



**The Effect of dietary supplementation of *Spirulina platensis* and *Chlorella vulgaris* algae on the growth and disease resistance of the sea bass (*Dicentrarchus labrax*)**

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**ABSTRACT**

The study was designed to investigate the protective effect of dietary *Spirulina platensis* and *Chlorella vulgaris* algal mixture (AM, 1:1), at three inclusion levels (3, 5 and 7%), in European sea bass diets as a supplement. 0.006 mg/l mixture of cadmium chloride and lead nitrate (TM, 1:1) was added to the rearing water as a pollutant. Results showed that the growth and feed utilization indices were not significantly different between fish fed diets containing algal mixture at inclusion levels in the presence of the pollutant and fish fed the control diet (Control<sub>1</sub>) meanwhile, fish fed control diet with heavy metals (Control<sub>2</sub>) addition showed the lowest growth and feed utilization indices. The body composition analysis showed no significant difference among groups. There was a significant decrease in the accumulation of Cd and Pb in white muscles. The lowest accumulations appear in fish fed diet<sub>3</sub>. A significant increase in the activity of antioxidant enzymes; SOD, Catalase and Gpx were recorded in white muscles of diet<sub>1</sub>, diet<sub>2</sub> and diet<sub>3</sub> fish groups as compared to the control<sub>2</sub> group and the highest activity is in diet<sub>3</sub>.

**INTRODUCTION**

Heavy metals damage the aquatic ecosystem and accordingly the human being who depend on aquatic products as food sources. Heavy metals cannot be destroyed by biological decay and have the ability to accumulate in the ecosystem and also in the tissues of aquatic organisms so they become harmful to both living organisms and humans (Shelke and Wani, 2016). Heavy metals enter the cell throughout the interaction between metal ions and functional groups on the cell surface and cause toxicity (Sunita *et al.*, 2015). Heavy metals (Cd and Pb) are toxic and highly poisonous at very low concentrations (Inthorn, 2001). They are commonly considered pollutants in aquatic habitats. Cd was considered an important global problem because of its persistence and its ability to bioaccumulate (Ruangsomboon

and Wongrat, 2006). Toxicity of Cd is associated with pulmonary, renal, hepatic, skeletal, reproductive and cardiovascular dysfunctions (Hong *et al.*, 2004, Koyu *et al.*, 2006 and Tellez-Plaza *et al.*, 2008). Also, Cd is classified as a Group I human carcinogen by IARC (1999). Lead is the second element (after arsenic) on the top 20 list of the most poisonous heavy metals. Pb exposure induces neurological and hematological dysfunctions, kidney and liver damage, reproductive disorders and autoimmune diseases such as rheumatoid arthritis in the human body (Sandhir, 1995, Ronis *et al.*, 1996, Bergdahl *et al.*, 1998, Lidsky and Schneider, 2003 and Patrick, 2006). The target organs of lead are the bones, brain, blood, kidney, reproductive, cardiovascular systems and the thyroid gland (Homady *et al.*, 2002 and Massadeh *et al.*, 2004). Fish is continuously contaminated with ambient Cd by both water and food in polluted environments. Cd can damage fish, including loss of appetite and reduce growth (Mcgeer *et al.*, 2000). The effect of cadmium on aquatic organisms is analogous to those in humans and includes skeletal deformities and impaired renal function in fish. Fish skeletal deformities can lead to an impaired ability of the fish to find food and avoid predators, making this sub-lethal effect to be lethal (Landis and Ming, 2003). Cd cause also a disorder of the respiratory tract of fish, change in hematology and disorder of the whole body or plasma ion regulation by the induction of a large number of reactive oxygen species (Zikic *et al.*, 2001 Chowdhury *et al.*, 2004, Atli and Canli 2010). Toxic heavy metals are responsible for the formation of reactive oxygen species (ROS) and reactive nitrogen species (NO) such as hydrogen peroxide, superoxide anion radicals, and hydroxyl radicals induced through various mechanisms such as Fenton and Haber-Weiss reactions and causes many disturbance as enzyme replacement co-factors and transcription factors, antioxidant enzyme inhibition, cellular redox imbalance, ionic transport imbalance, DNA damage and protein oxidation (Doherty *et al.*, 2010, Lushchak, 2011 and Olushola *et al.*, 2014). Oxidative damage is counteracted by antioxidant defense systems and repair mechanisms (Gill and Tuteja, 2010).

