Global Journal of Science Frontier Research: C
Biological Science
Volume 20 Issue 6 Version 1.0 Year 2020
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Effect of Sub Lethal Cadmium Concentrations on Serum Biochemical Parameters in Seabream (*Sparusaurata*) Fingerlings

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Abstract- Cadmium (Cd) is a heavy metal with limited biological function, is widely distributed in the aquatic environment as a result of natural and anthropogenic activities. The effect of exposure on gilthead sea bream (Sparusaurata) fingerlings to sub-lethal concentrations of Cd was evaluated as levels of Cd content. The main objective of this study was to determine the effects of cadmium at sub-lethal concentrations (1 and 3 µg/L) on serum biochemical parameters including enzymes, i.e. alkaline phosphatase (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT), glucose, triglyceride, cholesterol and total protein in seabream (S. aurata) fingerlings, sea bream were exposed to cadmium at intervals of 1, 15, and 30 days, selected parameters were evaluated. Triglyceride and cholesterol decreased transiently at day 15 and then increased at day 30.Total protein, AST, ALT and ALP increased linearly by time and Cd concentration. This investigation suggests that growth and serum biochemical parameters could be used as important and sensitive biomarkers in ecotoxicological studies concerning the effects of metal contamination and fish health.

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GJSFR-C Classification: *FOR Code: 300799p*

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I. Introduction

Various environmental conditions of marine ecosystems may cause several changes in the serum biochemical parameters of the sea bream (*Acanthopagruslatus*)fish[1].Heavy metals have received considerable attention due to their toxicity and accumulation. The increase in industrial activities, as well as in the use of chemical fertilizers and pesticides in the agricultural practice during the past few decades led to the marked rise of heavy metals in the environment. Fish serum may reflect the status of many biochemical processes in metabolism. Heavy metals, as environmental stressors, may alter serum biochemical parameters in fishes[2].Cadmium is a common environmental pollutants has a direct correlation with fish serum biochemical abnormalities[3].Some of the physiological effects of chronic exposure to waterborne cadmium at sub-lethal levels are manifested in the form of disturbances in respiration[4],[5],reduction in growth[6]disruption in whole-body or plasma ion regulation[7],[8],and changes in hematology, enzyme activity[9],[10],[11], and other blood parameters, such as glucose, total protein, triglyceride and cortisol that reveal the stress response in fish, the activity of total protein showed depletion. Glucose was significantly increased[1],[12],[13].Cadmium concentration at sub-lethal levels have been found to decrease in growth in juvenile and adult rainbow trout (*Oncorhynchus-mykiss*)[6],as well as to mortality and reduced growth in juvenile bull trout (*Salvelinus confluentus*)[14].and guppy (*Poecilia reticulate*)[15]. Serum enzymes such as alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) are important serum markers to study the health of animal species in question. The main objective of this study was to determine the effects of cadmium at sub-lethal concentrations (1 and 3 µg/L) on growth and serum biochemical parameters including enzymes (ALP, AST and ALT), glucose, triglyceride, cholesterol and total protein in sea bream(*S. aurata)*. Generally, an exposure concentration that is lower than LC_{50} is considered as sub-lethal. The preliminary experiment showed that 72 h-LC_{50} of cadmium was 9 µg/L. Therefore in this study, the sub-lethal doses of cadmium (1 and 3 µg/L) were determined according to 1/9th and 1/3th of the 72 h-LC_{50}.

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II. Materials and Methods

a) *Fish holding conditions and acclimation*

Seabream(*S.aurata*) fingerling, were collected during August 2014 from one farm at Mediterraneansea cost in Damietta city, matched for size (16.5±0.4 g; 10.5±0.3 cm), were transferred to the laboratory. They were kept in continuously aerated tanks (50 l). The physicochemical characteristics of experimental water were as shown in (Table 1). Temperature, DO (Dissolved Oxygen), TDS (total dissolved solids), and pH were monitored daily in all tanks. During an acclimation period of 2 weeks, the fish were handfed twice daily at random times. Any fish that showed abnormal behavior was removed immediately from the tanks.

