

## Cadmium Bioaccumulation and Toxicity in Tilapia Fish (*Oreochromis niloticus*)

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**Abstract:** The present study was undertaken to determine the bioaccumulation of cadmium in livers of tilapia fish (*Oreochromis niloticus*) and to assess the histological alteration of intestine, liver and kidney tissues due to cadmium toxicity. Fish (*Oreochromis niloticus*) were exposed to cadmium at different concentrations (5 and 10 mg L<sup>-1</sup>) for 7 days. Cadmium assayed by using AA220FS atomic absorption spectrophotometry. The concentration of cadmium in the liver tissues in the fish exposed to 10 mg L<sup>-1</sup> was found to be (9.09±0.51 µg g<sup>-1</sup> dry wt.) while it was 5.17±0.25 µg g<sup>-1</sup> dry wt. in the fish exposed to 5 mg L<sup>-1</sup>. There was a significant increase of cadmium chloride concentration in the liver tissues of the fish exposed to 10 mg L<sup>-1</sup> compared to the those exposed to 5 mg L<sup>-1</sup>. Histological alterations on liver tissues were in the form severe fatty vacuolations, generalised necrosis of hepatocytes, fatty change, congestion of liver sinusoids and central veins. Intestines showed severe congestion of submucosal blood vessels and sloughing of mucosal epithelium. Kidneys showed severe glomerular shrinkage and necrosis, lymphocytic infiltration in the distal renal convoluted tubules. Histopathological changes were more pronounced in fish exposed to 10 mg L<sup>-1</sup> cadmium chloride.

**Key words:** Cadmium, toxicity, *Oreochromis niloticus*, bioaccumulation, lymphocytic infiltration, necrosis

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### INTRODUCTION

Environmental problems have increased exponentially in recent decades mainly because of rapid growth in human population and increased demand for several household materials. Water is essential constituent of life support system and its quality play pivotal role in the maintenance of health. It is used for many purposes, especially for aquaculture industry, irrigation and domestic needs (Swarup *et al.*, 2006). Due to increased human activities and interests, cadmium has become one of the most hazardous heavy metals in aquatic environments and could threaten aquatic organisms including tilapia fish. Cadmium is a naturally occurring toxic heavy metal with no known biological function in humans and animals and therefore considered nonessential (USFDA, 1993; Pinot *et al.*, 2000). Cadmium is an extremely toxic metal commonly found in industrial workplaces. Pure cadmium is a soft, silver-white metal that occurs naturally in the earth's crust. It is usually found as a mineral combined with other elements such as oxygen, chlorine or sulfur. The 10% of total cadmium in the environment is derived from natural sources whereas

remaining 90% is derived from anthropogenic activity (Okada *et al.*, 1997). Cadmium is used in electroplating and galvanizing due to its non-corrosive and cumulative nature. It is used as color pigment for paints, plastics and as a cathode material for nickel-cadmium Batteries (Ahmad *et al.*, 2002; Khan *et al.*, 2003; Shukry, 2001; Midrar-ul-Haq *et al.*, 2005; Kalaiselvi *et al.*, 2010).

Industrial effluents including toxic metal compounds are major source of water pollution besides sewage, agricultural discharges and other household residues (Saxena and Garg, 2010).

A variety of contaminants including toxic heavy metals (cadmium, copper, mercury and zinc) are reported to be ubiquitously present in rivers, reservoirs and are disadvantageous for aquatic organisms (Olsson, 1998). Heavy metal contamination may have detrimental effects on the ecological balance of the aquatic environment (Vosyliene and Jankaite, 2006; Farombi *et al.*, 2007). They are generally not biodegraded and fishes are the main inhabitants that are directly suffered the resulting devastating effect and this has been reported in various localities around the world (Clarkson, 1998; Dickman and Leung, 1998; Olaifa *et al.*, 2004; Authman and Abbas, 2007).

