



Document heading

doi: 10.12980/JCLM.2.2014JCLM-2014-0015

© 2014 by the Journal of Coastal Life Medicine. All rights reserved.

## Effects of cadmium and copper on sialic acid levels in blood and brain tissues of *Cyprinus carpio* L.

Utku Güner<sup>1\*</sup>, Elvan Bakar<sup>2</sup><sup>1</sup>Trakya University, Faculty of Science, Department of Biology, Balkan Campus, Edirne, Turkey<sup>2</sup>Trakya University, Faculty of Pharmacy, Balkan Campus, Edirne, Turkey

### ARTICLE INFO

#### Article history:

Received 26 Jun 2014

Received in revised form 12 Jun 2014

Accepted 23 Jun 2014

Available online 31 Aug 2014

#### Keywords:

Carp

*Cyprinus carpio*

Copper

Cadmium

Sialic acid

Blood

Brain

Metal accumulation

### ABSTRACT

**Objective:** To investigate the effects of cadmium (Cd) and copper (Cu) on sialic acid levels of brain and blood tissues of *Cyprinus carpio*.

**Methods:** Adult carps were exposed to 0.1, 0.5 mg/L Cu, 0.1, 0.5 and 1.0 mg/L Cd and 0.1 mg/L Cu+0.1 mg/L Cd under static experiment conditions for 1 week. At the end of exposure period, heavy metal accumulations and sialic acid levels in blood and brain tissues of the test animals were analyzed.

**Results:** Cu and Cd accumulated in tissues in a dramatically increasing dose-dependent manner. Sialic acids level of the fish exposed to 0.1, 0.5 and 1.0 mg/L Cu and Cd and control groups for 1 week were 0.834, 1.427, 0.672, 0.934, 2.968, 4.714 mg/mL respectively. The results also showed that Cu has an antagonistic effect on tissue sialic acid level.

**Conclusions:** We propose that Cd and Cu make a complex with sialic acids of membranes in the tissues researched. This complex between metal ions and sialic acid might account for the cellular toxicity based on Cu and Cd.

## 1. Introduction

Sialic acids (N-acetylneuraminic acids) are negatively charged monosaccharides which are common constituents in oligosaccharides of vertebrates and some invertebrate species[1]. They are possibly the most biologically important monosaccharide units of glycoconjugates. The majority of sialic acid in higher animals is bound up in glycoconjugates. Sialic acid often occurs as the terminal monosaccharide of oligosaccharide chains of glycoproteins,

glycosphingolipids and glycoposphatidylinositol anchors. Both its negative charge and its terminal position make it critical in numerous biological processes. Sialic acids impart a net negative charge to the cell surface and are important in cell-to-cell and cell-to-matrix interactions. Because sialic acids have got negative charge, heavy metals (positive ions) such as copper (Cu) and cadmium (Cd) might affected sialic acids levels on different tissues.

Quantitative and qualitative differences in sialic acid are seen in health and disease and at different stages of cell growth, differentiation, aging and malignant transformation[2]. In recent years, it has been reported that levels of sialic acid are increased in certain types of cancer[3], and it has been proposed that sialic acid may be a useful tumor marker for some cancer types[4].

\*Corresponding author: Utku Güner, Department of Biology, Faculty of Art and Science, Trakya University, Edirne, Turkey.  
E-mail: uguner@trakya.edu.tr

The expansion of industrial activity in recent years has led to a remarkable increase in the presence of heavy metals in the environment[5]. Pollutants such as heavy metals enter living organisms through food chain, and they can accumulate in many tissues[6–8].

