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Research Article

Effects of nickel on liver and bone metabolic functions, biochemical and histopathological responses in common carp (*Cyprinus carpio*)

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Abstract

This study is performed to investigate the effects of water-borne Ni^{2+} on common carp (*Cyprinus carpio*) liver and bone metabolisms. Fish (*N*=60; 184.40±18.56g) were exposed to background concentrations of Ni^{2+} (based on measured LC_{50}-96h: 5.820 mg/L), including 0.058, 0.291, 0.580, 1.750, 2.910 mg/L for 30 continuous days. Ni^{2+}-exposed fish showed a rising trend in the case of serum aspartate transaminase (AST). Serum alkaline phosphatase (ALP) elevated (p<0.05) in all Ni^{2+} treatments. Serum total protein, globulin, and albumin showed a transient reduction in 0.058, 0.291 and 0.580 mg/L Ni^{2+} exposures (p<0.05). Although serum calcium level did not change significantly, serum inorganic phosphorus was elevated (p<0.05) in 0.580 mg/L Ni^{2+}. Bone isoenzyme of ALP observed in higher levels in all Ni^{2+} treatments than the control group (p<0.05). Pathological damages, such as focal necrosis, pycnosis and cytoplasm degeneration were observed in liver tissues of Ni^{2+}-exposed fish. A higher number of osteocytes as well as osteoclasts in bone of Ni^{2+}-exposed fish revealed dual effect of this metal in the case of bone metabolism. Generally, low level of nickel had no significant effect on metabolic parameters of liver and bone while highest nickel treatment had adverse effects, reflecting dual effects of this metal on carp.

Keywords: Nickel, Common carp, Liver histopathology, Bone histology, Blood chemistry

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Introduction

Aquatic environmental pollution is increasingly recognized due to the continuous introduction of various xenobiotics into aquatic ecosystems such as industrial effluents, mining activities, urban sewage, and agricultural fertilizers (da Silva and Martinez, 2014; Shamsaie Mehrgan *et al.,* 2019). Because of the improper idea of "self-cleaning capacity of waters", wastewaters were discharged untreated to the surrounding water ecosystems, even until the latter part of the twentieth century (Nikinmaa, 2014). Although pollutants enter aquatic ecosystems as a result of human lifestyles, their natural sources are currently responsible for most of them and together they are considered as one of the major threats to aquatic life (Ng *et al.,* 2003; Justino *et al.,* 2016).

Contamination of water ecosystems by heavy metals is increased, which in turn, results in greater pollution uptake by aquatic animals (da Silva and Martinez, 2014; Janbakhsh *et al.,* 2018). Low concentration of Ni^{2+} is detected nearly throughout the water ecosystem, which is reported to be 1.43 to 159.48 µg L^{-1} in different sampling areas, and possibly differ from time to time as well as different ranges of physicochemical parameters (Zhou *et al.,* 2020). In addition, some anthropogenic activities rise this metal in most surface waters (Yu, 2000). Therefore, fish possibly are exposed to different levels of Ni^{2+} during their life, consequently, different levels of adverse effects are expected in aquatic organisms (De Boeck *et al.,* 1995; Dreyfuss *et al.,* 2014).

Ni^{2+} can enter into the fish body through different routes, such as food, gills, drinking water, and skin; adsorbed through its uptake through gills accounted a prominent role in ion uptake and homeostasis (McGeer *et al.,* 2000). Ni^{2+} absorption from food or water regulates lipid metabolism and cell membrane, hormone secretion, and bone strength within the animals' body (Kumar *et al*., 2012). Furthermore, this metal can affect serum chemistry as well as whole-body metabolism and growth performance (Javed, 2013; Moeinnejad *et al.,* 2019). Adverse pathological degeneration in the liver of *Hypophthalmichthys molitrix* is reported at high concentrations of water-borne Ni^{2+} (Athikesavan *et al*., 2006). Research shows that Ni^{2+} accumulation in the plasma and tissues (gill, stomach, and intestine) that have direct contact with water, is roughly commensurate to Ni^{2+} concentration in ambient water (Pane *et al.,* 2006), and likewise leads to changes in metabolism, disturbances in the content of other trace metal in tissues (Misra *et al.,* 1990; Funakoshi *et al.,* 1996).

The effect of trace elements on fish bone metabolism are also reported (Lall and Lewis, 2007; Malekpouri *et al.,* 2011). Macro elements like Ca and Pi have an important role in the hard tissue of animals and their blood levels could reflect bone function, *i.e.,* mineral deposition and absorption (Burtis *et al.,* 2012). Alkaline phosphatase enzymes are accounted as multifunctional membrane enzymes, distributed in different tissues, such as the liver, bone, kidney, and

intestine. Moreover, liver enzymes, such as serum aspartate transaminase (AST) and alanine transaminase (ALT) are important parameters in assessing the state of liver tissue (Coz-Rakovac *et al*., 2005; Khodaei *et al.*, 2019), due to liver being rich in these enzymes and any changes in this organ tissue lead to release of them into the bloodstream (Taghavizadeh *et al*., 2020).

The aim of this study was to evaluate the effects of low levels of Ni^{2+} on serum biochemical parameters related to liver enzyme, protein, and carbohydrate metabolisms. In addition, possible changes in liver and bone histology in common carp, *Cyprinus carpio*, following 30 days of water-borne exposure were examined. In detail, we aimed to pursue the role of different concentrations of nickel (dose-dependent manner) in some physiological and biochemical functions of *C. carpio* to explore whether higher concentrations of nickel can weaken the organism (leading to adverse effects), or low levels induce beneficial effects.

Materials and methods

Chemicals and fish maintenance

All materials were purchased from Merck Chemical Company (Germany) unless otherwise stated. Nickel sulfate hexahydrate ($NiSO_4.6H_2O$) was obtained from ACROS Organics (USA). Common carp, *C. carpio* were purchased from a local fish farm and then transferred to the laboratory. All fish were treated using 5% saline bath upon arrival and were acclimated to laboratory conditions for at least 2 weeks prior to commencement of the experiment.

Experimental design

Acute toxicity test

Forty-eight fish (125.6±11.06 g) were subjected to the acute toxicity test according to OECD protocol, no 203 (OECD, 1994). Briefly, acclimated fish (30 days) were divided into 5 geometric serial concentrations of Ni^{2+} (0.058, 0.291, 0.580, 1.750, and 2.910 mg/L) for 96h and a control group (0 mg/L). Mortality rates were recorded daily. The tested fish did not feed throughout the experiment. Probit regression analysis was applied to estimate the concentration of Ni^{2+} that caused the death of 50% of the animals, i.e., LC_{50}. This was applied to the main experiment.

Nickel treatment

To investigate the effect of Ni^{2+} on the liver and bone functions of carp, a total of 60 fish weighing 184.4±18.56 g were randomly moved into 6 glass aquaria containing well-aerated tap water under natural photoperiod. Half of the water was replenished every other day. Fish were fed *ad libitum* with a diet, containing 31.3% crude protein, 11.67% crude fat, and 11.7% ash. The fish were exposed to background concentrations of Ni^{2+} for 30 continuous days, including 0.058 (1%), 0.291 (5%), 0.580 (10%), 1.750 (30%), and 2.910 mg/L (50%) of LC_{50}-96h.

Water quality parameters were determined daily according to American Public Health Association (APHA, 1998) method (Table 1).

Table 1: Physical and chemical parameters of water during the experiment.

