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Effects of Cadmium and Dimethoate on Some Biological and Biochemical Indices in Freshwater Green Algae, *Spirogyra* sp.

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ABSTRACT: The present study investigates the influence of an organophosphorus pesticide, namely Dimethoate, and cadmium on biomarkers of the green alga, *Spirogyra* sp., in a 14-day experiment. For so doing, it has exposed *Spirogyra* sp. to 0.0, 100, 200, and 400 μg L^{-1} of Dimethoate and/or 1 mg L^{-1} of cadmium chloride ($CdCl_2$) to observe a reduction in chlorophyll a and b level in *Spirogyra* sp., exposed to 200 and 400 μg L^{-1} of Dimethoate as well as algae treated with cadmium alone or in combination with Dimethoate. Levels of malondialdehyde (MDA) and total antioxidant in cells, as well as the activity of ascorbate peroxidase (APX) soar in *Spirogyra* sp., exposed to Dimethoate and/or cadmium (alone or simultaneously). Also *Spirogyra*'s exposure to cadmium and/or Dimethoate significantly increases catalase (CAT) activity. However, levels of carotenoids in *Spirogyra* sp., treated with both cadmium and Dimethoate, decline significantly, with no significant change found in catalase activity of *Spirogyra* sp., exposed to 100 and 200 μg L^{-1} of Dimethoate, in comparison to the control group. However, CAT activity rises significantly in *Spirogyra* sp., treated with 400 μg L^{-1} of Dimethoate. Cadmium can cause cytotoxicity in 1 mg L^{-1} concentration of the green algae (*Spirogyra* sp.). On the whole, investigating the biological and biochemical markers in *Spirogyra* sp., exposed to different concentrations of Dimethoate, has revealed some concentration-dependent toxicity. Furthermore, Dimethoate can synergistically increase toxicity and bioavailability of cadmium in *Spirogyra* sp.

Keywords: *Spirogyra* sp., Metal, Organophosphate pesticide, Bio-concentration, Oxidative stress

INTRODUCTION

Algae, such as phytoplankton and micro- and macro-algae, have a key role in the biogeochemical cycle of environmental pollutants in aquatic ecosystems (Wang et al., 2012; Qiu et al., 2017). Pollutants' bio-sorption by algae has a major impact on elevating the concentration of these compounds in water column (Qiu et al., 2017). Cell walls of algae usually contain a cellulose precursor and their cellular spaces are rich in sulphated polysaccharides. Although proteins, lipids, and nucleic acids might be found on the cell walls of macro-algae, these compounds are usually in cytoplasmic membrane and cell cytoplasm, easily absorbing lipophilic chemical

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compounds, which in turn increases both the rate of pollutants' distribution in the ecosystem and their bioaccumulation in the food chain (Wang et al., 2012; Farias et al., 2018). By absorbing such pollutants, algae play a key role in transferring these compounds from water to aquatic organisms (Wang et al., 2012). Algae have a high capacity to form a chemical bond with environmental pollutants; therefore, they can be regarded as an appropriate bio-indicator to evaluate and monitor pollution of aquatic ecosystems (Wang et al., 2012; Qiu et al., 2017).

In addition to conventional application of pesticides in agriculture, they are used in public health management (e.g. dealing with disease-carrying rodents and insects); are used both commercially (e.g. for water disinfection purposes) and domestically (Wee & Aris, 2017). Pesticides can also enter aquatic ecosystems through nonpoint sources (e.g. agricultural drainage) and point sources (e.g. domestic and industrial wastewater discharge) (Wee & Aris, 2017).

Dimethoate, O,O-dimethyl S-[2-(methylamino)-2- oxoethyl] phosphorodithioate, is an organophosphate pesticide, used to control a wide range of larval and adult insects (Dogan & Can, 2011); (Amorim, et al., 2012). Compared to other organophosphate pesticides, Dimethoate has a higher solubility in water and is very unstable in soil; therefore, it is easily leached away from soil to find its way into aquatic ecosystems via agricultural runoff (Fazilat et al., 2017).