Fish is widely consumed in many parts of the world by humans because it has high protein content, low saturated fat and also contains omega-3, calcium, phosphorus, iron, trace elements like copper and a fair proportion of the B vitamins known to support good health (Tucker, 1997). Beside good health benefits of fish, there have been many reports on contamination of fish by chemicals in the environment (Tuzen and Soylak, 2007). Heavy metals are considered the most important constituents of pollution from the aquatic environment and the sea because of their toxicity and accumulation by marine organisms such as fish (Inskip and Piotrowski, 1985; Khansari *et al.*, 2005).

Entry of cadmium into human and animal food chain due to anthropogenic activities such as smelting operations, use of phosphate fertilizers, pigment, cigarettes smokes and automobiles (WHO, 1992; Kumar *et al.*, 2007). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosyliene and Jankaite, 2006; Farombi *et al.*, 2007). Adverse effect on fish health is always encountered when pollutants are added to water (Kaushik *et al.*, 2003; Kumar *et al.*, 2008; 2009).

Fish are generally very sensitive to cadmium poisoning with exhibition of adverse effects. Cadmium in fish may disturb the biochemical processes leading to health abnormalities in some cases to fatal consequences. Increased levels of cadmium in foods may constitute a food safety risk.

The objective of this study is to determine the accumulation of cadmium in livers of fish (*Oreochromis niloticus*) exposed to different concentrations of cadmium and to assess the histological alterations in the kidneys intestines and livers due to cadmium toxicity.

## MATERIALS AND METHODS

Twenty one tilapia fry (*Oreochromis niloticus*) were obtained from the fish hatchery of King Abdulaziz for Science and Technology (KACST). Fish measured 6-8 cm in length (mean  $7\pm 1.5$ ) and weighed 8-11 g ( $9.30\pm 1.8$  g). They were divided into three groups each consists of 7 fishes. They were confined to large 10 L plastic aquaria with tap water for 4 days in the laboratory at the Department of Zoology, College of Science, King Saud University for acclimatization. The water in the aquaria was changed every day with well-aerated tap water at a temperature of  $25\pm 2^\circ\text{C}$  under standard fish rearing practices. They were fed daily on Grain Silos and Flour (Mills Organization, Riyadh, KSA) for the entire period of the experiment.

Group 1 was exposed to  $10\text{ mg L}^{-1}$  cadmium chloride solution ( $\text{CdCl}_2$ ) in tap water. Group 2 was exposed to  $5\text{ mg L}^{-1}$  cadmium chloride solution ( $\text{CdCl}_2$ ) in tap water. Group 3 was exposed to tap water without cadmium chloride ( $\text{CdCl}_2$ ) as a control group.

A fish was considered dead when observed to be immobile with no opercula movement seen when probed with a glass rod. Pieces of liver, kidney and intestine were fixed in 10% Neutral Buffered Formaldehyde (NBF), sectioned and stained with haemotoxylin and eosin for histological examination.

Cadmium concentration was determined in the liver tissues of dead fish. The accumulation of cadmium metal in fish was analyzed at the end of the experimental period. Pieces of livers from dead fish were acid digested in a closed vessel device using temperature control microwave (Milestone ETHOS PLUS labstation with HPR-1000/10s High Pressure Segment Rotor) and heated for the metal determination by spectroscopic methods using AA220FS atomic absorption spectrophotometer and the results were given as mg/L (ppm).

Data obtained from the experiments were analyzed and the results were expressed as mean $\pm$ SD. The results were compared using Student's t-test using the computer programme Sigmasat (Sigmasat Statistical Software, Version 2.03, SPSS Inc.),  $p < 0.001$  were considered significant (Sokal and Rohlf, 1981).

## RESULTS AND DISCUSSION

After 24 h after the start of the experiment, one fish died of each of Group 1 and 2. The 3 days later, all the individuals in Group 1 died together with four from Group 2. On day 7th after the start of the experiment all living fish including those in Group 3 were autopsied. The outer surface of skin of dead fish was covered by whitish mucus like especially in substance Group 1.

