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IMPORTANT OF *SPIRULINA PLATENSIS* TO RESTRAIN SULFUR TOXICITY IN SEA BASS *DICENTRARCHUS LABRAX*

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ABSTRACT

Background: Because of the potential antioxidant, anti-inflammatory, and immune-modulating properties of Spirulina platensis, there is an increased interest in its potential role in the prevention and control of sulfur toxicity in sea bass Dicentrarchus labrax. **Objective:** This study investigated the effects of sodium sulphate on sea bass (Dicentrarchus labrax) and protection effect of Spirulina platensis. **Material and methods:** A total of 260 sea bass exposed to 1/10 dose of sodium sulphate 96hr-LC_{50} (5.8 mg/L) and study the changes in antioxidant enzyme activities of serum. In addition to, study the amelioration of sodium sulphate toxicity by addition of S. platensis to the ration of exposed fish by the rate of 1%. **Results:** The results showed that the sodium sulphate at dose of 5.8 mg/L the level of SOD; CAT; GPx and TAC activity in the serum of sea bass were decreased. The SOD; CAT; GPx and TAC activity in sodium sulphate exposed group was found lower than the S. platensis supplemented groups. Exposure of sea bass to sodium sulphate for 8th weeks resulted in significant changes in mRNA abundance for a limited subset of the analyzed of hepatic sea bream genes. GSR; SOD and GPXs mRNA levels decrease significantly upon incubation with sodium sulphate, in liver, irrespectively to the length of the treatment, whereas the corresponding levels of sea bass feed with 1% S. platensis were indistinguishable from controls. **Discussion and conclusion:** S. platensis ameliorated the adverse biochemical effect of sodium sulphate toxicity in sea bass, likely by increasing and improving the antioxidant status.

KEYWORDS: Antioxidant Status, Spirulina Platensis, Sulfur Toxicity, Sea Bass.

INTRODUCTION

Fish are usually considered as organism of choice for assessing the effects of environmental pollution on aquatic ecosystem (Gernhöfer *et al.*, 2001; Olushola *et al.*, 2014). Pollution of aquatic environment with metals is common worldwide and under certain conditions aquatic fauna may concentrate large amount of some metals from water in their tissues (Kaoud and Rezk, 2011). The toxic effects of heavy metals have been reviewed, including bioaccumulations (Adami *et al.*, 2002; Waqar, 2006) and are surrounded with great care and special importance due to their highly toxic effects on fish as they affect survivability, growth and reproduction (Abdel-Tawwab *et al.*, 2004).

S. platensis is a photosynthetic, filamentous, blue–green microalgae and is generally regarded as a rich source of vitamins, essential amino acids, minerals, essential fatty acids (γ-linolenic acid), and antioxidant pigments such as carotenoids and phycocyanin (Jaime- Ceballos *et al.*, 2006). Hence, *S. platensis* may have potential to be used as a natural feed supplement for increasing fish growth. Estrada *et al.*, 2001 demonstrated the antioxidant activity of the phycobiliproteins, phycocyanin and alophycocyanin present in Spirulina biomass. The antioxidant activity of Spirulina has been very well documented by (Abd El-Baky *et al.*, 2006; Khan *et al.*, 2005; Athukorela *et al.*, 2006). The results of Arun *et al.*, 2012 revealed high percentage of NO scavenging activity in *S. platensis*. The antioxidant potential in the extract of Spirulina might be due to the total phycocyanin, triterpenoids and carotenoids present in the algal extracts. This study was aimed to investigate the effects of sodium sulphate on sea bass *(Dicentrarchus labrax)*, by determination of 96hr-LC_{50} of sodium sulphate. Chronic toxicity by exposed of sea bream to 1/10 dose of Sodium sulphate 96hr-LC_{50} values. The Changes in anti-oxidant enzyme activities of serum namely; activities of superoxide dismutase (SOD); catalase (CAT); the level of glutathione peroxidase (GPx) and total antioxidant

activity (TAC). Amelioration of Sodium sulphate toxicity by addition of *Spirulina platensis* to the ration of exposed fish by the rate of 1%, and detection of gene expressions of glutathion-s- transferase; Superoxide dismutase and Glutathione peroxidase in liver of toxicated fish after 8th weeks of toxicity.

MATERIALS AND METHODS

Fish for experimental work: A total of 260 apparently healthy sea bass fish were collected from private fish farms at Borg El-Arab, Alexandria governorate, Egypt and previously acclimated in indoor tanks in full glass aquaria measuring (70×50×60 cm). They seemed healthy and had a uniform size and weight with average body weight 40 ± 3 grams.