Studies, conducted on phytotoxic effects of organophosphate pesticides on phytoplankton, indicate that these pesticides negatively affect the growth rate, preventing the biosynthesis of chlorophyll, proteins, and carbohydrates (Chen et al., 2007). Changes in phytochemical compounds, reduced growth rate and biosynthesis of photosynthtic pigments, disruption of electron transport chain, lipid peroxidation, increased rate of oxygen consumption, redeuced carbon fixation, decreased adenosine triphosphate (ATP), total thiol, and total glutathione level are reported in *Aulosira fertilissima*, exposed to butachlor (Kumari et al., 2009). Dimethoate can influence the stability of cell membrane and physiology of microalgae significantly (Pandey et al., 2015). Microalgae's exposure to Dimethoate can highly reduce photosynthesis, activity of photosystem II, and phosphorylation, thus preventing electron transfer during photosynthesis and decreasing transpiration and stomatal conductance (Pandey et al., 2015).

Pesticides' introduction to aquatic ecosystems can significantly and unfavourably affect toxicity and bioavailability, not to mention the physiological response and effectiveness of nontarget species (Banaee et al., 2015a, b; Nematdoost Haghi & Banaee, 2017).

Cadmium (Cd) is another environmental pollutant, entering surface and underground waters through municipal and industrail wastewater (Zhang et al., 2015). By producing reactive oxygen species (ROS) at a cellular level and reducing the antioxidant capacity, it can cause oxidative stress (Zhang et al., 2015). This metal could also interact with other macromolecules within the cells, thus leading to DNA mutation, lipid peroxidation, and damage to proteins' structure and function (Zhang et al., 2015). When microalagae are exposed to heavy metals, the growth rate and carotenoids' synthesis will decline (Wang et al., 2012; Samadani et al., 2018). Decreased growth rate, biochemical disorders, and genetic damage are reported in the brown seaweed, Hijiki (*Sargassum fusiforme*), exposed to cadmium (Zhang et al., 2015).

A combination of pollutants, including pesticides and heavy metals, are often found in aquatic ecosystems, wherein they affect aquatic organisms (Wang, et al., 2018). Therefore, investigating the impact of two or more toxic compounds on aquatic

organisms' health is a key topic in toxicological studies (Wang, et al., 2018; Samadani et al., 2018). Toxicity of either Dimethoate or Cd has been reported; however, there is not much data on physiological response of algae to the combination of Dimethoate and Cd, the former of which may prevent detoxification and excretion of Cd from the green alga, *Spirogyra* sp., and vice versa. Thus, their detoxification can rise when they are simaltaneously present in the ecosystem. That is why, the present study has used *Spirogyra* sp. as a bioindicator to investigate the effects of Cd, both alone and in combination with Dimethoate, on pigments and oxidative stress in green algae.

MATERIALS AND METHODS

Spirogyra were collected from Maroon River, Khuzestan Province, Iran. They were washed thoroughly with distilled water so that their debris would be removed. Afterwards, to eliminate the bacterial contamination, algae were exposed to ultraviolet radiation for 10 minutes. The collected algae had a slippery mucilaginous texture, easily separable by hand into long hair-like threads. Under sterile conditions, algae samples were cultured on an agar medium (0.7 g agar per 100 ml of distilled water) in a petri dish. After 48 hours, they were removed from the solid surface of the culture medium and transferred to the Chu No.10 liquid medium (Table 1) for purification and propagation (Chu, 1942). Light microscopy studies identified the genus as the green algae *Spirogyra* sp.

Then, thalli of the green algae *Spirogyra* sp. were placed in Erlenmeyer flasks, equiped with aeration, containing liquid culture medium and were left inside a growth chamber (25±2°C, pH 7.5) under a 16:8 h light-dark cycle (under white fluorescent lamps with 3000-3500 lux) on a shaker (Mane & Bhosle, 2012).