b) *Exposure system*

Active groups of 20 fish were randomly transferred to 160l polyethylene exposure tanks with continuous aeration. The fish were exposed to: (i) control: nominally zero cadmium [actual measured 'in-tank' value: 0.051µg/L], (ii) low cadmium [1 µg/L] and

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(iii) high cadmium [3 μg/L] for 1, 15 and 30 days. Cadmium was added as $CdCl_2.H_2O$ (Merck, Germany), each with three replicates. The water was changed every two days to minimize metal loss and maintain the concentration of metal. The water quality parameters mentioned were assessed at collection days during the experimental period.

c) Sampling and biochemical processing

The fish were fasting for a 24-h period before sampling. Four fish were removed from each tank on days 1, 15 and 30. Fish blood samples were collected with a hypodermic syringe from the caudal vein. Blood samples were immediately kept in a refrigerator for four h. Serum was separated from cells by centrifugation of whole blood (10 min, 4000 g, 4°C) and stored at −48°C until the experimental assays. The levels of ALT, AST, ALP with concentrations of glucose, triglyceride, cholesterol and total protein in the serum were measured using a Gen Way biochemical analyzer.

d) Statistical analysis

Initially, the raw data were checked for normality of distribution by Kolmogorov- Smirnov tests. All values were expressed as means ± standard error. The analysis of differences between control and different sampling times in each exposure group and growth parameters was tested by one-way analysis of variance (ANOVA). The post hoc Duncan's multiple range tests was used among treatment means with SPSS 14. Significance was determined at $P < 0.05$.

III. Results

a) Serum biochemical parameters

After 15 days in both Cd- exposed groups, the concentration of glucose increased it reached 94.3±2.4mg/dl compared to 25.6±1.5mg/dl (control). This elevation in the glucose was transient and returned to the same level in the control group within 30 days(figure 1). After first and 15th days of exposure to different concentrations of Cd, the level of triglyceride decreased to its minimum value and reached to 285.3±6.5mg/dl (high Cd-exposed group) compared to511±8.7mg/dl (control group). These reductions were then followed by a rapid elevation in 30 days so that the level of triglyceride in serum in both Cd concentrations returned to the same level in the control group (figure 2). Serum cholesterol showed a significant reduction after first and 15th days in both Cd exposed groups and returned to the same level in the control group. In the first 15days, the cholesterol level decreased rapidly and reached to 256±10.3 mg/dl (low-dose) 192.6±1.6mg/dl (high-dose) compared to 328±8.2 mg/dl in control group but this reduction was transient (Figure 3). Total protein in serum exhibited a linear pattern and increased after 30 days. This elevation was not significant in 15 days but increased rapidly to 2.6±0.1g/dl (low-dose) and 3.8±0.1g/dl (high-dose) compared to 2.4±0.1 g/dl in control group after 30 days (Figure 4 & Table 2).

b) Serum enzymes

Both aspartate transaminase (AST) and alanine transaminase (ALT) activities exhibited a linear pattern and increased after 30 days (figures5&6). This increase was more remarkable in ALT activity for fish exposed to3 μg/l Cd so that after 30 days a 127% increase was observed. Following by a transient reduction, AST activity also increased after 30 days. Similarly, this elevation was greater in high concentration of Cd (3μg/L).The level of alkaline phosphatase (ALP) also exhibited remarkable increases in level from a mean control level, when the fish were exposed to the both sub-lethal concentrations of cadmium. This trend continued over time and with increasing Cd levels (figuer7).