Fish for determination of LC_{50}: At the first, the 60 apparently healthy sea bass fish were used for estimation of LC_{50} by addition of Sodium sulphate with different concentrations (0, 20, 40, 60, 80, 100 mg L-1) during 96 h. Sodium sulphate solutions were prepared by diluting of a stock solution with distal water. The concentration of dilution of heavy metal caused 50% mortality in fish for 96 h was taken as the LC_{50} value. During the toxicity test, the fish were not fed. The numbers of dead fish were counted daily and removed immediately from the aquaria. The lethal concentration of Sodium sulphate after 96 hour (96-h LC_{50}) of exposure was calculated according to Behrens and Karber, 1953.

Fish for chronic toxicity test

Chronic toxicity test were established by addition of 1/10 LC_{50} of Sodium sulphate (5.8 mg. /L.) with a trail of reducing impacts of Sodium sulphate toxicity by addition of *spirulina platensis* extract by 1% in ration. A total of 200 sea bass, mean initial weight of 40 ± 3 g. They were divided into four equal groups (each of 50 fish). Fifty fish were served as a control negative group. The groups were arranged as the following; G (2) exposed to Sodium sulphate (5.8 mg. /L.); G (3) Sodium sulphate (5.8 mg. /L.) plus *S. platensis* extract by 1% in ration and G (4) supplemented by *S. platensis* extract by 1% in ration only (table, 8). Each group was reared in a glass aquarium (70×50×60 cm) that was supplied with an aerator and acclimatized for two weeks. Fish were given a diet of 45% crude protein two times per day at feeding levels of 3% from the live body weight, 5 days per week.

Solutions, buffers, reagents and stains: Sodium sulphate was purchased from Lab Service Co. Egypt and dissolved in water. *Spirulina platensis* extract was purchased from Urgent ® Company, Alexandria branch, Semoha, Egypt and added to the ration by 1% in ration.

Kits for antioxidant parameters: The kits used for biochemical measurements of serum were catalase (CAT), Superoxide Dismutase, glutathione peroxidase (GPx) and total antioxidant capacity were purchased from Biodiagnostic and/or Biotechnology Co., ARE. All other chemicals were of reagent grade and were commercially available from local scientific distributors in Egypt.

Primer sequences used for RT-PCR

Table 1: Primer sequences used for RT-PCR.

Gene name	Short name	Forward primer	Reverse primer	Amplicon size (bp)	Accession number
b-Actin	β-actin[a]	CCTCACCCTCAAGTACCCCAT	TTGGCCTTTGGGTTGAGTG	153	AF082863
Glutathione S-transferase	GST	ATGATCTATGGCAACTATGA GACAGG	GAAGTACAAACAGATTGTAG TCCGC	152	EF194203
Superoxide dismutase	SOD	GACAGCATGGCTTCCATGTG	AGGAGCCCCGTGAGTTTTG	100	AY377970
Glutathione peroxidase	GPx	GAACGGCGTGGAGTTGATG	GAGGAAAATTCGGCACGAAA	71	DQ459994

[a] Housekeeping gene

Experimental design of determination of Lethal dose fifty (LC_{50})

Experimental design of LC_{50} of Sodium sulphate in sea bass

A total number of 60 apparently health sea bass, weighting 30 ± 2 grams were selected after the period of acclimation about two weeks and then divided into six equal groups; each group contained of 10 fish. The first five groups were consistently exposed to 20; 40; 60; 80 and 100 mg L^{-1} of Sodium sulphate while the control group (group 6) was act as a control group and the determine the LC_{50} were carried out according to Klassen (1991) (Table 2).

Table. 2: Experimental design of determination of LC_{50} of Sodium fluoride in sea bass.

Groups	Concentration of Sodium sulphate by mg $^{L-1}$	Number of sea bass
1	0 (control)	10
2	20	10
3	40	10
4	60	10
5	80	10
6	100	10

The data were also assessed according to Behrens Karber's method using the following formula Klassen (1991): $LC_{50} = LC_{100} \sum A \times B / N$ as mg/L. The dead fish were removed immediately. Behavioral changes, clinical toxic signs and postmortem lesions of tested fish were closely followed up and recorded daily.

Experimental design of chronic experiment

Four aquaria were used for experimented sea bass and divided to four equal groups (50 fish per each). The design of the chronic experiment was summarized in table 3.

Table. 3: Design of the chronic experiment.