Cd is a widespread and toxic heavy metal continuously introduced into the atmosphere and soil from a variety of sources, including the smelting of ores, the burning of fossil fuels, waste incineration, urban traffic and as a by-product of phosphate fertilizers[9]. Cd does not have a physiological role in living organisms. However, it can enter the food chain as a result of bioaccumulation and induce health problems in organisms. It may cause toxicity by disturbing the cellular homeostasis of essential metal ions, such as Cu, zinc and calcium. Of the hazards associated with exposure to Cd, central nervous system disorders have been reported in the case of Itai–itai disease and in various clinical studies on children and exposed workers[10,11]. Cd has a high affinity for zinc- and calcium-binding sites and can displace these metals from preexisting complexes[11]. The most common effects of acute and short-term exposure to Cd in animals are degenerative problems in liver and kidney, toxic effects on mice bone marrow and tissue damage[12–17]. It was reported that short-term and chronic exposure to Cu could alter many physiological parameters in the rainbow trout *Oncorhynchus mykiss*[18], the frog *Rana ridibunda*[19], *Haliotis rubra*[20], and in freshwater fish *Oreochromis niloticus*[21] and *Cyprinus carpio morpha*[22].

Cu is an essential trace element for living organisms, and it is used as a co-factor for structural and catalytic properties in a variety of enzymes including catalase, cytochrome oxidase and superoxide dismutase[23,24]. Though required as an essential trace metal, high Cu concentrations can be toxic[17,19,25]. Cu, which is a widespread pollutant in aquatic systems[11], is one of the most toxic heavy metals in freshwater biota and often accumulates and causes irreversible harm to some species at concentrations just above levels for growth and production[26]. Aquatic contamination of Cu has both natural and anthropogenic causes. In particular, Cu is frequently used to control aquatic vegetation in fish culture systems[27–29].

It was reported that exposure to metal toxicity may cause an increase in plasma and tissue sialic acid concentrations[29–33]. Recent studies have shown that some metal cations form complexes with the membrane-bound sialic acids under *in vivo* physiological conditions, and it was proposed that this interaction might be a cause of metal

toxicity[13,34].

Although accumulation of Cu and Cd in many fish species has been studied[18,28,35,36], there is no information in the literature concerning the *in vitro* effects of these metal ions on the concentration of sialic acid in tissues. Therefore, in the current study, accumulations of Cu and Cd were examined in blood and brain tissues of *Cyprinus carpio* (*C. carpio*). In addition, the relationships between these metals and blood and brain tissue sialic acid contents were investigated.

## 2. Materials and methods

The fish used in this study was obtained from The General Directorate of State Hydraulic Works ponds in Ipsala, Edirne, Turkey. Animals were transferred to a controlled laboratory environment and put in aquariums (50 cm×50 cm×100 cm) left free for 4 weeks to get acclimatized to laboratory conditions. The temperature and photoperiod of the laboratory during the experiments were (20±1) °C and 12 L:12 D, respectively.

Some physical and chemical parameters of the aquariums were listed below: pH: 8.17±0.10; total hardness: (268.7±4.8) mg/L CaCO<sub>3</sub>; dissolved O<sub>2</sub>: (6.67±0.60) mg/L.

A total of 7 aquariums, one of which was designed as a control, were used to conduct the experiments. CdCl<sub>2</sub>·H<sub>2</sub>O (Merck) salt and CuSO<sub>4</sub>·5H<sub>2</sub>O (Merck) salts were used for the preparation of stock metal solutions. Briefly, 6 aquariums were filled with 100 L filtered (active carbon) tap water and metal stock solutions were added to each so that the final solutions were 0.1, 0.5 mg/L Cu, 0.1, 0.5 and 1.0 mg/L Cd, and 0.1 mg/L Cu+0.1 mg/L Cd. The eighth aquarium was used as a control including solution free filtered tap water. About 5 fish were used for each experimental group in each aquarium (Table 1). The aquariums were continuously aerated and test animals were fed with fish bait during the experiments. The water in each aquarium was replenished once in two days to keep the metal concentrations constant.

**Table 1**

Body parameters of *C. carpio*.