Blue green algae are photosynthetic prokaryotes that capture sunlight for energy with chlorophyll and various accessory pigments. A microalgae strain must fulfill various criteria for being used in aquaculture feeding as simplicity of cultivation, lack of toxicity, high nutritional value with correct cell size and shape and a digestible cell wall to make nutrients available (Patil *et al.*, 2007).

*Chlorella vulgaris* is single-celled green algae that is found in both fresh and marine water and is widely used as a dietary supplement (Kay, 1991). *C. vulgaris* contains the highest levels of crude protein, carbohydrates, lipids, essential amino acids and minerals. (Radhakrishnan *et al.*, 2015). *Spirulina* sp. is a nutritious cyanobacterium and is used as a human food source for protein (Habib, 2008). It has gained considerable popularity in the human health, food industry and it is used as a protein supplement. As the use of synthetic antioxidants is reduced due to their presumed activity as carcinogenesis (Namiki, 1990) as well as the general refusal of the addition of synthetic food by the consumer (Mirada *et al.*, 1998). Recently, there has been a great demand for new natural sources of antioxidants to prevent oxidative damage of living cells and to reduce the destruction of food by oxidation. *Spirulina platensis* is the richest food source of antioxidants in nature and contains every known natural antioxidant, including the antioxidant vitamins B1, B5 and B6, and B12. The biochemical composition of spirulina indicates that *S. Platensis* has variety of essential nutrients such as provitamins, minerals as zinc, manganese, copper, the amino acid methionine, vitamin E and selenium (Kaur *et al.*, 2012). *S. platensis* has also polyunsaturated fatty acids such as  $\gamma$  Linolenic acid and phenolic acids,  $\beta$ -

carotene, which have antioxidant properties (Hirata *et al.*, 2000). On the other hand, it has high amino acid content up to (62%) and also has antiviral, anticancer, hypocholesterolemic, antidiabetic, anti-inflammatory and antimetastatic activities. These properties make *S. platensis* a potential drug for biomedical applications. In vitro studies show that *S. Platensis* polysaccharide content enhances the nuclear enzyme activity and DNA repair synthesis. The aqueous extract of *S. platensis* showed a protective effect against apoptotic cell death induced by free radicals (Estrada *et al.*, 2001, Joventino *et al.*, 2012).

The aim of the present study is to evaluate the effects of *Spirulina platensis* and *Chlorella vulgaris* algae supplementation as functional feed additives in sea bass diets on fish growth and disease resistance when TM mixture was added to fish culture medium.

## MATERIALS AND METHODS

**Chemicals :** The heavy metals in this work were obtained as metallic salts,  $Pb(NO_3)_2$  and  $CdCl_2$  from Sigma Co.

### *Spirulina platensis* growth

*Spirulina platensis* was cultivated and harvested at Hydrobiology Lab, National Institute of Oceanography and Fisheries, El-Qanater El-Khayria according to the method of Abou El-Kheir *et al.* (2008). The algal cells were aseptically cultured in modified Zarrouk's medium at the open air temperature. The cultures were scaled up serially from a 150 ml Erlenmeyer flask, 5 L carboy, 50 L inoculum pond and then to ponds of 1-5 m<sup>3</sup> capacity. The *S. platensis* culture was grown at ambient temperature in the pond. The culture growth was measured by the growth curve of algal chlorophyll concentration. The biomass were harvested by using 20 $\mu$  mesh size plankton net after 14 days. The harvested biomass was swaying and dried in open air for 48 hours. The dried Spirulina was used as a powder.

