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Heavy metal stress response of microalgal strains *Arthronema africanum* and *Coelastrella* sp. BGV

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Abstract

The present study compared the stress response of two microalgal strains – *Arthronema africanum* (Cyanoprokaryota) and *Coelastrella* sp. BGV (Chlorophyta), after heavy metals' treatment. Changes of algal growth, pigment and protein content were analyzed after adding Cu, Cd and Pb (50 µM and 100 µM) to the nutrition medium. It was found that Cd and Pb significantly inhibited growth and protein biosynthesis of microalgae, but the effect of Cu remained less pronounced. In both strains, a decrease of chlorophyll content was observed, while carotenoid content markedly increased, especially in *Coelastrella* sp. BGV biomass. The addition of 100 µM Cd and 100 µM Pb to the medium caused a strong enhancement of malondialdehyde in both microalgal strains, which corresponded to the significant increase of superoxide dismutase and catalase activity. The antioxidant enzymes appeared to be differently altered by heavy metals' exposure. The activity of SOD in the *Arthronema africanum* cells was most strongly affected by Cd, in contrast to *Coelastrella* sp. BGV that was highly increased by 100 µM Pb. The application of 100 µM Cd and 100 µM Pb increased in a similar manner catalase activity in both microalgae. The strains that were studied showed a high absorption capacity for metal ions, especially for Pb, which was absorbed largely than Cd and Cu. For that reason, we assumed that both microalga and, in particular, *Coelastrella* sp. BGV, could be successfully used for treatment of contaminated water bodies.

Keywords

Arthronema africanum, catalase, *Coelastrella* sp., Cu, Cd, Pb, pigments, superoxide dismutase

Introduction

Heavy metals are among the most common environmental pollutants nowadays. In addition to natural sources, heavy metal pollution often comes from anthropogenic activities – mining, refining, chemical and metallurgical industries, etc. (Sibi 2019). A large number of microorganisms, including bacteria, algae and fungi, have the ability to absorb and uptake metals and metalloids (Al-Amin et al. 2021; Spain et al. 2021). Microalgae (prokaryotic and eukaryotic species) may be used as an efficient and eco-friendly alternative to the existing physicochemical methods for heavy metal removal (Bestawi 2019; Cui et al. 2021). Cyanoprokaryotes and green microalgae have been recently studied, due to their high potential for disposal of various pollutants and their use in phytoremediation (Kalambate et al. 2019; Pham et al. 2020).

Heavy metals that have been usually tested for uptake were Cu, C, Ni, Pb, Zn, Hg, Cr (Kumar et al. 2015). Cu, Cd and Pb are known to be among the most common pollutants in natural ecosystems. Cd and Pb, being highly toxic metals, are nonessential for the growth and development of plant organisms, but Cu, although a necessary trace element, in elevated concentrations could be also damaging. High Cd content in the medium leads to significant inhibition of growth and photosynthesis, expressing its toxic effect via complexing to SH-groups of proteins and inhibiting cellular respiration. Excess Pb causes reduced growth and chlorosis, suppresses photosynthesis, mineral nutrition and water balance (Dao and Beardall 2016). Despite their micronutrient copper requirement most algae may be damaged by Cu, which decreases rates of photosynthesis and chlorophyll biosynthesis, imbalances cell division, etc. (Yruela 2005).

Arthronema africanum (Cyanoprokaryota) is a filamentous, nonheterocystous cyanoprokaryote manifesting some unique morphological and physiological characteristics (Komarek and Lukavsky 1988). The strain is highly adaptive, typical for extreme desert habitats, a very promising producer of phycobiliproteins (Chaneva et al. 2007). It is still unexplored and only a few studies are known examining its antitumor and antioxidant properties (Iliev et al. 2006; Gardeva et al. 2014).

Coelastrella sp. BGV, a newly isolated, fast growing Bulgarian strain, (Draganova 2018; Toshkova-Yotova et al. 2019; Toshkova-Yotova et al. 2020), has also focused the attention of researchers due to its high bioactive and antitumor capacity.

