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Suppressive of hepatotoxicity caused by lead and cadmium in the sea bass *Dicentrarchus labrax* by using an algal mixture of *Spirulina platensis* and *Chlorella vulgaris*

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ABSTRACT

Heavy Metals as Cadmium (Cd) and lead (Pb) are toxic and harmful to humans and animals. The present study aimed to investigate the protective effect of three concentrations of an algal mixture of *Spirulina platensis* and *Chlorella vulgaris* in chelating of the mixture of cadmium chloride and lead nitrate in the liver of seabass (*Dicentrarchus labrax*). Na^+/K^+-ATPase, the total cytochrome P450 (CYP 450), antioxidant enzymes Superoxide dismutase SOD, Catalase CAT, Glutathione peroxidase Gpx, and non-enzymatic antioxidant metallothionein MTs were measured as biomarkers. n the experimental diets, the first group was fed with a basal diet and lived in seawater (control 1); in other groups, heavy metals (0.003 mg/l of $CdCl_2$ + 0.003 mg/l $Pb(NO_3)_2$ were added and fed a basal diet (control 2), or 3% of diet supplemented with the algal mixture of *Spirulina* and *Chlorella* in the ratio (1: 1) (diet 1), 5% of a diet with the algal mix (diet 2), and 7% of a diet with the algal mixture (diet 3). Fish were fed 5% of the average body weight three times a day. There is a significant decrease in the activity of Na^+/K^+-ATPase ($P < 0.05$) between (control 1) and the other groups. The groups of diets 2 and 3 showed significant improvements in the value of Na^+/K^+-ATPase compared to the control "1" group. The CYP is highly induced in the control "2" compared to the control "1" or other groups. Diet "3" shows a minimum significance of CYP concentration than the control "2". There is a significant increase in SOD and Gpx activities in the liver of treated fishes (diets 2 and 3) compared to the control "2". The results reveal that the best supplement of the algal mixture appears in the concentration of 7% (diet 3). This concentration repairs the ionic disturbance and oxidative stress of lead and cadmium toxicity and increases the antioxidant activities responsible for reactive oxygen species ROS scavenging.

INTRODUCTION

Certain heavy metals are essential nutrients with low dose while others are relatively harmful and toxic elements in large quantities or certain chemical forms **(Mehrandish *et al.*, 2019)**. Some heavy metals have biological effects such as antimony used as anti-protozoa, bismuth for stomach ulcers, gold as anti-arthritis, and iron as anti-malaria. **(Lima *et al.*, 2013).** Some heavy metal derivatives act as anticancer agents. The heavy metals are not degradable, which makes them highly persistent in the

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environment. The continuous emission of toxic metals, as wastes, causes its increase in concentrations and redistribution in the ecosystem **(Awoyemi *et al.*, 2014).** Recently, due to the growing industrial activities, the release of Cd increased significantly in the environment above the normal limits with increasing exposure risk to Cd **(Cuypers *et al.*, 2010)**. Cadmium is a toxic metal that damages most of organs as liver, kidneys, bones, heart, brain and increase risk of cancer **(Nair *et al.*, 2013**). The mechanism of Cd toxicity is not very clear; however, (ROS) is the main suggested mechanism of Cd toxicity **(Dewanjee *et al.*, 2013).** ROS reacts with cellular macromolecules such as lipids, proteins, and nucleic acids, resulting in cell death **(Dewanjee *et al.*, 2013; Khanra *et al.*, 2015).** Antioxidants could be responsible for the possible mechanisms of toxic manifestations of Cd. Lead is toxic that is not biodegradable, and it is one of the pollutants that can be harmful at low concentrations due to its accumulatation in different parts of the human body **(Winiarska- Mieczan and Kwiecień 2016, Bede-Ojimadu *et al.*, 2018, Anyanwu *et al.*, 2018).**

Chelation is the extracting of heavy metals from cells with synthetic or natural agents and is considered the standard treatment for the toxicity of heavy metals. **Zhai *et al.*, (2015)** reported that $CaNa_2EDTA$ and meso-2,3-dimercaptosuccinic acid (DMSA) are synthetic chelators with a protective effects against Pb toxicity. However, synthetic chelators have a considerable disadvantage at high doses and cause a great disturbance in essential metals.