Table 1. Chu No.10 liquid medium

Compounds	g L^{-1}
$Ca(NO_3)_2$	0.04
K_2HPO_4	0.005-0.01
$MgSO_4.7H_2O$	0.025
Na_2CO_3	0.02
Na_2SiO_3	0.025
$FeCl_3$	0.001
Vitamin B_{12}, Biotin &Thiamine	3×10^{-7}

After 7-10 days, an algal mass appeared. Algae were separated from the initial culture medium by means of a centrifuge. The experiment exposed 2.5 g of *Spirogyra* sp. per liter in a 2×4 factorial design to 0.0, 100, 200, and 400 μg L^{-1} of Dimethoate (Aria Shimi Company, Iran, EC 40%) along with 0.0 and 1 mg L^{-1} of cadmium chloride ($CdCl_2$ (99%), Merck, Germany) in three replications and in 24 two-liter Erlenmeyer flasks, which contained the culture medium. The experiment also lasted for 14 days under standard conditions in a growth chamber. Fifty percent of the water was exchanged daily to reduce the build-up of metabolic wastes, followed by addition of the pesticide and $CdCl_2$ once more, in order to maintain dimethoate and cadmium concentrations constant. Thus, the compounds, needed to maintain alga *Spirogyra* sp. growth, were added to the culture medium every day.

At the end of the incubation period, 100 ml samples were taken from each Erlenmayer flask and then centrifuged at 5000 rpm for 15 minuteas. After collecting microalgae samples, physiological indices such as chlorophyll a, chlorophyll b, carotenoid, malondialdehyde, as well as the activity of ascorbate peroxidase (APX) and catalase (CAT) were measured. The cultured *Spirogyra* sp. were harvested through centrifugation at 5000 rpm for 10 min.

In this study, levels of chlorophyll a, b, and total carotenoids content of *Spirogyra* Sp. were determined spectrophotometrically, according to Lichtenthaler & Buschmann (2001) and Miazek & Ledakowicz (2013) with some modifications. Aliquots of the

extracts were prepared at a concentration of 1 mg/mL in acetone. Samples' absorbance was measured at 470, 645, and 664 nm by means of a spectrophotometer. Acetone was used as a blank. The pigment content (chlorophyll a, chlorophyll b, and total carotenoids) was calculated, using Lichtenthaler equations: (Chla: Chlorophyll a , Chlb: Chlorophyll b, V: volume of extract, W: weight of sample).

$$Chl_a = 13.36\left(A_{664}\right) - 5.19\left(A_{645}\right) \times \frac{V}{1000 \times W}$$

$$Chl_b = 27.43\left(A_{645}\right) - 8.12\left(A_{664}\right) \times \frac{V}{1000 \times W}$$

$$\text{Total Carotenoids} = \frac{1000\ A_{470} - 1.63\ Chl_a - 104.96\ Chl_b}{221}$$

Ascorbate peroxidase (APX) activity was assayed, using a modified method (Elavarthi & Martin, 2010) and APX activity was determined from decreased absorbance at 290 nm due to oxidation of ascorbate in the reaction. CAT activity was determined as a decrease in absorbance at 450 nm due to hydrogen peroxidase consumption, as described by Góth (Góth, 1991), though with some modifications. Malondialdehyde (MDA) content was assessed with modified thiobarbituric acid assay (Placer et al., 1996). The total antioxidant activity of the crude extract of *Spirogyra* Sp. was determined by the phosphomolybdneum method at 635 nm (Prieto et al., 1999). All biochemical indices were measured by UV/VIS spectrophotometer (Biochrom Libra S22).

For digesting the sample, a representative 1 g *Spirogyra* Sp. (wet weight) was digested with repeated additions of 5 ml nitric acid (HNO_3) and 5 ml of 30% hydrogen peroxide (H_2O_2). After 60 minutes, 5 ml of hydrochloric acid (HCl) was added to the initial digested material and the sample was refluxed. This digestate was filtered and the filter paper and residues were rinsed, first with HCl and then hot distilled water. The digestate was then diluted to a final volume of 15 mL. Concentrations of cadmium were measured, using ICP-OES-Perkin elmer (optima 7300-DV). After drawing a diagram to calibrate cadmium, metal levels in these prepared solutions were measured. Samples were filtered using whatman filter (0.22 µm) and at last Cd concentration in each sample was detected with ICP instrument. Cd levels were measured in triplicate and measurements were repeated three times.