Parameters	Range
pH	7.69-7.93
EC	448-488 µs/cm
DO	5.7-6.5 mg/L
Temperature	22.2-23.4°C
NO_2^-	0.057-0.080 mg/L
NO_3^-	7.52-9.34 mg/L
NH_4^+	<0.1 mg/L
Po_4^{3-}	78.92-79.62 µg/L
Hardness	232-284 mg $CaCO_3$/L
Ca^{2+}	188-246 mg/L
Mg^{2+}	16-32 mg/L
TS	349.2-382.6 mg/L
TDS	320.8-421.6 mg/L
TSS	5.80-6.18 mg/L

Water samples were collected randomly for measuring dissolved Ni^{2+} concentrations in each treatment using atomic absorption spectrophotometry (Perkin Elmer A Analyst 700). Background and measured concentrations of Ni^{2+} were summarized in Table 2.

Serum chemistry

At the end of the experiment, blood samples were withdrawn from the caudal vein of 5 starved fish (at least 24 h) from each treatment. The blood was then centrifuged at 3,500 ×g (10 min). Serum AST and ALT activities were determined using 2,4-dinitrophenyl-hydrazones in an alkaline solution at 505 nm (Reitman and Frankel, 1957).

Table 2: Background and measured concentrations of nickel in different treatments.

Background concentration (mg/L)	0	0.058	0.291	0.580	1.75	2.91
Measured concentration (mg/L)	<0.001	0.055± 0.027	0.275± 0.008	0.572± 0.012	1.687± 0.009	2.909± 0.037

Data are presented as mean ± standard deviation for 3 measurements randomly during the experiment. Nickel as $NiSO_4$. $6H_2O$ concentrations were measured by atomic absorption spectrophotometry.

Serum alkaline phosphatase (ALP) activity was measured at 405 nm using P-nitrophenyl phosphate as substrate (Malekpouri *et al.,* 2011). Triglycerides and glucose were determined by measuring formed H_2O_2 by adding phenol and 4-aminoantipyrine in the presence of peroxidase at 490 and 546 nm, respectively (Fossati and Prencipe, 1982). Total protein (TP) content of serum was determined according to the Biuret method as reported elsewhere (Gornall *et al.,* 1949), as a formation of a Cu^{2+}-protein complex in alkaline reagent at 540 nm and serum albumin (Alb) was measured at 540 nm using bromocresol green complex. Globulin (Glb) was calculated by subtracting Alb from TP. Serum pH was also measured by an electrical pH meter (Metrohm, UK).

The o-cresolphthalein complexone method was used to determine Ca level of serum at 570 nm (Moorehead and Biggs, 1974) and P_i was determined using the ammonium molybdate method (Fiske and Subbarow, 1925). For determining ALP isoenzyme, a heat stability test was performed. Briefly, different ALP isoenzymes have resistance to temperature (at 56 and 65°C). In this regard, fresh serum was heated in Bain Marie at 56°C for 10 min and transferred to an ice bath immediately to stop the reactions

(Romslo *et al.*, 1975). Finally, serum ALP activity was determined as described above. The bone-specific ALP (B-ALP) was determined due to higher stability to this temperature, *i.e*., other isoenzymes were deactivated.

Histopathology

At the end of blood sampling, three liver and bone tissues were sampled immediately from each treatment and were fixed in 10% neutral buffered formalin (pH=7.2). Serial sections with 5µm thickness were then processed and stained using hematoxylin and eosin method. Bone tissue was pretreated with 10% EDTA before the tissue was processed for staining.

Statistical analyses

If normality and homogeneity were achieved, analysis of variance (one-way ANOVA) was used for this study with a complimentary Duncan multiple test. Statistical analyses were carried out using SigmaPlot 12 program and data are presented as mean±standard deviation for all cases. Each treatment's mean value was compared with their specified control at *P*-value lower than 0.05.

Results

No difference in body weight was observed following 30 days' exposure among different treatments. The results of acute toxicity test are provided in Table 3. 96-h LC_{50} value of Ni^{2+} for *C. carpio* was found to be 5.82 mg/L. Among all treated groups, there was no mortality during the 30 days' experimental period.

Table 3: Determination of the LC_{50} for nickel in common carp (*Cyprinus carpio*).

Exposure time (h)	24	48	72	96
LC_{50} mg/L	31.97	20.13	12.38	5.82
(95% confidence limits)	-	(19.08-21.52)	(11.47-13.67)	(4.73-6.24)

Data (*n=8*) are presented as median and confidence intervals. Nickel was applied as $NiSO_4$. $6H_2O$.

The obtained results indicated that serum TP was reduced significantly ($p<0.05$) following low concentrations of Ni^{2+} (0.058, 0.291, and 0.580 mg/L), while there were no significant changes between the highest Ni^{2+} and the control treatment. Serum Alb did not show any significant change among the first three treatments as compared with the control. Fish exposed to 1.750 mg/L waterborne Ni^{2+} showed an increase in Alb level ($p<0.05$). Similar to TP, Glb content of carp serum indicated a significant decrease in the first three treatments, while 1.750 and 2.910 mg/L of Ni^{2+} treatments showed no significant difference as compared with the control. Serum TG reduced significantly ($p<0.05$) in 0.580 and 2.910 mg/L of Ni^{2+} treatments, whereas other treatments did not lead to any significant change as compared with the control. Although glucose level in carp serum did not change in 0.058 and 0.291 mg/L of Ni^{2+}treatments, other highest treatments led to a significant ($p<0.05$) increase compared to the control group. The serum pH level was detected to be high

following 0.291, 1.750, and 2.910 mg/L of Ni^{2+} as compared with other treatments. AST level of serum did not change significantly in 0.058 mg/L Ni^{2+} as compared with the control. Other treatments resulted in a higher level of AST in carp, with the highest level observed in 2.910 mg/L Ni^{2+} treatment (p<0.05). Serum ALT reduced (p<0.05) following 0.058 and 0.291 mg/L Ni^{2+}, while the middle treatment, *i.e*., 0.580 mg/L Ni^{2+} did not show any significant change. Higher levels of Ni^{2+} also showed a significant decrease in ALT level of serum in comparison with the control (p<0.05). Serum ALP was elevated following all Ni^{2+} treatments as compared with the control. A higher level of ALP was observed in 0.580 and 1.750 mg/L Ni^{2+} treatments (Fig. 1).