The cadmium concentration in the livers of individuals from Group 1 ranged between 8.30 and  $9.92\text{ }\mu\text{g g}^{-1}$  (ppm) with a mean of ( $9.09\pm 0.51$  ppm) while the concentration in Group 2 ranged from 3.9-6.4  $\mu\text{g g}^{-1}$  with a mean of ( $5.17\pm 0.25$  ppm). There was a significant accumulation of cadmium in the liver tissues of fish from Group 1 compared to those in Group 2 ( $p > 0.001$ ).

Histopathological changes observed in the livers intestines and kidneys sections prepared from fish which were exposed to  $10\text{ mg L}^{-1}$  were more pronounced compared to those exposed to  $5\text{ mg L}^{-1}$  cadmium chloride. The control group tissue did not show histopathological changes. The histopathological changes in livers were in the form of generalized fatty vacuolations, extensive necrosis of hepatocytes and congestion of liver

sinusoids, lymphocytic infiltration and congestion of the central veins (Fig. 1). Kidneys showed severe glomerular necrosis and shrinkage of glomeruli as well as lymphocytic infiltration between the distal convoluted tubules and tubular hyaline degeneration (Fig. 2). Intestinal mucosa showed marked desquamation and submucosal blood vessels congestion with inflammatory cells infiltrations (Fig. 3).

In the present study, exposing tilapia fish (*Oreochromis niloticus*) to 5 and 10 mg L<sup>-1</sup> cadmium chloride was lethal after 3 days. Bioaccumulation of cadmium in the liver tissues was high in both groups. It is likely that cadmium is also accumulated in other organs especially muscles which are the edible part of the fish, hence raising big concerns with regard to risk for human. Cadmium is listed by the US Environmental Protection

Agency as one of 126 priority pollutants. The most dangerous characteristic of cadmium is that it accumulates throughout a lifetime. Cadmium accumulates mostly in the liver and kidney and has a long biologic half-life up to 38 years in humans (ATSDR fact sheet). Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments (Schwaiger *et al.*, 1996; Korkmaz *et al.*, 2009).

Abdel-Baki *et al.* (2011) reported cadmium levels of 15.1 ppb in the liver tissues from tilapia in Wadi Hanifah in Saudi Arabia. Other studies have also reported the liver accumulate and concentrate the highest concentrations of cadmium (Jent *et al.*, 1998; Rashed, 2001). Kaoud and El-Dahshan (2010) reported mean cadmium concentrations in water of Tilapia farms in Egypt of 0.04 ppm which was above the limits recommended by WHO (1984) (0.005 ppm). They also found that the accumulation of cadmium in tissues was 1.21 ppm which again above the recommended limits indicated by FAO/WHO (1992) which is 0.05 ppm. However, the values indicated by Abdel-Baki *et al.* (2011) in Wadi Hanifah were within the allowed limits by FAO and WHO.

The presence of high level of cadmium chloride in the liver of fish accompanied by histological changes indicate that cadmium accumulated in liver tissues and interfere with the mitochondrial function of the hepatocytes. About 75% of the total accumulated cadmium in the organism is deposited in the liver and kidneys (Marafante, 1976; Kraal *et al.*, 1995). Fish exposure to pollutants will have several pathological alterations in tissue. Histopathological alteration can be used as biomarker to be able to detect cellular change in the affected organism (Velkova-Jordanoska and Kostoski,

