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RESEARCH PAPER

Cadmium and Lead Alter the Antioxidant and Osmoregulation Systems in the Erythrocyte of Fish (*Oreochromis niloticus*)

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Abstract

The erythrocytes deliver oxygen to all around body cells that make them extremely vital for the metabolism. Metals are transferred through the blood, meaning the erythrocytes face with metals continuously. The present study intended to explore the responses of osmoregulation (Na^+/K^+-ATPase, Mg^{2+}-ATPase, Ca^{2+}-ATPase) and antioxidant system parameters (CAT, SOD, GPX, GR, GST, tGSH, rGSH, GSSG, GSH/GSSG) in the erythrocytes of the Nile tilapia (*Oreochromis niloticus*) following acute (20 µM, 2 d) and sub-chronic (10 µM, 20 d) Cd and Pb exposures. ATPases were more sensitive compared to the antioxidant system parameters. All ATPase activities increased significantly in acute and sub-chronic metal exposures. Variations in antioxidant system parameters were recorded with metal and duration differences, Pb causing more alterations. GSH metabolism was mostly altered, especially after sub-chronic Pb exposure, though CAT and GPX activities decreased. Acute Pb exposure also caused a decrease in GPX activity and an increase in GST activity. There were differences in GSH/GSSG ratios between acute and sub-chronic Pb exposures. Cd caused few alterations in the antioxidant system parameters (GSSG and GSH/GSSG ratio decrease and GST increase). The present study demonstrated that the osmoregulation and antioxidant systems of the erythrocyte are sensitive to metal exposures and may provide useful data about the stress fish face.

Keywords: Antioxidant, GSH, ATPase, Metal, *Oreochromis niloticus.*

Kadmiyum ve Kurşunun Balık (*Oreochromis niloticus*) Eritrositlerinde Antioksidan ve Ozmoregülasyon Sistemlerini Değiştirmesi

Özet

Eritrositlerin tüm vücut hücrelerine oksijen taşımaları metabolizma için onları son derece hayati yapar. Metallerin kan yollarıyla taşınmaları eritrositlerin metallerle sürekli karşı karşıya kalması anlamındadır. Bu nedenle bu çalışma Nil çuprası (*Oreochromis niloticus*) eritrositlerinde akut (20µM, 2 gün) ve sub-kronik (10 µM, 20 gün) Cd ve Pb etkisi sonrası ozmoregülasyon (Na^+/K^+-ATPase, Mg^{+2}-ATPase, Ca^{+2}-ATPase) ve antioksidan sistem parametrelerinin (CAT, SOD, GPX, GR, GST, tGSH, rGSH, GSSG, GSH/GSSG) cevabını araştırmayı amaçlamaktadır. ATPaz'ların antioksidan sistem parametreleri ile karşılaştırıldığında daha duyarlı oldukları görülmüştür. Bütün ATPaz aktiviteleri akut ve sub-kronik metal etkilerinde önemli düzeyde artmıştır. Metal ve etki sürelerine bağlı olarak antioksidan sistem parametrelerinde farklılık olduğu ve Pb'nin daha çok etkiye neden olduğu gözlenmiştir. En çok GSH metabolizmasının özellikle de sub-kronik Pb etkisi sonrası değişmesine karşın CAT ve GPX aktiviteleri de azalmıştır. Akut Pb etkisi de azalan GPX ve artan GST aktivitelerine neden olmuştur. Akut ve sub-kronik Pb etkileri arasında GSH/GSSG oranı bakımından farklılıklar vardır. Cd, akut etki sonrası GSSG düzeyi ve GSH/GSSG oranındaki azalış ile sub-kronik etki sonrası GST aktivitesindeki azalış dışında antioksidan sistem parametrelerinde çok etkili olmamıştır. Bu çalışma, eritrosit osmoregülasyon ve antioksidan sistem parametrelerinin metal etkilerine duyarlı olduğunu ve stresle yüzleşen balıklar hakkında yararlı veriler sağlayabileceğini belirtmektedir.