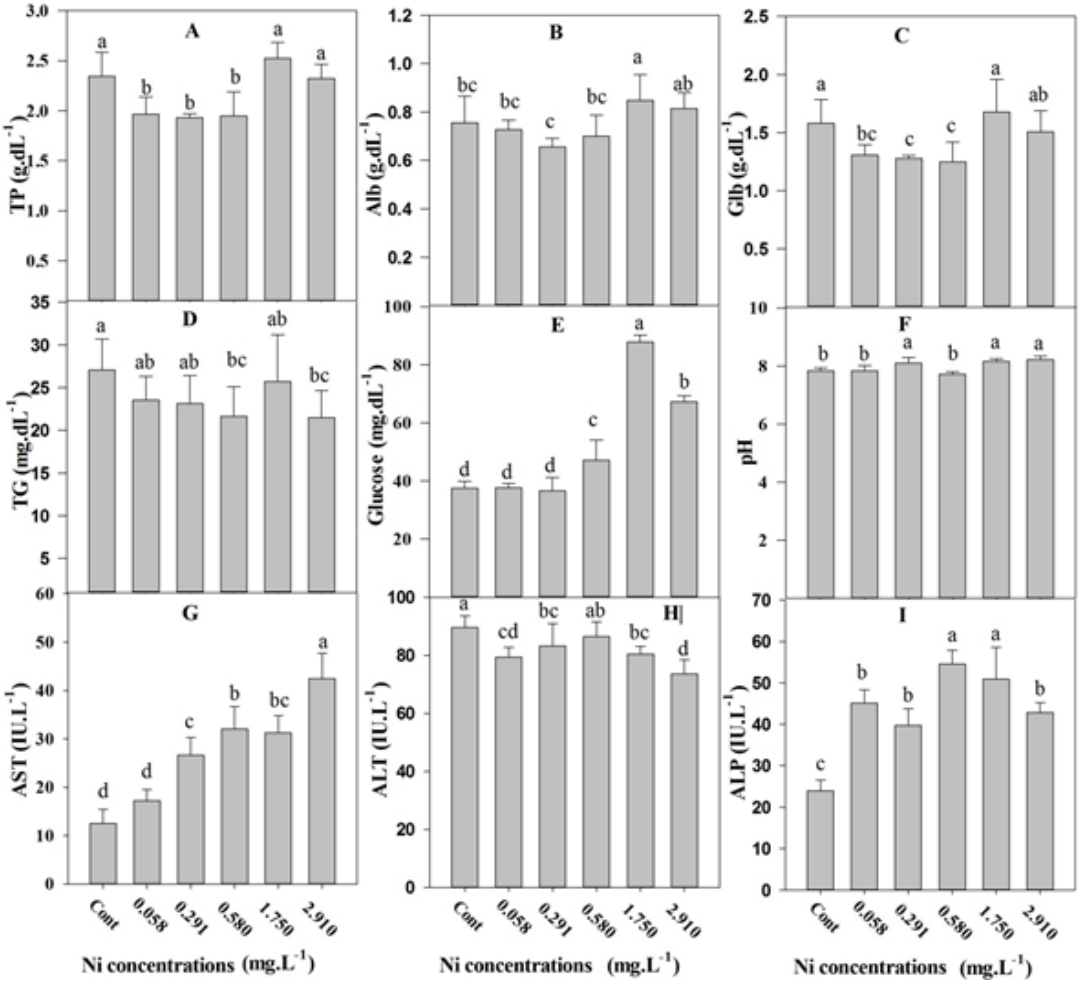


Figure 1: Serum biochemical parameters of *C. carpio* after 30 days of nickel ($NiSO_4$. $6H_2O$) exposures. Each value represents mean ± standard deviation of five separated samples, *i.e*, a total of 60 fish with 184.4±18.56 g initial weight were applied in this experiment. Different letters indicate significant differences among treatments at p<0.05. TP: total protein, Alb: albumin, Glb: globulin, TG: triglyceride, AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase.

Serum parameters related to bone metabolism were monitored and the results showed that there was no significant change in the case of serum Ca level following all Ni^{2+} treatments. Serum P_i elevated (p<0.05) only in 0.580 mg/L Ni^{2+}, while other changes in this parameter were not significant as compared with the control. Bone ALP isoenzyme elevated (p<0.05) following all treatments in comparison with the untreated fish (control). The highest level observed in 0.580 and 1.750 mg/L Ni^{2+}treatments, showing a maximum level in those treatments (Fig. 2).

Liver histopathological investigations indicated focal necrosis, lateral nuclei, pycnosis, and cytoplasm degeneration in all Ni^{2+} treatments but with different degrees (Fig. 3). Number of osteocytes elevated as fish exposed to 0.058 mg/L Ni^{2+}. The higher number of osteocytes as well as osteoclasts was detected, when the fish were exposed to a higher level of Ni^{2+}, *e.g.*, 1.750 and 2.910 mg/L (Fig. 4).

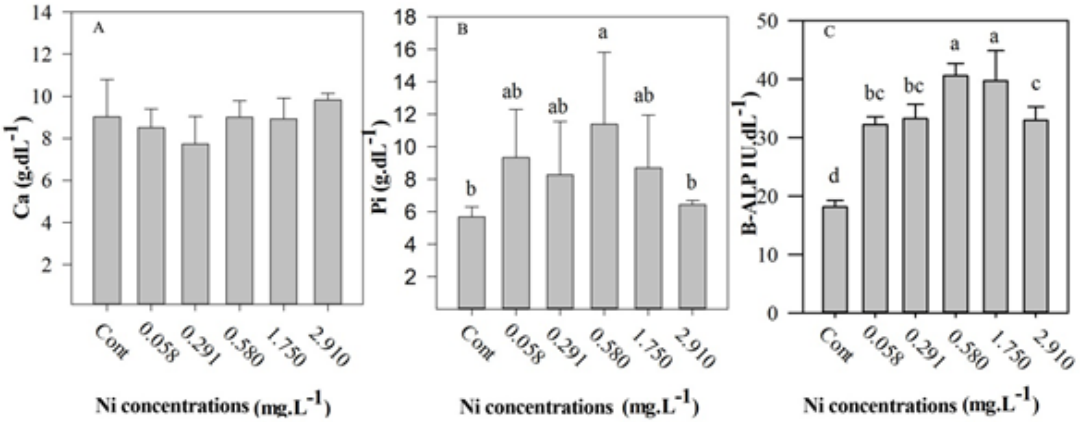
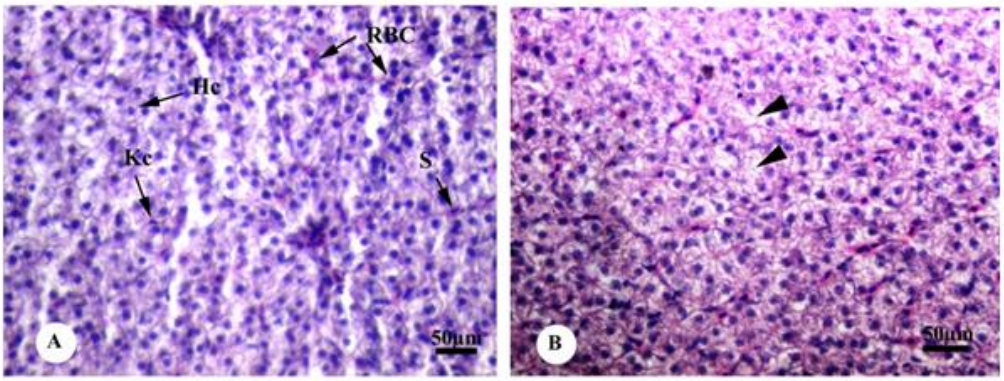


Figure 2: Serum biochemical parameters of bone metabolism in *C. carpio* after 30 days of nickel ($NiSO_4$. $6H_2O$) exposures. Each value represents mean ± standard deviation of five separated samples, *i.e*, a total of 60 fish with 184.4±18.56 g initial weight were applied in this experiment. Different alphabetical letters indicate significant differences among treatments at *p<0.05*. Ca: calcium, P_i: inorganic phosphorus, B-ALP: bone-specific alkaline phosphatase.



