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# Oxidative stress status of freshwater fish (*Oreochromis niloticus*) exposed to cadmium in differing calcium levels

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## Abstract

Xenobiotic exposures cause an alteration in oxidative stress in fish and metals are one of the most toxic xenobiotics present in the environment. Thus, freshwater fish (*Oreochromis niloticus*) were exposed to Cd in different calcium levels (30, 60 and 120 mg/L), resembling the waters with different hardness. Experiments were conducted in 2 different durations, denoted acute (25 μM Cd, 3 days) and chronic (5 μM Cd, 30 days) and the serum of fish was used to measure the oxidative status. For this aim, total oxidant status (TOS) and total antioxidant status (TAS) were measured in the serum and associated oxidative stress indicator values (OSI) were calculated. Data showed that Cd exposures, at all calcium levels, did not cause any fish mortality or changes in feeding behaviour. Likewise, the oxidative stress parameters did not change significantly ($p>0.05$) among controls. However, the mean TOS values between controls and Cd-exposed fish differed significantly ($p<0.05$), as there were increases in TOS values in fish. Similarly, the mean TAS values between controls and Cd-exposed fish also differed significantly ($p<0.05$), as there were decreases in TAS values. OSI values significantly increased in Cd-exposed fish, suggesting oxidative stress. Data showed that significant alterations in the measured parameters were seen more at the lower calcium levels, emphasizing the protective roles of calcium ions against the toxic effects of Cd. This study demonstrated the effective and fast determinations of metal toxicity in fish regarding oxidative stress status and suggested that be used in environmental monitoring.

**Keywords:** Metal, Cadmium, Calcium, Fish, Oxidative, Antioxidant.

## 1. Introduction

The aquatic environments are contaminated by different xenobiotics worldwide, causing serious environmental problems. Therefore, these systems must be controlled using different methods, including the biological responses of aquatic animals. In particular, rivers and lakes seem to be more affected by industrial or domestic effluents, as they have small volumes and are located in human settlement areas [1,2]. Metals or metal-containing products are a group of xenobiotics which threaten the aquatic biota, causing serious problems [3-5].

Several biomarker molecules belonging to different metabolic systems are used to detect the stress that fish face after metal exposures, naming them as "early warning systems" before hazardous effects occur [6,7,8,9]. There is a natural balance between free radicals and antioxidant defence systems in aerobic organisms. If this balance shifts towards free radicals, oxidative stress occurs. There are very important enzymes or non-enzymatic substances synthesized in cells to eliminate oxidant substances either taken from outside (e.g. heavy metals and pesticides) or produced by the organism itself (reactive oxygen derivatives, etc.). One of the important antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase. On the other hand, substances such as alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), beta-carotene, flavonoid, coenzyme Q and lycopene have non-enzymatic antioxidant properties. In addition to measuring many enzymatic and non-enzymatic parameters one by one, scientists may achieve quick and easy methods to determine oxidative stress by measuring TOS and TAS together with OSI values [10,11].

Thus, the present study was undertaken to investigate the effects of cadmium on the oxidative stress status of freshwater fish (*O. niloticus*). Experiments were carried out in acute (3 days 25 μM Cd) and chronic (30 days, 5 μM Cd) durations at different calcium levels (30, 60 and 120 mg Ca/L). Then, the TOS and TAS values in the serum were measured and associated OSI values (arbitrary unit) were calculated to determine the oxidative stress status of fish.

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## 2. Material and Method

### 2.1. Experimental Protocol

Freshwater fish (*O. niloticus*) are continuously cultured in the pools of the Fisheries and Aquatic Sciences Faculty at Cukurova University (Adana, Turkiye). Fish were transferred from the pools to the ecotoxicology laboratory situated in the Biology Department and placed in glass aquariums (40x40x100 cm) to let them accustomed to the new conditions for one month. The physical and chemical conditions in the laboratory are presented in Table 1. The experimental room was air-conditioned (21±1.0 °C) and enlightened with fluorescent lamps (daylight 65/80 W) for 12 hours. The tab water used for the experiment had a pH value of 8.0±0.3 and a total hardness of 231±2.2 $CaCO_3$, oxygen levels of 5.7±0.87 mg $O_2$/L. Fish were fed once a day with fish food supplied from Pinar Yem (Izmir, Turkiye). The mean length (15.4±1.33cm) of fish used in the experiments did not differ significantly (P>0.05) among controls and exposure groups. The chemicals used in the present study were supplied from Merk Company (Germany).