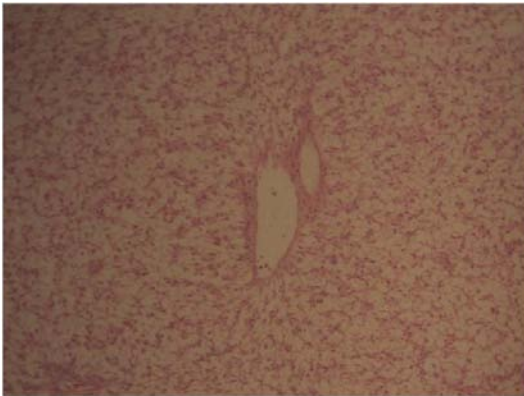


Fig. 1: Histological section of a liver from fish exposed to 10 mg L<sup>-1</sup> CdCl<sub>2</sub> showing generalised fatty change and necrosis of hepatocytes. H and E x400

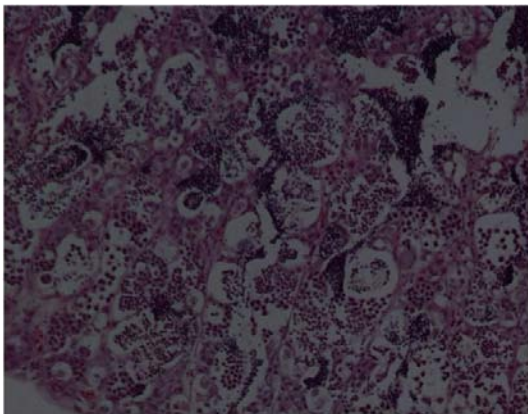


Fig. 2: Histological section of a kidney from a fish exposed to 10 mg L<sup>-1</sup> CdCl<sub>2</sub> showing infiltration of lymphocytes, glomerular atrophy and expansion of the space inside the Bowman's capsule. H and E x400

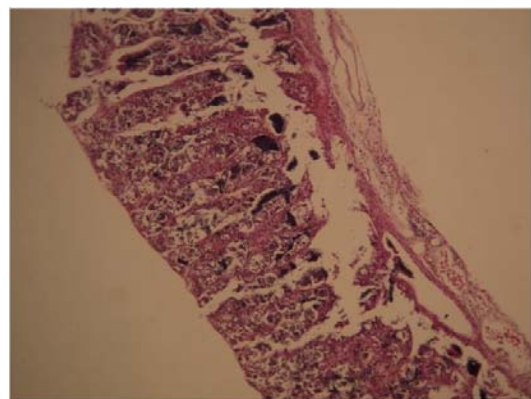


Fig. 3: Histological section of intestine from a fish exposed to 10 mg L<sup>-1</sup> CdCl<sub>2</sub> showing congestion of blood vessels and desquamation of the intestinal villi. H and E x200

2005). Al-Nasser (2000) examined the effect of cadmium on isolated liver mitochondria and found out that cadmium resulted into swelling and inhibition of respiration and loss of inner mitochondrial membrane and loss of preaccumulated calcium. The fatty change detected in livers of fish exposed to cadmium can be explained by the ability of cadmium to induce lipid peroxidation in the hepatocytes which might probably lead to necrosis (Bagchi *et al.*, 1996). Keating *et al.* (2007) was the first to document hepatopancreatic histological changes from shrimps (*Litopenaeus vannamei*) such changes were similar to what has been reported from tilapia in the present study. Hepatopancreas was listed as one of the tissues known to accumulate the highest cadmium levels in the pink shrimps, *Penaeus duorarum* (Nimmo *et al.*, 1977).

Kidneys of fish receive much the largest proportion of postbranchial blood hence, renal lesions are expected to be good indicators of environmental pollution. Histopathological changes in the glomeruli and tubules reported in the present study are probably due to high concentrations of cadmium accumulated in the kidneys and cause such effect. The hyaline droplets in the proximal tubules encountered in fishes exposed to cadmium often results in displacement of the nuclei and it represented protein which had been reabsorbed from the glomerular filtrate. Such changes have been reported in the present study and they are always associated with cases of intoxication with heavy metals including herbicides (Chaudhuri *et al.*, 1999; Jiraungkoorskul *et al.*, 2002).