Anahtar Kelimeler: Antioksidan, GSH, ATPaz, Metal, *Oreochromis niloticus.*

Introduction

Biomonitoring of aquatic biota has still considerable interest depending upon the increased several anthropogenic facilities such as agricultural and industrial etc. (Heath, 1987). In natural waters, Pb was in the range of 0.6–120 µg/L though Cd levels were <0.1µg/L. However, these metal concentrations can be many folds of their natural levels at sites where excess amounts of anthropogenic inputs exist (Mance,

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1987; Jorgensen, 2012). In this respect, biochemical parameters have gain importance to characterize the effects of metals on aquatic organisms. Enzymatic and non-enzymatic parameters of antioxidant system have significant functions to prevent the hazardous effects of reactive oxygen species (superoxide anion radical, hydroxyl radical and hydrogen peroxide) resulted by oxidative stress after metal exposures (Barata *et al.*, 2005; Atli and Canli, 2010). ROS can be able to oxidize proteins, lipids and nucleic acids followed by damage to cell structure or even cell death (Nagalakshmi and Prasad, 1998; Cao *et al.*, 2010). Cd and Pb known as redox inactive metals can cause major antioxidant depletion in the cell, thiol containing ones specially (Pinto *et al.*, 2003). Significant antioxidant enzymes include catalase (CAT; eliminates hydrogen peroxide to water), superoxide dismutase (SOD; converts superoxide anion radical into hydrogen peroxide), glutathione peroxidase (GPX; detoxifies hydrogen and organic peroxides), glutathione reductase (GR; reduces oxidized glutathione (GSSG) to reduced glutathione (rGSH) and glutathione S-transferase (GST; catalyzes glutathione (GSH) and xenobiotic conjugation). In addition, a non-enzymatic antioxidant GSH acting as a reductant in with xenobiotic conjugation (Pena-Llopis *et al.*, 2001). Increased GSSG values and also decreased GSH/GSSG ratio have been known as oxidative stress indicators (Ramos-Vasconcelos and Hermes-Lima, 2003; Sevgiler *et al.*, 2007). Various responses of antioxidant enzyme activities have been observed in the aquatic organisms exposed to metals in both field and laboratory experiments and these have been shown to be either induced or inhibited by metals depending on the dose, the species or the route of exposure (Sanchez *et al.*, 2005; Atli and Canli, 2008; Atli and Canli, 2010). ATPases are important membrane bound enzymes depending upon their pivotal role in transporting ions across the cell and maintaining osmotic balance in fish. Na^+/K^+-ATPase functions in the active electrolyte transport across the membrane. Mg^{2+}-ATPase acts an important role in oxidative phosphorylation and ionic transport and is responsible for the trans-epithelial regulation of Mg^{2+} ions (Parvez *et al.*, 2006). Ca^{2+}-ATPase involves in Ca^{2+} ions remove from the cytoplasm low Ca^{2+} level protection (Watson and Beamish, 1981). Previous studies have been demonstrated that ATPase activities altered considerably in fish, suggesting their potential as sensitive biomarkers for the assessment of the membrane rupture (Canli and Stagg, 1996; Grosell *et al.*, 2004; Atli and Canli 2007, 2011a).

Alterations of enzymatic activities in red blood cells can be a good signal for environmental stress factors due to their responsibility for transport and excretion of important nutrients and also to be influenced by a variety of factors (Roche and Boge, 1996). A freshwater fish *O. niloticus* is shown to be a good tolerant with a strong immune system and sensitive bio-indicator organism based on the studies suggesting the toxicity and physiological effects of several metals including Cu and Pb on this species (Almeida *et al.*, 2002; Atli *et al.*, 2006). Nevertheless, there is still required data showing the relation of metal toxicity with several biomarker responses to understand the physiological and biochemical role of these systems in this sensitive species. Therefore, the current study was attempted to investigate both antioxidant and osmoregulatory system response in Nile tilapia after acute and sub-chronic Cd and Pb exposures.

Materials and Methods

Experimental Protocol

One year old *O. niloticus* (Perciformes: Cichlidae) were obtained from Çukurova University fish culturing pools. They were transferred to the laboratory illuminated for 12 h with fluorescent lamps (daylight 65/80 W) for acclimatization (20± 1°C) for one month. The experiments were carried out in glass aquaria (40 x 40 x 100 cm) containing 100 L contaminated test solution or only dechlorinated test water for controls. The chemical quality of water was tested daily using a multiple measurement apparatus (Orion-5-star). During experiments, the tap water pH, total hardness (with EDTA titration method), alkalinity (acidimetry method) and conductivity were measured as 7.65±0.02, 330.4±2.63 mg $CaCO_3$/L and 228.0±6.83 mg $CaCO_3$/L, 605.0±2.39 µS/cm, respectively. The aquariums were aerated to saturate with oxygen using air pumps and measured as 5.93±0.28 mg O_2/L. Fish were fed (2% of their weight) with commercial fish feed (Pinar Sazan, Izmir, Turkey) just 1 h before the cleaning of aquaria every 2 days.

