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Assessment of the effects of zinc on the growth and antioxidant enzymes in *Scenedesmus ellipsoideus* Chodat

by

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Abstract

This study explores the activity of total superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR), biomass accumulation and chlorophyll *a* content in *Scenedesmus ellipsoideus* Chodat grown under conditions of varying zinc (Zn) concentrations. In addition, the activity of different SOD isozymes (MnSOD, FeSOD and CuZnSOD) was measured separately to determine the intracellular extent of oxidative stress resulting from Zn toxicity. We found that the activity of FeSOD and MnSOD was induced by lower Zn concentration (2 μg ml^{-1} and 4 μg ml^{-1}, respectively), whereas CuZnSOD activity was not affected, which indicates that chloroplasts are the first location in *S. ellipsoideus* cells where superoxide accumulation is accelerated by Zn toxicity. The activity of total SOD and APX was significantly increased by moderate Zn concentrations, probably due to some oxidative stress caused by Zn toxicity. The higher level of Zn application, however, led not only to the inhibition of total SOD and APX activity, but also to the reduction of biomass accumulation and chlorophyll *a* content. As a result, it can be concluded that the accumulation of superoxide radicals and H_2O_2 in *S. ellipsoideus* cells induced by Zn toxicity may be responsible for the reduced growth rate and the impairment of photosynthetic pigments.

Key words: ascorbate peroxidase, glutathione reductase, superoxide dismutase, zinc, *Scenedesmus ellipsoideus*

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Introduction

The category of heavy metals includes approximately 53 metals and metalloids with an atomic density greater than 4 g cm^{-3} (HMs; Duruibe et al. 2007). Some of the HMs are micronutrients (e.g. Zn, Cu, Ni, Mn and Co) for plants and have vital functions in cell metabolism, while others (e.g. Cd, Hg and Pb) have no biological function (Gaur & Adholeya, 2004). Both groups of HMs, however, are considered to be toxic and harmful to organisms at high concentrations. According to Lasat (2000), human activities have contributed to toxic levels of HMs in many ecosystems. Kumar et al. (2015), on the other hand, reported that Cd, Cr, Cu, Pb, Hg, Sn and Zn are the most common HM contaminants in aquatic environments. HMs are unique among other chemicals due to their higher stability in environmental conditions. It has been well established that industrial and domestic effluents are primarily responsible for HM pollution in the environment and adverse effects on aquatic organisms (De Filippis & Pallaghy 1994). According to Pinto et al. (2003), the oxidation degree and toxicity of HMs may greatly vary in the natural environment. Choudhary et al. (2007), for example, found that higher Pb concentration strongly inhibits the growth rate and dry mass accumulation in *Spirulina platensis* (Gomont) Geitler. Önem et al. (2018), on the other hand, investigated the toxic effects of some HMs (Hg, Sn and Zn) in the *Arthrospira platensis*-M2 strain and concluded that Sn is the most toxic HM as it causes a permanent cessation of biomass accumulation and chlorophyll-a synthesis. Similarly, some researchers reported that higher HM concentrations lead to a reduction in the chlorophyll content in higher plants and algae (Surosz & Palinska 2004; Lamai et al. 2005; Bajguz 2010). According to Surosz and Palinska (2004), higher Cu and Cd levels reduce photosynthetic activity of *Anabaena flosaquae* Brébisson ex Bornet & Flauhault as a result of structural damage to thylakoid membranes and PSII reaction centers. Moreover, it has been reported that HMs can affect biological systems as a result of bioaccumulation through the food chain (Baş & Demet 1992).

It has been well established that HM toxicity may interfere with electron transport reactions and accelerate the production rate of reactive oxygen species (ROS) in plant cells. These highly toxic compounds, such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), may lead to the oxidation of proteins, fatty acids and nucleic acids (Cho & Park 2000; Cargnelutti et al. 2006; Chen et al. 2009). However, plants have developed an effective antioxidant defense system responsible for the detoxification of ROS. This system consists of both enzymatic and non-enzymatic components. Superoxide dismutase (SOD), for example, constitutes the first line of the enzymatic defense system and converts O_2^- to H_2O_2 and O_2 (Valentine et al. 1998). There are three different isozymes of SOD in eukaryotic plants (Asada 1999). CuZnSOD is located in thylakoids and cytosol of some higher plants and certain algae belonging to Miozoa and Charophyta. MnSOD and FeSOD are present in mitochondria and chloroplasts, respectively (Fridovich 1997). Ascorbate peroxidase (APX) is responsible for the conversion of H_2O_2 to H_2O and O_2, while glutathione reductase (GR) maintains the pool of reduced glutathione, using NADPH as a reductive substrate (Foyer et al. 1994; Urso & Clarkson 2003). Recent studies have demonstrated that HM toxicity can affect the activity of certain antioxidant enzymes in plant cells in different ways. For example, Choudhary et al. (2007) and Bajguz (2010) found that an increase in HM concentrations enhances the activity of SOD and GR, respectively, in various algal species. Önem et al. (2018), on the other hand, reported that the activity of SOD and GR was increased by 50 and 100 µg ml^{-1} Sn concentrations, while higher Sn levels led to a reduction in SOD and GR activity.