The most commonly used therapeutic strategy for heavy metal poisoning is chelation therapy to promote the excretion of metals. However, the chelators for Cd and Pb toxicity have several safety and efficacy problems. No chelation therapies approved for Cd toxicity in clinical use **(Goyer and Clarkson, 2001 and McCarty, 2012).** The development of research to find safe and efficient strategies against the toxicity of Cd and Pb. **Zhai *et al*. (2015)** reported that dietary supplements play a paramount role in relieving or preventing the toxicity of Cd and Pb. Dietary supplement strategies are advantageous**.**

Medicinal algae have been essential for living to fight against diseases since the dawn of civilization **(Nayak *et al.*, 2011**). *Spirulina platensis* is a species of cyanobacterium inhabiting highly alkaline, which incorporated into experimental fish feeds **(Palmegiano *et al.*, 2005).** *Spirulina platensis* has antiviral, anticancer, hypocholesterolemic, antidiabetic, antioxidant, anti-inflammatory, and antimetastatic activities; due to its content of virtually all known natural antioxidants, vitamins (B1, B5, B6, and E), the minerals (zinc, manganese, and copper), the amino acid methionine, and the super oxidants B-carotene, and the trace element selenium **(Kaur *et al.*, 2012; Colla *et al.*, 2007)**. The polysaccharide content of *S. platensis* increases the activity of the nuclear enzyme and the DNA repair synthesis. *Chlorella vulgaris* is a unicellular green alga

found in both fresh and marine water widely used as a food supplement due to its content of the supreme level of crude protein, carbohydrates, lipids, essential amino acids, and minerals **(Radhakrishnan *et al.*, 2015).**

ATPases are membrane-bound enzymes that regulate cellular volume, osmotic pressure, and membrane permeability **(Li *et al*., 2011).** ATPases are concerned with the immediate release of energy and are responsible for a large part of basic metabolic and physiological activities **(Dogan *et al.*, 2015)**. Therefore, the activity of ATPase transports as indicator of cellular activity and forms a useful toxicological tool **(Atli and Canli, 2007).**

The environmental pollution assessment includes antioxidant enzymes because of their ability under conditions of mild oxidative stress and their potential role in adaptation to pollutant-induced stress **(Adams and Greeley, 2000).** The main antioxidant defense enzymes in marine fish are SOD, CAT, and Gpx **(Sladjan *et al*., 2010).** Metallothioneins MTs are non enzymatic antioxidants have four main functions in the aquatic vertebrate: bioaccumulation of toxic metals and detoxification, homeostatic regulation of metals, protection against oxidative stress, and neuroprotective mechanism **(Wang *et al*., 2014).** MTs are proteins with low molecular weight (6-7 kDa) that contain up to 30% of cysteine can bind to heavy metal ions as Cd^{2+} and Pb^{2+} from 7-12 atom for each molecule and act as a trap for free radicals **(Takahashi, 2012).**
The purpose of this study is to examine the use of three concentrations of the algal mixture of *Spirulina platensis* and *Chlorella vulgaris* as a natural antidote to chelate the mixture of lead and cadmium-induced hepatic toxicity of seabass (*Dicentrarchus labrax*).