c) Serum biochemical parameters

After 15 days in both Cd- exposed groups', the concentration of glucose increased and reached 94.3±2.4mg/dl compared to 25.6±1.5mg/dl (control). This elevation in the glucose was transient and returned to the same level in the control group within 30 days(figure1). After the first and 15th days of exposure to different concentrations of Cd, the level of triglyceride decreased to its minimum value and reached to 285.3±6.5mg/dl (high Cd-exposed group) compared to 511±8.7mg/dl (the control group). These reductions were then followed by a rapid elevation in 30 days so that the level of triglyceride in serum in both Cd concentrations returned to the same level in the control group (figure 2). Serum cholesterol showed a significant reduction after first and 15th days in both Cd exposed groups and returned to the same level in the control group. In the first 15days, the cholesterol level decreased rapidly and reached to 256±10.3 mg/dl (low-dose) 192.6±1.6mg/dl (high-dose) compared to 328±8.2 mg/dl in the control group but this reduction was transient (Figure 3). Total protein in serum exhibited a linear pattern and increased after 30 days. This elevation was not significant in 15 days but increased rapidly to 2.6±0.1g/dl (low-dose) and 3.8±0.1g/dl (high-dose) compared to 2.4±0.1 g/dl in the control group after 30 days (Figure 4 & Table 2).

d) Serum enzymes

Both aspartate transaminase (AST) and alanine transaminase (ALT) activities exhibited a linear pattern and increased after 30 days (figures 5 & 6). This increase was more remarkable in ALT activity for fish exposed to 3μg/LCd so that after 30 days a 127% increase was observed. Following by a transient reduction, AST activity also increased after 30 days. Similarly, this elevation was greater in high concentration of Cd (3μg/L). The level of alkaline phosphatase (ALP) also exhibited remarkable increases in level from a mean control level, when the fish were exposed to both sub-lethal concentrations of cadmium. This trend continued over time and with increasing Cd levels (figure 7).

Table 1: Water quality parameters, cations, anions, and background metals in acclimatio

Parameter	Acclimation water
Temperature (°C)	25±1°C
pH	8.1±0.2
Dissolved oxygen (mg/l)	8.2-8.9
Total Hardness (mg/l as $CaCO_3$)	106.2
Total alkalinity(mg/l as $CaCO_3$)	42.8
Total dissolved solids(mg/l)	173
Sodium (mg/l)	5.0
Calcium (mg/l)	31.0
Potassium (mg/l)	0.6
Magnesium (mg/l)	6.1
Cl- (mg/l)	10.5
NH_3(mg/l)	0.034
SO_4 (mg/l)	13.0
PO_4 (mg/l)	0.03
Copper(µg/l)	0.63
Cadmium (µg/l)	0.051
Zinc (µg/l)	0..35

Table 2: Biochemical effects of cadmium in low exposed (1µg/L) and high exposed (3 µg/L) sea bream (*Sparusaurata*) fingerlings in comparison with control group over the experimental period of 1, 15 and 30 days

Parameters	Day	Control groups	LowCd	HighCd
Glucose(mg/dl)	1	31.6±0.7	31±1.3	38.6±1.5
	15	25.6±1.5	70±7.5	94.3±2.4
	30	32±1.1	61±2.5	35.3±1.8
Triglyceride(mg/dl)	1	511±8.7	290.6±13.3	284.6±4.4
	15	480.6±29.5	301.3±3.7	285.3±6.5
	30	556.6±31.1	490.3±4.1	614±24.3
Cholesterol(mg/dl)	1	328±8.2	231.3±5.1	255.6±5.5
	15	282.6±23	256±10.3	192.6±1.6
	30	340.3±21.1	327.3±5.7	383±6.2
Total Protein(g/dl)	1	2.1±0.3	2.2±0.1	2.7±0.1
	15	2.2±0.1	2.6±0.1	3.8±0.1
	30	2.4±0.1	3.1±0.1	4.1±0.1
AST(IU/L)	1	1640.3±31.2	1785.6±21	1925.3±42.4
	15	1830.3±63	1617.3±39.1	1850.3±32.1
	30	18.30±51.2	2140.6±36.5	2464.3±40
ALT(IU/L)	1	124.3±4	120.6±4.1	131±10.5
	15	140.3±15.2	174±4.1	203.6±3.1
	30	160±4.5	251.3±15.2	304.6±8.7
ALP(IU/L)	1	440±30.3	386.6±20.8	453±21.8
	15	503±51.6	566±21.5	615.3±25.1
	30	503.6±14.4	620.3±13.2	880.6±24.6

Figures (1-7): Effects of different sub-lethal cadmium concentrations on biochemical parameters andserum enzymes activities in sea bream (*Sparusaurata*) fingerlings.