Although Zn belongs to HMs, it is the second most abundant element in living organisms after Fe. Zn is very important to plants and plays an important role in their physiological and biochemical processes. For example, it has been reported that Zn is the only HM found in enzymes of all six enzyme classes, including oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Sousa et al. 2009). In addition, Zn is used by plants to activate or modulate a large number of enzymes (Marschner 2012). The important role of Zn in DNA, RNA and carbohydrate metabolism, in cell division, protein and indole acetic acid synthesis, and membrane integrity has been well documented (Marschner 2012). According to Benavides et al. (2005), however, HM levels in aquatic environments above the critical concentrations may cause serious problems. Metal casting and mining have been identified as the most important anthropogenic source of Zn enrichment in nature (ATSDR 2005) and Zn concentration must not exceed 0.12 mg l^{-1} in freshwater in order to protect aquatic life (USEPA 2006). In the case of crop plants, Zn toxicity is observed very rarely and occurs mainly in the contaminated soils (Broadley al. 2007). The inhibitory effect of Zn accumulation on the root growth, chlorophyll synthesis, mineral nutrition and photosynthesis has been well documented for higher plants (Ruano et al. 1988; Marshcner 2012; Sagardoy et al. 2009; Van Assche & Clijsters 1986a). In addition, it has been reported that

the increased activity of antioxidant enzymes reflects the extent of oxidative stress triggered by HM toxicity and the tolerance degree of organisms (Zhou et al. 2007). Studies addressing the effects of Zn toxicity on algal metabolism are, however, very limited and are now receiving increasing attention.

It has been reported that Zn pollution is rapidly increasing in some freshwater ecosystems located around Sakarya and İzmit, Turkey (Yalçın & Sevinç 2001; Pekey et al. 2004; Dündar & Altundağ 2007; Atici et al. 2008; Saygı & Yiğit, 2012). Therefore, we investigated the effect of different levels of Zn toxicity on *S. ellipsoideus* isolated from Lake Ketence (İzmit). Biomass and chlorophyll-*a* content were measured to determine metabolic damage caused by Zn toxicity. The activity of antioxidant enzymes (total SOD, APX and GR), on the other hand, was examined to clarify the cellular antioxidant defense response to Zn toxicity. In addition, the activity of MnSOD, FeSOD and CuZnSOD was measured separately to determine the intracellular extent of oxidative stress resulting from O_2^- accumulation induced by Zn toxicity.

Materials and methods

Algae culture and treatment

S. ellipsoideus was isolated from Lake Ketence (İzmit) and was cultured in BG11 medium. Prior to the heavy metal treatment, 250 ml culture of *S. elipsoideus* was prepared and allowed to grow under the conditions of 5000 lux photosynthetically available radiation with full-spectrum lamps in a 12:12 h light/dark cycle at 25 ± 1°C. After 10 days, various concentrations of zinc ($ZnCl_2$; 0 µg ml^{-1}, 1 µg ml^{-1}, 2 µg ml^{-1}, 4 µg ml^{-1}, 6 µg ml^{-1}, 8 µg ml^{-1}) were added to *S. ellipsoideus* Chodat cultures that contained 0.5 µg ml^{-1} of chlorophyll-*a*. Zn concentrations used in this study were determined by the growth inhibition test (OECD, 2006). For this purpose, various concentrations of $ZnCl_2$ were added to the culture medium for 7 days. At the end of the study, the EC50 (half maximal effective concentration) value for Zn was calculated on the basis of the growth rate (OD560) using Origin Pro 8.5 (Origin Lab, Northampton, MA, USA). The EC50 value was 7.29 µg ml^{-1}.