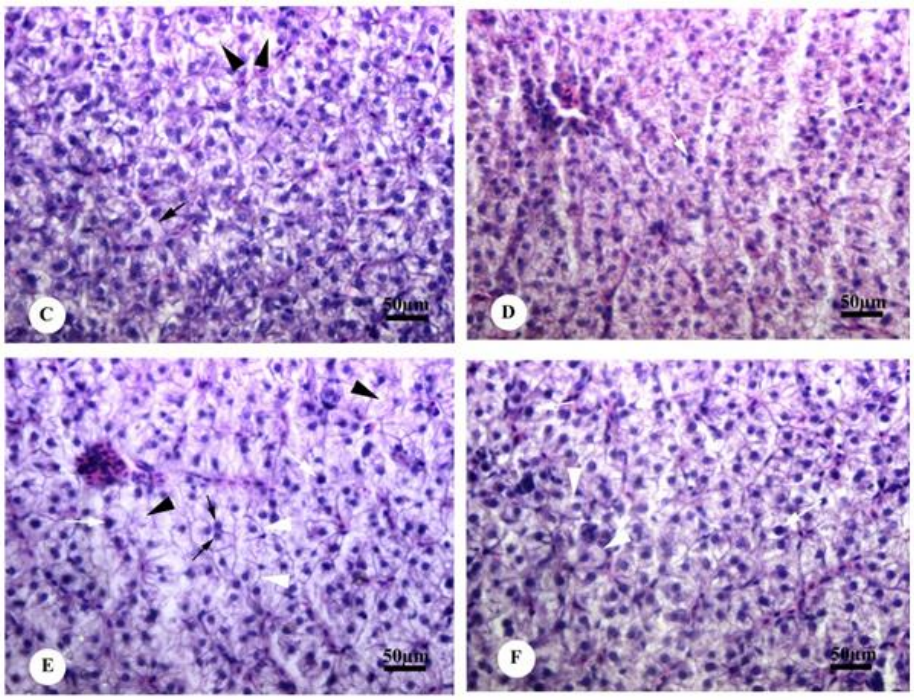


Figure 3: Photomicrograph of common carp histological sections of liver (hematoxylin and eosin), under control condition (A) showing normal structure of liver tissue, (B) exposed to 0.058 mg/L Ni^{2+} (as NiSO4. 6H2O) showing focal necrosis (black arrow head), (C) exposed to 0.291 mg/L Ni^{2+} showing focal necrosis (black arrow head) and lateral nuclei (black arrow), (D) exposed to 0.580 mg/L Ni^{2+} pycnosis (white arrow), (E) exposed to 1.750 mg/L Ni^{2+} showing focal necrosis (black arrowhead), later nuclei (black arrow), cytoplasm degeneration (white arrowhead) and pycnosis (white arrow) and (F) exposed to 2.910 mg/L Ni^{2+} showing cytoplasm degeneration (white arrowhead) and pycnosis (white arrow) following 30 continuous days. RBC: red blood cells, Hc: hepatocytes, Kc: Kupffer cells, S; sinus. Nickel was applied for 30 days as $NiSO_4$. $6H_2O$.

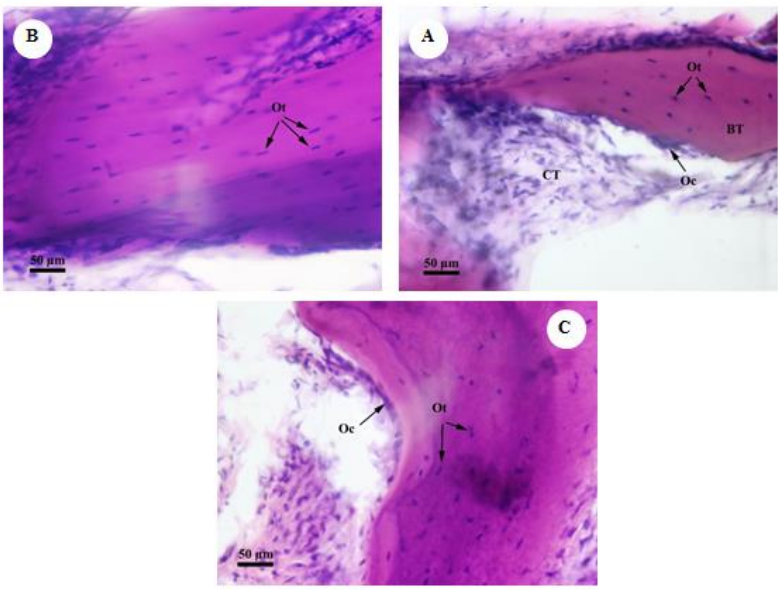


Figure 4: Selected photomicrographs of histological sections of bone (hematoxylin and eosin) in common carp under control condition (A), showing a normal structure of bone tissue, (B) exposed to 0.058 mg/L Ni^{2+} showing higher number of osteocytes, (C) exposed to 1.750 mg/L Ni^{2+} showing a higher number of osteocytes as well as osteoclasts. BT: bone tissue, CT: connective tissue, Ot: osteocytes, Oc: osteoclasts. X40. Nickel was applied for 30 days as $NiSO_4$. $6H_2O$.

Discussion

Trace elements play different biological roles in the body of living organisms. Extensive and detailed studies are needed, since interactions between the elements and physiological activities are complex (Sauliutė and Svecevičius, 2015). Therefore, attention to interactions between toxic elements and essential elements in biochemical pathways and the physiological function of aquatic animals is increased (Ali *et al.*, 2019). Nickel is an essential metal that is not shown to play a biological role in high doses, while in small amounts it can be used as an essential element in many bodily functions, including ossification, synthesis, and activation of metalloenzymes (Chowdhury *et al.*, 2008).

Serum protein content of common carp decreased only in nickel treatment at low concentrations. It may be related to increase protease activity and free amino acids in gills of the exposed fish to the lethal concentration of nickel. This possibly suggests the predominance of proteolytic sensitivity following metal exposure (Sreedevi *et al.*, 1992). Because fish gill tissues are in direct contact with the ambient water, high concentrations of nickel can destroy its resistance by disrupting cellular components. In addition, a high level of soluble protein in the kidneys indicates the dissolution of enzymes necessary to detoxify and eliminate the metal. Sharma and Davis (1980) reported that methylmercury disrupts carp protein synthesis. Most serum proteins are synthesized in liver, and therefore total serum protein is used as an indicator of liver dysfunction. Rivarola and Balegno (1991) reported that pesticides can decrease plasma protein owing to changes in protein and free amino acid metabolism and synthesis. Generally, a decrease in blood protein may be due to loss of protein through decreased protein synthesis or increased proteolytic activity or degradation as mentioned above. Decreasing in total protein can be partly attributed to the effects of the metal on liver cells, which is confirmed by increasing the serum AST and ALT activities observed in this study.

Blood glucose levels are shown to increase in fish exposed to a variety of environmental changes that are considered stressful. Higher level of carbohydrate in fish blood is well evidenced to be a general secondary response to acute intoxication and is considered a reliable indicator of environmental stress (Mazeaud *et al.*, 1977). Al-Attar (2007) suggested that high blood glucose levels could be a reliable indicator of nickel toxicity in fish. In the present study, low levels of nickel tested did not induce any significant change in the serum glucose concentration. Ptashynski *et al*. (2002) also found no difference in glucose concentration between nickel dietary treatment and the control group. In contrast, a high level of nickel exposure led to a significant increase in the blood glucose of this study. Eisler and Jacknow (1985) observed that blood glucose level can be elevated in nine species of freshwater fish following exposure to plating effluent containing cyanide and

Ni, chrome, copper, and zinc salts. Generally, exposure to Ni caused a significant amount of stress in fish, which may have led to a decrease in energy storage, followed by an increase in blood glucose. During the stress period, fish increased levels of glucocorticoids and catecholamines, which raise blood glucose (Reid *et al.,* 1998).

The glycemic response shown in the present study is a sign of impaired carbohydrate metabolism, possibly due to increased hepatic glucose 6-phosphatase activity, increased hepatic glycogen breakdown, or glucose synthesis from extra-hepatic tissue proteins and amino acids (Kubrak *et al.,* 2012). Combined exposure to metals (Nickel, Cadmium, and Lead) increases blood glucose content due to intense glycogenolysis and glucose synthesis from extra-hepatic tissue proteins and amino acids (Vinodhini and Narayanan, 2009). Fırat and Kargın (2010) suggested that elevated blood glucose during pesticide treatment may indicate impaired carbohydrate metabolism due to increased hepatic glycogen breakdown, possibly resulting in increased adrenocorticotrophic and glucagon hormones or decreased insulin activity.

Changes in the activity of hepatic enzymes indicate liver cell damages or a disruption in the metabolic process. Therefore, the study of enzyme activity as an important biochemical indicator is considered an important strategy to assess environmental conditions and the presence of toxic compounds (Baghshani and Shahsavani, 2013). ALT, AST, and ALP play very important roles in the metabolic processes of the body and fish health and are introduced as appropriate biomarkers in toxicological studies (Benincá *et al*., 2012; Kaviani *et al*., 2018; 2020). These enzymes are present in cells of various tissues, such as liver, heart, kidneys, muscles, and brain. Some physiological conditions, such as liver damage and skeletal disorders change (as observed here) the activity of these enzymes (Bogé *et al.*, 1992). Similarly, Öner *et al*. (2008) observed that levels of ALT and AST in the blood increased due to cell damage in liver and concluded that high levels of these enzymes in serum usually indicate disease and necrosis in animals' liver.