Data are expressed as mean ±standard error (SE). Means with different letters are significantly different from each other ($P < 0.05$). Values with the same letters are not significantly different.

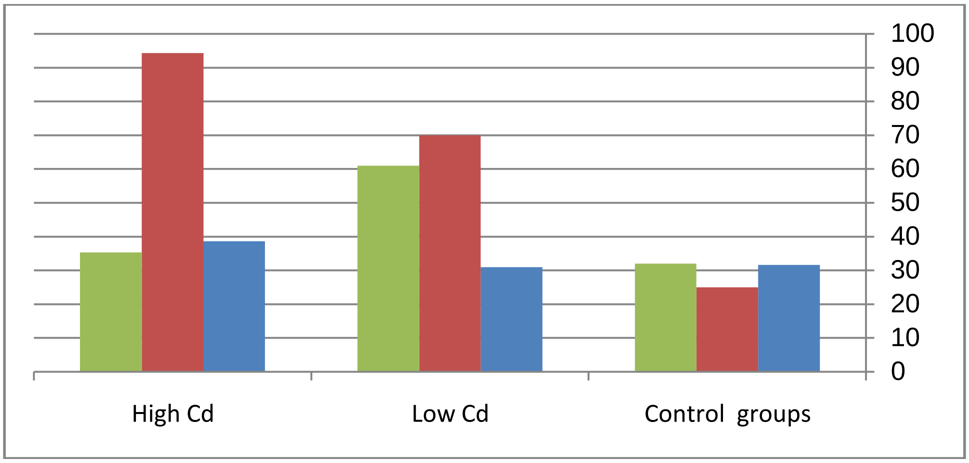

Fig. 1: Serum glucose level; after 15 days, the concentration of glucose increased but this elevation was transient so that after 30 days, the level of glucose returned to the same level in the control group

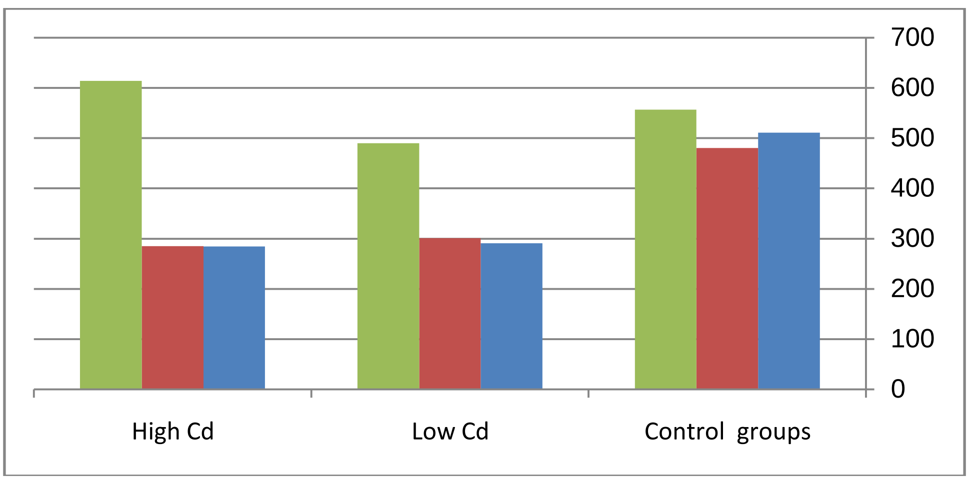

Fig. 2: Serum triglyceride level; there was a decrease in the level of triglyceride in the first and 15th days but it followed by a rapid elevation in 30 days