### CONCLUSION

The pathological effects of cadmium deposits included skin irritation and severity increases in the gills and respiratory lamellae hence, interfering with gills to carry oxygen for respiration (Hu, 1998; Tawari-Fufein *et al.*, 2008). The mucus material which was found in the skin are probably due to irritation of the skin which reveal that cadmium can cause poison to external parts of the body. Exposed fishes probably died of intoxication due to respiratory failure intestinal hypermotility, liver and kidney dysfunction. Intestinal hypermotility was demonstrated by the histopathological changes seen in the intestinal tissues whereas liver and kidney dysfunction was also shown by the histopathological changes in tissues.

This study indicated that high levels of cadmium in fish can cause death of the exposed fish as well as constitutes a potential risk concern on human consumer's health.

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### REFERENCES

- Abdel-Baki, A.S., M.A. Dkhil and S. Al-Quraishy, 2011. Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of Wadi Hanifah, Saudi Arabia. *Afr. J. Biotechnol.*, 10: 2541-2547.
- Ahmad, B., S. Zakir, B. Nawab, A. Jehangir and D. Farhan, 2002. Monitoring of wastewater quality of gadoon amazai industrial estate NWFP Pakistan. *Asian J. Plant Sci.*, 1: 70-72.
- Al-Nasser, I.A., 2000. Cadmium hepatotoxicity and alterations of the mitochondrial function. *J. Toxicol. Clin. Toxicol.*, 38: 407-413.
- Ashraj, W., 2005. Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. *Environ. Monit. Assess.*, 101: 311-316.
- Authman, M.M.N. and H.H.H. Abbas, 2007. Accumulation and distribution of copper and zinc in both water and some vital tissues of two fish species (*Tilapia zillii* and *Mugil cephalus*) of lake Qarun, Fayoum Province, Egypt. *Pak. J. Biol. Sci.*, 10: 2106-2122.
- Bagchi, D., M. Bagchi, E.A. Hassoun and S.J. Stohs, 1996. Cadmium induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Sprague-Dawley rats. *Biol. Trace Elem. Res.*, 52: 143-154.
- Chaudhuri, B.N., G.J. Kleywegt, J. Bjorkman, L.S. Lehman-McKeeman, J.S. Oliver and T.A. Jones, 1999. The structures of  $\alpha$ 2u-globuline and its complex with a hyaline droplet inducer. *Acta Cryst.*, 55: 753-762.
- Clarkson, T.W., 1998. Human toxicology of mercury. *J. Trace Elem. Exp. Med.*, 11: 303-317.
- Dickman, M.D. and K.M. Leung, 1998. Mercury and organo chlorine exposure from fish consumption in Hong Kong. *Chemosphere*, 37: 991-1015.
- FAO/WHO, 1992. Food monitoring and assessment programme. WHO, Geneva 5, UNEP, Nairobi. 52. Report of the Third Meeting of the GEMS/Food.
- Farombi, E.O., O.A. Adelowo and Y.R. Ajimoko, 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. *Int. J. Environ. Res. Public Health*, 4: 158-165.