Groups	Treatments	Number of fish	Dose of Sodium sulphate and other additives	Reference
G (1)	Control (without treatment)	50	0	
G (2)	Sodium sulphate only	50	5.8 ppm	1/10dose of LC_{50}
G (3)	Sodium sulphate plus *S .platensis* extract	50	5.8 ppm+ *S .platensis* extract by 1% in ration	
G (4)	*S .platensis* extract	50	*S .platensis* extract by 1% in ration	

Bleeding and serum collection

At 2nd, 4th, 6th and 8th weeks during the experimental period, 4 ml blood samples were collected from different groups via the caudal vessels from 3 fish using disposable syringe (Hawak *et al.*, 1965). Also, sampling was carried out for the analysis of blood parameters. Half the blood sample was then transferred immediately to sterile penicillin vial containing a pinch of lithium heparin powder, shaken gently and kept at 4°C; and for serum separation. The serum was collected with a micropipette and then was stored in sterile Eppendorf tubes at -20°C until used for assay (Lied *et al.*, 1975) for measurement of different antioxidant parameters from different groups.

Anti-oxidant enzymes assay

- Catalase activity assay: Catalase (CAT) activity was determined by measuring the decrease of H_2O_2 concentration at 410 nm according to Koroliuk *et al.*, (1988).
- Superoxide dismutase activity assay: (SOD) activity was determined according to Kostiuk *et al.*, (1990).
- Glutathione peroxidase activity assay were determined by detecting the nonenzymatic utilization of GSH as the reacting substrate at an absorbance of 412 nm after incubation with DTNB according to the method of Moin (1986).
- Total antioxidant capacity (TAC) assay: The TAC level was estimated spectrophotometrically at 532 nm following the method with Tween 80 oxidation (Galaktionova *et al.*, 1998).

Transcript expression analysis of glutathion-s-transferase; Superoxide dismutase and Glutathione peroxidase in liver of different treatments

At the end of the experiment (8th week) another liver samples were collected from different treatments for Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis of mRNA expression of antioxidant related gene (glutathion-s transferase; Superoxide dismutase and Glutathione peroxidase) was performed. The total mRNA was extracted from tissue samples from the control and treated groups (n=6 per group) using an mRNA extraction kit according to the manufacturer's instructions. The quality of the extracted RNA was confirmed with 2% agarose electrophoresis. A total of 2 µg total RNA from the liver was reverse transcribed to first-strand cDNA using an oligo (dT) primer and M-MLV Reverse Transcriptase (Go Script™ Reverse Transcription System), according to the manufacturer's instructions. The cDNAs were used as the template for RTPCR using QPCR SYBR® Green Master Mix and specific primers for the indicated antioxidant-related genes: β- actin and glutathion-s-transferase; Superoxide dismutase and Glutathione peroxidase (Table, 1) (Abd El-Rahim *et al.*, 2010 and Puerto *et al.*, 2011). Real-time PCR was conducted using Mx3005P Real-time PCR system. The relative differences in gene expression between the controls and treated groups were calculated using threshold cycle (CT) values that were first normalized to those of the housekeeping gene, β- actin as the endogenous control in the same sample. The relative differences in gene expression compared with control CT values were calculated using the $2^{-\Delta\Delta C}{}_{T}$ method as previously described Livak and Schmittgen (2001).

Statistical analysis

All data are expressed as the mean ± standard deviations (SDs), and the levels of significance are cited. SPSS statistical package version 17.0 for Windows (IBM, Armonk, NY, USA) was used for all data analysis. Differences in values were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests. Differences were deemed significant when $p<0.05$.

RESULTS

Results of determination of LC_{50} sodium sulphate in sea bass

Results of LC_{50} of Sodium sulphate in sea bass was summarized in Table (4) and Figure (1). The obtained results showed that the lethal concentration $_{50}$ (LC_{50}) of sodium sulphate in sea bream was 58 mg/L; so the 1/10 dose of LC_{50} of sodium sulphate in sea bream to induce chronic toxicity was 5.8 mg/L.

Table. 4: Results of determination of LC_{50} sodium sulphate in sea bass.

Sodium sulphate dose (mg/L)	No. of exposed fish	No of dead fish				Overall deaths within 96 h	A	B	AB
		D1	D2	D3	D4				
0 (control)	10	0	0	0	0	0	0	0	0
20	10	0	0	0	0	0	20	0	0
40	10	0	0	1	1	2	20	1.0	20
60	10	1	1	2	2	6	20	4.0	80
80	10	1	2	2	3	8	20	7.0	140
100	10	2	2	3	3	10	20	9.0	180
									∑AB =420

$LC_{50} = LC_{100} - \sum (A \times B)/N = 100 - 420/10 = 58.0$ ppm. Or (mg/L)
$LC_{50)}$ of Sodium sulphate in *O.niloticus* =58 mg/L.