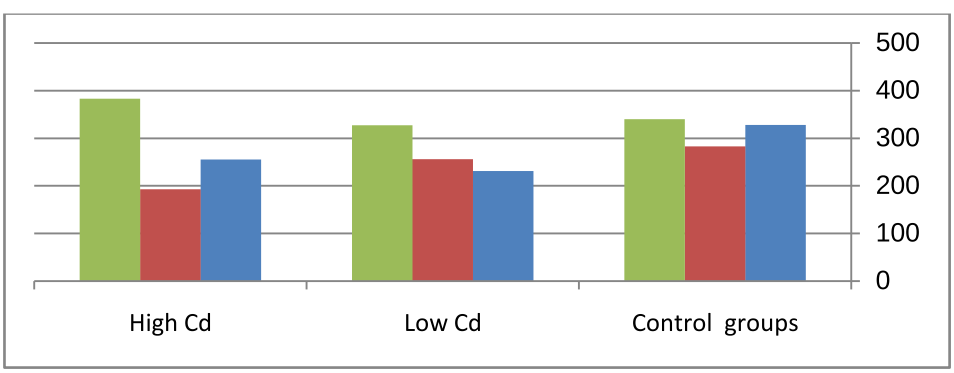

Fig. 3: Serum cholesterol level; a significant reduction was observed after the first and 15th days

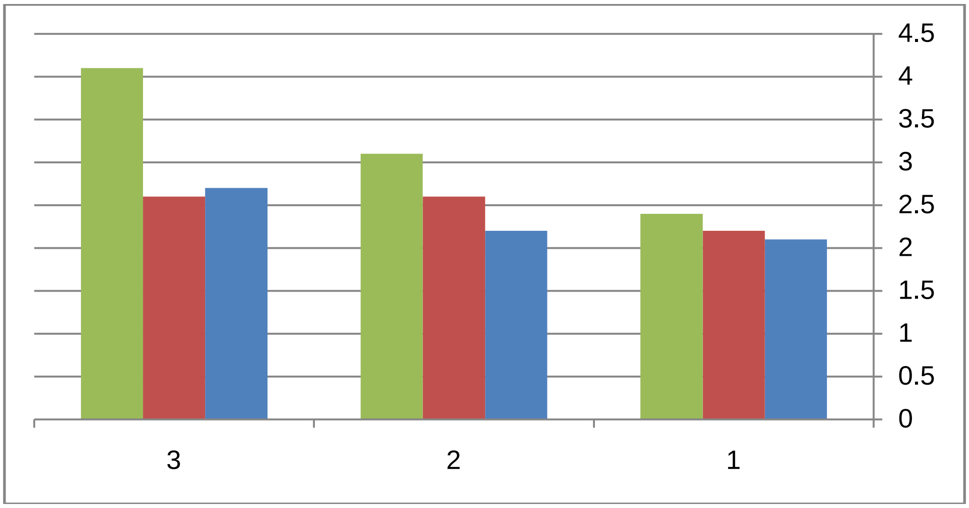

Fig. 4: Serum total protein level; there was a significant increase after 30 days

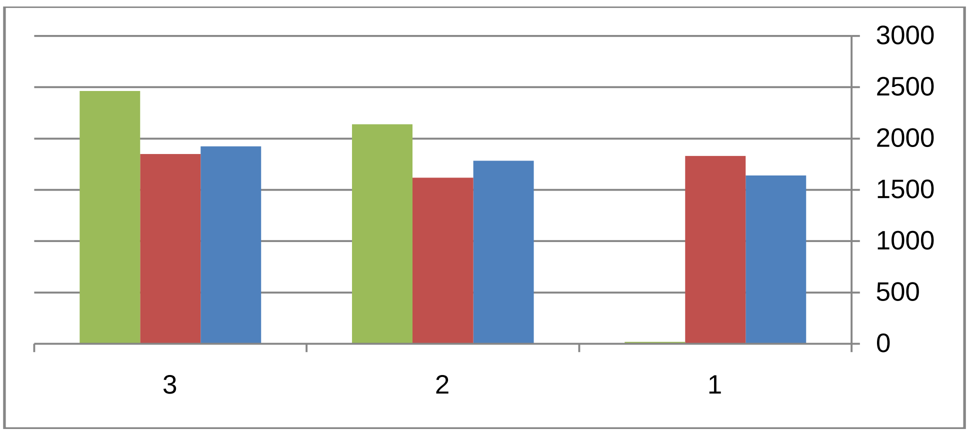

Fig. 5: Serum aspartate transaminase (AST). The AST increased and exhibited a liner pattern.