- Hu, H., 1998. Heavy Metal Poisoning. In: Harrison's Principles of Internal Medicine, Fauci, A.S., E. Braunwald, K.J. Isselbacher, J.D. Wilson and J.B. Martin *et al.* (Eds.). McGraw-Hill, New York, pp: 2564-2569.
- Inskip, M.J. and J.K. Piotrowski, 1985. Review of the health effects of methylmercury. *J. Applied Toxicol.*, 5: 113-133.
- Jent, S., J.S. Heing and C.M. Tate, 1998. Concentration distribution and composition of selected trace metals in bed sediment and fish tissue in the South Platte River Basin, USA, 1992-1993. National Water-Quality Assessment (NAWQA) program report.
- Jiraungkoorskul, W., E.S. Upatham, M. Kruatrachue, S. Sahaphong, S. Vichasri-Grams and P. Pokethitiyook, 2002. Histopathological effects of roundup, a glyphosate herbicide, to Nile tilapia (*Oreochromis niloticus*). *ScienceAsia*, 28: 121-127.
- Kalaiselvi, P., S. Mahimairaja, P. Srimathi and G.S. Kumar, 2010. Impact of industrial effluents in seed invigouration: A review. *Asian J. Plant Sci.*, 9: 249-255.
- Kaoud, H.A. and A.R. El-Dahshan, 2010. Bioaccumulation and histopathological alterations of the heavy metals in *Oreochromis niloticus* fish. *Nature Sci.*, 8: 147-156.
- Kaushik, A, S. Jain, J. Dawra and P. Sharma, 2003. Heavy metal pollution in various canals originating from river Yamuna in Haryana. *J. Environ. Biol.*, 24: 331-337.
- Keating, J., M. Delaney, D. Meehan-Meola, W. Warren and A. Alcivar *et al.*, 2007. Histological findings, cadmium bioaccumulation and isolation of expressed sequence tags (ests) in cadmium-exposed, specific pathogen-free shrimp, *Litopenaeus vannamei* postlarvae. *J. Shellfish Res.*, 26: 1225-1237.
- Khan, M., K. Hammad Naqi and A. Hania, 2003. Hudiera drain. A case of trans-boundary water pollution between India and Pakistan. *Pak. J. Biol. Sci.*, 6: 167-175.
- Khansari, F.E., M.G. Khansari and M. Abdollahi, 2005. Heavy metals content of canned tuna fish. *Food Chem.*, 93: 293-296.
- Korkmaz, N., E.I. Cengiz, E. Unlu, E. Uysla and M. Yanad, 2009. Cypermethrin-induced histopathological and biochemical changes in Nile tilapia (*Oreochromis niloticus*) and the protective and recuperative effect of ascorbic acid. *Environ. Toxicol. Pharma.*, 28: 198-205.
- Kraal, M.H., M.H.S. Kraak, C.J. DeGroot and C. Davids, 1995. Uptake and tissue distribution of dietary and aqueous cadmium by carp (*Cyprinus carpio*). *Ecotoxicol. Environ. Saft.*, 31: 179-183.
- Kumar, P., Y. Prasad, A.K. Patra and D. Swarup, 2007. Levels of cadmium and lead in tissues of freshwater fish (*Clarias batrachus*) and chicken in Western UP (India). *Bull. Environ. Contam. Toxicol.*, 79: 396-400.
- Kumar, P., Y. Prasad, A.K. Patra, R. Ranjan and R.C. Patra *et al.*, 2009. Ascorbic acid, garlic extract and taurine alleviate cadmium-induced oxidative stress in freshwater catfish (*Clarias batrachus*). *Sci. Total Environ.*, 407: 5024-5030.
- Kumar, P., Y. Prasad, R. Ranjan, D. Swarup and A.K. Pattanaik *et al.*, 2008. Accumulation pattern of cadmium in tissues of indian catfish *Clarias batrachus*. *Anim. Nut. Feed Technol.*, 8: 115-119.
- Marafante, E., 1976. Binding of mercury and zinc to cadmium-binding protein in liver and kidney of goldfish (*Carassius auratus* L.). *Experientia*, 32: 149-150.
- Midrar-ul-Haq, R.A. Khattak, H.K. Puno, M.S. Saif, K.S. Memon and N.B. Sial, 2005. Heavy metals accumulation in potentially contaminated soils of NWFP. *Asian J. Plant Sci.*, 4: 159-163.
- Nimmo, D.W.R., D.V. Lightner and L.H. Bahner, 1977. Effects of cadmium on shrimp *Penaeus duorarum*, *Palaemonetes pugio* and *Palaemonetes vulgaris*. In: Physiological responses of marine biota to pollutants, Vernberg, F.J., A. Calabrese, F.P. Thurberg and W.B. Vernberg (Eds.). Academic Press, New York, pp: 131-184.
- Okada, I.A., A.M. Sakuma, F.D. Maio, S. Dovidemskas and O. Zenebon, 1997. Evaluation of lead and cadmium in milk due to environmental contamination in Paraiba Valley region of South Eastern Brazil. *Revista-de-Saude-Publica.*, 31: 140-143.
- Olaifa, F.G., A.K. Olaifa and T. E. Onwude, 2004. Lethal and sublethal effects of copper to the African Cat fish (*Clarias gariepinus*). *Afr. J. Biomed. Res.*, 7: 65-70.
- Olsson, P.E., 1998. Disorders associated with heavy metal pollution. In: Fish Diseases and Disorders (Non-infectious Disorders), 2nd Edn., CABI International, U.K., 105-131.
- Pinot, F., S.E. Kreps, M. Bachelet, P. Hainaut, M. Bakonyi and B.S. Polla, 2000. Cadmium in the environment: Sources, mechanisms of biotoxicity and biomarkers. *Rev. Environ. Health*, 15: 299-323.
- Rashed, M.N., 2001. Monitoring of environmental heavy metals in fish from nasser lake. *Environ. Int.*, 27: 27-33.
- Saxena, R. and P. Garg, 2010. Vitamin E provides protection against *In vitro* oxidative stress due to pesticide (Chlorphrifos and Endosulfan) in goat RBC. *GERF Bull. Biosci.*, 1: 1-6.