The aim of that study was to perform a comparative analysis of the stress response of two microalgal strains, *Arthronema africanum* (Cyanoprokaryota) and *Coelastrella* sp. BGV (Chlorophyta), after heavy metal treatment. This would contribute these strains to find effective application in phycoremediation.

Methods

The microalgal strains were maintained as batch cultures at the Algal Culture Collection, Department of Experimental Algology, IFRG, BAS. The cyanoprokaryote *Arthronema africanum*, strain Lukavsky, 1981/01 (Komárek and Lukavský 1988), CCALA (Tøeboò Collection of Autotrophic Organisms, Czech Republic) was cultivated at 28–30 °C, on the nutrition medium based on the Allen and Arnon (1955) and Zehnder (Staub 1961) media, modified by Chaneva et al. (2007).

The green alga *Coelastrella* sp. BGV, newly isolated Bulgarian strain (Dimitrova et al. 2017), was grown at 28 °C, on the Setlik nutrition medium (Setlik 1967) modified by Georgiev et al. (1978). Both strains were intensively cultivated in 200 ml vessels, under continuous illumination by white light, 150 μmol phot m^{-2} s^{-1}. Carbon source was provided by bubbling sterile 2% (v/v) CO_2 in air (100 l h^{-1}). The algal cultures were centrifuged at the end of the exponential phase of growth and the biomass was further re-suspended in a fresh nutrition medium at an inoculum 0.5 mg ml^{-1} DW (dry weight). The 10-day treatment was performed by adding to the medium 50 μM and 100 μM of Cu^{2+}, Cd^{2+} and Pb^{2+} (added as $CuSO_4$, $CdCl_2$ and $(CH_3COO)_2Pb$). These concentrations were chosen according to data received in our previous study (Marinova et al. 2018).

Growth and physiological changes of the algal cultures were determined on the 3rd, 7th and 10th day of the treatment. Algal growth was monitored by the changes of dry weight and total protein content (measured according to Lowry et al. 1951). Pigment content was determined spectrophotometrically after methanol extraction and calculated after McKinney (1941). Malone dialdehyde (MDA) content was determined according to Dhindsa et al. (1981). Superoxide dismutase (SOD) activity was measured after the method of Beauchamp and Fridovich (1971) and catalase (CAT) activity – after Aebi (1984). The content of Cu, Cd and Pb in dry algal biomass was analyzed by atomic absorption spectrophotometer Perkin-Elmer. The experimental data were averaged of triplicate measurements and only statistically significant results (as defined by a $p\text{-value} < 0.05$) were discussed.

Results

All heavy metals (Cu, Cd and Pb) that were studied inhibited *A. africanum* growth. The application of 100 μM Cu and 100 μM Cd led to a significant decrease in dry biomass, which was by 55%–57% lower than the control variant. In the initial stages of treatment, on the 3th day, 50 μM Cu stimulated, albeit slightly, the growth of *A. africanum* (Fig. 1). The green microalga *Coelastrella* sp. BGV also suffered the t toxic effect of heavy metals, however, less pronounced than in the cyanoprokaryote. The most significant influence was observed at 100 μM Cd and 100 μM Pb when the dry algal biomass was 30%–33% reduced on the 10th day. 50 μM Cu led to an increase of dry weight on the 3rd and 7th day. However, on the 10th day of the experiment, the accumulation of biomass slowed and remained 17% lower than the control (Fig. 2). The lowest protein content in *A. africanum* biomass was measured on the 10th day after 100 μM Cd and 100 μM Pb treatment. The least inhibition of protein biosynthesis was observed after Cu treatment – 8%–22% decrease (Table 1). Both Cd concentrations caused maximal reduction of protein biosynthesis in *Coelastrella* sp. (~36%). The effect of copper ions was the weakest and a decrease of about 5% was observed at 50 μM Cu (Table 1).