Fish were exposed to 20 µM Cd ($CdCl_2.H_2O$) and Pb ($PbNO_3$) for 2 days (acute) and 10 µM Cd and Pb for 20 days (sub-chronic). Two control groups were used for acute and sub-chronic durations. A total of eight fish were used for each group as duplicate aquaria. Mean length (16.2±0.29 cm) and weight (70.3±3.43 g) of fish did not differ significantly ($P>0.05$) among different exposure groups and controls. Metal concentrations in the exposure medium were controlled using Atomic Absorption Spectrophotometer (Perkin Elmer 3100). Metal levels in the tap water were below the detection limits as 0.001 and 0.015 µg/mL for Cd and Pb, respectively. Accuracy of the AAS and validity of measurements were tested with a reference material (TORT 1 lobster hepatopancreas, National Research Council, Canada). Mean values and standard deviations of the reference material were 5% of the ranges.

At the end of each experimental period, fish were killed by transaction of spinal cord, according to the decision of the Ethic Committee of Çukurova University. Fish blood was obtained from the caudal vessel (Congleton and La Voie, 2001) and centrifuged at 3000 g (4 ^oC) for 5 min. Red blood cells were

washed 3 times with 0.9 % NaCl. 20 mM Tris–HCl (pH 8.0) was added in hemolysates (1:3 v/v) and kept frozen for 2–3 h. After centrifugation at 5000 g for 10 min, the supernatants were stored at -80 °C until the analysis (Marcon and Filho, 1999). All chemicals used in this study were obtained from Sigma or Merck (Germany).

Enzyme Activity and Glutathione Assay

CAT activity was measured at 240 nm for 1 min by monitoring the H_2O_2 decrease according to the method of Bergmeyer *et al.* (1974). It was calculated as µmol H_2O_2/mg prot./min., respectively. SOD activity was measured by the indirect method involving the inhibition of cytochrome c reduction at 550 nm for 1 min (McCord and Fridovich, 1969). The SOD activity was calculated as Unit/mg prot. GPX and GR activities were measured by the NADPH decrease though GST activity was calculated by NADPH increase at 340 nm for 1 min. GPX, GR and GST activities were given as µmol/mg prot./min. according to the method of Livingstone *et al.* (1992), Carlberg and Mannervik (1975) and Habig *et al.* (1974), respectively. For GSH analysis, samples were centrifuged at 9,500 g for 5 min (+4 °C) after 10% 5-sulfocyclic acid addition. Total oxidized and reduced GSH were measured according to the method of Griffith (1980) at 412 nm for 1 min and given as µmol GSH/mg prot./min. Brief explanations of methods were described in our previous study (Atli and Canli, 2010). ATPase activity was measured by inorganic phosphate analysis (Atkinson *et al.*, 1973) at 390 nm described briefly in our previous research (Atli and Canli, 2011a). KH_2PO_4 was used as Pi standard. The assays were carried out in triplicate. The total protein levels were determined by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Statistical Analysis

Statistical analysis of data (Mean±Standard error) was carried out using SPSS 15.0 statistical package program (SPSS, Chicago, IL). One-way Anova was used to compare data and significant differences ($P<0.05$) were reanalyzed by Duncan tests to determine which individual group was significantly different from controls. All data from and acute and sub-chronic exposures were compared individually.

Results

ATPase Activity

In acute duration (Figure 1A); all ATPase activities increased after Cd exposure (Mg^{2+}-: 3.46±0.52; Na^+/K^+-: 0.93±0.19; Ca^{2+}-ATPase: 1.10±0.17 µmol Pi/mg prot./h.) in contrast to their control values (Total-: 0.74±0.18; Mg^{2+}-: 0.57±0.17; Na^+/K^+-: 0.42±0.05; Ca^{2+}-ATPase: 0.29±0.04 µmol Pi/mg prot./h.). However, only Na^+/K^+- increased (1.11±0.17 µmol Pi/mg prot./h.) after Pb exposure.