- Schwaiger, J., K. Fent, H. Stecher, H. Ferling and R.D. Negele, 1996. Effects of sublethal concentrations of triphenyltinacetate on rainbow trout (*Oncorhynchus mykiss*). *Arch. Environ. Contam. Toxicol.*, 30: 327-334.
- Shukry, W.M., 2001. Effect of industrial effluents polluting the river Nile on growth, metabolism and productivity of *Triticum aestivum* and *Vicia faba* plants. *Pak. J. Biol. Sci.*, 4: 1153-1159.
- Sokal, R.R. and F.J. Rohlf, 1981. *Biometry, the principles and practice of statistics in biological research*. 2nd Edn., Freeman, W.H. and Company, New York, 859-859.
- Swarup, D., R.C. Patra, Naresh, Ram and K. Puneet *et al.*, 2006. Deficiency of copper and cobalt in goats reared around lead zinc smelter. *Small Rumin. Res.*, 63: 309-313.
- Tawari-Fufein, P., J. Igetei and M.E. Okoidgun, 2008. Changes in the catfish (*Clarius gariepinus*) exposed to acute cadmium and lead poisoning. *Biosci. Res. Comm.*, 20: 271-276.
- Tucker, B.W., 1997. Overview of current seafood nutritional issues: Formation of potentially toxic products. In: *Seafood safety, processing and biotechnology*. Shahidi F., Y. Jones and D.D. Kitts (Eds.). Technomic Publishing Co. Inc., Lancaster, PA (USA).
- Tuzen, M. and M. Soylak, 2007. Determination of trace metals in canned fish marketed in Turkey. *Food Chem.*, 101: 1378-1382.
- USFDA, 1993. United States Food and Drug Administration. Center for Food Safety and Applied Nutrition. Guidance document for cadmium in shellfish.
- Velkova-Jordanoska, L. and G. Kostoski, 2005. Histopathological analysis of liver in fresh (*Barbus meridionalis petenyi*/I> Hekel) in reservoir Trebenista. *Natura Croatica*, 14: 147-153.
- Vosyliene, M.Z. and A. Jankaite, 2006. Effect of heavy metal model mixture on rainbow trout biological parameters. *Ekologija*, 4: 12-17.
- WHO, 1984. Guidelines for drinking water quality. WHO, Geneva, No.111.
- WHO, 1992. Environmental Health Criteria. No. 134, Environmental aspects, Geneva WHO.