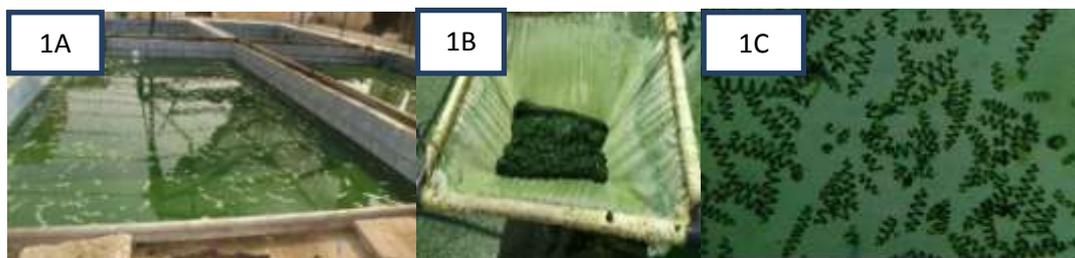


Fig. (1A) *Spirulina platensis* 5m<sup>3</sup> pond  
(1B) *S. platensis* harvest  
(1C) Microscopic photo to *S. platensis*

### *Chlorella vulgaris*

#### 1.3. Culture of *Chlorella vulgaris*

*Chlorella vulgaris* cultures were obtained from the marine hatchery in National Institute of Oceanography and fisheries Alexandria, Egypt.

#### **Inoculum and Culture conditions:**

*Chlorella vulgaris*, was grown in Amaral's medium (do Amaral and Freire, 2012). The growth medium was prepared by using analytically grade reagents supplied by sigma (St -Louis Mo, USA). Sterilization was carried out by autoclaving the flasks containing the media at 120°C for 20 minutes to prevent bacterial contamination. Indoor algal culture takes place in three stages. Culture volumes range from 2-liter flasks to 20-liter carboys. The temperature of the alga culture room was 20-25 °C.

Illumination was constant; provided by cool fluorescent lamps (5000 Lux) in a 12:12 h light dark regime. The cultures were incubated for 15 days (early stationary phase).

Culture in 200 L Cylinders:

Fiberglass semitransparent cylinders were used as intermediate culture, cleaned well with bleach and re-rinsed until bleach smell had totally gone off after addition of sodium thiosulphate solution, then were filled with tap water up to desired volume and aerate with the vigorous air through the blower before adding components of culture medium then inoculate with about 20% of the total water volume from indoor pure *C. vulgaris* culture vessel.

#### Culture in fiberglass tanks:

Culture tanks of 2m<sup>3</sup> were well cleaned, rinsed and sun dried for 24 h. They were filled with filtered tap water enriched with the pure, nutrient medium and the mother culture of pure *C. vulgaris* was inoculated with about 20% of the tank water volume. The tanks were aerated to supply with required quantity of oxygen and to keep cells and media in suspension. The required concentration of algae was developed after 20 days of inoculation. The tanks were kept open under 100% outdoor light exposures and the temperature average from 25-30°C .



Fig. 2 (a): Mass production of *Chlorella vulgaris* in National Institute of Oceanography and Fisheries (in Marine hatchery) (b) Dry Biomass, *Chlorella vulgaris*.

#### The Amaral's Medium for culturing *Chlorella vulgaris*:

This medium contained the following constituents (per two liter tap water) (do Amaral and Freire, 2012):

Urea	1.1123 g
KH <sub>2</sub> PO <sub>4</sub>	0.2400 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.2195
CaCl <sub>2</sub> .2H <sub>2</sub> O	0.1144
C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>8</sub> · 2H <sub>2</sub> O	0.0408
Tap water	To 2000 ml

#### Solution Trace metal stock (mg L<sup>-1</sup>) consists of:

Zn	0.686 ppm
Cu	0.909 ppm
Mn	0.488 ppm
Fe	8.158 ppm

#### Harvesting the algal biomass

The cells of *C. vulgaris* of 20 days old cultures (at the stationary phase) were concentrated through precipitation technique and then dried.