	Total weight (g)	Total length (cm)	Dorsal length (cm)
Mean	41.72	12.54	4.03
Standard error	2.26	0.22	0.08
Standard deviation	16.45	1.63	0.55

At the end of seven days, fish were anaesthetized with MS 222 (tricane methanesulphonate, 75 mg/L) for tissue (brain and serum) samplings[37].

The chemicals used for spectrophotometrical

determinations were purchased from Merck.

All fish were dissected to obtain their blood and brain for evaluation of Cd and Cu accumulation. Before dissections, body weight and length of all animals were measured. All tissues were then weighed and digested by 1 mL:1 mL in concentrated nitric/perchloric acids (Merck) at closed falcon tube with 120 °C in autoclave (about 3 h) until they return yellow clear liquid.

The tissues were digested with concentrated nitric acid and perchloric acid (1:1 v/v) at 120 °C for 2 h in an autoclave. Following acid digestion, all samples were analyzed for the two elements by atomic absorption spectrometry (UNICOM 929 AA). All digested samples were analyzed three times for each metal[38].

Tissue samples were frozen at -70 °C until being used for sialic acid determinations. After melting, tissues were homogenized in phosphate buffer, pH 7. Sialic acid was liberated with perchloric acid hydrolysis according to Neyra[39], Sydow[40] and Karacali *et al*[41,42]. Spectrophotometric determinations were carried out using a Schmadzu UV/visible spectrophotometer operated at 525 nm.

Statistical analysis of data was performed using the SPSS statistical package program.

### 3. Results

No mortality was observed at control group while the animals in aquariums containing 5 mg/L of Cu died after five days of exposure.

#### 3.1. Metal accumulation in the tissues

The results of the metal accumulation in the fish tissues exposed to Cu, Cd and Cu/Cd are presented in Tables 2 and 3. In comparison with the control group, Cu and Cd accumulated in the tissues, dramatically increased in a dose-dependent manner (Figures 1 and 2).

**Table 2**

Sialic acid level in the blood tissue of the fish exposed to 0.1, 0.5 and 1.0 mg/L Cu and Cd for 1 week.

Dose Blood	Mean	Std. error	Decrease or increase
0.1 mg/L Cu	0.834	0.110	↓
0.5 mg/L Cu	1.427*	0.084	↑
0.1 mg/L Cd	0.672	0.069	↓
0.5 mg/L Cd	0.934***	0.107	↓
1.0 mg/L Cd	2.968	0.354	↑
0.1 mg/L Cu+0.1 mg/L Cd	4.714*	0.980	↑
Control (Cu level)	0.978	0.160	

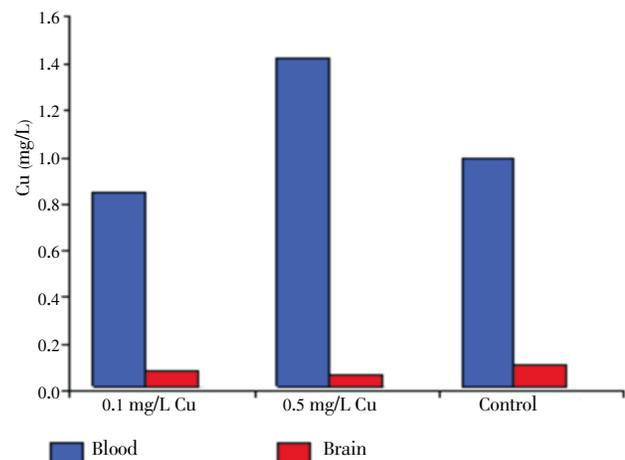
↓ ↑ indicate a decrease or increase, respectively, with respect to the control. Two sample paired *t* test (\*\**P*<0.01, \**P*<0.1, \**P*>0.5) according to control.