MATERIALS AND METHODS

1. Chemicals : Metallic salts, $Pb(NO_3)_2$, and $CdCl_2$ Analytical grade.

2. Algae*:* *Spirulina platensis* was cultivated and harvested according to the methods of **(Abou El-Kheir *et al*. 2008).** *Chlorella vulgaris* was cultivated and harvested according to do **(Amaral and Freire 2012; and Hasanein *et al*. 2018).**

3. Preparation of the diet: Formulated basal practice diet that contains approximately 47% raw protein and 12% crude lipids, needed for the nutritional requirements of juvenile European seabass, were used in the current study as basal diet. In addition, we added three concentrations of *Spirulina platensis* and *Chlorella vulgaris* in a ratio (1:1) to prepare the experimental diets. The addition of supplements has consisted of 30, 50, and 70 g kg^{-1} to the basal diet **(Hasanein *et al*., 2018).**

4. Animals: European juvenile sea bass was obtained from the marine hatchery of the Alexandria Fisheries Authority and acclimated to experimental conditions for a week. After the acclimation of fish, the average weight of fish (about 5.3g) was distributed to a density of 15 fish per aquarium, in triplicate, in 15 glass aquariums (100 L each) that were supplied with filtered natural seawater (23°C).

5. Experimental treatment and sample preparation: After the acclimation of fish, they randomly divided into five groups as follows: 1st group was fed with basal diet and lived in fresh seawater without any addition of heavy metals (control 1). In the other four groups, heavy metals (0.003 mg/l of $CdCl_2$ + 0.003 mg/l Pb $(NO_3)_2$ were added as water pollutant to the seawater and fed a basal diet (control 2), a 3% of a diet supplemented with an algal mixture of *Spirulina platensis* and *Chlorella Vulgaris* in the ratio (1: 1) (diet 1), a 5% of a diet supplemented with an algal mixture (diet 2), and a 7% of a diet supplemented with an algal admixture (diet 3). Fish were fed with 5% of the average body weight three times daily. The feces were diverted daily before the first feeding to avoid the accumulation of wastes. Filtered seawater of 37.5 ppt of salinity at temperature "23.4 ± 1.2°C" was added daily. Oxygen was supplied by aeration around 5.93 mg L^{-1}. The parameters of water quality were recorded during the entire experimental period, and the total ammonia and nitrite were analyzed according to the standard methodology of (APHA, 1995). The pH was adjusted at 7.6 ± 0.31, The natural photoperiod was ten hours of light: 14 hours of the darkness during the feeding experiment. Freshly prepared solution of heavy metals was added after the residual siphon to maintain the constant concentration of heavy metals in each aquarium. The experiment lasted for five weeks. At the end of the experiment, fishes were killed rapidly, below their head, by using a sharp knife. Six fishes from each aquarium in which liver and gills were dissected on ice, excised, and rinsed in isotonic NaCl saline. The tissue was rapidly homogenized in 10% w / v of phosphate buffer (pH 7.4). An electric homogenizer at 22,000 rpm for 20 seconds with 10-second intervals was used to get the supernatant for ELISA and antioxidant assay in liver samples. The homogenate was centrifuged at 1000 ×*g* in a refrigerated centrifuge at 4°C for 15 min then the supernatant was separated. For the antioxidant assay, the supernatant was frozen, thawed twice for complete disruption of the mitochondria **(Salach, 1978),** and then centrifuged again at 6000 ×*g* in a refrigerated centrifuge at 4°C for 15 minutes. Finally, the supernatant that contained the cytosolic and mitochondrial enzymes was saved for the immediate analysis of enzymatic activities.

6. Assay of Gill Na^+/K^+- Activity : The gill samples were weighed and homogenized by an electric homogenizer at 35,000 rpm at 65 mM L^{-1} in imidazole buffer (pH 7.4). The ATPase activity was tested in the crude homogenate by determining the inorganic phosphate (Pi) released from the hydrolysis of the ATP substrate at 25°C according to **(Uner *et al*. 2005).** The incubation media were prepared as described by **(Ames and Dubin 1956).** The final assay concentrations of the chemicals used for the incubation

media: Tris-HCl (pH 7.4) 30 mML^{-1}, NaCl 100 mML^{-1}, KCl 5 mML^{-1}, MgCl 25 mML^{-1}, ATP (vanadium free) 3 mML^{-1} and EDTA 0.1 mML^{-1}. After preincubation of the medium for 5 min at 25°C, the reaction was started by adding the samples and ATP appropriately, and the reaction continued for 30 min. The reaction was stopped after putting the samples on ice for 10 minutes and adding a mixture of ascorbic acid and molybdic acid (1:6, v/v). The samples were kept at room temperature for 10 min. The inorganic phosphate was determined at 390 nm using 1.0 ml aliquots of the incubated mixtures. The activity of ATPase was calculated from the slope of the graph. The tests, in triplicate, were carried out against the reaction target, and the ATPase activity was expressed as μM Pi/min/g of wet tissue. The change in absorbance was monitored at 390 nm using a UV/VIS spectrophotometer.