In subchronic duration (Figure 1B); similar to acute Cd exposure, all ATPase activities (Mg^{2+}-: 2.50±0.15; Na^+/K^+-: 1.57±0.38; Ca^{2+}-ATPase: 0.71±0.07 µmol Pi/mg prot./h.) increased after Cd exposure compared to their control values (Total-: 1.22±0.24; Mg^{2+}-: 0.57±0.14; Na^+/K^+-: 0.38±0.05; Ca^{2+}-ATPase: 0.41±0.06 µmol Pi/mg prot./h.). Nevertheless, Pb exposure caused only Mg^{2+}-ATPase (1.30±0.28 µmol Pi/mg prot./h.) increase.

Antioxidant Enzyme Activities

In acute duration (Figure 2A); CAT and SOD activities were not altered by metal exposures ($P>0.05$) though only decreased GPX (13.7±2.81 nmol/mg prot./min.) and increased GST (3.49±0.29

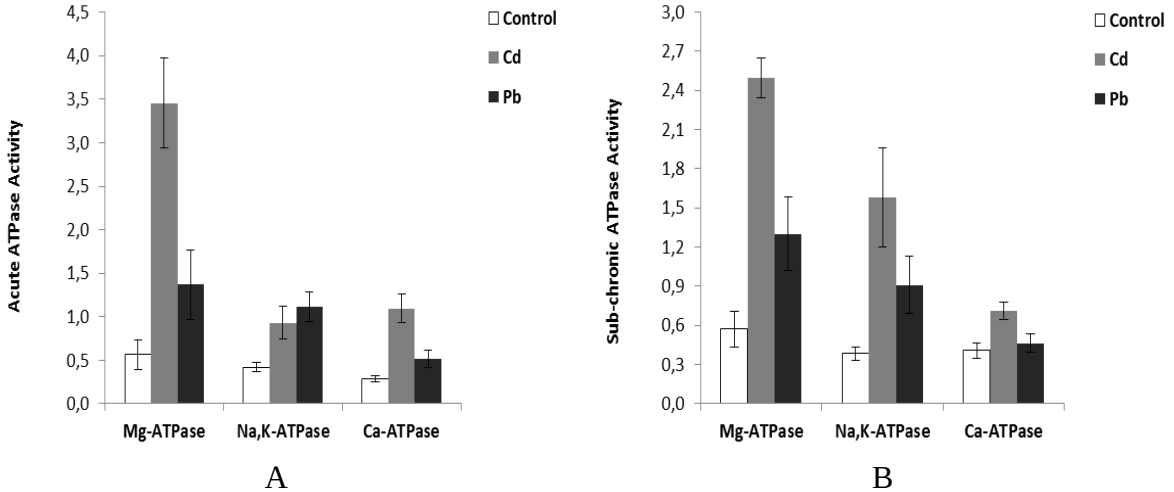

Figure. 1 ATPase activities in the erythrocytes of *O. niloticus* upon Cd and Pb exposures during acute (A) and sub-chronic (B) durations. The units of ATPase activities are given as µmol Pi/mg prot./h. Data are expressed as mean (n=8)±standard error and asterisks indicate significant differences ($P<0.05$) between control and metal exposed groups.

nmol/mg prot./min.) activities were observed after Pb exposure compared to their control values (GPX: 24.3±1.86 nmol/mg prot./min.; GST: 2.56±0.25 nmol/mg prot./min.). There was no change in GR activity upon both metal exposures (P>0.05).

In subchronic duration (Figure 2B); Cd exposure caused a decrease in GST activity (1.59±0.20 nmol/mg prot./min.) compared to the control group (2.84±0.28 nmol/mg prot./min.). CAT decrease (6.42±0.50 µmol H_2O_2/mg prot./min.) and GST increase (5.17±0.25 nmol/mg prot./min.) were observed after Pb exposure in contrast to the control group (CAT: 8.86±0.52 µmol H_2O_2/mg prot./min.; GST: 2.84±0.28 nmol/mg prot./min.).

GSH Levels

In acute duration (Figure 3A); tGSH and rGSH levels did not change following metal exposures (P>0.05). However Cd caused decreases in GSSG level (32.9±3.82 µmol/mg prot./min.) and GSH/GSSG ratio (2.56±0.41) in comparison with their control values (GSSG: 21.3±3.43 µmol/mg prot./min.; GSH/GSSG: 5.08±0.80). Pb also decreased the ratio of GSH/GSSG (1.91±0.47).