All data were examined for normality (Shapiro-Wilk test). Statistical tests were performed with SPSS (IBM, 19) software by means of one way analysis of variance (ANOVA). The significant means were compared via Tukey test, wherein $P < 0.01$ was considered statistically significant. Data were presented as mean ± S.D in each experimental group and significant differences between values were indicated by $P < 0.01$. The effects of the treatments with Dimethoate and Cd on biochemical indices were evaluated with two-way ANOVA.

RESULTS AND DISCUSSION

Algae are very sensitive to environmental stress, quickly responding to any alteration in their conditions. Besides, biomarkers such as chlorophyll a and chlorophyll b, carotenoid content, the activity of enzymes and oxidative stress can demonstrate the effect of pollutants on algae (Pan, et al., 2017). *Spirogyra's* exposure to 200 and 400 µg L^{-1} of Dimethoate significantly reduced levels of chlorophyll a and b. Furthermore, levels of chlorophyll a and b in algae, treated either with Cd alone or with Cd in combination with dimethaote were significantly lower than those of the control. An increase in the production rate of free radicals in *Spirogyra* sp., exposed to Dimethoate and/or Cd, can be the main factor for the destruction of chlorophyll a and b in chloroplast; therefore, a significant reduction in chlorophyll a and b in *Spirogyra sp.*, treated with Dimethoate and/or Cd, can adversely affect the photosynthetic capacity of this alga (Table 2).

Table 2. Biological index of freshwater green alga, *Spirogyra* sp., exposed to dimethoate, both alone and in combination with cadmium chloride

Treatments	Chlorophyll a (g L^{-1})	Chlorophyll b (g L^{-1})	Carotenoids (g L^{-1})
Control	0.88 ± 0.30^{b}	0.94 ± 0.09^{c}	10.82 ± 1.32^{c}
100 µg/L Dimethoate	0.57 ± 0.17^{ab}	0.75 ± 0.14^{bc}	8.17 ± 2.27^{abc}
200 µg/L Dimethoate	0.46 ± 0.13^{a}	0.63 ± 0.13^{ab}	7.54 ± 1.63^{ab}
400 µg/L Dimethoate	0.36 ± 0.12^{a}	0.50 ± 0.12^{a}	9.30 ± 0.94^{bc}
1 mg/L Cadmium	0.30 ± 0.10^{a}	0.47 ± 0.08^{a}	9.02 ± 1.22^{abc}
100 µg/L Dim × 1 mg/L Cd	0.34 ± 0.08^{a}	0.51 ± 0.07^{a}	6.80 ± 1.13^{ab}
200 µg/L Dim × 1 mg/L Cd	0.33 ± 0.03^{a}	0.43 ± 0.06^{a}	6.37 ± 0.37^{a}
400 µg/L Dim × 1 mg/L Cd	0.39 ± 0.05^{a}	0.48 ± 0.08^{a}	7.21 ± 0.73^{ab}

- Data are presented as mean ± S.D. Significant differences between values, when compared with the control group, are characterized by alphabetical symbols ($P < 0.01$).
- Dim: Dimethoate
- Cd: Cadmium

Although there was no significant difference in cartenoid levels of *Spirogyra sp.*, treated with 100 and 400 µg L^{-1} of Dimethoate, compared to the control group, cartenoid level of *Spirogyra sp.*, treated with 200 µg L^{-1} of Dimethoate, was significantly lower than that of the control. Moreover, cartenoid level in *Spirogyra sp.*, treated with both Cd and Dimethoate, was considerably lower than that of the control. In these treatments, carotenoids production was decreased in comparison to control (Table 3). However, there was no significant change in cartenoid levels of *Spirogyra* sp., treated with Cd, compared to the control group (Table 3). Cartenoids, as non-enzymatic antioxidants, play an important role when neutralizing free radicals in *Spirogyra* sp.; therefore, the reduced levels of cartenoids in *Spirogyra* sp., treated with Dimethoate and/or Cd, can be attributed to their oxidation by ROS. A decrease in levels of chlorophyll a, chlorophyll b, and cartenoids is reoprted in the green microalga *Chlorella vulgaris* treated with cadmium, copper, and lead (Piotrowska-Niczyporuk et al., 2012), and in *Chlamydomonas reinhardtii* treated with triclosan, aclonifen, and dichlofluanid (Almeida et al., 2017). Decreased chlorophyll a in microalgae, viz. *Chlorella pyrenoidosa*, *Merismopedia* sp., *Scenedesmus obliquus*, and *Chlorella pyrenoidosa*, treated with chlorpyrifos (Chen, et al., 2016) and carbamazepine (Zhang, et al., 2012) suggests the role of free radicals in destroying chlorophylls of chloroplasts. Any decline in the synthesis level of chlorophyll in microalgae, exposed to pesticides, can be another factor that contributes to a lower chlorophyll level (Zhang, et al., 2012). Therefore, environmental tensions can reduce levels of chlorophyll a, chlorophyll b, and cartenoids in algae (Pan, et al., 2017).