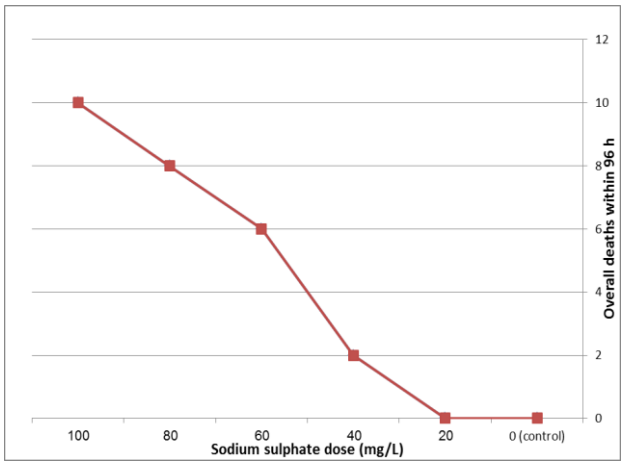

Fig. 1: LC_{50} of sodium sulphate in sea bream.

Effect of sodium sulphate and/or *S.platensis* on glutathione peroxidase activities (GPx) in the serum of sea bass

GPX activity varied among the experimental groups in the serum of sea bass. GPX activity in group exposed to 5.8 mg. /L sodium sulphate for 8th weeks was lower comparing to both feeding group on *S.platensis* and control group respectively. In group feed on *S.platensis* increased its activity of GPx while a decrease was observed after 2nd weeks of sodium sulphate exposure. On the other hand, Sodium sulphate exposures induce severe decrease of GPX activity in the serum, gills, liver, kidney and muscular tissue of sea bream compared to its control group (P>0.05) table (5) Figures (2 and 3).

Table. 5: Effect of sodium sulphate and / or *S.platensis* on glutathione peroxidase activities (GPx, U/ml.) in serum of sea bass.

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	15.66±0.25 A a	15.04±0.38 A b	15.41±0.49 A b	14.90±0.39 A b
SS	13.93±0.34 A b	12.05±0.20 B c	10.56±0.40 B d	8.06±0.45 C d
SP	15.16±0.20 A a,b	13.94±0.12 A,B b	12.61±0.28 B,C c	11.14±0.33 C c
SE	16.29±0.33 D a	19.30±0.18 C a	24.99±0.89 B a	28.50±0.67 A a

Different superscript small letters within the same column indicate significantly different mean values ($p< 0.05$) between different groups.

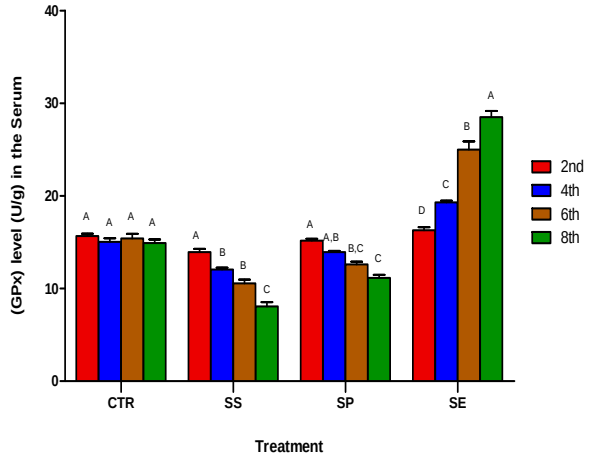

Fig. 2: Glutathione peroxidase activities (GPx, U/ml.) in the serum of sea bass.

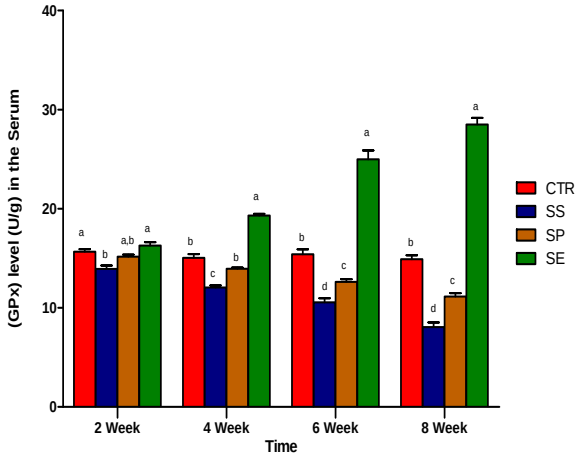

Fig. 3: Glutathione peroxidase activities (GPx, U/ml.) in the serum of sea bass.