**Table 3**

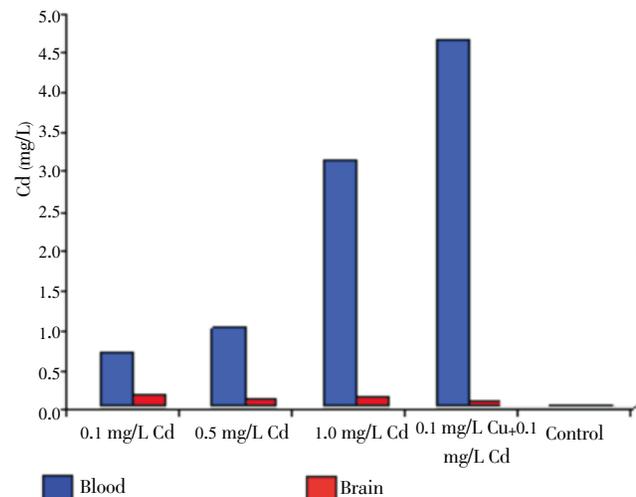
Sialic acid level in the brain tissue of the fish exposed to 0.1, 0.5 and 1.0 mg/L Cu and Cd for 1 week.

Dose Braid	Mean	Std. error	Decrease or increase
0.1 ppm Cu	0.579*	0.032	↓
0.5 ppm Cu	0.701	0.124	↓
0.1 ppm Cd	1.820**	0.000	↑
0.5 ppm Cd	0.933***	0.072	↓
1.0 ppm Cd	1.010	0.157	↑
0.1 mg/L Cu+0.1 mg/L Cd	0.641	0.089	↓
Control (Cu level)	1.007	0.185	

↓ ↑ indicate a decrease or increase, respectively, with respect to the control. Two sample paired *t* test (\*\**P*<0.01, \**P*<0.1, \**P*>0.5) according to control.



**Figure 1.** The results of carp blood and brain Cu levels on different heavy metal concentrations.



**Figure 2.** The results of carp blood and brain Cd levels on different heavy metal concentrations.

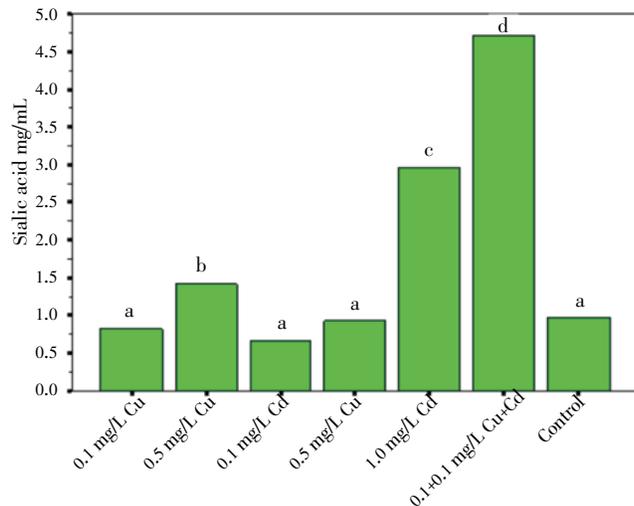
#### 3.2. Sialic acid levels in blood tissues

Sialic acid levels of blood samples of animals exposed to Cu, Cd and Cu/Cd were shown in Figure 3.

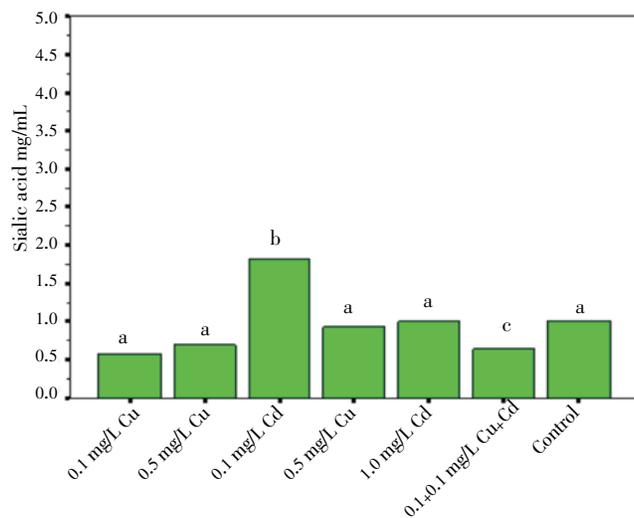
#### 3.3. Sialic acid levels in brain tissues

Sialic acid levels of brain tissue samples of animals

exposed to Cu, Cd and Cu/Cd were shown in Figure 4.