Table 3. Bio-concentrations of cadmium in freshwater green alga, *Spirogyra* sp. exposed to dimethoate, both alone and in combination with cadmium chloride

Treatments	Bio-accumulation of cadmium (mg g^{-1})
Control	Nd
Dimethoate 100 µg L^{-1}	Nd
Dimethoate 200 µg L^{-1}	Nd
Dimethoate 400 µg L^{-1}	Nd
Cadmium 1 mg L^{-1}	0.078 ± 0.032^{a}
Dim 100 µg L^{-1} × Cd 1 mg L^{-1}	0.183 ± 0.105^{a}
Dim 200 µg L^{-1} × Cd 1 mg L^{-1}	0.473 ± 0.089^{b}
Dim 400 µg L^{-1} × Cd 1 mg L^{-1}	0.606 ± 0.060^{b}

- Data are presented as mean ± S.D. Significant differences between values, in comparison with the control group, are characterized by alphabetical symbol ($P < 0.01$).
- Nothing has been detected.
- Dim: Dimethoate
- Cd: Cadmium

Levels of MDA in *Spirogyra* sp., treated with Dimethoate and/or Cd, were considerably higher than those of the control group (Figure 1). An increase in MDA level in *Spirogyra* sp., exposed to Dimethoate and/or Cd, may suggest the increased rate of free radicals as well as the occurance of lipid peroxidation, which was reportedly higher in microalga *Chlorella vulgaris*, treated with cadmium, copper, and lead (Piotrowska-Niczyporuk et al., 2012). Researchers found that oxidative stress in the green alga, *Chlamydomonas reinhardtii*, treated with triclosan, aclonifen, and dichlofluanid, can increase MDA level, reduce cellular glutathione, and alter other biochemical indices (Pan, et al., 2017 ; Almeida et al., 2017).

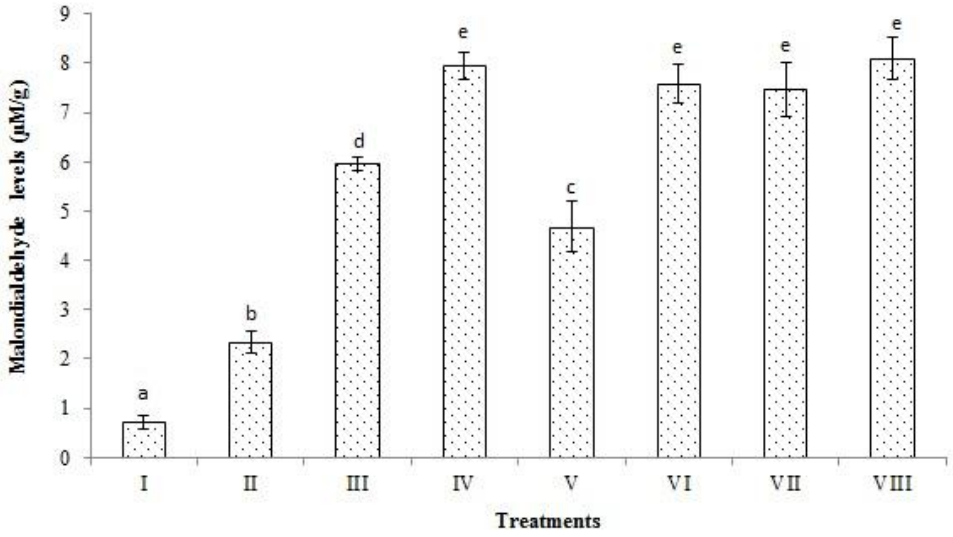

Fig. 1. Levels of malondialdehyde in *Spirogyra* sp., exposed to Dimethoate and/or cadmium chloride. Data are presented as mean ± S.D with significant differences indicated by alphabetical symbol ($P < 0.01$)