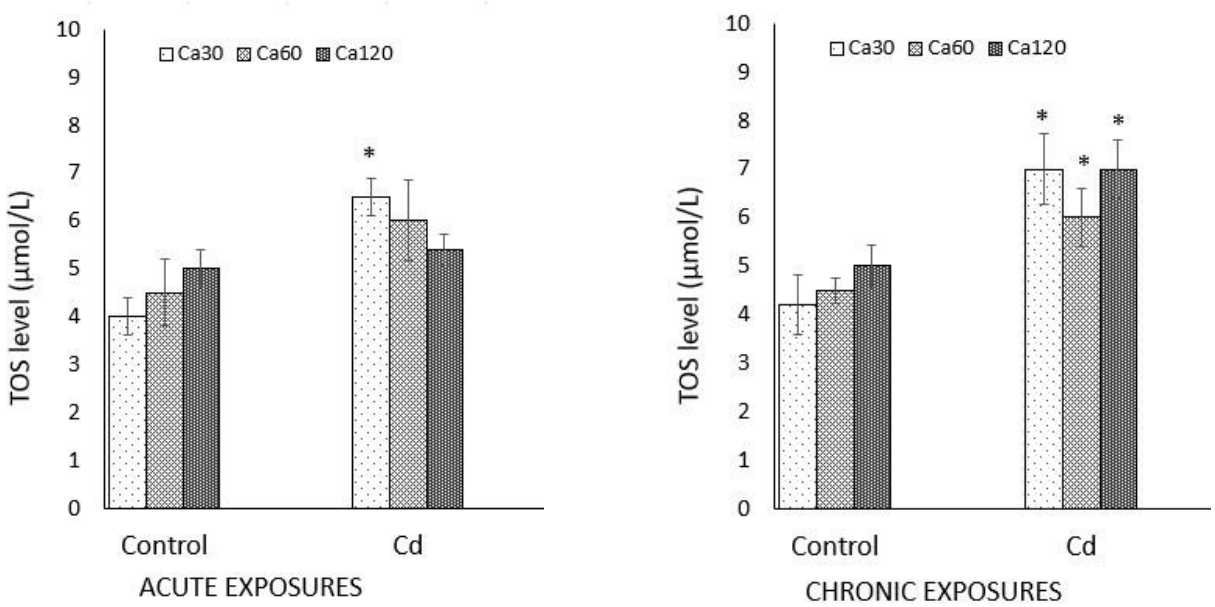
**Table 1.** Data (mean±sd) show the physical and chemical conditions of 3 control waters depending on their calcium levels ($Ca_{30}$, $Ca_{60}$ and $Ca_{120}$) during the experiments

| Parameters | $Ca_{30}$ | $Ca_{60}$ | $Ca_{120}$ |
|---|---|---|---|
| Calcium (mg Ca/L) | 28.3±0.56 | 58.3±0.86 | 115.8±1.52 |
| Tot. hardness (mg $CaCO_3$/L) | 98.6±6.76 | 192±7.54 | 355±16.5 |
| Alkalinity (mg $CaCO_3$/L) | 99.0±2.34 | 98.9±4.23 | 103±5.22 |
| Conductivity (µS/cm) | 170±5.55 | 303±4.34 | 610±9.97 |
| Oxygen (mg/L) | 5.90±0.28 | 6.02±0.26 | 6.21±0.28 |
| Temperature (°C) | 20.5±0.33 | 21.0±0.55 | 19.9±0.25 |
| pH | 7.08±0.07 | 7.10±0.06 | 6.99±0.04 |

Experiments were carried out using spring waters supplied by Nestle company (Nestle Pure Life), as they had very low Ca levels (30 mg/L). This water was used as background calcium levels and then, calcium levels of the water were increased to 60 and 120 mg Ca/L by adding $CaCl_2$ appropriately, to obtain soft, mild and hard water. Thus, there were 3 controls with different calcium levels ($Ca_{30}$, $Ca_{60}$ $Ca_{120}$). Finally, fish were exposed to Cd (as CdCl) for 3 days (25 µM Cd) for acute experiments and 30 days (5 µM Cd) for chronic experiments. For each exposure concentration and control, 6 fish (a total of 60 fish) were used and the media were changed every 3rd day to supply clean water and re-load Cd and calcium. After acute and chronic durations, fish were removed from the aquariums and killed by the transaction of the spinal cord (permission of the Ethic Committee of Cukurova University). Blood samples were immediately taken by puncture of the caudal vessel and centrifuged at 3000 × g (Hettich Universal 30 RF, Germany) for 5 min (4 °C) to obtain the serum. Then, the serum samples were frozen at -85 °C (Esco UUS-480A) for later measurements of TAS and TOS values. Total oxidant and total antioxidant levels in serum were measured by spectrophotometer (Shimadzu UV-1800) using Rel Assay Diagnostic kits. The oxidative effects of cadmium and/or calcium were determined by the method of Erel [12,13].