## Diet Preparation

The basal practical diet was formulated (Table 1) to contain about 47% Crude protein and 12% crude lipid, to fulfilled Juvenile European sea bass nutritional requirements. For preparing the experimental diets, three concentrations of dry *Spirulina platensis* and *Chlorella vulgaris* mixture (AM, 1:1) were added at 30, 50 and 70g kg<sup>-1</sup> diet and the basal diet was used as control.

**Table 1: Ingredients and chemical proximate composition of experimental diets**

Ingredient (g/kg)	Control	Diet1	Diet2	Diet3
Fish meal	500	500	500	500
Soybean meal	200	200	200	200
Shrimp meal	40	40	40	40
Yellow corn	20	20	20	20
Wheat bran	30	30	30	30
Starch	100	70	50	31
Fish oil	70	70	70	69
Vitamin and minerals premix	40	40	40	40
<i>Spirulina platensis</i>	-	15	25	35
<i>Chlorella vulgaris</i>	-	15	25	35
<b>Proximate composition (%)</b>				
Crude protein	46.5	46.9	47	46.6
Crude lipid	12.0	11.9	12.5	12.4
Ash%	11.2	11.5	10.9	11.8
Moisture	8.7	8.5	8.2	8.6

## Experimental Protocol

Juvenile European sea bass was obtained from the Marine hatchery of the National Institute of Oceanography and Fisheries in Alexandria and were acclimated to experimental conditions for one week before starting. After fish acclimation, uniform fish ( $IW = 5.3 \pm 0.2$  g), at a stocking density of 15 fish per aquarium, were distributed on triplicate basis, in 15 glass aquaria (100 L each) and supplied with filtered naturally seawater (23°C). The five experimental groups are as follows:

1<sup>st</sup> group: fed on the control diet and live in fresh filtered sea water without any pollutant addition (C1). In the other 4 groups, TM was added to the rearing sea water and fish were fed as follows: control diet (C2), 3% AM supplemented diet (D1), 5% AM supplemented diet (D2) and 7% AM supplemented diet (D3). Fish were fed at 5% of body weight 3 times a day. Water was changed daily and the faeces were siphoned before the first feeding to prevent accumulation of wastes. Filtered sea water of 37.5 ppt salinity and temperature  $23.4 \pm 1.2^{\circ}$  C. Oxygen was supplied by aeration with the minimum level observed during trial being 5.93 mg L<sup>-1</sup>. Water quality parameters were registered throughout the experimental period. Total ammonia and nitrite were analyzed according to the standard methodology of APHA (1995). PH was  $7.6 \pm 0.31$ , ammonia  $0.05 \pm 0.01$ , nitrite 1.4 mg/l, and the natural photoperiod was 10 h light: 14 h dark throughout the feeding experiment. Filtered naturally sea water was added. Fresh prepared stock solution of heavy metals was added daily to rearing water after the residual siphon to remain the constant concentration of heavy metals in each aquarium. The experiment lasted for 5 weeks.

## Growth and feed utilization

Fish growth was evaluated depending on weight gain (WG, g), weight gain rate (WG, %), specific growth rate (SGR, % day<sup>-1</sup>) parameters.

Feed utilization efficiency was expressed as follows: feed conversion ratio (FCR), feed intake (FI, % BW day<sup>-1</sup>), protein efficiency ratio (PER).

The following formulae were used:

WG = final fish weight (Wf) - initial fish weight (WI)

WG (%) = [(Wf - WI) / WI] × 100

SGR = [(ln Wf) - (ln WI)] / feeding trial days × 100

FI = 100 × Total feed consumed / [(WI + Wf) / 2] / feeding trial days.