Cell growth and chlorophyll *a* assay

Optical density (OD) and the growth rate were measured spectrophotometrically at 750 nm. Cultures were diluted at 1/10 ratio with BG11 medium and BG11 medium is used as blank and each measurement was conducted every 24 h for 7 days. Chlorophyll-*a* content was estimated by methanol extraction and measured spectrophotometrically for 7 days (MacKinney 1941).

Antioxidant enzyme activities

At the end of the 7th day, 2 ml of cultures were centrifuged and then pellets were extracted by 100 mM K_2HPO_4 buffer (pH 7.0), 2% PVP and 1 mM Na_2EDTA buffer for SOD and GR activity, respectively, and by 50 mM Tris–HCl (pH 7.2), 2% PVP, 1 mM Na_2EDTA, and 2 mM ascorbate buffer for APX activity assays. After centrifugation at 14 000 rpm for 20 min at 4°C, the resulting supernatants were used to measure protein concentration and antioxidant enzyme activity. The protein concentration of leaf crude extracts was determined according to Bradford (1976), using BSA as a standard.

The activity of superoxide dismutase (SOD; EC 1. 15. 1. 1) was determined by the method of Beyer & Fridovich (1987), based on the photoreduction of NBT (nitroblue tetrazolium). The reaction mixture consisted of 100 mM K_2HPO_4 buffer (pH 7.8), containing 9.9×10^{-3} M methionine, 5.7×10^{-5} M NBT, 1% triton X-100 and enzyme extract. The reaction was started by adding 0.9 µM of riboflavin and the mixture was exposed to light with an intensity of 375 µmole m^{-2} s^{-1}. After 15 min, the reaction was stopped by switching off the light and the absorbance was read at 560 nm. The SOD activity was calculated using a standard graphic and expressed as unit mg^{-1} protein. The activity of different SOD isozymes (MnSOD, FeSOD and CuZnSOD) was determined based on the sensitivity to inhibition of 2 mM KCN or 5 mM H_2O_2 (Fridovich 1986).

The activity of ascorbate peroxidase (APX; EC 1.11.1.11) was determined according to Wang et al. (1991) by estimating the decreasing rate of ascorbate oxidation at 290 nm. The reaction mixture consisted of 50 mM K_2HPO_4 buffer (pH 6.6), 2.5 mM ascorbate, 10 mM H_2O_2 and an enzyme extract containing 100 µg of protein in a final volume of 1 ml. The enzyme activity was calculated from the initial rate of the reaction, using the extinction coefficient of ascorbate (E = 2.8 mM cm^{-1} at 290 nm).

The activity of glutathione reductase (GR; EC 1. 6. 4. 2) was measured using the method of Sgherri et al. (1994). The reaction mixture (total volume of 1 ml) contained 100 mM K_2HPO_4 buffer (pH 7.8), 2 mM Na_2EDTA, 0.5 mM oxidized glutathione (GSSG), 0.2 mM NADPH and an enzyme extract containing 100 µg of protein. A decrease in the absorbance at 340 nm was recorded. A correction was made for the non-enzymatic oxidation of NADPH by recording the decrease at 340 nm without adding GSSG to the

assay mixture. The enzyme activity was calculated from the initial rate of the reaction after subtracting the non-enzymatic oxidation using the extinction coefficient of NADPH (E = 6.2 mM cm^{-1} at 340 nm).

Statistical analysis

The experimental design was a complete randomized block with three independent replicates. The significance of a difference between mean values was determined by one way ANOVA at a 95% confidence level, using the SPSS 20.0 statistical program for Windows. In the graphical presentations, significant changes in relation to the control levels were indicated by asterisks.

Results

Biomass and chlorophyll-*a* content

OD750 measurements and chlorophyll-*a* content in *S. ellipsoideus* cultures treated with different zinc concentrations are presented in Fig. 1 and Fig. 2, respectively. Zn added to the culture medium for 7 days caused a significant decrease in both biomass accumulation and chlorophyll-*a* content in *S. ellipsoideus* cells in a dose-dependent manner. It has been found that the highest Zn concentration (8 µg ml^{-1}) leads to the most significant reduction in biomass accumulation and chlorophyll-*a* content. In addition, we found that the biomass accumulation in *S. ellipsoideus* cells increased progressively for 7 days in a time-dependent manner. Chlorophyll-*a* content, however, was significantly reduced by all Zn concentrations on the 5th day of the application