- Control: Group I; 100 µg L^{-1}, 200 µg L^{-1}, and 400 µg L^{-1} of Dimethoate: Groups II, III, & IV, respectively; 1 mg L^{-1} of Cadmium chloride: Group V; 100 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VI; 200 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VII; 400 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VIII

Levels of total antioxidant in *Spirogyra* sp., treated with Dimethoate and/or Cd, were significantly higher than the control group (Figure 2). This increase in total antioxidant levels of the cells can be due to increased activity of antioxidant enzymes, involved in detoxification. An increase in the total antioxidant level was observed in the green microalga *Chlorella vulgaris*, treated with cadmium, copper, and lead (Piotrowska-Niczyporuk et al., 2012). Pan, et al., (2017) reported higher activity of enzymes in the antioxidant system of green alga *Chlamydomonas reinhardtii*, treated with triclosan.

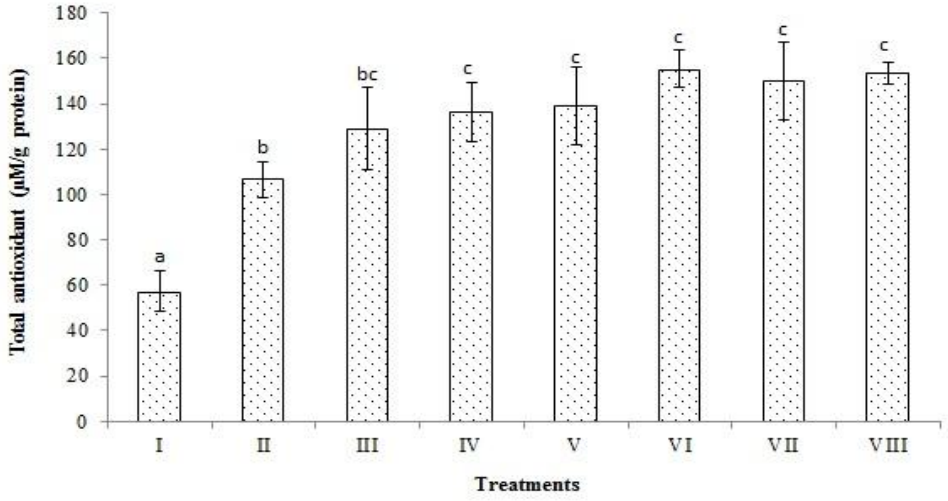

Fig. 2. Levels of total antioxidant in *Spirogyra* sp., exposed to Dimethoate and/or cadmium chloride. Data are presented as mean ± S.D with significant differences indicated by alphabetical symbols ($P < 0.01$)

- Control: Group I; 100 µg L^{-1}, 200 µg L^{-1}, and 400 µg L^{-1} of Dimethoate: Groups II, III & IV, respectively; 1 mg L^{-1} of cadmium chloride: Group V; 100 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VI; 200 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VII; 400 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VIII

Although catalase activity in *Spirogyra* sp., exposed to 400 µg L^{-1}, of Dimethoate was considerably higher than that of the control, there was no significant difference in catalase activity of *Spirogyra* sp., exposed to 100 and 200 µg L^{-1} of Dimethoate, compared to the control. *Spirogyra*'s exposeure to Cd or both Cd and Dimethoate significantly increased CAT activity, compared to the control group (Figure 3). An increase in CAT activity was found in microalgae, exposed to lead (Pb) (Bajguz, 2010), chlorpyrifos (Chen, et al., 2016), and petroleum compounds (Ramadass et al., 2017).