FCR = feed consumed / WG

PER = WG / protein consumed

### Tissue preparation

At the end of the experiment, the fish were collected and weighed, six fish from each tank were rapidly killed by decapitation and then incised below the head; an incision was made along the left of the dorsal fin. Avoiding tissue squeezing, the skin was carefully and quickly peeled down. Parts of white muscles were dissected on ice, then excised, rinsed in isotonic NaCl saline, finally plotted and weighed. The tissue was quickly homogenized 10% W/V in ice-cold 50 Mm phosphate buffer (pH 7.4) contained 1 % Triton X100, using electric Homogenizer (USA) at 22,000 r.p.m for 20 Sec. each with 10s intervals. The homogenate was centrifuged at 1000 xg in a cooling centrifuge (Hettich, Germany) at 4° C for 15 min and the supernatant was separated. The supernatant was frozen-thawed twice for the complete disruption of mitochondria (Salach, 1978) then it was again centrifuged at 6000xg in a cooling centrifuge at 4 °C for 15 min. At last, the supernatant which contains the cytosolic and mitochondrial enzymes, was saved for immediate assay of enzyme activities. The concentrations of heavy metals (Cd and Pb) were measured in muscles, according to Oregioni and Aston (1984). A sample of tissue was digested using a mixture of nitric, perchloric and hydrofluoric acids in a previously cleaned and dried Teflon beaker, then evaporated to near dryness at 80°C. After complete digestion, the residue was transferred to 25 ml volumetric flask with 0.1 M HCl. The concentration of trace metals was measured using atomic absorption spectrophotometer (AAS), Shimadzu model (6800), metals were determined and measured in µg/g wet weight. All chemicals used in this study were obtained from Sigma Co.

### Enzymatic Activity and Glutathione (GPx) assay

SOD activity was measured by total superoxide dismutase, (mitochondrial Mn-SOD and cytosolic Cu/Zn- superoxide dismutase). The activity assayed according to (Paoletti and Mocali 1990). Catalase (CAT) activity was measured at 240 nm for 1 min by decreasing H<sub>2</sub>O<sub>2</sub> using the method of Bergmeyer *et al.* (1974). The activity of GPX was measured by NADPH decrease at 340 nm for 1 minute.

### Statistical analysis

Each reading parameter is represented as mean ±SE. Data were analyzed by one-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test using a software program (GraphPad 6 In Stat Software, Inc). P ≤ 0.05 was regarded as statistically significant (Snedecor and Cochran, 1967).

## RESULTS

### Growth performance and feed utilization efficiency

Growth performance and feed utilization indices are presented in Table (2). The values of WG, WG% and SGR showed that adding TM to rearing water affect

negatively ( $P \leq 0.05$ ) all growth parameters and causing sever regression in all values.

**Table 2: Growth performance and feed utilization parameters of *Dicentrarchus labrax* fed the**

Parameters	C1	C2	D1	D2	D3
IW	5.24±0.03	5.14±0.07	5.25±0.03	5.51±0.03	5.36±0.06
FW	8.39±0.02a	6.35±0.1b	8.22±0.45a	8.11±0.25a	8.83±0.24a
%WG	60.10±0.13a	23.51±2.15b	56.90±8.23a	54.73±3.81a	64.65±5.58a
SGR	1.34±0.02a	0.60±0.05b	1.28±0.15a	1.25±0.07a	1.42±0.10a
FI	2.31±0.1a	1.69±0.03b	2.34±0.08a	2.37±0.05a	2.27±0.03a
FCR	1.75±0.04a	3.55±0.46b	1.93±0.31a	1.94±0.12a	1.65±0.13a
PER	1.19±0.03a	0.47±0.04b	1.13±0.16a	1.09±0.08a	1.28±0.11a

experimental diets ( mean ± SE).

Also, for feed utilization indices the same trend was observed. Results also indicate that using AM at all inclusion levels enhance growth and feed utilization indices and group (D3) showed the best results, although they were not significantly different when compared with other groups which fed either control or diets supplemented with 3 and 5% AM.

### Body composition

The fish biochemical composition at the end of the experimental trial is illustrated in Table (3). The data showed no significant difference in ( $P \leq 0.05$ ) between C<sub>1</sub> and all parameters. Significantly, the lowest values were recorded in C<sub>2</sub> fish group.