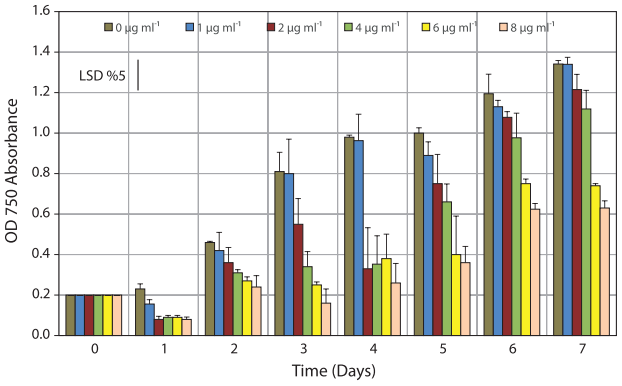

Figure 1

OD750 absorbance of *S. ellipsoideus* supplemented with 0–8 µg ml^{-1} zinc for 7 days. Data are means ± SD of three replicates.

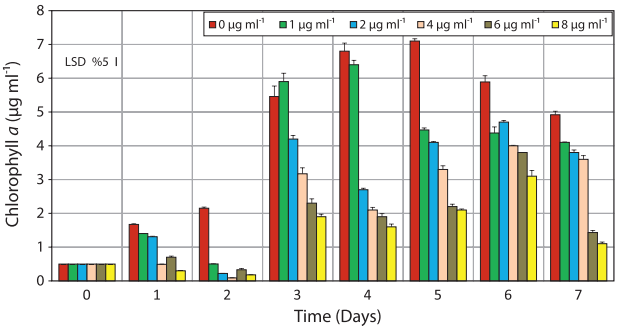

Figure 2

Chlorophyll-*a* content in *S. ellipsoideus* supplemented with 0–8 µg ml^{-1} zinc for 7 days. Data are means ± SD of three replicates.

and then remained almost constant throughout the study, probably due to a disturbance in chlorophyll metabolism in *S. ellipsoideus* cells as a result of Zn toxicity.

Antioxidant enzyme activity

Changes in the activity of total SOD and MnSOD in *S. ellipsoideus* cells represented almost the same trend in a dose-dependent manner (Figs 3a and b). Our results showed that 4 µg ml^{-1} of Zn increased the activity of total SOD and MnSOD by 46% and 28%, respectively, compared to the control levels. Further increase in Zn concentration, however, significantly reduced the activity of enzymes, especially at the highest Zn concentration (8 µg ml^{-1}), when the activity of total SOD and MnSOD in *S. ellipsoideus* cells was significantly lower compared to the control levels (36% and 51%, respectively). Our results showed that Zn toxicity had a stronger effect on FeSOD in *S. ellipsoideus* cells compared to the activity of total SOD and MnSOD. For example, at a Zn concentration of 2 µg ml^{-1}, the FeSOD activity was about 220% higher than the control and at a concentration of 4 µg ml^{-1} – 464% higher (Fig. 4a). Higher Zn treatments, on the other hand, led to a decrease in FeSOD activity to the control level. Contrary to these results, the CuZnSOD activity in *S. ellipsoideus* cells was significantly reduced by 2 µg ml^{-1} and 4 µg ml^{-1} Zn treatments compared to the control level (86% and 73%, respectively; Fig 4b). While 6 µg ml^{-1} Zn increased the activity of CuZnSOD in *S. ellipsoideus* cells up to the control level, the 8 µg ml^{-1} Zn treatment decreased it by 45% compared to the control.

Similarly to total SOD and MnSOD, the activity of total APX in *S. ellipsoideus* cells was stimulated by 44% and 45% as a result of 4 µg ml^{-1} and 6 µg ml^{-1} Zn applications compared to the control