Effect of sodium sulphate and/or *S.platensis* on Glutathione Catalase (CAT) activity in the serum, of sea bass

In groups feed on *S.platensis* and at the same times exposed to 5.8 mg. /L of sodium sulphate for 8th weeks the CAT of serum, of sea bass catalase activity were gradually decreased compared to the control and treated groups (Table, 6) (Figures, 4 and 5). In serum, of sea bass, there were significant ($p>0.05$) changes in CAT activity at 2nd weeks, 4th weeks, 6th weeks and 8th weeks of exposure period compared with the control. However, CAT activity significantly ($p<0.05$) increased in sodium sulphate exposed fish at 8th weeks days of exposure period. And in gills, there were no significant ($p>0.05$) changes in CAT activity at all experimental period.

Table. 6: Effect of sodium sulphate and / or *S.platensis* on Catalase (CAT) activity (µmol O2/min/mg protein /ml.) in serum of sea bass.

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	2.66±0.07 A a,b	2.47±0.06 A b	2.49±0.14 A b	2.52±0.08A b
SS	2.26±0.04 A c	2.08±0.02 A,B c	1.95±0.04 B c	1.28±0.05 C d
SP	2.47±0.09 A b,c	2.28±0.04 A,B b,c	2.21±0.12 B b,c	1.89±0.15 C c
SE	2.85±0.04 D a	3.11±0.07 C a	3.77±0.06 B a	4.34±0.16 A a

Different superscript small letters within the same column indicate significantly different mean values ($p< 0.05$) between different groups.

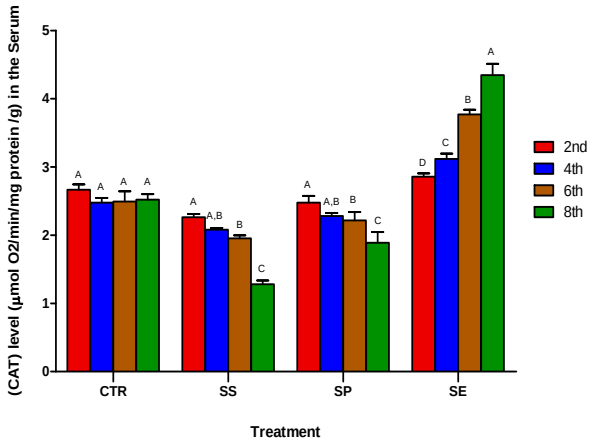

Fig. 4: Catalase (CAT) activity (µmol O2/min/mg protein /ml.) in serum of sea bass.

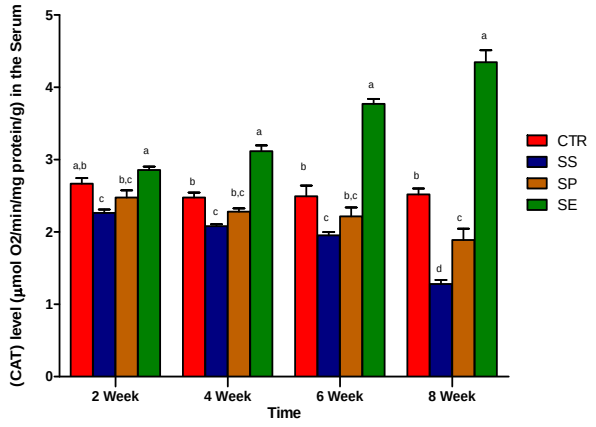

Fig.5: Catalase (CAT) activity (µmol O2/min/mg protein /ml.) in serum of sea bass.

Effect of sodium sulphate and/or *S.platensis* on Super oxide dismutase (SOD) activity in the serum, of sea bass

Levels of Super oxide dismutase (SOD) activity in the serum of sea bass after chronic exposure to 5.8 mg./L for 8th weeks to sodium sulphate and / or *S.platensis* are summarized in (Table,7) (Figure,6 and 7). The levels of SOD activity in the serum of sea bream after chronic exposure to sodium sulphate group were higher, but not significantly ($P > 0.05$) compared to the control group. However, in general, the activities of SOD enzymes in the *S.platensis* treated groups were higher than those in the toxicated and controls groups, but the SOD activities and the extent of changes were not more obvious than those in the sodium sulphate exposed group.