**Table 3: The body biochemical composition of sea bass at the end of the experimental trial (expressed as mg/ml based on the fresh weight).**

Parameters	C <sub>1</sub>	C <sub>2</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
Protein	118.6± 17.7a	109.4± 17.3b	128.4± 7.7a	126.4± 37.3a	142.6± 25.4a
Lipid	19.0±2.4a	17.6±4.4b	20.4±3.9a	22.0±3.8a	26.8±10.5a

Values are expressed as mean ± SE.

### Bioaccumulation of heavy metals

The heavy metal concentrations in fish muscles are illustrated in Fig (3). From the figure it is clear that there is a significant decrease ( $P \leq 0.05$ ) in the accumulation of Cd and Pb in white muscles of D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub> fish groups relative to the C<sub>2</sub> and the lowest heavy metals accumulation is recorded in D<sub>3</sub>.

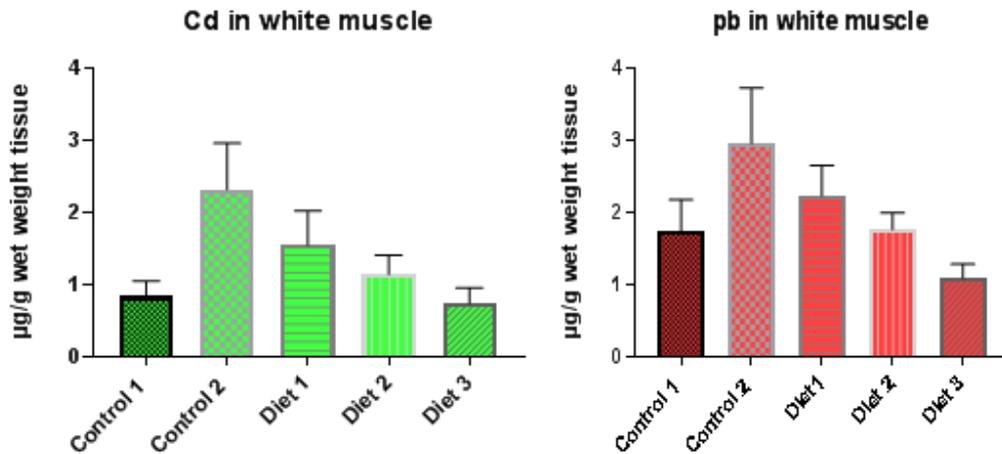


Fig. 3: Accumulation of Cd and Pb in white muscle

### Antioxidation enzymes

There was a significant increase ( $P \leq 0.05$ ) in the activities of SOD, CAT and Gpx in white muscle of treated fish (D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub>) compared to the C<sub>2</sub>. The highest activities were recorded in D<sub>3</sub> relative to C<sub>1</sub> and C<sub>2</sub> as shown in Fig (4).

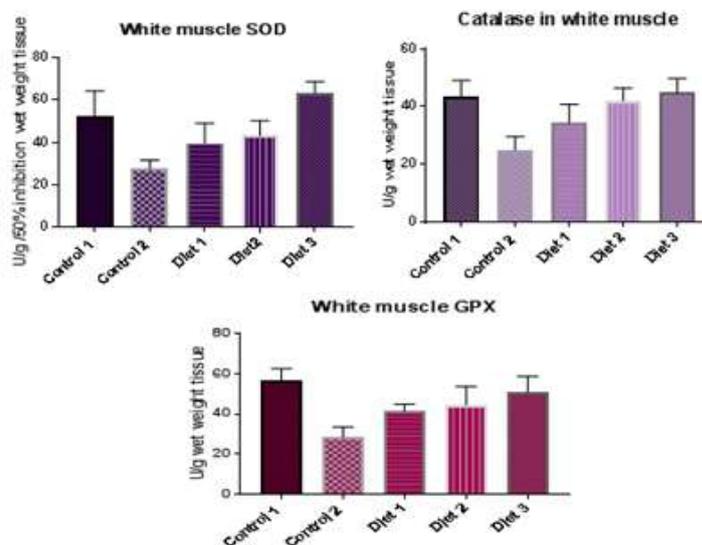


Fig. 4: Enzymatic antioxidant activities in white muscle of Seabass

## DISCUSSION

The present results indicate that using AM in fish diet has positive effects on fish growth and feed utilization when TM is added to rearing medium and the best results are recorded in D<sub>3</sub> fish group. This indicates chelation and reducing the harmful effect of TM when AM was used. The present results indicate the remedial effect of *Spirulina platensis* and *Chlorella vulgaris* that revealed signs of recovery in fish growth and feed utilization and indicating the positive potential of this algal mixture in fish toxicity overcome. Kaur *et al.* (2012) mentioned that *Spirulina* and