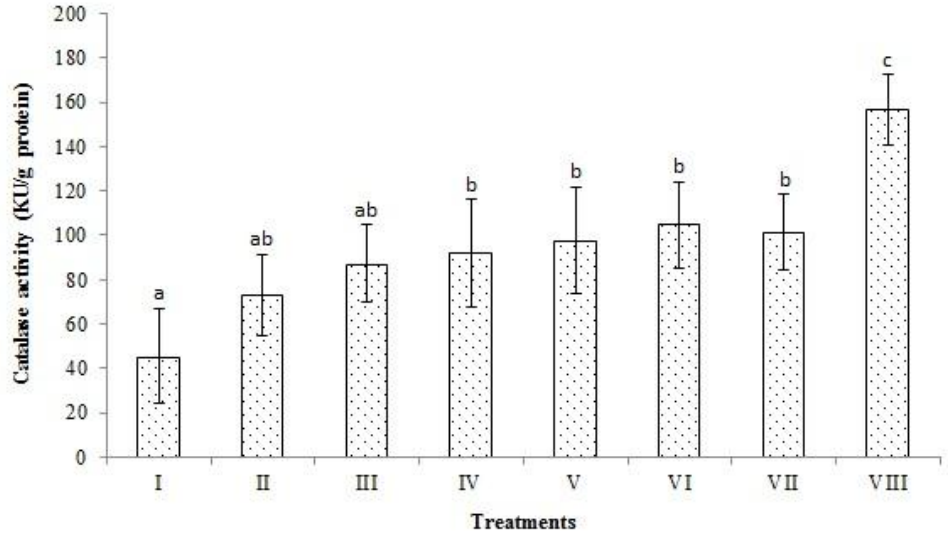

Fig. 3. Catalase activities in *Spirogyra* sp., exposed to Dimethoate and/or cadmium chloride. Data are presented as mean ± S.D with significant differences indicated by alphabetical symbols ($P < 0.01$)

- Control: Group I; 100 µg L^{-1}, 200 µg L^{-1}, and 400 µg L^{-1} of Dimethoate: Groups II, III & IV, respectively; 1 mg L^{-1} of cadmium chloride: Group V; 100 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VI; 200 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VII; 400 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VIII

Spirogyra sp's exposure to Dimethoate and/or Cd significantly increased ascorbate peroxidase (APX) activity, compared to the control (Figure 4). When the green microalga *Chlorella vulgaris* was exposed to cadmium, copper, and lead, APX and CAT activity were increased (Piotrowska-Niczyporuk et al., 2012). APX and CAT are two of the most important enzymes in the antioxidant system of plant cells and participate in decomposition of hydrogen peroxide to water and oxygen (De Tullio et al., 2013). APX detoxifies H_2O_2 (produced in chloroplast) via glutathione-ascorbate cycle. Ascorbate peroxidase controls environmental tensions in plants by regulating H_2O_2 concentration, thus regulating their transpiration and stomatal conductance (De Tullio et al., 2013). The increased activity of CAT and APX in *pirogyra* sp. could be a physiological response to hydrogen peroxide (Pan, et al., 2017).

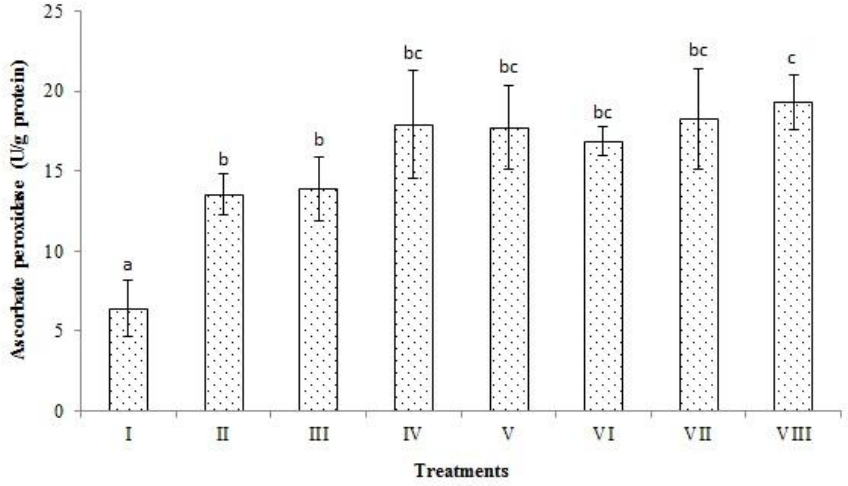

Fig. 4. Ascorbate peroxidase activities in *Spirogyra* sp., exposed to Dimethoate and/or cadmium chloride. Data are presented as mean ± S.D with significant differences indicated by alphabetical symbols ($P < 0.01$)