Fish showed different enzymatic responses (including decreasing or increasing enzyme activity) to heavy metal contamination, depending on species, metals, concentrations, and physicochemical conditions of water as contributing factors (Jiraungkoorskul *et al*., 2003; Sanchez *et al*., 2005). Fırat and Kargın (2010) showed that heavy metal poisoning can increase the activity of AST in tilapia liver, but the activity of ALT might be lessened due to poisoning in this fish. Some studies reported no significant change in these two enzymes in tissues and blood serum of some fish under the influence of heavy metals (De Smet and Blust, 2001). ALP is made up of several isozymes that are found in almost all tissues of the body, especially in cell membranes. This enzyme accelerates the hydrolysis of monophosphate esters and plays an important role in transporting substances through cell membranes and is also

effective in bone formation (Molina *et al.,* 2005). ALP enzyme is considered a suitable indicator due to sensitivity to cell toxicity due to xenobiotic substances (Lohner *et al.,* 2001).

The results of this study showed that water-borne nickel (at levels, applied here) does not change serum Ca concentration in common carp. However, a decreasing trend in serum Ca is somewhat evident in different treatments of nickel. Serum Ca concentration at the highest concentration of nickel (2.910 mg/L) showed a change compared to the control group but this change was not statistically significant. The route of uptake for essential or even non-essential elements (such as cadmium, zinc, nickel, copper, etc.) from water is the same as that of Ca. Therefore, it is expected that there is a possible interaction between nickel and Ca in uptake through the gills, although the present study did not show any significant change. In the studies of Knox *et al.* (1982) who examined different levels of copper and zinc in the diet of rainbow trout (*Oncorhynchus mykiss*) and Grosell *et al.* (2004) who examined changes in copper concentration in water, there was no significant difference in serum calcium concentration. Berntssen *et al.* (2003) also reported no change in serum Ca levels of Atlantic salmon (*Salmo salar*) fed with cadmium supplementation compared with the control group. Overall, antagonistic effects between the elements on Ca metabolism in common carp may have inhibited some of Ca uptake from the water, although no significant negative effect of nickel was observed here.

However, another study showed that feeding red drum, *Sciaenops ocellatus*, with a minimum level of zinc can increase Ca levels (Gatlin *et al.,* 1991). There are also several reports of decreased plasma Ca concentrations due to the presence of lead, cadmium, and copper in water (Dhanapakiam and Ramasamy, 2001; Pizent *et al.,* 2003; Alves and Wood, 2006). In the present study, the amount of Ca was reduced to some extent, therefore, the development of hypocalcemia under the influence of water-soluble nickel in common carp is somewhat predictable. Hypocalcemia may not be far from expectation as the duration of nickel exposure increases or the concentration changes (using higher concentrations). In fish, hypocalcemia can occur as a result of competition between metal ions and Ca for absorption through gills or competition for replacement in bone structure. In this regard, we can refer to the study conducted by Muramoto (1981), showing that cadmium ions can affect the metabolism of bone tissue and damage its structure and even lead to hypocalcemia. In addition, Zohouri *et al.* (2001) reported hypocalcemia in rainbow trout due to cadmium exposure. Similarly, Malekpouri *et al.* (2011) declared that hypocalcemia is the most prominent effect of toxic elements in common carp.

In the present study, increases (significant or insignificant) in serum concentrations of P_i were observed in common carp following Ni^{2+} treatments. Bone tissue contains an organic bone

matrix with minerals. The organic matrix of bone tissue often contains collagen, hydroxyapatite, a hydroxylated polymer of calcium phosphate $Ca_{10}(PO_4)_6(OH)_2$ (Mondal *et al.,* 2016). Therefore, phosphate is one of the main components of bone tissue. Studies showed that toxic elements such as aluminum and cadmium can damage bone tissue (Rodríguez and Mandalunis, 2018). Nickel can also disrupt kidney function, upsetting the balance of calcium and phosphorus, as the reabsorption and excretion of these ions from the renal tubules may also be impaired (Guo *et al.,* 2016).

In the present study, the level of serum ALP in fish was elevated. This enzyme, acting as a transphosphorylase in alkaline environments, plays a key role in bone mineralization. Thus, the highest concentration of that can be found in bone osteoblast cells (Leung *et al.,* 1993). As previously stated, toxic metals destroy bone tissue and it can be concluded that bone destruction causes separation of this enzyme from bone cells and its release into the blood (He *et al.,* 2020). As a result, the concentration of ALP in the serum of fish increases owing to the effect of nickel. On the other hand, synthesis of this enzyme takes place in hepatocytes; hence any damage to the liver cells can disrupt the release of this enzyme into the bloodstream (Muriel, 1998).

ALP is one of the metalloenzymes containing zinc and magnesium in its structure and increasing the amount of zinc in the diet increases the activity of this enzyme (within essential concentration). This enzyme was observed in fish for the first time to determine the amount of zinc required in the diet of channel catfish, *Ictalurus punctatus* (Wilson and Poe, 1974). Besides, a significant decrease in the activity of this enzyme was observed in the plasma of *I. punctatus* and Nile tilapia (*Oreochromis niloticus*) fed with zinc-free diet (Huang *et al.,* 2015). In the present study, it is possible to attribute increase of this enzyme in fish serum following nickel exposure to enhancing effect of this metal in synthesis of ALP. However, in the present study, this increase was not significant in many cases. another research showed that ALP is raised in the serum, liver, and intestine of Nile tilapia following exposure to zinc, cadmium, copper, and lead (Atli and Canli, 2007).

Studies to date have shown that there is a direct linear relationship between serum ALP activity and serum phosphorus concentration. Overall, it is believed that physiological changes resulting from exposure to heavy metals can alter the activity of the enzyme alkaline phosphatase.

Bone is a connective tissue that is constantly changing, and these changes involve three types of cells in bone tissue; Osteoblasts (bone-forming cells), osteoclasts (multinucleated bone-reabsorbing cells), and osteocytes (enclosed within the bone matrix). Osteocytes appear to be involved in the preservation of bone material and the exchange of ions with body fluids. In fact, the number of osteocytes in histopathological observations indicates the amount of metabolic activity of bone

tissue (Aarden *et al.,* 1994). The number of osteocytes in low concentration of nickel treatments was elevated compared to the control treatment while number of osteocytes reduced and osteoclasts increased in high nickel levels. The increase in osteoclast cell density is evidence of an increase in serum mineral phosphorus (Koyama *et al.,* 2002; Mohammadi *et al*., 2018). Therefore, the increased ALP activity, indicates a disruption of bone formation, can itself increase serum phosphorus. It is already shown that number of osteoclasts in *Barbus grypus* with signs of bony deviation increased compared to healthy fish (Malekpouri *et al*., 2015).

In conclusion, nickel within the range used in this experiment appears to be somewhat toxic to fish. Comparing the results obtained for different parameters, it is shown that small amounts of nickel had no significant effect on the metabolic parameters of liver and bone, and intermediate treatments (0.580 and 1.750 mg/L) improved the function of bone and liver. Finally, the highest concentration of nickel treatment significantly reduced the number of parameters, which in a way reflects toxic effects of this element. Therefore, it can be concluded that this element in very small amounts has not played an effective role in the physiological and biochemical processes of carp but high concentrations can show toxic effects. Of course, to reveal such a dual effect, creating treatments with closer concentration intervals and longer experimental periods along with a careful examination of physiological stress parameters should be addressed.