In subchronic duration (Figure 3B); tGSH (215.4±17.4 µmol/mg prot./min.), rGSH level (207.9±18.8 µmol/mg prot./min.) and GSH/GSSG ratio (42.2±9.53) increased after Pb exposure compared to the control group (tGSH: 150.7±10.1 µmol/mg prot./min.; rGSH: 129.8±8.18 µmol/mg prot./min.; GSH/GSSG: 3.99±0.59). However Pb caused a decrease in GSSG level (GSSG: 3.35±0.91 µmol/mg prot./min.) compared to the control group (GSSG: 20.3±2.89 µmol/mg prot./min.).

Discussion

Previous studies based on both field and laboratory experiments demonstrated the disruptive effects of Cd and Pb in a wide range of fish metabolism including biochemical, physiological and hormonal system parameters (Heath, 1987; Zikic *et al.*, 2001; Oner *et al.*, 2008; Atli and Canli, 2011a). It is known that the metal toxicity varies according to the metal structure as their essential or nonessential nature. In addition, antioxidant system response can differ due to their redox active and inactive nature which Cd and Pb are considered among the redox inactive metals (Pinto *et al.*, 2003). Osmoregulatory and antioxidant systems have pivotal functions as being key mechanisms where metal toxicity occurs (Grosell *et al.*, 2004; Atli and Canli, 2010; Eroglu *et al.*, 2015). Fish erythrocytes has shown to provide useful tool in several aspects; a) specific tissue for oxidative stress because of their membrane structure rich in long chain n-3 polyunsaturated fatty acids which can be a target for oxidation by toxicants b) based on its oxygen transport function, erythrocytes is an important site for the ROS production, c) they come into contact with metals directly due to its transportation role in carrying chemicals to other tissues (Heath, 1987; Roche and Boge, 1996; Ruas *et al.*, 2008). In light of these, the objective of the current study based on the varied responses of both ATPase and antioxidant system parameters in the erythrocytes of tilapia due to the Cd and Pb effects in different exposure durations.

Recent data demonstrated main findings that (I) ATPases were responded more than antioxidant system parameters, (II) when considering antioxidant system, GSH metabolism and also including GPX and GST were affected mostly after Pb exposure, (III) Cd was found more effective on ATPase activity alteration as increases at all conditions.

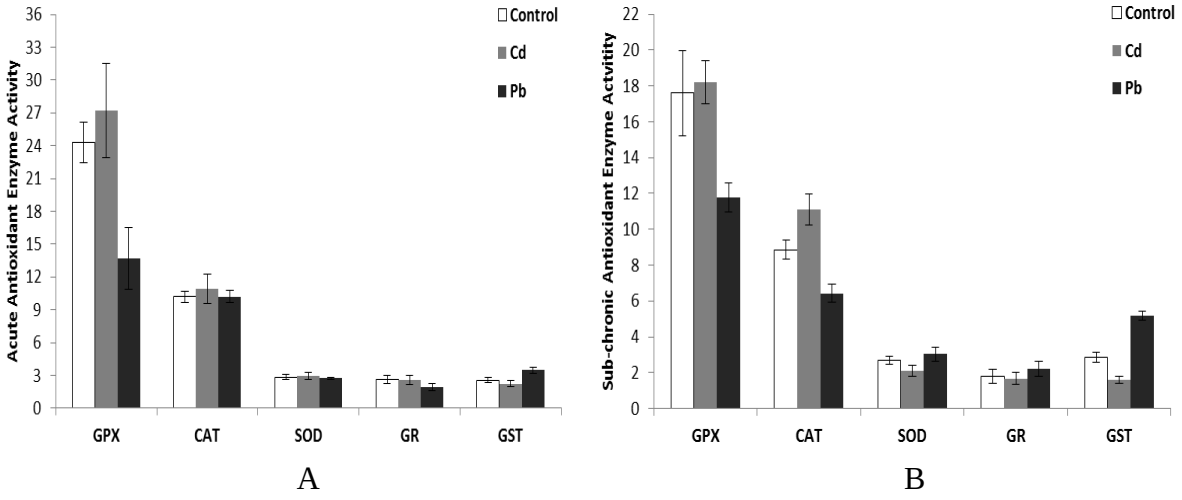

Figure. 2 Antioxidant enzyme activities in the erythrocytes of *O. niloticus* upon Cd and Pb exposures during acute (A) and sub-chronic (B) durations. The units of enzyme activities are given as follows; GPX: nmol/mg prot./min., CAT: µmol H_2O_2/mg prot./min., SOD: U/mg prot., GR: nmol/mg prot./min., GST: nmol/mg prot./min. See Fig.1 for details.