- Control: Group I; 100 µg L^{-1}, 200 µg L^{-1}, and 400 µg L^{-1} of Dimethoate: Groups II, III & IV, respectively; 1 mg L^{-1} of cadmium chloride: Group V; 100 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VI; 200 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VII; 400 µg L^{-1} of Dimethoate + 1 mg L^{-1} of cadmium chloride: Group VIII

The bioavailability of heavy metals in freshwater algae depends on physical and chemical conditions of water. Most freshwater algae have high capacities for Cd bioavailability (Shamshad, et al., 2015). In green algae, Cd penetrates via cell membranes, getting accumulated as intermitochondrial granules. Consequences of green algae' exposure to cadmium chloride are swelling, vacuolization and destruction of mitochondrial membrane, prevention of cell division and proliferation, and decreased ATP, chlorophyll, and oxygen production (Das et al., 1997; Hasan et al., 2009). Also, a decrease in cellular potassium and disturbance in Zn absorption was reported in green algae, exposed to cadmium chloride (Das et al., 1997). Our findings indicate that with an increase in Dimethoate concentration, the bioavailability of Cd in

Spirogyra sp. has increased as well (Table 3), which can be attributed to reduced efficiency of detoxification system as well as destruction of algae's cellular walls. The impact of pesticides on higher bioavailability of heavy metals in these aquatic organisms demonstrates a lower ability to dispose metals from their body. Changes in metallothionein level in pesticide-exposed organisms could be an effective way to detoxify heavy metals. Banaee, et al. (2015a,b) found that the higher the Permethrin concentration, the more enhanced the accumulation of cadmium and mercury in zebrafish. Reduction in metallothionein level in organisms, exposed to pesticides, can account for decreased Cd bioaccumulation (Das et al., 1997; Banaee et al., 2015a).

Table 4. Statistical significance of biological and biochemical modulations; Results of two-way ANOVA, performed on biological index and biochemical parameters in *Spirogyra* Sp., considering treatments with dimethoate and Cadmium. Differences are considered significant for $p < 0.05$

Biochemical parameters	Treatments	Sig
Chlorophyll a (g L^{-1})	Dimethoate	<0.0001
	Cadmium	<0.0001
	Dimethoate × Cadmium	<0.0001
Chlorophyll b (g L^{-1})	Dimethoate	<0.0001
	Cadmium	<0.0001
	Dimethoate × Cadmium	<0.0001
Carotenoids (g L^{-1})	Dimethoate	<0.0001
	Cadmium	<0.0001
	Dimethoate × Cadmium	0.124
Malondialdehyde (µmol g^{-1} tissue)	Dimethoate	<0.0001
	Cadmium	<0.0001
	Dimethoate × Cadmium	<0.0001
Total antioxidant (µmol g^{-1} protein)	Dimethoate	<0.0001
	Cadmium	<0.0001
	Dimethoate × Cadmium	<0.0001
CAT (KU g^{-1} protein)	Dimethoate	<0.0001
	Cadmium	0.150
	Dimethoate × Cadmium	<0.0001
Ascorbate peroxidase (U g^{-1} protein)	Dimethoate	<0.0001
	Cadmium	<0.0001
	Dimethoate × Cadmium	<0.0001

CONCLUSION

Results of this study indicate that Cd is toxic for *Spirogyra* sp. at 1 mg L^{-1} and algae's exposure to Cd can bring about significant changes to their biological and biochemical markers. Moreover, toxicity of Dimethoate for *Spirogyra* sp. depends on its concentration in the environment. Furthermore, an increase in the concentration of this organophosphate pesticide (200-400 µg L^{-1}) can cause oxidative stress, altering biochemical markers. According to the results of the present study, the influence of Dimethoate on the bioavailability of Cd in *Spirogyra* sp. is synergistic.

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