7. Total cytochrome P450 (CYP) : The microsomal liver fractions were obtained from the livers according to **(Stegemann *et al.*, 1979).** Livers' samples were thawed and homogenized in four volumes of homogenization buffer (0.08M Na2HPO4; 0.02M KH2PO4; 0.15M KCl, pH 7.4). Homogenate was centrifuged at 10,000 ×*g* for 20 min at 4°C, and the supernatant was then ultracentrifuged at 100,000 ×*g* for 60 min at 4°C. The pellet resuspended in 1.0 ml buffer (homogenization buffer with 20% glycerol, v/v) to obtain the hepatic microsomal fraction. The microsomal fraction for all spectroscopic CYP analyses of CYP was based on the methods described by **(Omura and Sato 1964 and Johannesen and Depierre 1978).**

8. Antioxidant enzymes: SOD activity was measured according to **(Paoletti and Mocali 1990).** Gpx. activity was measured according to the method of **(Paglia and Valentine 1967).** CAT activity was measured according to the procedure of **(Xu *et al.* 1997).**

9. Metallothionein ELISA assay: The assay of MT was measured by sandwich ELISA techniques at wavelength 450 nm. The micro-ELISA strip plate had been pre-coated with antibodies specific to Fish MT Standards or samples were added to the appropriate micro-ELISA strip plate wells and combined with the specific antibody. Then peroxidase-conjugated antibody specific for MT was added to each micro-ELISA strip plate well and incubated. Free components were washed away. The concentration of MT was determined by comparing the optical density of the samples to the standard curve.

10. Statistical Analysis: One-way analysis of variance (ANOVA) was used the level of $P \leq 0.05$ was adjusted as a statistically significant analytical grade.

RESULTS

1. Gill Na^+/K^+-ATPase Activity

There is a significant decrease in the activity of Na^+/K^+-ATPase ($P < 0.05$) between the control "1" and other groups (Fig. 1). The least value of Na^+/K^+-ATPase appears in control "2". The exposure to 0.003 mg/l of $CdCl_2$ + 0.003 mg/l Pb $(NO_3)_2$ causes an ionic disturbance and depletion in Na^+/K^+-ATPase. The groups of diets "2 and 3" appear significant improvements in the activities of Na^+/K^+-ATPase *vs.* the control "2".

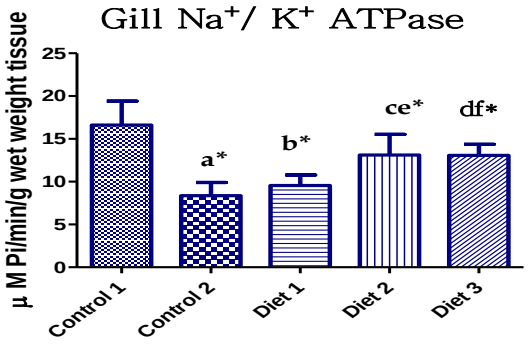

Figure 1: Gill Na^+/K^+-ATPase activity in *Dicentrarchus labrax*.

Data represent mean ± SD. *Significant at $p < 0.05$. n = 6; **a, b, c, and d:** represent a significant decrease between the control "1" and other groups; **e and f:** represent a significant increase between diet "2" and diet "3" *vs.* the control "2".

2. Total cytochrome P450 (CYP)

There is a significant increase ($P < 0.05$) in the concentrations of CYP between the control "2", diet "1", and diet "2" *vs.* the control "1". There is a significant decrease in the concentration of total CYP between diet "3" *vs.* the control "2". The CYP increased significantly in the control "2". Diet "3" shows the least values of CYP concentration (Fig. 2).