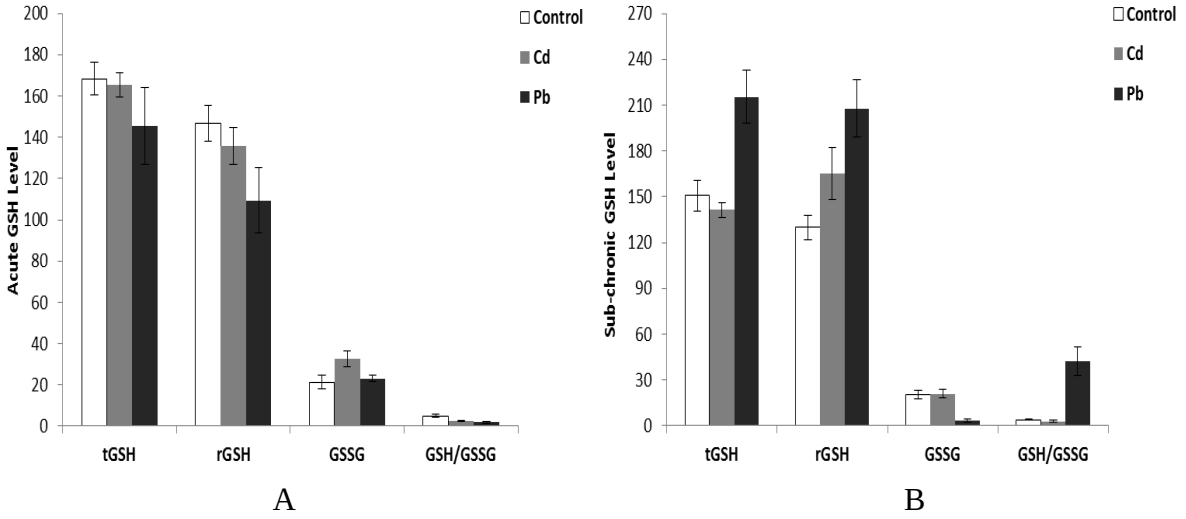

Figure. 3 GSH levels in the erythrocytes of *O. niloticus* upon Cd and Pb exposures during acute (A) and sub-chronic (B) durations. The units of GSH levels (tGSH, RGSH and GSSG) and GSH/GSSG are given as µmol/mg prot./min. and ratio, respectively. See Fig.1 for details.

ATPase Activity

Data indicated that Cd increased the all ATPase activities after both acute and sub-chronic durations, though Pb caused increases only in Na^+/K^+-ATPase activity after acute and Mg^{2+}-ATPase activities upon sub-chronic exposure. It should be emphasized that erythrocytes were also found to be very sensitive to metal exposures beside the general acceptance of other tissue specificity to ATPase activities such as gill (Mg^{2+}- and Na^+/K^+) and muscle (Ca^{2+}-ATPase).