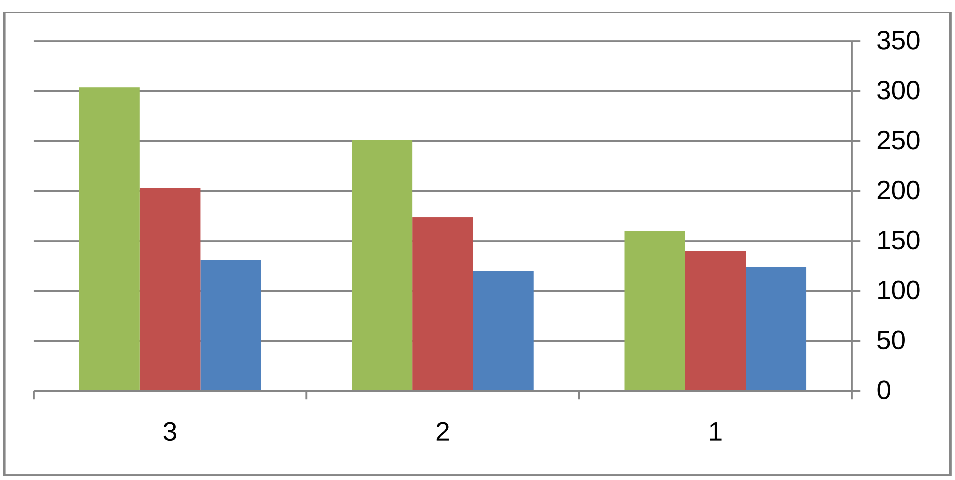

Fig. 6: Serum alanine transaminase (ALT). The ALT increased and exhibited a liner pattern

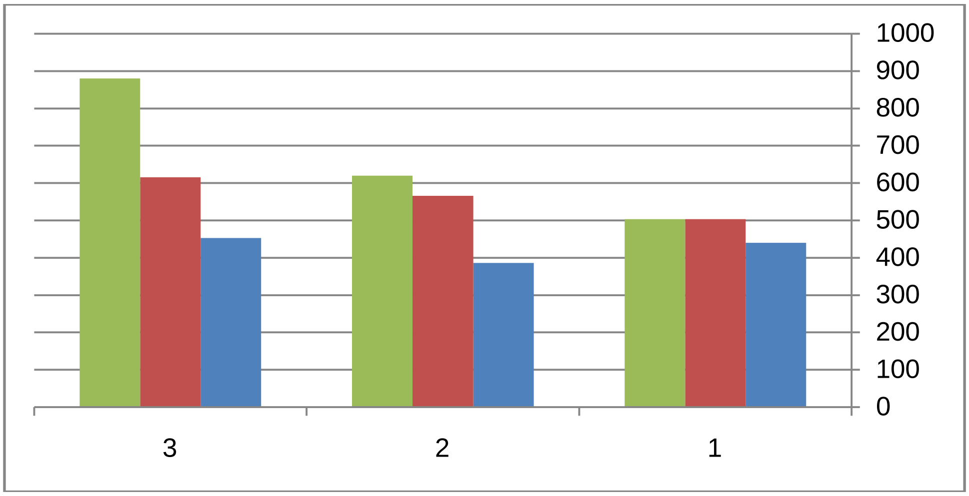

Fig. 7: Serum alkaline phosphatase (ALP) in sea bream (Sparusaurata) fingerlings exposed to cadmium. After 30 days. The level of the ALP also showed a significant increase compared to the control group, when the fish were exposed to the both concentrations of cadmium