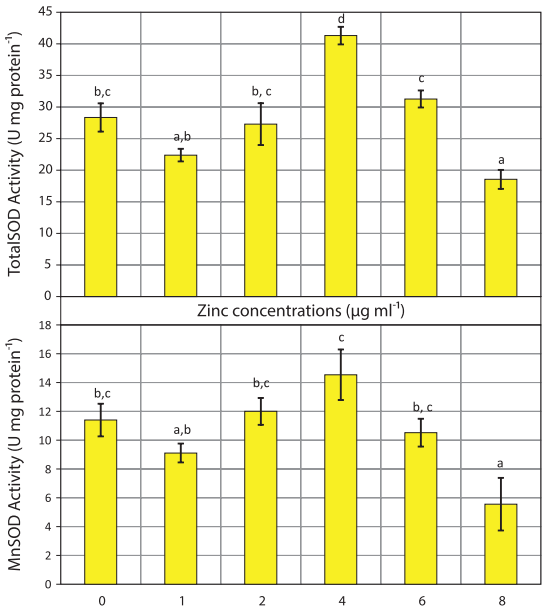

Figure 3

Total superoxide dismutase (SOD) (a), MnSOD (b) activity in *S. ellipsoideus* treated with 0–8 µg zinc ml^{-1}. Data are means ± SD of three replicates. Mean values in columns with different letters are significantly different at the 5% level according to the least significant difference (LSD) test.

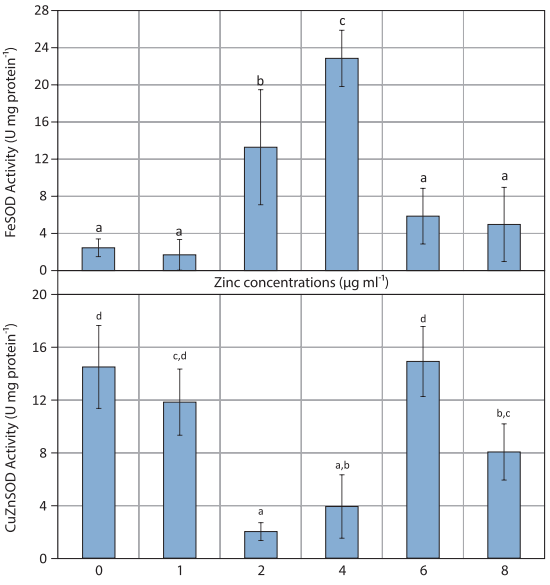

Figure 4

FeSOD (a) and CuZnSOD (b) activity in *S. ellipsoideus* treated with 0–8 µg zinc ml^{-1}. Data are means ± SD of three replicates. Mean values in columns with different letters are significantly different at the 5% level according to the least significant difference (LSD) test.

(Fig. 5a). However, 8 µg ml^{-1} concentration of Zn led to a significantly lower APX activity (22% of the control). Changes in GR activity caused by all Zn concentrations tested in this study were statistically insignificant (Fig. 5b).

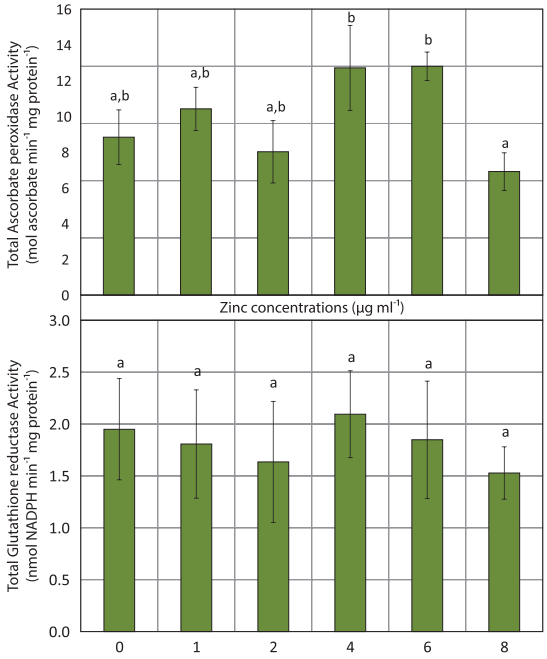

Figure 5

Ascorbate peroxidase (APX) (a) and glutathione reductase (GR) (b) activity in *S. ellipsoideus* treated with 0–8 µg zinc ml^{-1}. Data are means ± SD of three replicates. Mean values in columns with different letters are significantly different at the 5% level according to the least significant difference (LSD) test.