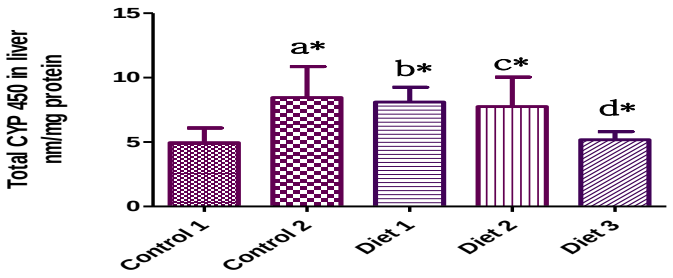

Figure 2. Total cytochrome P450 (CYP) in the liver of *Dicentrarchus labrax*.

Data represent mean ± SD. * Significant at $p < 0.05$. $n = 6$; **a:** represents a significant increase between the control "2" *vs.* the control "1", **b:** represents a significant increase between the diet "1" *vs.* the control "1", **c:** represents a significant increase between the diet "2" *vs.* the control "1". **d:** represents a significant decrease between the diet "3" *vs.* the control "2".

3. Enzymatic antioxidants

There are a significant decrease ($P < 0.05$) in the activities of SOD and Gpx for the control "2", diet "1", and diet "2", compared with control the "1"; the lower activities appear in the control "2", while the highest activities appear in the diet "3" compared with the control "2", as shown in (Fig. 3). There is a significant increase in catalase activity ($P < 0.05$) between the control "2" *vs.* the control "1", and a significant decrease between the diet "1" and diet "2" *vs.* the control "2". There is a significant decrease between the diet "3" *vs.* the control "1" and the control "2". *S. platensis* and *C. vulgaris* dietary supplements increase the activity of SOD and Gpx, but reduce the activity of catalase, in the diet "3". These results show that the best supplement of the algal mixture appears in the concentration of 7% algal mixture (diet 3).

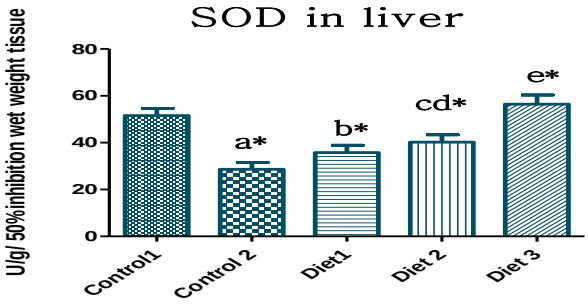

Figure 3.1 SOD activity in the liver of *Dicentrarchus labrax*.

Data represent mean ± SD. ***** Significant at $p < 0.05$. $n = 6$. **a,b,c:** represent significant decrease between the control "2", diet "1", diet "2" *vs.* the control "1"**. d and e:** represent a significant increase between the diet "2" and diet "3" *vs.* the control "2".

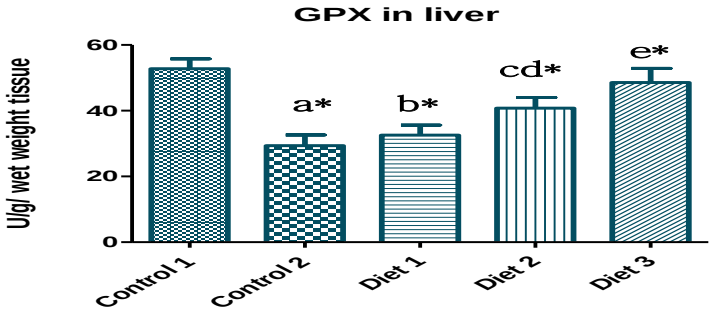

Figure 3.2. GPX activity in the liver of *Dicentrarchus labrax:*

Data represent mean ± SD. ***** Significant at $p < 0.05$. $n = 6$; **a, b, and c:** represent a significant decrease between the control "2", diet "1", and diet "2" *vs.* the control "1", **d** and **e:** represent a significant increase between the diet "2" and diet "3" *vs.* the control "2".