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References

Aarden, E.M., Nijweide, P.J. and Burger, E.H., 1994. Function of osteocytes in bone. *Journal of Cellular Biochemistry*, 55(**3**), 287–299. https://doi.org/10.1002/jcb.240550304

Al–Attar, A.M., 2007. The influences of nickel exposure on selected physiological parameters and gill structure in the teleost fish *Oreochromis niloticus*. *Journal of Biological Sciences* 7(**1**), 77–85. https://doi.org/10.3923/jbs.2007.77.85

Ali, H., Khan, E. and Ilahi, I., 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, 2019(**4**), 6730305. https://doi.org/10.1155/2019/6730305.

Alves, L.C. and Wood, C.M., 2006. The chronic effects of dietary lead in freshwater juvenile rainbow trout (*Oncorhynchus mykiss*) fed elevated calcium diets. *Aquatic Toxicology*, 78(**3**), 217–232. https://doi.org/10.1016/j.aquatox.2006.03.005.

APHA, 1998. *Standard methods for the examination of water and wastewater*, 20th edition. American Public Health Association, American Water Work

Association, Water Environment Federation, Washington, DC, USA.

Athikesavan, S., Vincent, S., Ambrose, T. and Velmurugan, B., 2006. Nickel induced histopathological changes in the different tissues of freshwater fish, *Hypophthalmichthys molitrix* (Valenciennes). *Journal of Environmental Biology*, 27(**2Suppl**), 391–395.

Atli, G. and Canli, M., 2007. Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 145(**2**), 282–287. https://doi.org/10.1016/j.cbpc.2006.12.012.

Baghshani, H. and Shahsavani, D., 2013. Effects of lead acetate exposure on metabolic enzyme activities in selected tissues of common carp (*Cyprinus carpio*). *Comparative Clinical Pathology*, 22(**5**), 903–907. https://doi.org/10.1007/s00580-012-1497-3.

Benincá, C., Ramsdorf, W., Vicari, T., de Oliveira Ribeiro, C.A., de Almeida, M.I., De Assis, H.C.S. and Cestari, M.M., 2012. Chronic genetic damages in *Geophagus brasiliensis* exposed to anthropic impact in estuarine lakes at Santa Catarina coast-southern of Brazil. *Environmental Monitoring and Assessment*, 184(**4**), 2045–2056. https://doi.org/10.1007/s10661-011-2098-3.

Berntssen, M., Waagbø, R., Toften, H. and Lundebye, A.K., 2003. Effects of dietary cadmium on calcium homeostasis, Ca mobilization and bone deformities in Atlantic salmon (*Salmo salar* L.) parr. *Aquaculture Nutrition*, 9(**3**), 175–183. https://doi.org/10.1046/j.1365-2095.2003.00245.x.

Bogé, G., Leydet, M. and Houvet, D., 1992. The effects of hexavalent chromium on the activity of alkaline phosphatase in the intestine of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, 23(**3-4**), 247–260. https://doi.org/10.1016/0166-445X(92)90056-S.

Burtis, C.A., Ashwood, E.R. and Bruns, D.E., 2012. *Tietz textbook of clinical chemistry and molecular diagnostics-e-book.* Elsevier Health Sciences, Amesterdam, the Netherlands.

Chowdhury, M.J., Bucking, C. and Wood, C.M., 2008. Pre-exposure to waterborne nickel downregulates gastrointestinal nickel uptake in rainbow trout: indirect evidence for nickel essentiality. *Environmental Science and Technology*, 42(**4**), 1359-1364. https://doi.org/10.1021/es071889n.

Coz-Rakovac, R., Strunjak-Perovic, I., Hacmanjek, M., Popovic, N.T., Lipej, Z. and Sostaric, B., 2005. Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrarchus labrax*) in the North Adriatic Sea. *Veterinary Research Communications*, 29(**8**), 677–687. https://doi.org/10.1007/s11259-005-3684-z.

da Silva, A.O. and Martinez, C.B., 2014. Acute effects of cadmium on osmoregulation of the freshwater teleost *Prochilodus lineatus*: Enzymes activity and plasma ions. *Aquatic Toxicology*, 156, 161–168. https://doi.org/10.1016/j.aquatox.2014.08.009.

De Boeck, G., De Smet, H. and Blust, R., 1995. The effect of sublethal levels of copper on oxygen consumption and ammonia excretion in the common carp, *Cyprinus carpio*. *Aquatic Toxicology*, 32(**2-3**), 127–141. https://doi.org/10.1016/0166-445X(94)00086-6.

De Smet, H. and Blust, R., 2001. Stress responses and changes in protein metabolism in carp *Cyprinus carpio* during cadmium exposure. *Ecotoxicology and Environmental Safety*, 48(**3**), 255–262. https://doi.org/10.1006/eesa.2000.2011.

Dhanapakiam, P. and Ramasamy, V.K., 2001. Toxic effects of copper and zinc mixtures on some haematological and biochemical parameters in common carp, *Cyprinus carpio* (Linn). *Journal of Environmental Biology*, 22(**2**), 105–111.

Dreyfuss, J., Geyer, J., Stamper, M.A., Baldessari, A. and Lewbart, G.A., 2014. Zinc toxicosis in a brook trout, *Salvelinus fontinalis* Mitchill. *Journal of Fish Disease*, 37(**4**), 397–399. https://doi.org/10.1111/jfd.12130.

Eisler, R. and Jacknow, J., 1985. *Toxaphene hazards to fish, wildlife, and invertebrates: A synoptic review.* Contaminant Hazards Reviews Report 4; Biological Report 85(1.4). U.S. Department of the Interior Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD, USA.

Fırat, Ö. and Kargın, F., 2010. Individual and combined effects of heavy metals on serum biochemistry of Nile tilapia *Oreochromis niloticus*. *Archives of Environmental Contamination and Toxicology*, 58(**1**), 151–157. https://doi.org/10.1007/s00244-009-9344-5.

Fiske, C.H. and Subbarow, Y., 1925. The colorimetric determination of phosphorus. *Journal of Biological Chemistry*, 66, 375–400.

Fossati, P. and Prencipe, L., 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*, 28(**10**), 2077–2080. https://doi.org/10.1093/clinchem/28.10.2077.

Funakoshi, T., Kuromatsu, K. and Kojima, S., 1996. Effect of nickel on enzymatic activities in the mouse pancreas. *Research Communications in Molecular Pathology and Pharmacology*, 92(**2**), 245–252.

Gatlin III, D.M., O'Connell, J.P. and Scarpa, J., 1991. Dietary zinc requirement of the red drum, *Sciaenops ocellatus*. *Aquaculture*, 92, 259–265. https://doi.org/10.1016/0044-8486(91)90027-5.

Gornall, A.G., Bardawill, C.J. and David, M.M., 1949. Determination of serum proteins by means of the biuret

reaction. *Journal of Biological Chemistry*, 177(**2**), 751-766. https://doi.org/10.1016/S0021-9258(18)57021-6.

Grosell, M., McDonald, M.D., Walsh, P.J. and Wood, C.M., 2004. Effects of prolonged copper exposure in the marine gulf toadfish (*Opsanus beta*). II: copper accumulation, drinking rate and Na+/K+–ATPase activity in osmoregulatory tissues. *Aquatic Toxicology*, 68(**3**), 263–275. https://doi.org/10.1016/j.aquatox.2004.03.007.

Guo, H., Chen, L., Cui, H., Peng, X., Fang, J., Zuo, Z., Deng, J., Wang, X. and Wu, B., 2016. Research advances on pathways of nickel–induced apoptosis. *International Journal of Molecular Sciences*, 17(**1**), 10. https://doi.org/10.3390/ijms17010010.