Discussion

Petersen (1982) reported that Zn is an essential trace metal for algal growth and the growth rate strictly depends on the Zn concentration in the growth medium. Omar (2002) found that lower concentrations of zinc increased not only the dry weight but also chlorophyll *a* and *b* content in *Scenedesmus obliquus* and *Scenedesmus quadricauda*. However, zinc is toxic when present in the environment in excessive concentrations. Guang et al. (2012), for example, observed that high Zn concentrations suppressed the growth of *Scenedesmus obliquus*. Similarly, it has been reported by several authors that high Zn concentrations caused a reduction in the growth of different algae species, including *S. platensis*-S5 and

Arthrospira platensis-M2 (Choudhary et al. 2007; Önem et al. 2018). In our study, we found that an exogenous addition of Zn inhibited the growth of *S. ellipsoideus* cells in a concentration-dependent manner. It has been reported that Zn may practically inhibit cell division in plant cells (Sentsova & Maksimov 1985). Also Rosko & Rachlin (1977) and Önem et al. (2018) reported that the reduction in growth could be due to the inhibition of normal cell division caused by higher concentrations of Zn in *Chlorella vulgaris* Beyerinck and *A. platensis*-M2, respectively. According to these findings, we can conclude that cell division in *S. ellipsoideus* is inhibited by higher Zn concentrations used in this study. It has been well established that a reduced rate of cell division can be attributed to HMs that bind to sulfhydryl groups, which is important in the regulation of cell division (Fisher et al. 1981; Visviki & Rachlin 1991). In addition, our results show that 1, 2, 4 and 6 μg ml^{-1} Zn concentrations did not cause chronic inhibition in the growth of *S. ellipsoideus* cells in a time-dependent manner, as indicated by a progressive increase in biomass accumulation. These results may suggest the development of tolerance to Zn in *S. ellipsoideus* cells at a lower Zn concentration. However, 6 and 8 μg ml^{-1} Zn application inhibited the growth of *S. ellipsoideus* cells. This situation occurred on the 5th day of the cultivation and then persisted throughout the study, possibly due to the irreversible inhibition of cell division in *S. ellipsoideus* caused by higher Zn concentrations applied in our study.

Photosynthesis is a physiological process that is highly sensitive to HMs, especially in unicellular organisms (Lu et al. 2000). Aravind & Prasad (2004) and Bajguz (2010) reported that a low chlorophyll content and the inhibition of chlorophyll biosynthesis were primarily responsible for the reduced photosynthetic activity in plants exposed to HM toxicity. In accordance with these studies, our research has demonstrated that the chlorophyll-*a* content in *Scenedesmus ellipsoideus* cells was reduced in a concentration-dependent manner as a result of Zn toxicity. Our results are also consistent with the findings of Surosz & Palinska (2004), who reported a reduced level of chlorophyll-*a* content in *A. flosaquae* due to Cu and Cd toxicity. As indicated by several authors (Prasad & Strzałka 1999; Mysliwa-Kurdziel & Strzałka 2002; Mysliwa-Kurdziel et al. 2003; Surosz & Palinska 2004; Schoefs & Bertrand 2005), this result can be attributed to the enzymatic inhibition of chlorophyll biosynthesis and/or accelerated degradation of photosynthetic pigments, or disruption of thylakoid integrity due to HM toxicity. It has also been reported that some HMs can replace Mg in chlorophyll molecules, which may reduce the photosynthetic efficiency (Solymosi et al. 2004). In addition, we determined a positive correlation between biomass accumulation and chlorophyll-*a* content in *S. ellipsoideus* cells in a concentration-dependent manner as a result of Zn toxicity. Such a positive correlation was also confirmed by Surosz & Palinska (2004) and Önem et al. (2018), who reported both higher chlorophyll-*a* concentration and the optimal growth of *A. flosaquae* in the presence of Cu and Cd toxicity. However, we did not observe a correlation between biomass accumulation and chlorophyll-*a* content in *S. ellipsoideus* cells in a time-dependent manner. Unlike the biomass accumulation, the chlorophyll-*a* content decreased significantly after the 6th day of the study, probably due to the selective inhibitory effect of Zn toxicity on chlorophyll metabolism rather than cell division.

