the mouse forced swim test: involvement of glutamatergic and serotonergic receptors. Pharmacological Reports **60,** 991- 995.

Prasath PMD, Arivoli S. 2008. Biochemical study of freshwater fish *Catla catla* with reference to mercury chloride. Iranian Journal of Environmental Health Science and Engineering **5,** 109- 116.

Sanjay P, Ravindra K, Shilpi S, Nagpure SN, Satish K, Srivastava S, Verma SM. 2006. Acute toxicity of Cr exposed *Channa marulius*. Indian Journal of Environmental sciences **38,** 118- 121.

Selvam A, Anandhan R, Kavitha V. 2014. Acute toxicity of trivalent chromium to freshwater fish *Labeo rohita* (ham). International Journal of Research in Fisheries and Aquaculture **4,** 104- 107.

Shaukat T, Javed M. 2013. Acute toxicity of chromium for *Ctenopharyngodon idella*, *Cyprinus carpio* and *Tilapia nilotica*. International Journal of Agriculture and Biology **15,** 590- 594. http://dx.doi.org/12–589/2013/15–3–590–594

Singh AP, Singh S, Bhartiya P, Yadav K. 2010. Toxic effect of hexavalent chromium on the serum biochemical parameters of snake headed fish *Channa punctatus* (Bloch). Advanced Biomedical research **1,** 177- 181.

Singh D, Singh A. 2002. Biochemical alteration in freshwater fish *Channa punctatus*. Environmental Toxicology andPharmacology **12,** 129- 136. http://dx.doi.org/10.1016/S1382-6689(02)00016-9

Steinhagen D, Helmas D, Maurers S. 2004. Effect of hexavalent carcinogenic chromium on carp, *Cyprinus carpio* immune cells. Diseases of Aquatic Organisms **23,** 155- 161.

Valko M, Morris H, Cronin TM. 2005. Metals toxicity and oxidative stress. Current Medicinal Chemistry **12,** 1161- 1208. http://dx.doi.org/0929-8673/05$50.00+.00

Wepener V, Vurer VJHJ, Depreez HH. 2001. Uptake and distribution of a copper, iron and zinc mixture in gill, liver and plasma of a freshwater teleost, Tilapia sparrmani. Water **27,** 99- 108.