It is interesting to observe the general increased activities of erythrocytes ATPases in *O. niloticus* despite their decreases in most previous studies (Atli and Canli, 2007; Atli and Canli, 2011a; Baysoy *et al.*, 2013). This could be explained by tissue specific differences in ATPase enzyme kinetics and also metal dose and duration can determine the ATPase response. Possible reasons of increased ATPase activities could be related with a period of adaptation processes and/or increased number of enzyme molecules or turnover rates of the enzyme to maintain the ion flux during metal toxicity. One of the significant hazardous effects of metals is the disturbances of the ion balances in the vital osmoregulatory organs (Larsson *et al.*, 1985; Canli and Stagg, 1996; Grosell *et al.*, 2004). It was also mentioned that ion levels were significantly changed in the different tissues of *O. niloticus* after acute and chronic metal treatments (Atli and Canli, 2011b). High dietary Pb exposure lead an increase in intestine Na^+/K^+-ATPase activity of *Oncorhynchus mykiss* associated with the compensation to balance the ion levels (Alves and Wood, 2006). Gill Na^+/K^+-ATPase activity was also increased in *O. niloticus* after 20 µM Pb exposure (Atli and Canli, 2007), concurring similar response with the present Pb data. Nevertheless, insignificant Pb effects in some cases of this study might be the result of antioxidant system role particularly GSH in preventing the Pb toxicity. Generally, Cd is accepted as its negative influence on the ATPase activities, though variable responses exist in Cd exposed fish (De la Torre *et al.*, 2000; Atli and Canli, 2007). Triggered other physiological mechanisms can be the reason for such variation to counteract the osmoregulatory system impairment. Alteration in antioxidant system parameters following sub-chronic Pb exposure might support this observation in this study. Stimulated kidney Mg^{2+}-ATPase activity associated with the detoxification role of the tissue was also found after Cu exposure in Nile tilapia. It was demonstrated that metal exposures can activate the homeostatic control mechanisms in these situations (McGeer *et al.*, 2000; Grosell *et al.*, 2004). Impaired gill ATPase activities were also recorded in *Cyprinus carpio* after Cd exposure showing Na^+/K^+-ATPase inhibition and Mg^{2+}-ATPase activation. Present data revealed the osmoregulatory disturbance linked with the membrane permeability and active ion uptake alterations by increased ATPase activities. Thus activity of ATPases has still created interest due to their high importance in the reflection of ion-regulatory capacity status.

Antioxidant Enzyme Activities

Antioxidant enzymes did not change after metal exposures, in several cases. Further, Pb exposure seemed to cause more changes than Cd on several antioxidant enzymes such as CAT decrease after sub-chronic duration with GPX and GST alterations after both acute and sub-chronic durations. It is interesting to see that the SOD and CAT were not altered by metal exposures except for one case, despite their significant role as first line antioxidant enzymes against metal toxicity. Other mechanisms such as

GSH metabolism could be one of the possible reasons for this situation which is significantly affected particularly by sub-chronic Pb exposure. Also redox active nature of metals might be another reason that they act in an indirect way not through Fenton and Haber-Weiss reactions. Nevertheless, unchanged GR activity based on the data is in accordance with previous data (Saglam *et al.,* 2014; Eroglu *et al.,* 2015).

GST, GPX and GSH levels are the most affected parameters particularly by Pb exposure and increased GSH levels were observed mostly after sub-chronic duration. Duration effect was also emphasized once again on the basis of getting different responses of the system. Reduced GSH increase and decreased GSSG levels accompanying to this could be the result of enhanced GST activity that uses the GSH as a substrate. It is also shown in our previous data that sub-chronic Pb exposure caused significant alterations in serum biochemistry in *O. niloticus* (Atli *et al.,* 2015). Min and Kang (2008) recorded an increase in GST activity after fungicide benomyl exposure in the liver of *O. niloticus* which caused plasma alanine- and aspartate-amino transferase increases. This was associated with this study showing enhanced GST activity and also linked with increased ALT and AST levels in serum of *O. niloticus* in our recent published data (Atli *et al.,* 2015). Increased GST activity could be due to protection against oxidative stress which this phase II enzyme functions in conjugation and detoxification of xenobiotics as a significant biomarker. Increases in antioxidant enzyme activities could also be correlated to the coping with the oxidative stress followed by the metal exposure.

In general, it might be said that effective response was given by non-enzymatic antioxidant parameters with an exception of GPX and GST linked with changes in GSH levels. On the other hand, decreased CAT activity after sub-chronic Pb exposure possibly occurred due to the high hydrogen peroxide concentration which led to the enzyme inhibition and/or direct binding of metal on the enzyme structure. Increased high amount of hydrogen peroxide concentration could be the possible reason of the decreased GPX activity which its one of the major role is hemoglobin protection from the oxidative breakdown (Mills, 1959). GPX activity can be also considered complementary to CAT activity that was also supported with the present data with their different responses in different tissues. This variation could be occurred depending upon the nature, concentration and different localization of ROS (peroxisomes for CAT and cytosol for GPX) (Orbea *et al.,* 2000; Barata *et al.,* 2005). Nevertheless the trend of these two enzyme activities was in similar way as decreases due to the enhanced oxidative stress. On the other hand, Ruas *et al.,* (2008) also recorded the increased GPX activity in the blood of cichlid species from the polluted site. The authors indicated the oxidative stress induction in the blood of several fish species under chronic metal exposures and also complex responses to metals is probably due to specific sensitivity of species and/or potential of antioxidants. Variable effects of metals could be dependent on the induction of different antioxidant/pro-oxidant responses depending on their ability to produce ROS which was also mentioned by Barata *et al.,* (2005). These results were also in accordance with our previous studies (Atli *et al.,* 2006; Atli and Canli, 2010; Eroglu *et al.,* 2015). It is also important to declare that similar pattern of sub-chronic Pb effects was observed on both hematological and antioxidant system including enzymatic and non-enzymatic parameters. This point may be a reflection of providing a protection by antioxidant system as a result of hematological changes.