IV. Discussion

a) *Glucose*

Changes of blood glucose are a good indicator of metal stress in fish[16], and alterations in the glucose level might be related to renal injury, liver damage, and lack of nutrition[17]. This study showed a dose-dependent increase of glucose level after first 15-days. Similarly, serum glucose levels of *C.* carpio exposed to sublethal concentrations of Cd for ten days increased with increasing concentrations of Cd in the water[18]. Nevertheless, the level of glucose reduced for the next 30 days of the current study. The higher level of glucose established on the first days of exposure might be a result of glycogenolysis (a release of glucose into the blood from energy resources stored as glycogen in muscles and liver), initiated by hormones (cortisol and catecholamines) when the organism was in unfavorable condition. The reduced level of glucose established at the end of exposure probably reflected the exhaustion of the energy reserves of the organism and impaired capacity of fish to restore them and acclimatized conditions[19]. Another study showed that glucose was significantly increased compared to the control treatment. This study showed that cadmium has a direct correlation with sea bream (*Acanthopagruslatus*) serum biochemical abnormalities.

b) *Triglyceride*

Triglyceride functions primarily in providing cellular energy and can be used as an indicator of nutritional status. Triglyceride concentration in the serum of cadmium exposed fish also showed a different pattern. The present study showed a reduction in serum triglyceride concentration in 15 days and returned to the control level after 30 days due to acclimation to the toxicant over time. Serum triglyceride concentration in *Oreochromisniloticus* exposed to 0.05 mg/L Cd during 30 day did not change when compared to control value [20].Variations in serum triglyceride concentrations might be due to differences in exposure concentration, lipid metabolism, and glycogen storage impairment in different fish species. Another study determined the LC_{50} value in *Oreochromisniloticus*fry; they found 50 % mortality of fish fry at 1.6 mg/L[21]. LC_{50} value of cadmium chloride for the fish *Oreochromisniloticus* was determined by Probit-regression analysis using SPSS 15, and LC_{50} was found to be 35.848 mg/L. This value obtained was lower than the LC 50 values determined in other tilapia species, i.e. 200 mg/l in *Tilapia mossambica* [22]and 80mg/L as determined by[23]. This shows that *Oreochromisniloticus* is are less resistant to cadmium toxicity as compared to other tilapia species. The difference may be due tothe size of fish as large size fish are more resistant to the toxicity as compared to fish fry[24].

c) *AST and ALT*

In the present study, the level of both AST and ALT increased linearly over a 30 day period, and the higher concentration caused a more significant effect on fish. Similarly, continuous exposure to sub-lethal cadmium concentrations resulted in significantly elevated levels of both AST and ALT activity in *Oreochromisniloticus* exposed to 0.05 mg/L Cd of 20-days[20].Transaminases like ALT and AST play a significant role in amino acid, and protein metabolism and they may release into the plasma following tissue damage and dysfunction. The activity of AST and ALT enzymes in blood may also be used as a stress indicator. The significant changes in the activities of these enzymes in blood plasma indicate tissue impairment caused by stress[25],[26]. In the present study, there were significant changes in AST and ALT activities in plasma of fish exposed to cadmium compared to the control group. The increase in the concentration of AST and ALT in blood plasma indicates impairment of parenchymatous organs, mainly the liver. In addition, the increase of plasma AST and ALT may be attributed to the hepatocellular damage or cellular degradation in liver, spleen or muscles [27]. These results are in agreement with those reported by [28]who found that sub-lethal concentration of Cd caused significant increases in AST and ALT of Common Carp after 7 and 25 days. Some studies showed that plasma enzymes (AST and ALT) were greatly affected by exposure to Cd in marine fish *Mugilsepheli* and after a transient reduction during the first two days, activity of enzymes increased to reach levels similar to the control value[29] Similarly,[30] showed that the levels of AST and ALT activity increased in the tissues of *Oreochromismossambicus* exposed to cadmium chloride due to necrosis and increases in the permeability of cell membrane resulting in the damage of tissues after 7 and 14 days. Different factors such as life history, water quality, and exposure duration and cadmium concentration influence ALP activity.