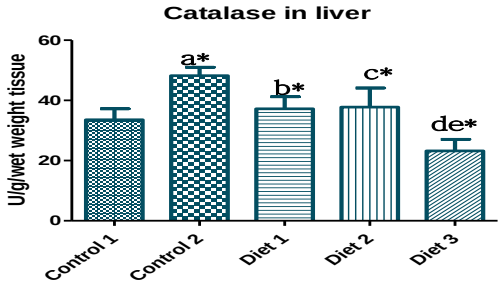

Figure 3.3. Catalase in the liver of *Dicentrarchus labrax:*

Data represent mean ± SD. ***** Significant at $p < 0.05$. $n = 6$. **a:** represents a significant increase between (control 2) vs. (control 1), **b** and **c**: represent the significant decrease between diet1 and (diet 2) vs. (control 2). **d** and **e**: represent a significant decrease between (diet 3) vs. (control 1 and control 2), respectively.

4. Non Enzymatic antioxidants

There was a significant increase ($P < 0.05$) in the concentration of metallothionein content in the liver of the control "2" and diets "1,2 and 3" compared to the control "1". The highest value appeared in the diet "3" compared with the control "2", as shown in Fig. "3.4".

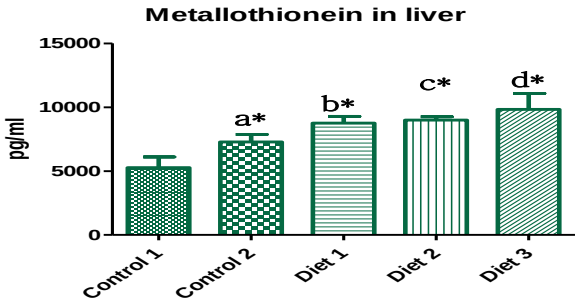

Figure 3.4. Non-enzymatic antioxidant "metallothionein" content in the liver of *Dicentrarchus labrax*.

Data represent mean ± SD. ***** Significant at $p < 0.05$. $n = 6$.

a: represents a significant increase between the control "2" *vs.* the control "1"; **b:** represents a significant increase between the diet "1" *vs.* the control "1", **c:** represents a significant increase between the diet "2" *vs.* the control "1"; **d:** represents a significant increase between the diet "3" *vs.* the control "1".

DISCUSSION

Our previous study **Hasanein *et al.*, (2018)** showed that the algal mixture of *Spirulina platensis* and *Chlorella Vulgaris* at a 7% supplemented diet has positive effects on the growth and feed utilization of *Dicentrarchus labrax*. This supplemented diet decreased the accumulation of Cd and Pb in the white muscles in the presence of a 0.006 mg/l mixture of cadmium chloride and lead nitrate. This algal mixture also had improvements in the antioxidant defense system of white muscles of *Dicentrarchus labrax*. The present study illustrates the effect of 3 concentrations of this algal mixture of *Spirulina platensis* and *Chlorella vulgaris* to chelate Cd and Pb from the gill and liver of *Dicentrarchus labrax*.

The exposure of seabass to these mixture metals causes ionic disturbance and oxidative stress that appears clearly in the values of the control "2" for Na^+ /K^+-ATPase and the total CYP450. The protective effect of the algal mixture repairs these disturbances by increasing the antioxidant activities. In agreement with **Assis *et al.*, (2009),** who reported that the exposure of fish "*Ancistrus multispinis*" to sublethal doses of deltamethrin inhibited the activity of the gills and heart Na^+/K^+-ATPase, and increased the total CYP450 in the liver. In addition, it agrees with **Atli and Canli (2011)** and **Kaya and Abulut (2015)** who illustrated that Pb toxicity caused inhibition in all ATPase enzymes and explained that the inhibition of Na^+/K^+-ATPase occurred because the enzyme molecule is very susceptible to the metals.