**Figure 3.** The results of carp blood sialic acid analysis are shown at different heavy metal concentrations.



**Figure 4.** The results of carp brain tissue sialic acid analysis are shown at different heavy metal concentrations.

#### 4. Discussion

In this study, accumulation patterns of the heavy metals Cd and Cu and the relationship between these metals and sialic acid were investigated in brain and blood tissues of *C. carpio* after 7-day exposure. The results of metal analysis showed that the accumulations of Cd and Cu in blood and brain lower than other tissue such as the kidney, liver, muscle and gills[29]. Blood and brain were not target tissues.

Heavy metals released to aquatic systems have toxic effects on fish populations in proportion with their concentrations. Many heavy metals, *i.e.* Cd and Cu, that enter the food chain are not discharged from fish and therefore are accumulated in their body[43]. A number of

earlier studies demonstrated the importance of the free metal ion activity for the uptake of trace metals by aquatic organisms[11]. The complex interactions of immunocompetent cells, which are required to produce an immunological response of fish, are susceptible to many biochemical and physiological disturbances which can be induced by heavy metals[44]. It was reported that Cd accumulation in liver and kidney of *Carassius auratus* increases depending on the dose[17]. Chronic exposure of fish to Ni, Zn, Cu and Cr has a detrimental effect on the immunological response in trout and carp and methyl mercury and copper at sub-lethal doses applied singly and jointly in fish blue gourami[44,45].

The present results showed that exposure of carp to Cd and Cu simultaneously had no significant effect on Cu accumulation in brain and blood tissues of the animals. However, Cd levels were significantly increased in blood tissues. This difference in individual metal accumulations may be due to the inhibitory effect of Cd on Cu absorption[29].

Cd accumulation in tissues appeared to increase significantly depending on the dose exposed. Similarly, sialic acid values of blood tissues increased, but its increase was reduced together with Cd increase. According to these findings, it might be pronounced that sialic acid in tissues under *in vitro* conditions are complexed with the metal ions. The results of sialic acid in the Cu+Cd group are parallel with the findings of Cd group. These results also suggest that Cu absorption were prevented by Cd.

Sialic acid is one of the carbohydrates of the oligosaccharide units in glycoproteins composing cellular membranes. It contains an  $\alpha$ -hydroxycarboxylate moiety, which is known to chelate cations. In an nuclear magnetic resonance, potentiometric and spectroscopic study, Saladini *et al.* reported that sialic acid has great affinity for the toxic bivalent metals Cd and Pb, near physiological conditions, and the high stability of the complex species formed with these metals may account for the mechanism of toxicity[13]. In another study, it was reported that Al at physiological pH values (*in vivo* conditions) is complexed with sialic acid[46]. According to these researchers, the toxic effect of Al towards cellular membranes may be due to its coordination by protein-bound sialic acid. In birds and mammals, heavy metal levels in the blood varies depending on the level of sialic acid found in the blood[47].

The results of the present study are similar to the findings of above studies. Cu accumulation in the experimental group of 2.5 mg/L Cu increased, but sialic acid was reduced probably due the the complex formed by Cu with sialic acid

in the tissues sampled.