Table. 7: Effect of sodium sulphate and / or *S.platensis* on Super oxide dismutase (SOD) activity (U/min/mg protein /ml.) in serum of sea bass

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	0.46±0.05 A a,b	0.45±0.09 A,B b	0.43±0.10 B b	0.44±0.09 B b
SS	0.41±0.03 A c	0.37±0.08 B d	0.33±0.08 C d	0.27±0.05 D d
SP	0.44±0.05 A b	0.42±0.01 B c	0.40±0.02 B c	0.38±0.04 C c
SE	0.47±0.04 B a	0.48±0.03 B a	0.49±0.07 A,B a	0.50±0.06 A a

Different superscript small letters within the same column indicate significantly different mean values ($p< 0.05$) between different groups.

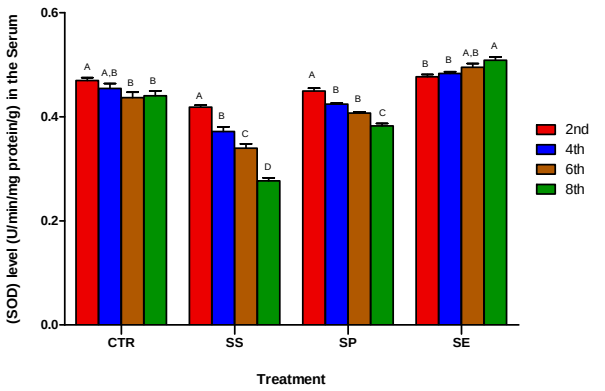

Fig. 6: Super oxide dismutase (SOD) activity (U/min/mg protein /ml.) in serum of sea bass.

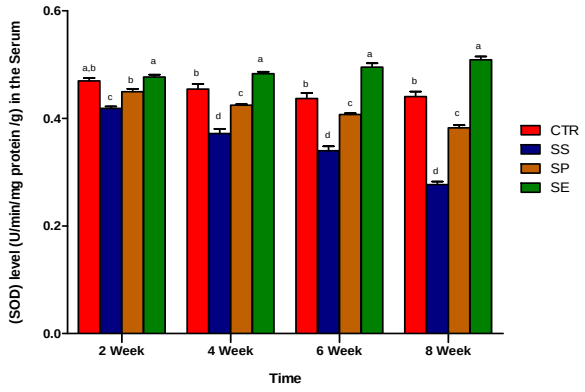

Fig. 7: Super oxide dismutase (SOD) activity (U/min/mg protein /ml.) in serum of sea bass.

Effect of Sodium sulphate and/or *S.platensis* on Total antioxidant capacity in the serum of sea bass

Table (8) and Figures (8 and 9) showed that there are no significant ($p>0.05$) changes in Total antioxidant capacity levels in the serum of sea bass at 2nd of exposure to 5.8 mg. /L. of sodium sulphate between groups; however, they are markedly ($p>0.05$) decreased upon sodium sulphate exposure at 4th, 6th and 8th weeks in a time dependent manner. *S.platensis* supplementation significantly ($p<0.05$) increased total antioxidant capacity levels in the serum of sea bass levels at only 6th and 8th weeks of exposure.

Table. 8: Effect of sodium sulphate and/or *S.platensis* on Total antioxidant capacity (mML-1/ml.) in serum of sea bass.

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	0.89±0.01 A b	0.85±0.005 A b	0.79±0.01 B b	0.71±0.02 C b
SS	0.79±0.02 A c	0.74±0.01 A,B c	0.69±0.01 B c	0.61±0.02 C c
SP	0.82±0.01 A c	0.78±0.01 A c	0.71±0.01 B c	0.67±0.01 B b,c
SE	0.95±0.01 D a	1.04±0.01 C a	1.17±0.02 B a	1.26±0.01 A a

Different superscript small letters within the same column indicate significantly different mean values ($p< 0.05$) between different groups.

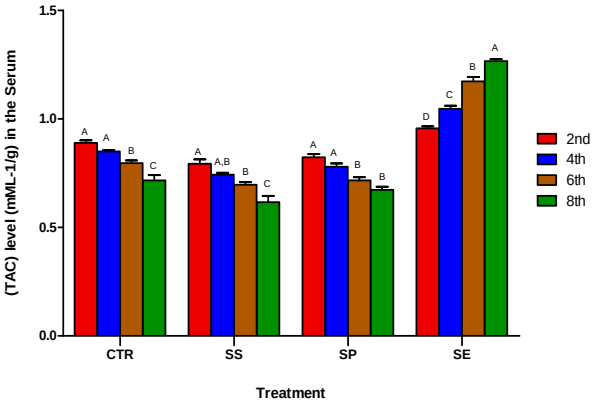

Fig. 8: Total antioxidant capacity (mML-1/ml.) in serum of sea bass.