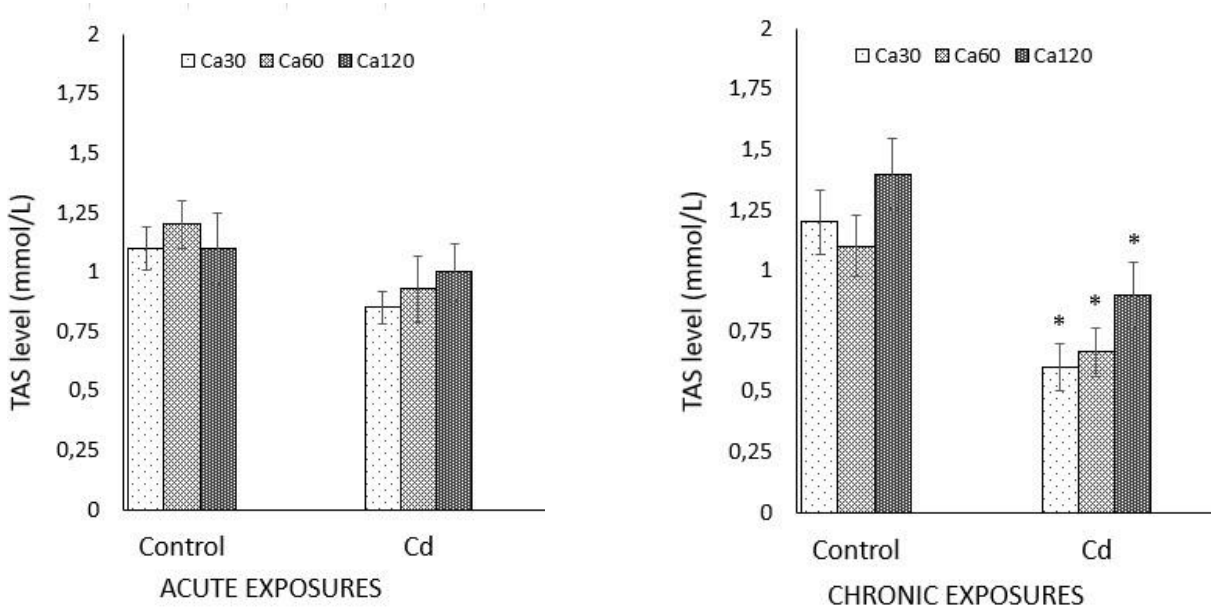
*Statistical Analyses*

A statistical package program (SPSS 20) was performed to analyze the data. Acute and chronic exposures were handled separately and results were accepted if $p<0.05$. First, data were checked for their normality. Considering the results of this test, One-way ANOVA test or Kruskal-Wallis One-Way ANOVA test was applied to the data to estimate differences among control and cadmium exposure groups. Data showing significant differences were re-tested by Tukey or Mann-Whitney U test to determine group differed from the individual control group. Mean values and associated standard errors were shown in figures (Figures 1-3), indicating the results of statistical tests.




(A) (B)

**Figure 1.** Total oxidant levels (mean±se) in the serum of fish (*O. niloticus*) exposed to cadmium in differing calcium levels after acute (A) and chronic (B) exposures. Asterisks indicate significant ($p<0.05$) differences between controls and associated cadmium exposures




(A) (B)

**Figure 2.** Total antioxidant levels (mean±se) in the serum of fish (*O. niloticus*) exposed to cadmium in differing calcium levels after acute (A) and chronic (B) exposures. Asterisks indicate significant ($p<0.05$) differences between controls and associated cadmium exposures