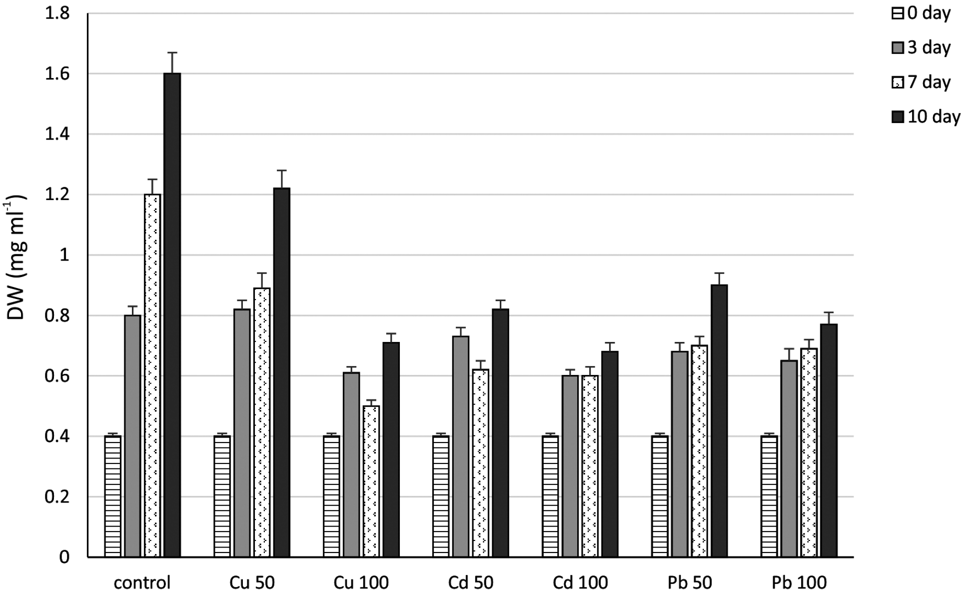

Figure 1. Influence of heavy metals (Cu, Cd, Pb) on the *Arthronema africanum* growth (DW, mg ml^{-1}), measured on the 3rd, 7th and 10th day after treatment by Cu, Cd and Pb (each metal added in concentrations 50 μM and 100 μM).

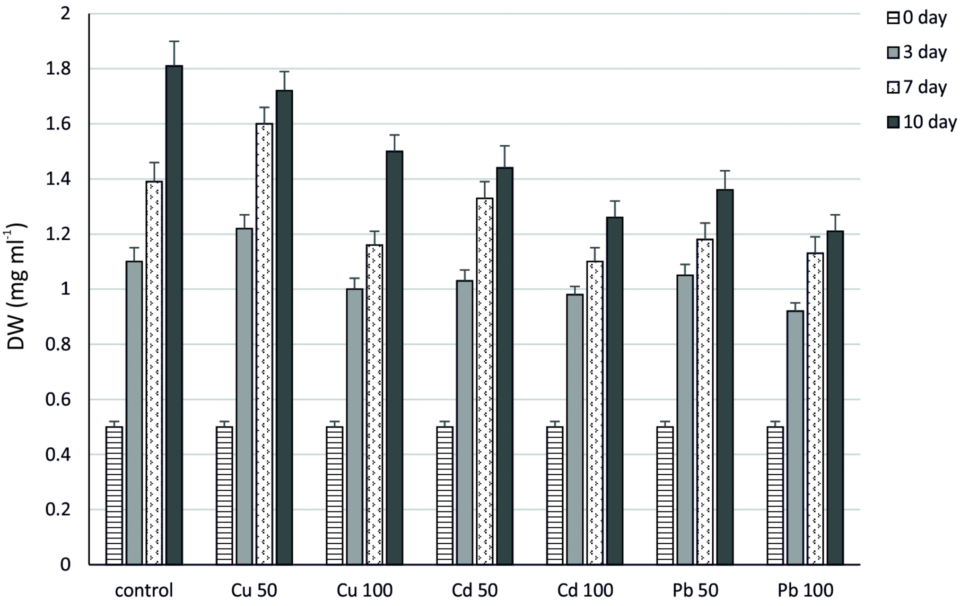

Figure 2. Influence of heavy metals (Cu, Cd, Pb) on the *Coelastrella* sp. BGV growth (DW, mg ml^{-1}), measured on the 3rd, 7th and 10th day after treatment by Cu, Cd and Pb (each metal added in concentrations 50 μM and 100 μM).