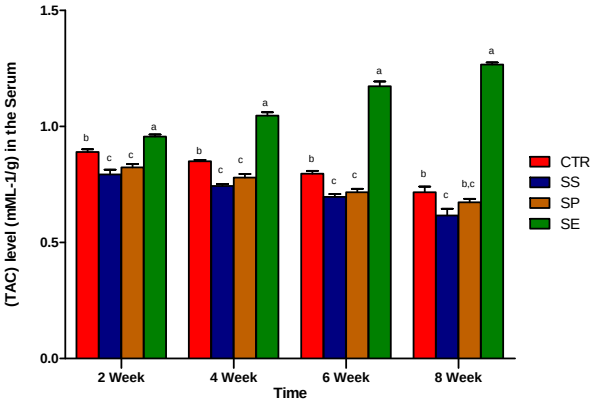

Fig. 9: Total antioxidant capacity (mML-1/ml.) in serum of sea bass.

Quantitative analysis of mRNA abundance of stress genes in liver tissues of sea bass

Exposure of sea bass to sodium sulphate (5.8 mg. / L) for 8th weeks resulted in significance change in mRNA abundance for a limited subset of the analyzed of hepatic sea bream genes (Figure, 10). GSR; SOD and GPXs mRNA levels decrease significantly upon incubation with sodium sulphate, in liver, irrespectively to the length of the treatment, whereas the corresponding levels of fish feed with 1% *S.platensis* were indistinguishable from controls. A similar pattern of response was observed in SOD in liver of fish mRNA levels also decrease in the presence of heavy metals, especially in at the end of experiment 8th week (100–50 fold) after exposure, but this response faded after 8th weeks of supplemented with 1% of *S.platensis*.

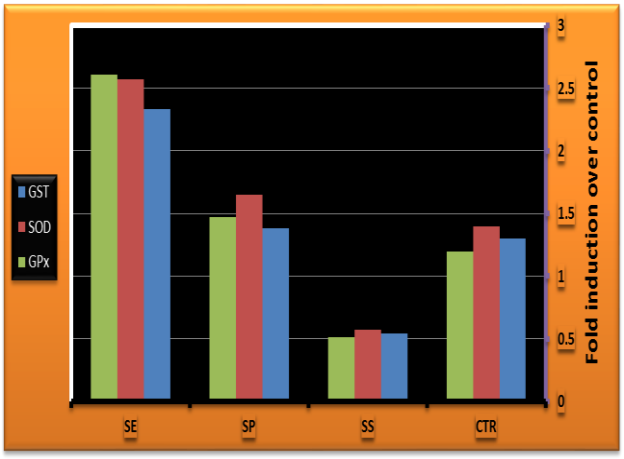

Fig. 10: Relative mRNA transcript expression of the hepatic antioxidant related gene Glutathione S-transferase; Superoxide dismutase and Glutathione peroxidase in sea bass 8th weeks of consecutive exposure to CTR: Control; SS: 5.8 ppm sodium sulphate; SP: 5.8 ppm sodium sulphate + 1% *S .platensis* and SE: *S .platensis* extract by 1% in ration. The mRNA transcript levels are normalized relative to that of β-actin, housekeeping gene, and represented as fold induction over control. Triplicate samples were analyzed to obtain an average concentration for each treatment