He, S., Zhuo, L., Cao, Y., Liu, G., Zhao, H., Song, R. and Liu, Z., 2020. Effect of cadmium on osteoclast differentiation during bone injury in female mice. *Environmental Toxicology*, 35(**4**), 487–494. https://doi.org/10.1002/tox.22884.

Huang, F., Jiang, M., Wen, H., Wu, F., Liu, W., Tian, J. and Yang, C., 2015. Dietary zinc requirement of adult Nile tilapia (*Oreochromis niloticus*) fed semi–purified diets, and effects on tissue mineral composition and antioxidant responses. *Aquaculture*, 439, 53–59. https://doi.org/10.1016/j.aquaculture.2015.01.018.

Janbakhsh, S., Hosseini Shekarabi, S.P. and Shamsaie Mergan, M., 2018. Nutritional value and heavy metal content of fishmeal from the Southwest Caspian Sea. *Caspian Journal of Environmental Sciences*, 16(**4**), 307–317. https://doi.org/10.22124/IJES.2018.3200.

Javed, M., 2013. Chronic effects of nickel and cobalt on fish growth. *International Journal of Agriculture and Biology*, 15(**3**), 575–579.

Jiraungkoorskul, W., Upatham, E.S., Kruatrachue, M., Sahaphong, S., Vichasri-Grams, S. and Pokethitiyook, P., 2003. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). *Environmental Toxicology*, 18(**4**), 260–267. https://doi.org/10.1002/tox.10123.

Justino, C.I.L., Duarte, K.R., Freitas, A.C., Panteleitchouk, T.S.L., Duarte, A.C. and Rocha–Santos, T.A.P., 2016. Contaminants in aquaculture: Overview of analytical techniques for their determination. *Trends in Analytical Chemistry*, 80, 293–310. https://doi.org/10.1016/j.trac.2015.07.014.

Kaviani, E.F., Naeemi, A.S. and Salehzadeh, A., 2018. Short term effects of zinc oxide nanoparticles on hematological parameters and metabolic enzymes of juvenile Caspian trout (*Salmo trutta caspius*). *Iranian Scientific Fisheries Journal*, 26(**5**), 43-50, in Persian. https://doi.org/10.22092/ISFJ.2017.114914.

Kaviani, F.E., Naeemi, A.S. and Salehzadeh, A., 2020. Acute toxicity and effects of titanium dioxide nanoparticles (TiO2 NPs) on some metabolic enzymes and hematological indices of the endangered Caspian trout juveniles (*Salmo trutta caspius* Kessler, 1877). *Iranian Journal of Fisheries Sciences*, 19(**3**), 1253-1267. https://doi.org/10.22092/ijfs.2019.119319.

Khodaei, S., Naeemi, A.S. and Nazarhaghighi, F., 2019. Effects of copper oxide nanoparticles on the tissue and metabolic enzymes of liver and kidney of common carp (*Cyprinus carpio*). *Veterinary Researches and Biological Products*, 32(**3**), 82-96, in Persian. https://doi.org/10.22092/VJ.2018.116362.1388.

Knox, D., Cowey, C.B. and Adron, J.W., 1982. Effects of dietary copper and copper: zinc ratio on rainbow trout *Salmo gairdneri*. *Aquaculture*, 27(**2**), 111–119. https://doi.org/10.1016/0044-8486(82)90130-2.

Koyama, H., Nakade, O., Takada, Y., Kaku, T. and Lau, K.H.W., 2002. Melatonin at pharmacologic doses increases bone mass by suppressing resorption through down-regulation of the RANKL-mediated osteoclast formation and activation. *Journal of Bone and Mineral Research*, 17(**2**), 1219–1229. https://doi.org/10.1359/jbmr.2002.17.7.1219.

Kubrak, O.I., Husak, V.V., Rovenko, B.M., Poigner, H., Mazepa, M.A., Kriews, M., Abele, D. and Lushchak, V.I., 2012. Tissue specificity in nickel uptake and induction of oxidative stress in kidney and spleen of goldfish *Carassius auratus*, exposed to waterborne nickel. *Aquatic Toxicology*, 118-119, 88-96. https://doi.org/10.1016/j.aquatox.2012.03.016.

Kumar, N.R., Solanki, R. and Kumar, J.I.N., 2012. Geochemistry of Sabarmati River and Kharicut Canal, Ahmedabad, Gujarat. *International Journal of Environmental Science and Technology*, 2(**4**), 1909-1919. https://doi.org/10.6088/ijes.00202030074.

Lall, S.P. and Lewis-McCrea, L.M., 2007. Role of nutrients in skeletal metabolism and pathology in fish — an overview. *Aquaculture*, 267(**1-4**), 3-19. https://doi.org/10.1016/j.aquaculture.2007.02.053.

Leung, K.S., Fung, K.P., Sher, A.H., Li, C.K. and Lee, K.M., 1993. Plasma bone–specific alkaline phosphatase as an indicator of osteoblastic activity. *Journal of Bone and Joint Surgery British*, 75(**2**), 288–292. https://doi.org/10.1302/0301-620X.75B2.8444951.

Lohner, T.W., Reash, R.J. and Williams, M., 2001. Assessment of tolerant sunfish populations (*Lepomis* sp.) inhabiting selenium–laden coal ash effluents: 2. Tissue biochemistry evaluation. *Ecotoxicology and Environmental Safety*, 50(**3**), 217–224.

https://doi.org/10.1006/eesa.2001.2098.

Malekpouri, P., Moshtaghie, A.A., Kazemian, M. and Soltani, M., 2011. Protective effect of zinc on related parameters to bone metabolism in common carp fish (*Cyprinus carpio* L.) intoxified with cadmium. *Fish Physiology and Biochemistry*, 37(**1**), 187–196. https://doi.org/10.1007/s10695-010-9430-7.

Malekpouri, P., Mesbah, M. and Rezaie, A., 2015. Aetiology of skeletal deformity in a *Barbus grypus* (Heckel, 1843). fish: clinical and radiological studies. *Comparative Clinical Pathology*, 24(**1**), 201–206. https://doi.org/10.1007/s00580-014-1932-8.

Mazeaud, M.M., Mazeaud, F. and Donaldson, E.M., 1977. Primary and secondary effects of stress in fish: some new data with a general review. *Transactions of the American Fisheries Society*, 106(**3**), 201–212. https://doi.org/10.1577/1548-8659(1977)106<201:PASEOS>2.0.CO;2.

McGeer, J.C., Szebedinszky, C., McDonald, D.G. and Wood, C.M., 2000. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout. 1: Iono–regulatory disturbance and metabolic costs. *Aquatic Toxicology*, 50(**3**), 231–243. https://doi.org/10.1016/S0166-445X(99)00105-8.

Misra, M., Rodriguez, R.E. and Kasprzak, K.S., 1990. Nickel induced lipid peroxidation in the rat: correlation with nickel effect on antioxidant defense systems. *Toxicology*, 64(**1**), 1–17. https://doi.org/10.1016/0300-483X(90)90095-X.

Moeinnejad, S., Naeemi, A.S., Nazarhaghighi, F. and Nasr, E., 2019. Sub-acute effects of copper oxide nanoparticles on enzymes and hematological parameters of the juvenile beluga (*Huso huso*). *Veterinary Researches and Biological Products*, 32(**1**), 84-96, in Persian. https://doi.org/10.22092/vj.2018.120724.1438.

Mohammadi, N., Hosseini Shekarabi, S.P. and Shamsaie Mehrgan, M., 2018. Effect of dietary phytase and wheat bran on some growth performances and phosphorus absorption function of common carp (*Cyprinus carpio*) fry. *Iranian Scientific Fisheries Journal*, 27(**3**), 75-83, in Persian. https://doi.org/10.22092/ISFJ.2018.117016.