In conclusion, the present results showed that Cu and Cd accumulation in *C. carpio* increased in a dose dependant manner. It was observed that these metals have different effects on sialic acid contents of tissues, under *in vitro* conditions. Cu, the useful ion for tissues has an antagonistic effect on tissue sialic acid levels while Cd which is not involved in any physiological function has a synergistic effect. The results indicated that Cd and Cu are complexed with sialic acids of membranes in tissues researched, and this complexation, as formerly explained by Aktac *et al.* might be the cause of cellular toxicity depended on metals<sup>[28]</sup>. On the other hand, it might be thought that these results are due to the direct effects of metal ions on sialic acid metabolism. However, for the clarified toxicity mechanism of Cu and Cd, more detailed *in vitro* studies are needed.

### Conflict of interest statement

We declare that we have no conflict of interest.

### References

- [1] Tatsuta T, Hosono M, Miura Y, Sugawara S, Kariya Y, Hakomori S, et al. Involvement of ER stress in apoptosis induced by sialic acid-binding lectin (lectzyme) from bullfrog eggs. *Int J Oncol* 2013; **43**(6): 1799–1808.
- [2] Bassaganas S, Perez-Garay M, Peracaula R. Cell surface sialic acid modulates extracellular matrix adhesion and migration in pancreatic adenocarcinoma cells. *Pancreas* 2014; **43**(1): 109–117.
- [3] Nakano T, Ozimek L. A sialic acid assay in isolation and purification of bovine kappa-casein glycomacropeptide: a review. *Recent Pat Food Nutr Agric* 2014 Jan 31. Epub 2014 Jan 31.
- [4] Goodarzi MT, Shafiei M, Nomani H, Shahriarahmadi A. Relationship between total and lipid-bound serum sialic acid and some tumor markers. *Iran J Med Sci* 2005; **30**: 124–127.
- [5] Falahi-Ardakani A. Contamination of environment with heavy metals emitted from automatives. *Ecotoxicol Environ Saf* 1984; **8**: 152–161.
- [6] Witeska M, Sarnowski P, Ługowska K, Kowal E. The effects of cadmium and copper on embryonic and larval development of ide *Leuciscus idus* L. *Fish Physiol Biochem* 2014; **40**(1): 151–163.
- [7] Kargin F. Metal concentrations in tissues of the freshwater fish *Capoeta barroisi* from the Seyhan River (Turkey). *Bull Environ Contam Toxicol* 1998; **60**(5): 822–828.
- [8] Aktaç T, Bakar E. The histopathological changes in the mouse thyroid depending on the aluminum. *J Cell Mol Biol* 2002; **1**: 69–72.
- [9] Trinchella F, Riggio M, Filosa S, Volpe MG, Parisi E, Scudiero R. Cadmium distribution and metallothionein expression in lizard tissues following acute and chronic cadmium intoxication. *Comp Biochem Physiol C Toxicol Pharmacol* 2006; **144**(3): 272–278.
- [10] Shukla GS, Singhal RL. The present status of biological effects of toxic metals in the environment: lead, cadmium, and manganese. *Can J Physiol Pharmacol* 1984; **62**(8): 1015–1031.
- [11] An YJ, Kampbell DH. Total, dissolved, and bioavailable metals at Lake Texoma marinas. *Environ Pollut* 2003; **122**(2): 253–259.
- [12] Mitsumori K, Shibutani M, Sato S, Onodera H, Nakagawa J, Hayashi Y, et al. Relationship between the development of hepato-renal toxicity and cadmium accumulation in rats given minimum to large amounts of cadmium chloride in the long-term: preliminary study. *Arch Toxicol* 1998; **72**(9): 545–552.
- [13] Saladini M, Menabue L, Ferrari E. Binding ability of sialic acid towards biological and toxic metal ions. NMR, potentiometric and spectroscopic study. *J Inorg Biochem* 2002; **88**(1): 61–68.
- [14] Mueller PW, Price RG, Finn WF. New approaches for detecting thresholds of human nephrotoxicity using cadmium as an example. *Environ Health Perspect* 1998; **106**(5): 227–230.
- [15] Lu DY, Xu J, Lu TR, Wu HY, Xu B. Inhibitions of several antineoplastic drugs on serum sialic acid levels in mice bearing tumors. *Sci Pharm* 2013; **81**(1): 223–231.
- [16] Damek-Poprawa M, Sawicka-Kapusta K. Damage to the liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. *Toxicology* 2003; **186**(1–2): 1–10.
- [17] Zhang YM, Huang DJ, Wang YQ, Liu JH, Yu RL, Long J. Heavy metal accumulation and tissue damage in goldfish *Carassius auratus*. *Bull Environ Contam Toxicol* 2005; **75**(6): 1191–1199.
- [18] Dethloff GM, Schlenk D, Hamm JT, Bailey HC. Alterations in physiological parameters of rainbow trout (*Oncorhynchus mykiss*) with exposure to copper and copper/zinc mixtures. *Ecotoxicol Environ Saf* 1999; **42**(3): 253–264.
- [19] Papadimitriou EA, Loumbourdis NS. Copper kinetics and hepatic metallothionein levels in the frog *Rana ridibunda*, after exposure to CuCl<sub>2</sub>. *Biometals* 2003; **16**(2): 271–277.
- [20] Gorski J, Nugegoda D. Sublethal toxicity of trace metals to larvae of the blacklip abalone, *Haliotis rubra*. *Environ Toxicol Chem* 2006; **25**(5): 1360–1367.
- [21] Atli G, Alptekin O, Tukul S, Canli M. Response of catalase