DISCUSSION

The obtained results showed that the lethal concentration $_{50}$ (LC_{50}) of sodium sulphate in sea bass was 58 mg/L; so the 1/10 dose of LC_{50} of sodium sulphate in sea bass to induce chronic toxicity was 5.8 mg/L. The Mount *et al.*, 1997 reported that the acute toxicity of sodium sulphate for fish is very low, with LC_{50} values far above 1,000 mg/l for species, *Lepomis macrochirus* and *Pimephales promelas*. On the other hand, the acute toxicity (LD_{50}) of sodium sulfate has not been reliably established but is probably far in excess of 5000 mg / kg Wang et al. (2015). Variations of LC_{50} may be attributed to some differences in standard techniques that were adopted in the experiments such as the larger size of the test-organisms (Ishikawa *et al.*, 2007). *S. platensis* is a photosynthetic, filamentous, blue–green microalgae and is generally regarded as a rich source of vitamins, essential amino acids, minerals, essential fatty acids (γ-linolenic acid), and antioxidant pigments such as carotenoids and phycocyanin (Jaime- Ceballos *et al.*, 2006). The antioxidant activity of Spirulina has been very well documented by (Abd El-Baky *et al.*, 2006; Khan *et al.*, 2005; Athukorela *et al.*, 2006). The activities and expression levels of antioxidant enzymes and metabolic were used as biomarkers to evaluate the influence of pollution on the biochemical pathway and enzymatic function in fish (Correia *et al.*, 2007) and also for monitoring unacceptable levels of environmental contamination. The results are reported in the present study showed that the adverse effect of sodium sulphate on the activities superoxide dismutase (SOD); catalase (CAT); the level of glutathione peroxidase (GPx) and total antioxidant activity (TAC). This could be due to the high hydrogen peroxide concentration which associates with CAT activity (Pinto et al. 2003; Trenzado et al. 2006). GPX activity can be also considered complementary to CAT activity that was also supported with the present data. Garcia Sampaio *et al.*, 2008 also emphasized the secondary capacity to decompose the peroxides by GPX than CAT in the liver. Sensitivity of SOD and CAT activities to metal exposures were also supported with our previous results Atli and Canli (2008). Decreased SOD activity might be an indicator of damage in the antioxidant mechanisms caused by metal exposure and water hardness. Barata *et al.*, 2005 also found variations in SOD and CAT activity in Daphnia magna after Cd and Cu exposures depending upon metal concentrations. They concluded that toxicants may induce different antioxidant/prooxidant responses depending on their ability to produce ROS. The SOD-CAT system, the first line of defense system against oxidants varied according to the response of fish antioxidant system to counteract with the toxicity of hardness and metal exposures (Garcia *et al.*, 2008; Atli and Canli, 2010).

Supplementation of dried S. platensis at 10 g/kg of diet for 2 months in feeding of sea bream during sodium sulphate toxicity is highly improving glutathione, CAT and SOD) activity in the serum of sea bass. Sharma *et al.*, 2007; Dartsch, 2008) found that the S.platensis inactivation of free superoxide radicals (antioxidant effect) as well as the different extracts from the microalga S. platensis has antioxidant activity. The antioxidant activity of Spirulina has been very well documented by (Abd El-Baky *et al.*, 2006; Khan *et al.*, 2005; Athukorela *et al.*, 2006).

A large cluster of oxidative metabolism genes (GSR; SOD and GPXs) showed a tight correlation in their mRNA levels in our analysis, particularly in the liver. These genes are under the control of AREs in many systems (Jones *et al.*, 2007) and their coordinate expression may reflect variations in the redox status of the fish. Variations on mRNA abundance of these genes

were largely unaffected by the presence of metals, suggesting a rather weak oxidative stress in sea bream upon metal exposure.

Therefore, we consider likely that the observed expression pattern was associated to metabolic differences among individuals, although the exact nature of the factors responsible for this coordinated regulation is currently unknown. It is most interesting to observe that mRNA abundance of GSR; SOD and GPXs, on the other, mutually correlated in larvae, suggesting that the putative factors co-regulating these genes (presumably, through ARE or similar elements) are already present at the fish. The similar results obtained by (Faria *et al.*, 2010b; Wang *et al.*, 2014) who found that the Glutathione peroxidases (GPxs) are key enzymes in the antioxidant defense system of living organisms, and protect organisms against oxidative stresses. The results suggest that MgcGPx and MgGPx4 perhaps play an important role in maintaining cellular redox homeostasis and protecting M. galloprovincialis against cadmium and arsenate toxicity. Their differential expression as compared to normal expression pattern can indicate exposure to cellular stress and adverse cellular effects (Dash *et al.*, 2006). In common carp, the mRNA expression of GPx4b was greatly down-regulated following Cd (10 mg L^{-1}) exposure for 21 d (Hermesz and Ferencz, 2009). Also, the obtained results are consistent with those of (Huynh Thi *et al.*, 2012) who reported the induction of GPx activity in the black tiger shrimp Penaeus monodon treated with deltamethrin. Also, (Mohamed *et al.*, 2015) stipulated that pyrethroids have the potential to induce antioxidant enzymes such as GPx. The liver is found to be stronger in view of oxidative stress than the other tissues with the highest SOD and CAT activities (Heath, 1987; Schlenk and Benson, 2001; Atli *et al.*, 2006).

CONCLUSION

From the obtained results, we can conclude that *Spirulina platensis* to restrain sulfur toxicity in sea bass *Dicentrarchus labrax* possessed hepatoprotective effects against sulfur toxicity alterations. Furthermore, sulfur toxicity up-regulates the gene expression of glutathione S-transferase; superoxide dismutase and glutathione peroxidase due to the presence of biologically active antioxidant compounds. Ultimately, we recommend the aquaculture farmers of marine fish suffering from diseases condition the *Spirulina platensis* used as a food supplement in their diet.

COMPETING INTERESTS

The authors have no conflict of interest.

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