Molina, R., Moreno, I., Pichardo, S., Jos, A., Moyano, R., Monterde, J.G. and Cameán, A., 2005. Acid and alkaline phosphatase activities and pathological changes induced in Tilapia fish (*Oreochromis* sp.) exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. *Toxicon*, 46(**7**), 725–735. https://doi.org/10.1016/j.toxicon.2005.07.012.

Mondal, B., Mondal, S., Mondal, A. and Mandal, N., 2016. Fish scale

derived hydroxyapatite scaffold for bone tissue engineering. *Materials Characterization*, 121, 112–124. https://doi.org/10.1016/j.matchar.2016.09.034.

Moorehead, W.R. and Biggs, H.G., 1974. 2–Amino–2–methyl–1–propanol as the alkalizing agent in an improved continuous–flow cresolphthalein complexone procedure for calcium in serum. *Clinical Chemistry*, 20(**11**), 1458–1460. https://doi.org/10.1093/clinchem/20.11.1458.

Muramoto, S., 1981. Vertebral column damage and decrease of calcium concentration in fish exposed experimentally to cadmium. *Environmental Pollution Series A, Ecological and Biological*, 24(**2**), 125–133. https://doi.org/10.1016/0143-1471(81)90074-X.

Muriel, P., 1998. Nitric oxide protection of rat liver from lipid peroxidation, collagen accumulation, and liver damage induced by carbon tetrachloride. *Biochemical Pharmacology*, 56(**6**), 773–779. https://doi.org/10.1016/S0006-2952(98)00220-2.

Ng, J.C., Wang, J. and Shraim, A., 2003. A global health problem caused by arsenic from natural sources. *Chemosphere*, 52(**9**), 1353–1359. https://doi.org/10.1016/S0045-6535(03)00470-3.

Nikinmaa, M., 2014. *An introduction to aquatic toxicology*. Elsevier Inc., Oxford, UK. https://doi.org/10.1016/C2012-0-07948-3.

OECD, 1994. *OECD Guidelines for the Testing of Chemicals.* Organization for Economic Cooperation and Development. OECD, Paris, France.

Öner, M., Atli, G. and Canli, M., 2008. Changes in serum biochemical parameters of freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. *Environmental Toxicology and Chemistry*, 27(**2**), 360–366. https://doi.org/10.1897/07-281R.1.

Pane, E.F., Glover, C.N., Patel, M. and Wood, C.M., 2006. Characterization of Ni transport into brush border membrane vesicles (BBMVs). isolated from the kidney of the freshwater rainbow trout (*Oncorhynchus mykiss*). *Biochimica et Biophycica Acta (BBA)-Biomembranes*, 1758(**1**), 74–84. https://doi.org/10.1016/j.bbamem.2005.12.003.

Pizent, A., Jurasović, J. and Telišman, S., 2003. Serum calcium, zinc, and copper in relation to biomarkers of lead and cadmium in men. *Journal of Trace Elements in Medicine and Biology*, 17(**3**), 199–205. https://doi.org/10.1016/S0946-672X(03)80026-3.

Ptashynski, M.D., Pedlar, R.M., Evans, R.E., Baron, C.L. and Klaverkamp, J.F., 2002. Toxicology of dietary nickel in lake whitefish (*Coregonus clupeaformis*). *Aquatic Toxicology*, 58(**3-4**), 229–247. https://doi.org/10.1016/S0166-445X(01)00239-9.

Reid, S.G., Bernier, N.J. and Perry, S.F., 1998. The adrenergic stress response in fish: control of catecholamine storage and release. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 120(**1**), 1-27. https://doi.org/10.1016/S0742-8413(98)00037-1.

Reitman, S. and Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(**1**), 56–63. https://doi.org/10.1093/ajcp/28.1.56.

Rivarola, V.A. and Balegno, H.F., 1991. Effects of 2,4–dichlorophenoxyacetic acid on polyamine synthesis in Chinese hamster ovary cells. *Toxicology Letters*, 56(**1-2**), 151–157. https://doi.org/10.1016/0378-4274(91)90101-B.

Rodríguez, J. and Mandalunis, P.M., 2018. A review of metal exposure and its effects on bone health. *Journal of Toxicology*, 2018, 4854152. https://doi.org/10.1155/2018/4854152.

Romslo, I., Sagen, N. and Haram, K., 1975. Serum alkaline phosphatase in pregnancy: I. A comparative study of total, l-phenylanine-sensitive and heat-stable alkaline phosphatase at 56 degrees C and 65 defrees C in normal pregnancy. *Acta Obstetricia et Gynecologica Scandinavica*, 54(**5**), 437–442. https://doi.org/10.3109/00016347509157106.

Sanchez, W., Palluel, O., Meunier, L., Coquery, M., Porcher, J.M. and Aït-Aïssa, S., 2005. Copper–induced oxidative stress in three–spined stickleback: relationship with hepatic metal levels. *Environmental Toxicology and Pharmacology*, 19(**1**), 177–183. https://doi.org/10.1016/j.etap.2004.07.003.

Sauliutė, G. and Svecevičius, G., 2015. Heavy metal interactions during accumulation via direct route in fish: a review. *Zoology and Ecology*, 25(**1**), 77–86. https://doi.org/10.1080/21658005.2015.1009734.

Shamsaie Mehrgan, M., Hosseini Shekarabi, S.P., Hassanzadeh, B. and Seyed Alhosseini, S.H., 2019. Seasonal variations of cadmium and lead concentrations in water, sediments, and tissues of fish in Mellat artificial lake, Iran. *Journal of Human Environment and Health Promotion*, 5(**4**), 177–182. https://doi.org/10.29252/jhehp.5.4.6.

Sharma, D.C. and Davis, P.S., 1980. Effect of methylmercury on protein synthesis in liver of the European carp *Cyprinus carpio*. *Indian Journal of Experimental Biology*, 18(**9**), 1054-1055.

Sreedevi, P., Sivaramakrishna, B., Suresh, A. and Radhakrishnaiah, K., 1992. Effect of nickel on some aspects of protein metabolism in the gill and kidney of the freshwater fish, *Cyprinus carpio* L. *Environmental Pollution*, 77(**1**), 59–63.

https://doi.org/10.1016/0269-7491(92)90158-7.

Taghavizadeh, M., Hosseini Shekarabi, S.P., Shamsaie Mehrgan, M. and Rajabi Islami, H., 2020. Efficacy of dietary lysophospholipids (Lipidol™) on growth performance, serum immuno-biochemical parameters, and the expression of immune and antioxidant-related genes in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 525, 735315. https://doi.org/10.1016/j.aquaculture.2020.735315.

Vinodhini, R. and Narayanan, M., 2009. The impact of toxic heavy metals on the hematological parameters in common carp (*Cyprinus carpio* L.). *Journal of Environmental Health Science and Engineering*, 6(**3**), 23–28.

Wilson, R.P. and Poe, W.E., 1974. Nitrogen metabolism in channel catfish, *Ictalurus punctatus*—III. Relative pool sizes of free amino acids and related compounds in various tissues of the catfish. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 48(**4**), 545-556. https://doi.org/10.1016/0305-0491(74)90134-5.

Yu, M.H., 2000. E*nvironmental Toxicology: Impacts of Environmental Toxicants on Living Systems.* Lewis Publishers, Boca Raton, FL, USA.

Zhou, Q., Yang, N., Li, Y., Ren, B., Ding, X., Bian, H. and Yao, X., 2020. Total concentrations and sources of heavy metal pollution in global river and lake water bodies from 1972 to 2017. *Global Ecology Conservation*, 22, e00925. https://doi.org/10.1016/j.gecco.2020.e00925.

Zohouri, M.A., Pyle, G.G. and Wood, C.M., 2001. Dietary Ca inhibits waterborne Cd uptake in Cd–exposed rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 130(**3**), 347–356. https://doi.org/10.1016/s1532-0456(01)00260-5.