- activity to Ag<sup>+</sup>, Cd<sup>2+</sup>, Cr<sup>6+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup> in five tissues of freshwater fish *Oreochromis niloticus*. *Comp Biochem Physiol C Toxicol Pharmacol* 2006; **143**(2): 218–224.
- [22] Radi AA, Matkovic B, Csengeri I. Effects of various oxygen concentrations on antioxidant enzymes and the quantity of tissue phospholipid fatty-acids in the carp. *Acta Biol Hung* 1988; **39**(1): 109–119.
- [23] Gravato C, Teles M, Oliveira M, Santos MA. Oxidative stress, liver biotransformation and genotoxic effects induced by copper in *Anguilla anguilla* L.–the influence of pre-exposure to beta-naphthoflavone. *Chemosphere* 2006; **65**(10): 1821–1830.
- [24] World Health Organization. Environmental health criteria 200. Geneva: World Health Organization; 1998. [Online] Available from: <http://www.inchem.org/documents/ehc/ehc/ehc200.htm> [Accessed on 8th April, 2014]
- [25] Papadimitriou E, Loumbourdis NS. Glycogen, proteins, and aminotransferase (GOT, GPT) changes in the frog *Rana ridibunda* exposed to high concentrations of copper. *Bull Environ Contam Toxicol* 2005; **74**(1): 120–125.
- [26] Hall LW, Anderson RD. A deterministic ecological risk assessment for copper in European saltwater environments. *Mar Pollut Bull* 1999; **38**(3): 207–218.
- [27] Fainerman–Melnikova M, Szabó–Plánka T, Rockenbauer A, Codd R. Coordination modes between copper(II) and N-acetylneuraminic (sialic) acid from a 2D-simulation analysis of EPR spectra. Implications for copper mediation of sialoglycoconjugate chemistry relevant to human biology. *Inorg Chem* 2005; **44**(7): 2531–2543.
- [28] Nor YM. Ecotoxicity of copper to aquatic biota—a review. *Environ Res* 1987; **43**(1): 274–282.
- [29] Aktac T, Bakar E, Guner U. The effects of short-term exposure to cadmium and copper on sialic acid in carp (*Cyprinus carpio*) tissues. *Fresenius Environ Bull* 2010; **19**(3): 432–437.
- [30] Patel AB, Venkatakrisnabhath H. Effect of lead on the blood-serum, liver and brain sialoglycoconjugate levels in rats. *Hum Exp Toxicol* 1992; **11**(2): 89–92.
- [31] Babal P, Janega P, Cerná A, Kholová I, Brabencová E. Neoplastic transformation of the thyroid gland is accompanied by changes in cellular sialylation. *Acta Histochem* 2006; **108**(2): 133–140.
- [32] Zayed J, Philippe S. Acute oral and inhalation toxicities in rats with cadmium telluride. *Int J Toxicol* 2009; **28**(4): 259–265.
- [33] Kikkeri R, Padler–Karavani V, Diaz S, Verhagen A, Yu H, Cao HZ, et al. Quantum dot nanometal surface energy transfer based biosensing of sialic acid compositions and linkages in biological samples. *Anal Chem* 2013; **85**(8): 3864–3870.