Differences in the response of erythrocyte SOD to different toxicants were found as decreases in *Cyprinus carpio* (Dimitrova *et al.,* 1994) and increases in *Dicentrarchus labrax* (Akbarsha *et al.,* 2001). Nevertheless, SOD activity did not change after metal exposures in acute and sub-chronic protocols in the current study, suggesting the high tolerance capacity of tilapia species. Metals are known to affect the relative proportion of the saturated and unsaturated fatty acids in erythrocytes membranes leading hemolysis and decreased thiol content (Gabryelak *et al.,* 2000). ROS production can be insufficient to produce oxidative damage since there was a lack of antioxidant enzyme response particularly at least during acute duration and Cd exposure in this study. Similarly, Woo *et al.* (2006) observed neither significant changes in antioxidant enzymes nor lipid peroxidation in fish *Rhabdosarga sarba* upon exposure to toxicants. In addition, Roche and Boge (1996) observed unchanged SOD and CAT activities in the red blood cell of sea bass *Dicentrarchus labrax* after Cu exposure, though increases and decreases were also observed after Cr and Zn exposures. They concluded that decreased activities seemed to be balanced by other antioxidant enzyme activities. In another study, highest GST activity in the scorpion fish *Scorpaena porcus* (L.) blood was noted in the most polluted site in Ukraine (Rudneva *et al.,* 2012) which shows similarities with the current data. They also linked the lack or unchanged enzyme activities with the summary effects of the pollutant mixture in the field and concluded that erythrocyte GST measurement might provide sensitive tool for anthropogenic pollution and the response of biomarkers was not uniform.

Cd impact was indicated as changes in SOD and CAT activities in the erythrocytes of fish *Carassius auratus gibelio* accompanied by hemoglobin decrease (Zikic *et al.,* 2001). They also indicated that enzymes can response differently upon to the exposure duration which is also in accordance with our data. Variation of SOD and CAT response can be due to the differences in species, tissues and also doses due to

the capacity limit to counteract with metal toxicity. One can conclude that the estimation of the changes in these enzyme activities could provide useful data for biomonitoring the aquatic environment to early detection of the pollution. Unchanged antioxidant enzyme activities could be attributed to the significant changes particularly in GPX, GST and GSH levels. Therefore it can be concluded that sufficient response was provided with these parameters for this condition. Lima *et al.* (2006) indicated that increased GSH levels in *O. niloticus* exposed to a contaminated effluent appear to be an antioxidant adaptation to chronic exposure. Our previous data also showed the significant inductions of the liver GSH levels in Nile tilapia upon Cu and Cd treatments (Atli and Canli 2008). It was suggested that GSH primarily provide a rapid protection against oxidative stress through GSH redox cycle or directly detoxifying the ROS (Barata *et al.*, 2005, Ruas *et al.*, 2008).

In this context, it has still gain importance to investigate these parameters due to their responses in different ways depending upon the differences in metal type, their nature and also duration of the exposure. The data emphasized that using a set of biomarkers function in different systems including defense and specific damage parameters can give opportunity to comment the fish health under stress in a wide range. From another point, it has still retained vital importance to study with the sensitive and valid biomarker in the bioindicator organisms for evaluating the ecological status of the aquatic biota.

Conclusion

Observed results present the impairment of enzyme activities as key physiological roles in significant vital systems could cause fish metabolism dysfunctions. It seems that initiation of integrated responses associated with compensation and adaptation of metabolic systems to tolerate the metal toxicity. Therefore, it gains importance to select biomarkers as indicator of homeostatic mechanisms based on their physiological and biochemical significance in assessment of environmental quality. According to the data, ATPase and GSH related antioxidant parameters can be considered as important index of the osmoregulatory and antioxidant system status during metal toxicity.

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