Vitamin E (Tocopherol acetate) revealed some signs of recovery in the haemopoietic tissues, but Spirulina found to be comparatively much effective than Vitamin E (Tocopherol acetate) when Catfish *Clarias gariepinus* were subjected to mercuric chloride in rearing water.

The study conducted by Ayyappan *et al.* (1991) clearly demonstrated the advantages of spirulina meals as additives in the diet for different carp species. The results of Abu-Elala *et al.* (2016) indicate that adding spirulina to Nile tilapia diets significantly improves growth and food utilization in terms of FCR and PER as compared with the control group. In addition, Teimouri *et al.* (2013) concluded that the inclusion of *Spirulina platensis* as a feed additive in the Nile tilapia diet may improve feed utilization and attributed that to the increment of gut bacterial colonization. James *et al.* (2006) suggested that *S. platensis* improves the intestinal flora that break down of indigestible feed components to extract more nutrients from the diet and also stimulates the enzyme production that incorporated fats in fish metabolism instead of to be stored. In contrast, Ungsethaphand *et al.* (2010) showed that the growth performance of hybrid red tilapia, fed on *S. platensis* supplemented diets, was not significantly differing with the control group. Sirakov *et al.* (2012) indicated that weight gain and average daily growth of rainbow trout fed with 10% spirulina meal as feed additive were higher than those from the group fed with basal diet, but the differences were not statistically proven ( $p > 0.05$ ). The potential of Spirulina as a nutrient source in diets for *Haliotis midae* (Britz, 1996) and *Haliotis asinina* (Bautista-Teruel *et al.*, 2003) was reported. The present results indicate that adding of AM does not significantly affect protein and lipid contents and these results are parallel to Amer (2016) results that showed no significant difference in protein content between control and mono-sex Nile tilapia which fed graded levels of *S. platensis*, but he recorded a significant decrease in fat content between control and other groups. Abdel-Latif and Khalil (2014) showed that no significant differences among treatments in lipid content, but the protein content in the muscle of fish fed 10% spirulina diet was the highest. The contradiction in results may be attributed to the difference in species and experimental conditions.

*Spirulina platensis* and *Chlorella vulgaris* are microalgae that have unique therapeutic properties. The present results indicate significant decrease in Cd and Pb accumulation in white muscles of fish groups, D1, D2 and D3 compared to C2. The lowest accumulation of Cd and Pb is recorded in D3 as AM was supplemented at a level of 7% in fish diet. This finding agrees with the previous studies where Hegazi *et al.* (2014) reported that *spirulina* decreased the accumulation of lead in bone, muscle and liver of Nile tilapia. Kaoud (2012) showed that, adding of dried *S. platensis* ameliorate the hematological parameters (RBCs, Hb and Hct) and the toxic effect of Hg (mercury) which indicates the capability of *S. platensis* to chelate Hg from the reared water of Catfish *Clarias gariepinus*. The chelation effect of Spirulina is due to the presence of B-carotene as a bioactive compound that maintains the mucous membrane firmly and prevents entry of toxic element into the body and else chlorophyll acts as a cleansing and detoxifying phytonutrient against the toxic substances (Henrikson, 1994). On the other hand, the chelation effect of *C. vulgaris* stimulates toxicant excretion in the urine and feces of rats (Miranda *et al.* 2001, Mustafa, 2015). *C. vulgaris* else has defensive mechanisms against Lead acetate trihydrate in rats by eliminating heavy metal through the digestive function and affecting the absorption or excretion of metals. Also, *C. vulgaris* is rich in iron, zinc, calcium, phosphorus, and magnesium leading to a competition at shared absorptive receptors in the intestinal mucosa and consequence may prevent lead absorption from