- [34] Oto G, Ekin S, Ozdemir H, Levent A, Berber I. The effect of *Plantago major* Linnaeus on serum total sialic acid, lipid-bound sialic acid, some trace elements and minerals after administration of 7,12-dimethylbenz(a)anthracene in rats. *Toxicol Ind Health* 2012; **28**(4): 334–342.
- [35] Cirillo T, Amodio Cocchieri R, Fasano E, Lucisano A, Tafuri S, Ferrante MC, et al. Cadmium accumulation and antioxidant responses in *Sparus aurata* exposed to waterborne cadmium. *Arch Environ Contam Toxicol* 2012; **62**(1): 118–126.
- [36] Dhanapakiam P, Ramasamy VK. Toxic effects of copper and zinc mixtures on some haematological and biochemical parameters in common carp, *Cyprinus carpio* (Linn). *J Environ Biol* 2001; **22**(2): 105–111.
- [37] Wagner GN, Singer TD, McKinley RS. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquac Res* 2003; **34**(13): 1139–1146.
- [38] Guner U. Freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823) accumulates and depurates copper. *Environ Monit Assess* 2007; **133**(1–3): 365–369.
- [39] Neyra C, Paladino J, Le Borgne M. Oxidation of sialic acid using hydrogen peroxide as a new method to tune the reducing activity. *Carbohydr Res* 2014; **386**: 92–98.
- [40] Sydow G. A simplified quick method for determination of sialic-acid in serum. *Biomed Biochim Acta* 1985; **44**(11–12): 1721–1723.
- [41] Karacali S, Deveci O, Deveci R, Onat T, Gurcu B. Spectrophotometrical determination of sialic acid in several tissues of isolated and crowded *Locusta migratoria* (Orthoptera). *Istanbul Univ Fen Fak Biyoloji Der* 1995; **58**: 47–57.
- [42] Karacali S. [Glycobiology current molecular biology]. *Turk J Vet Anim Sci* 2003; **27**(3): 489–495. Turkish.
- [43] Baby J, Raj JS, Biby ET, Sankarganesh P, Jeevitha MV, Ajisha SU, et al. Toxic effect of heavy metals on aquatic environment. *Int J Biol Chem Sci* 2010; **4**(4): 939–952.
- [44] Oneill JG. The humoral immune response of *Salmo trutta* L and *Cyprinus carpio* L. exposed to heavy metals. *J Fish Biol* 1981; **19**(3): 297–306.
- [45] Roales RR, Perlmutter A. Effects of sublethal doses of methylmercury and copper, applied singly and jointly, on immune-response of blue gourami (*Trichogaster trichopterus*) to viral and bacterial antigens. *Arch Environ Contam Toxicol* 1977; **5**(3): 325–331.
- [46] Di Marco VB, Károly–Lakatos A, Venzo A, Bertani R, Seraglia R, Kiss T. The aluminium(III)–sialic acid interaction: a potential role in aluminium-induced cellular membrane degeneration. *Inorg Chim Acta* 2006; **359**(13): 4227–4234.
- [47] Merhan O, Ozcan A. [Investigation of serum ceruloplasmin and total sialic acid levels in geese]. *J Fac Vet Med Univ Kafkas Kars* 2004; **10**(2): 137–142. Turkish.