Abiotic stress factors, including heavy metal toxicity, are known to lead to ROS accumulation in cells as well as to oxidative stress in plants. In plant cells, chemical or physical activation of molecular oxygen (O_2) may induce the production of certain ROS such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$). ROS accumulation may result in yield loss and death of plants. However, plants have evolved an effective antioxidant defense system to cope with oxidative stress. SOD, for example, is responsible for dismutation of superoxide radicals to H_2O_2. APX, on the other hand, is responsible for the degradation of H_2O_2 to H_2O and O_2. In our study, the activity of total SOD and total APX was significantly increased by 4 and 6 μg ml^{-1} Zn concentrations, while the highest Zn concentration led to a significant decrease. Tripathi et al. (2004) treated *Scenedesmus* sp. with Cu and Zn for a short (6 h) and long period (7 days) and reported that 2.5 ve 10 μM Cu and 5 ve 25 μM Zn increased the SOD activity. Since the concentrations used in the study by Tripathi et al. (2004) are lower than in our case, it is possible that the inhibitory effects of metals were not observed. In the above-mentioned study, 2.5 μM Cu ve 25 μM Zn increased the APX activity. Our results are consistent with the findings of Rai et al. (2013), who reported that total SOD activity in *C. vulgaris* increased up to 1 μg ml^{-1} Cr treatment and then decreased at a higher concentration. Similarly, Önem et al. (2018) found higher total SOD activity in *Arthrospira platensis*-M2 cells under moderate Zn (2 μg ml^{-1}) and Sn concentrations (50 and 100 μg ml^{-1}), whereas severe Zn and Sn toxicity led to the reduced total SOD activity. In our study, the enhanced activity of total SOD and total APX in *S. ellipsoideus* cells under moderate Zn toxicity clearly demonstrates an increased production and elimination rate of superoxide radicals and H_2O_2, respectively. In addition, the impairment of total SOD

and total APX activity by zinc toxicity (at 8 µg ml^{-1}) and accumulation of both superoxide radical and H_2O_2 may be responsible for the decreased chlorophyll-*a* content and the decreased growth rate in *S. ellipsoideus* cells at the same Zn concentration. However, Tripathi et al. (2004) reported that the application of Cu and Zn on *Scenedesmus* sp. decreased the GR activity, which maintains the reduced glutathione pool in plant cells and was not affected by any of the Zn concentrations used in this study.

SOD has three different isozymes located in different cell compartments (Raychaudhuri & Deng 2000). FeSOD and MnSOD, for example, are present in chloroplasts and mitochondria, respectively, while CuZnSOD is located in the cytosol (Raychaudri & Deng 2000). It has been reported that different SOD isozymes protect different proteins found in different parts of the cell from damage caused by superoxide radicals (Lesser & Stochaj 1990). In our study, changes observed in the activity of MnSOD and total SOD in *S. ellipsoideus* cells, resulting from Zn toxicity, were very similar. For example, 2 µg ml^{-1} Zn application increased and 8 µg ml^{-1} Zn decreased the MnSOD activity. However, FeSOD activity was enhanced by 2 µg ml^{-1} Zn concentration, which clearly indicates that the production of superoxide anions is triggered by a lower Zn level in *S. ellipsoideus* chloroplasts compared to mitochondria. As previously reported, chloroplasts are the major source of ROS in plant cells and they account for 40% of the cell volume in *Scenedesmus* algae (Edreva 2005; Tukaj et al. 1998). Furthermore, Bowler et al. (1992) reported that a very high proportion of SOD (more than 90%) in plants appears to be located in chloroplasts as FeSOD, which is responsible for protecting the photosynthetic apparatus against oxidative damage. Therefore, the inhibition of FeSOD activity in *S. ellipsoideus* cells using 8 µg ml^{-1} Zn may explain the reduced chlorophyll-*a* content at the same Zn level due to the accumulation of superoxide radicals. The CuZnSOD activity, on the other hand, was reduced by moderate Zn levels (2 and 4 µg ml^{-1}) and then reached the control level at 6 µg ml^{-1} Zn concentration, probably due to insignificant superoxide production in the cytosol. Similarly to MnSOD and FeSOD activity, CuZnSOD activity is suppressed by 8 µg ml^{-1} Zn concentration in *S. ellipsoideus* cells.

In conclusion, our study demonstrated that FeSOD is the most predominant isozyme, because it was stimulated at a lower Zn concentration (2 µg ml^{-1}) compared to MnSOD and CuZnSOD. The increased level of total SOD and total APX activity at moderate Zn concentrations clearly indicates that the accumulation of superoxide radicals and H_2O_2 in *S. ellipsoideus* cells is accelerated as a result of Zn toxicity. Higher Zn concentrations inhibited the activity of total SOD and total APX in *S. ellipsoideus* cells. These results may lead to the conclusion that the superoxide radical and H_2O_2 induced by Zn toxicity may be responsible for the reduced growth rate and the impairment of photosynthetic pigments.

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