Among the mechanisms of toxicity, oxidative stress (defined as an imbalance between the antioxidant and prooxidant cellular forces) causes oxidative damage to tissues. Oxidative stress occurs mainly in the endoplasmic reticulum of liver cells, where the activities of CYP can generate ROS as by-products of detoxification processes **(Halliwell and Gutteridge, 1999).**

Initially, the "phases" of detoxification are described as the functionalization (or phase I) or the addition of oxygen to form a reactive site on the toxic compound, and the conjugation (phase II), or the process of addition of a water-soluble group at this new reactive site. In phase I CYP450 superfamily, the body's first defense to biotransformation of xenobiotics, steroid hormones, and pharmaceuticals. These microsomal membrane-bound heme-thiolate proteins are located mainly in the liver, kidneys, lungs, and brain. It is responsible for the oxidation, peroxidation, and reduction of several endogenous and exogenous substrates.

The function of CYP450 enzymes is to add a reactive group such as a hydroxyl, carboxyl, or amino group through oxidation, reduction, and (or) hydrolysis reactions. These initial reactions have potentially created oxidative damage in cellular systems due to the resulting formation of reactive electrophilic species. Therefore, it is recognized that any variability in the number of CYP450 enzymes could benefits and/or consequences for how an individual responds to the effects of one or more toxins **(Danielson, 2002).** After a xenobiotic has gone through the operation of becoming hydrophilic by reactions supervised by CYP450 enzymes, its reactive site can conjugate with an endogenous hydrophilic substance. This reaction is often named "phase II detoxification." Conjugation involves the transfer of several hydrophilic compounds (*via* their corresponding enzymes), including glucuronic acid (glucuronyl transferases), sulfate (sulfotransferases), glutathione (glutathione transferases), amino acids (D transferases' amino acids), an acetyl group (N-acetyltransferases), and a methyl group (N- and O-methyltransferases) **(Xu *et al.*, 2005).**

The result of the collective activity of these enzymes is an increase in the hydrophilicity of the metabolite, theoretically leading to increased excretion in the bile and/or urine **(Xu *et al.*, 2005).** Genetic polymorphisms have a profound influence on the function of CYP450 enzymes **Ginsberg *et al.*, (2010**) with the potential implications in the development of several forms of cancer (**Jancova *et al.*, 2010).** It is conceivable that the modulation of phase II enzymes by bioactive compounds of food origin may benefit patients who have altered enzymatic activity due to genetic polymorphisms or who have a high toxic load due to chronic exposure to environmental pollutants, overactive phase I activity, or hormonal imbalance. ROS can inflict irreversible damage to cellular components. In organisms, the expression of CYP increases, which tends to raise ROS levels in cells (**Schlezinger *et al.*, 2000).** This production of ROS adds to that of the

mitochondrial electron transport chain. To cope with this, organisms possess antioxidant defenses **(Cadenas and Davies, 2000).**

In the present study, there was a significant increase in the activities of SOD, Gpx, and metallothionein content in the liver of treated fish in diets "1, 2 and 3" compared to the control "2". The highest activities of them appeared in diet "3". The catalase activity increased in the control "2" and reversed again in the diet "3" due to the high contents of essential metals and fibers in the algal mixture that compete with toxic metals of cadmium. These results agreed is in agreement with **Upasani and Balaraman (2001)** who reported that *spirulina* attenuated the toxicity of Cd or Pb in the liver, the kidneys, and brain of the rat. In addition, **Shim *et al*. (2008)** reported that *Chlorella Vulgaris* had protective effects on liver toxicity in cadmium-treated rats.

The results are in agreement with **Castro *et al*. (2011),** who reported that *Spirulina* had marked anti-teratogenic effects in pregnant mice injected with Cd. Results showed that *Spirulina* had marked anti-teratogenic effects in pregnant mice injected with Cd. Furthermore, the oral administration of a high dose of *Spirulina* significantly decreased the frequency of fetuses with exencephaly, micrognathia, and skeletal abnormalities induced by Cd.