d) ALP

The present study showed a linear pattern of increasing ALP over time with Cd exposure resulting in recognizable physiological and functional alterations after 30-days. In contrast, *Oreochromisniloticus* exposed to 0.05 mg/L Cd during 30-days showed a reduction in ALP activity[20].The decrease in ALP activity might be a result of disturbance of the membrane transport system, although the increase in the activity may be related to tissue damage[31].

e) Cholesterol

The present study showed a reduction in cholesterol within 15-days, possibly due to tissue damage in the kidney. On the contrary, in *Oreochromisniloticus*, an increase in cholesterol was seen during a 21 days to cadmium [20].This alteration in cholesterol concentration could be due to the hazardous effects of metals on the cell membrane. Thus, an increase in cholesterol levels is good indicators of environmental stress in fishes.

f) Total protein

Total protein (TP) measurements from previous studies also exhibited no consistent pattern of response; protein levels decreased or were unaffected by cadmium exposures. The present study showed an increase in a 30 days. In contrast, when *Oreochromisniloticus* was exposed to 0.05 mg/L Cd during 30 days, no significant alteration occurred in protein concentration[20].Changes in the serum TP may be due to liver damage, reduction absorption, and protein loss and thus may be a good indicator of the health status of fish. Anotherresults showed that the amount of protein for polluted creeks was lower than clean creek that means the occurrence of hypoproteinemia. Hypoproteinemia results from either decreased production or increased loss of protein. In dietary toxicity studies, decreased protein production can result from effects on food consumption, digestion, or absorption. Because of the reserve capacity of the liver, the hepatic injury must be fairly severe before protein synthesis is notably diminished [32]. However, in large studies, small differences between the control and treated groups might be apparent with mild to moderate hepatotoxicity [33]. Hypoproteinemia, like anemia, can be masked by dehydration. A small, statistically significant decrease in serum albumin concentration is one of the most frequent findings in toxicology studies. The exact mechanism is usually not apparent but a combination of factors, similar to those causing mildly lower glucose, are probably responsible[35]. Some studies showed that in the controls, Cd content was significantly higher in gills compared to in the liver, still the treatment of fish with 0.1mg/L Cd induced a stronger accumulation of metal in the liver depending on the length of the exposure period[1]. Cd traces were found in plasma, muscle, and kidney. Cd forms complexes in the cytosol with MT only in the liver still Cd–MT content significantly increased after 11 days of exposure to the metal, while after four days of treatment, the protein level was similar to the control. The ''comet assay'' performed on S. aurata erythrocytes isolated from fish treated for four and 11 days with 0.1mg/L. Cd showed that there was no DNA damage at both exposure periods. Recorded data for the activity of total protein showed depletion in all treatments. Some studies indicated a decrease in total protein content during heavy metal exposure. Such decreases were, for example, found in the edible crab *(Scylla Serrata*) exposed to cadmium or in the common carp exposed to mercury [34]. Depletion in the protein content of the *Catlacatla* exposed to mercury chloride sub-lethal concentrations was estimated [36]. The rapid decrease in total protein content was associated with the active degradation of proteins under stress. This fact is correlated to the development of resistance toward toxic stress. Proteins being involved in the architecture and physiology of the cell, they seem to occupy a key role in cell metabolism. Catabolism of proteins makes a major contribution to the total energy production in fishes. Under stress, situations may constitute a physiological mechanism with an important role in providing energy to cope with the stress full situation. Therefore, a depletion of total protein content might also be attributed to the destruction or necrosis of cellular function, and consequent impairment in protein synthetic machinery[37]. When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted[38].The depletion of the total protein content may be due to the breakdown of protein into free amino acid under the effect of mercury chloride at the lower exposure period[39]. Reduction in protein content in liver of exposed fish might be due to either arrested metabolism in the liver or to use it to build up new cells or enzymes to reduce the stress [40].

V. Conclusions

Results of the present investigation showed that pollutants have a direct correlation with fish serumbiochemical abnormalities and also indicated that the difference in the environmental conditions of marine ecosystems might cause several changes in the serum biochemical parameters of the studied fish.

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