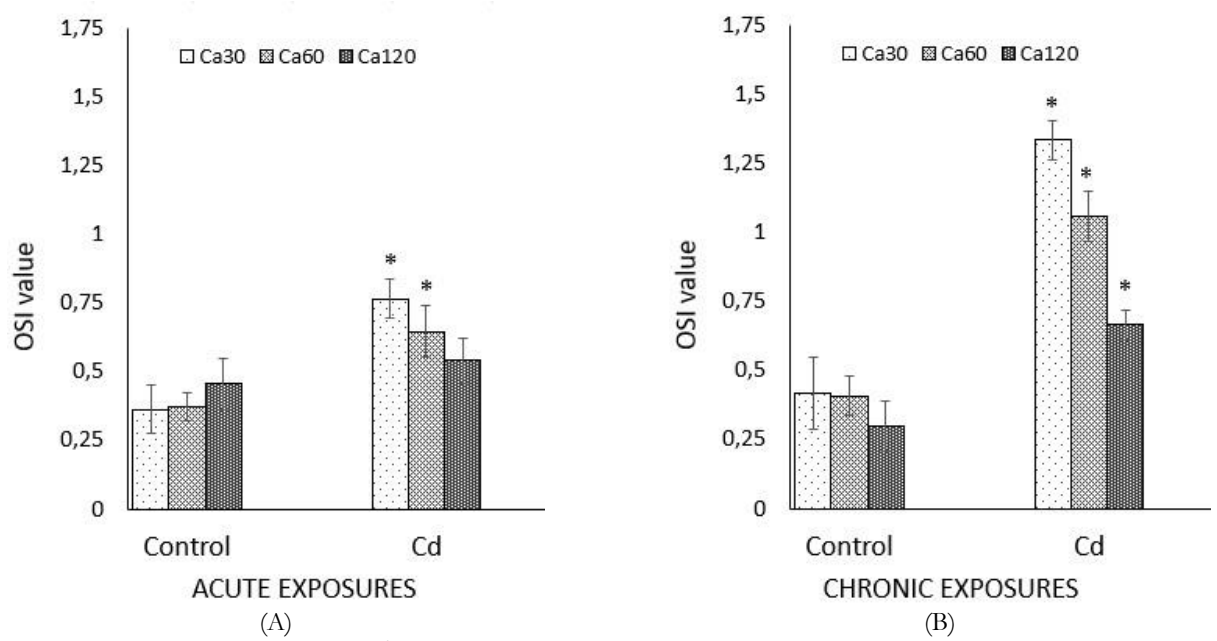



**Figure 3.** Oxidative status Index values (mean±se) in the serum of fish (*O. niloticus*) exposed to cadmium in differing calcium levels after acute (A) and chronic (B) exposures. Asterisks indicate significant ($p<0.05$) differences between controls and associated cadmium exposures.

## 3. Results and Discussion

There were no fish deaths after acute and chronic Cd exposures at any calcium level. Likewise, fish did not show any symptoms of appetite loss or abnormality in swimming. This indicates that the exposure conditions and the levels of Cd were not too high and relevant to the environmental situations. The Nile tilapia is known for its tolerance and therefore they are accepted as a good species to study toxicology. This suitability is possibly due to their adaptive capacity to different waters or strong osmoregulation systems to different waters with different chemical qualities [14,15]. In the present study, the results of the measurements showed that TAS levels in the serum of O. niloticus decreased significantly ($p<0.05$) in chronic exposures, though there was no significant change ($p>0.05$) in acute exposures (Figure 1). Oppositely, data showed that TOS levels in the serum of fish increased significantly ($p<0.05$) in both acute and chronic exposures (Figure 2). The changes in TAS and TOS levels also altered the OSI values, as there were significant increases ($p<0.05$) in OSI values in both acute and chronic exposures (Figure 3). When data were investigated in terms of calcium levels, it can be seen that the most significant alterations occurred at the lower calcium levels, emphasizing the protective roles of calcium against Cd toxicity. Nevertheless, the levels of TAS, TOS or OSI did not differ significantly ($p>0.05$) among controls ($Ca_{30}$, $Ca_{60}$ $Ca_{120}$), suggesting calcium in water does not have predominant effects on these parameters.

There are considerable amounts of literature data regarding the toxic effects of metals in freshwater fish. Metals seem to affect many physiological metabolisms in fish, changing their activities or levels [1,2,4]. Literature data and our previous studies also showed that metals, including Cd, had toxic effects in different systems of *O. niloticus* such as the antioxidant and osmoregulation systems [16-24]. The above literature data generally demonstrated that chemical or physical qualities of exposure waters such as hardness, salinity and temperature also play direct roles in metal toxicity. Likewise, one of our previous studies also demonstrated that the toxic effects of Cu were more evident in waters with lower conductivity values [25]. In freshwaters, the conductivity levels mainly depend on water calcium levels. Therefore, the authors suggested that environmental monitoring studies in freshwater should the conductivity levels to take into account for meaningful comparison. Because the aquatic systems are generally the final stops of most contaminants discharged by man-made activities, they must be checked often for their metal contaminations. Cadmium is a nonessential metal with no known biological metabolisms and occurs in the environment as a result of anthropogenic activities or from natural sources. After discharge to the aquatic environment, Cd can be accumulated by fish, exerting adverse effects on different metabolisms. Studies also showed that Cd can cause haematological effects, impaired Ca homeostasis, histological and morphological deformation [26-28]. Hazardous effects of Cd in ion-regulating tissues (e.g. kidney, gills and intestine) can lead imbalance of ion concentration in extracellular fluid and an alteration of the osmoregulatory capacity of fish [4,29]. All kinds of toxic effects of metals in different metabolic systems eventually can cause oxidative stress in fish

## 4. Conclusion

The present data showed that Cd exposures cause an increase in TOS levels and a decrease in TAS levels, affecting OSI values in both acute and chronic durations. However, calcium alone (controls) did not cause any significant change in studied parameters, though calcium played significant roles in the toxic effects of Cd. In this context, data suggested that the toxic effects of metal may be much higher in soft waters compared to hard waters and thus, water hardness levels should be taken into account in environmental monitoring studies.

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