The current results are in agreement with **Yun *et al*. (2011)** that explained the protective effects of *Chlorella vulgaris* against Pb-induced oxidative stress in rat brains. Its content of dietary antioxidants such as vitamin C, vitamin E, phycocyanin and carotenoids, chlorophyll, protein (all the essential amino acids), and dietary fiber (along with minerals) allow them to relieve oxidative stress induced by toxic metals. In addition, the algal supplementation of *Spirulina platensis* and *Chlorella vulgaris* has essential metals such as zinc, calcium, iron, and magnesium. A deficiency in these metals leads to increase absorption and toxicity of Cd and Pb **(Reeves and chanery 2004).** Therefore, the supplementation with essential metals can provide protective effects against Cd and Pb poisoning. Zinc is one of the best-studied fundamental metals for the relief of heavy metal toxicity. Since zinc has chemical and physical properties similar to Cd and Pb, it competes for the binding sites of enzymatic proteins and metal absorption **(Bridges and Zalups 2005).** Zinc intake also induces the synthesis of metallothionein **(Suzuki *et al*., 1990).** Metallothionein plays an important role not only in the homeostasis of physiological metal ions (such as zinc, copper, and selenium), but also in protection from the toxicity of heavy metals (such as cadmium, mercury, lead, and arsenic) **(Yang and Shu 2015).**

In the present study, all the diet groups reveal high contents of MTs, especially diet "3". Our study agreed with **Prasanthi *et al.* (2010)** that reported the chronic exposure of rodents to cadmium chloride led cadmium to bind to the albumin in the circulation system and form the complex of metal-protein, that especially taken up by the liver. In the liver, cadmium acts as a stimulus for the synthesis of MT and bounds to MTs as a

protective mechanism for the body to sequester xenobiotic cadmium. Cadmium-metallothionein (Cd-MT) complex released to the circulation system. Due to the small size of MTs, the complex easy filtered through the glomerulus and reabsorbed by the proximal tubular epithelial cells in the kidney, then gets rid of this complex. The algal supplementation of *Spirulina platensis and Chlorella vulgaris* also rich in selenium. In the present study, the activity of GPx increased in diet "3", which may be due to the presence of high supplied with selenium, this agreed with the previous reports **Luches *et al*., (2007) and Liu *et al*., (2013)** illustrated that taking selenium prevents the toxicity of Cd and Pb in different organs in mice, including the brain, liver, lungs, kidneys, and blood. Selenium is a cofactor in the antioxidant enzyme "Gpx" that contributes to the antioxidant defense system, which can reduce the toxicity of Cd and Pb by reducing the Cd/Pb-induced oxidative stress and enhanced antioxidant capacity. The algal supplementation of *Spirulina platensis* and *Chlorella Vulgaris* also rich in vitamins C and E (**Yun *et al*., 2011).** Vitamins C and E are natural, non-enzymatic antioxidants that can eliminate free radicals and decrease lipid peroxidation. **El-Sokkary and Awadalla (2011)** reported that vitamin C attenuates oxidative damage and histopathological changes induced by $CdCl_2$ in the lungs and brain of rats. **El-Neweshy and El-Sayed (2011)** reported that vitamin C has similar protective effects on the liver, kidney, brain, and testes of rats exposed to Pb. In addition **Goyer and Cherian (1979)** reported that vitamin C acts as a Pb chelating agent, with a potency similar to that of EDTA.

CONCLUSION

The dietary supplement of *S. platensis* and *C. Vulgaris* of seabass (*Dicentrarchus labrax*) at a concentration of 7% supplement have natural chelation properties for the mixture of lead and cadmium in the gill and liver toxicity. The dietary supplement easily and inexpensively added to the daily diet. This dietary supplement is rich in essential metals that provide a protective effect against Cd and Pb poisoning. The dietary supplement repaired the ionic disturbance in the gills (that resulted from exposure to toxic metals), also increased the antioxidant activities in the liver (that responsible for ROS scavenging).

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