the alimentary tract, (Wright *et al.* 2003, Mustafa, 2015). Shaker *et al.* (2008) reported that fish survived in a mixture of Pb, Cd and Hg and fed with hyacinth and *Chlorella* as feed additives, showed reduction in the negative effects of pollutants and additionally, the residual effects. On the other hand, *C. vulgaris* has dietary fibers, which constitute approximately 10% may be used to treat humans exposed to lead (Wright *et al.* 2003). This study concluded that dietary supplement of *C. vulgaris* and *spirulina platensis* have the ability to chelate Cd and Pb and consequently, decrease their accumulation in white muscles of Juvenile European sea bass.

Antioxidant enzyme activities of SOD, CAT and Gpx in white muscle of fish consumed AM at all inclusion levels were significantly increased as compared with results recorded in the control group. The highest activities appear in D3 when compared to D1 and D2. This finding agrees with the previous studies (Kaoud, 2012 and Hegazi *et al.*, 2014). The antioxidant defense systems include a number of enzymes, which act as scavengers of the highly reactive intermediates generated in cells during hydrocarbon metabolism to maintain cell homeostasis. Antioxidant enzymes include SOD, CAT, GST, GPx and GR. Superoxide dismutase, (SOD) is a metalloprotein that catalyzes the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen, so it reduces the risk of the most effective OH, through the metal-catalyzed Haber-Weiss reaction (Gill and Tuteja, 2010). SOD represents the first line of defense against ROS, which formed in various organelles such as mitochondria. Also catalase (CAT), removes the hydrogen peroxide by conversion to water and oxygen. Also, glutathione-S-transferase (GST); glutathione peroxidase and glutathione reductase, all involved in the removal of hydrogen peroxide from the system in conjunction with reduced glutathione (GSH) (Gülüzar *et al.*, 2016).

Choudhary *et al.* (2007) investigated the effect of heavy metals (Pb, Cu, and Zn) in *Spirulina platensis* and observed increased SOD activity with increasing concentrations of metals, indicating the appearance of the scavenging mechanism of ROS ( $O_2^{\cdot-}$ ) and the resistance of *Spirulina platensis*. A bell-shaped dose-response curve of SOD activity was detected in *Chlorella vulgaris* in the presence of chromium, which is proposed as a biomarker for Cr contamination in water (Rai *et al.*, 2013). *Spirulina* also has a pronounced antiteratogenic effect in Cd-injected pregnant mice. Oral administration of a high dose of spirulina significantly reduces the incidence of fetuses with exencephaly, micrognathia, and skeletal abnormalities induced by Cd (Paniagua-Castro, 2011) In addition, it has been reported that spirulina reduces the amount of microsuclear polychromatic erythrocytes and microkernel-containing normochromatic erythrocytes in blood cells of Cd-exposed mice (both mother and fetus) (Argüelles-Velázquez, 2013). *Spirulina* also has many dietary antioxidants, such as vitamin C, vitamin E, phycocyanobilin and carotenes that allow them to relieve toxic metal-induced oxidative stress (Yun *et al.*, 2011). Cyanobacteria catch ROS with their antioxidant systems. *Spirulina platensis* has two important phycobiliproteins known as phycocyanin and allophycocyanin. Previous studies have shown that phycocyanin has potential antioxidant and anti-inflammatory properties by trapping peroxy, hydroxyl and superoxide radicals (Estrada *et al.* 2001, Bhat and Madyastha, 2001, Bermejo *et al.*, 2008).

## CONCLUSION

The present study concluded that the dietary supplement of *Chlorella vulgaris* and *spirulina platensis* has the ability to chelate and reduce the harmful effect of Cd

and Pb, consequently, decrease their accumulation in white muscles and increase antioxidant defense mechanism of Juvenile European sea bass. This cheap dietary supplement can act as a therapeutic adjuvant agent for the problems associated with the aquaculture of sea bass.

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