Table 1. Effect of heavy metals (Cu, Cd, Pb) on the protein and pigment content of *Arthronema africanum* and *Coelastrella* sp. BGV, 10th day.

Variant	**Proteins** (% DW)	**Chlorophyll *a*** (% DW)		**Carotenoids** (% DW)
		Arthronema africanum		
control	46.8 ± 1.8	1.71 ± 0.06		0.29 ± 0.01
Cu 50 µM	43.5 ± 1.9	1.59 ± 0.07		0.30 ± 0.01
Cu 100 µM	36.6 ± 1.4	0.97 ± 0.04		0.22 ± 0.01
Cd 50 µM	33.9 ± 1.4	0.81 ± 0.04		0.17 ± 0.01
Cd 100 µM	28.3 ± 1.3	0.62 ± 0.03		0.18 ± 0.01
Pb 50 µM	36.5 ± 1.6	1.04 ± 0.04		0.34 ± 0.01
Pb 100 µM	30.7 ± 1.3	0.65 ± 0.02		0.33 ± 0.01
		***Coelastrella* sp. BGV**		
	Proteins (% DW)	**Chlorophyll *a*** (% DW)	**Chlorophyll *b*** (% DW)	**Carotenoids** (% DW)
control	44.8 ± 1.6	1.57 ± 0.07	0.64 ± 0.03	0.28 ± 0.01
Cu 50 µM	42.9 ± 1.8	1.19 ± 0.05	0.49 ± 0.02	0.25 ± 0.01
Cu 100 µM	33.4 ± 1.3	1.23 ± 0.06	0.44 ± 0.02	0.35 ± 0.01
Cd 50 µM	29.0 ± 1.2	0.82 ± 0.04	0.34 ± 0.01	0.39 ± 0.02
Cd 100 µM	28.5 ± 1.1	0.63 ± 0.03	0.31 ± 0.01	0.54 ± 0.02
Pb 50 µM	37.1 ± 1.8	0.96 ± 0.04	0.38 ± 0.01	0.50 ± 0.02
Pb 100 µM	32.2 ± 1.5	0.87 ± 0.03	0.36 ± 0.01	0.51 ± 0.02

It was found a significant decrease of chlorophyll *a* biosynthesis in the *A. africanum* cells. The severe inhibitory effect was observed at 100 µM Cd and 100 µM Pb – a strong decrease by 62%–64%. The negative effect of Cu ions was the least pronounced (Table 1). Cd also caused a significant decrease in the carotenoid content of *A. africanum* – a decrease of about 40% compared to the control. In contrast, Cu ions enhanced the level of carotenoids – by 24% at 100 µM Cu (Table 1). *Coelastrella* sp. reacted by an extreme reduction of the amounts of chlorophyll *a* and chlorophyll *b* after Cd adding to the medium. Similar, though not so pronounced, was the effect of Pb. Both metals led to a decrease of about 60% of chlorophyll *a* content and 50% of chlorophyll *b*. Copper ions caused the least change in the chlorophyll content (Table 1). The addition of Cd and Pb to the nutrition medium led to a strong increase in the level of carotenoids in *Coelastrella* sp. After 100 µM Cd and 100 µM Pb treatment, a 93% and 82% increase in carotenoids was registered (Table 1).

Examining changes in the levels of malondialdehyde (MDA), one of the most commonly used markers for the degree of lipid peroxidation in cells, it was found that all studied heavy metals led to a sharp increase in MDA in *A. africanum*, especially Cd and Pb (Fig. 3A). As for *Coelastrella* sp., the strain showed a similar trend of MDA increase, although to a lesser extent – it was about 3 times higher in response to the addition of Cd and Pb (Fig. 3B).

SOD activity of *A. africanum* increased more than twice at both Cd concentrations. Less pronounced changes were observed under Cu and Pb treatment (Fig. 4A). The activity of SOD in *Coelastrella* sp. has been changed in different ways – the highest values were registered under Pb treatment (80% – 90% enhancement), as well as at 100 µM Cu. The enzyme activity remained lower than the control variant at 50 µM

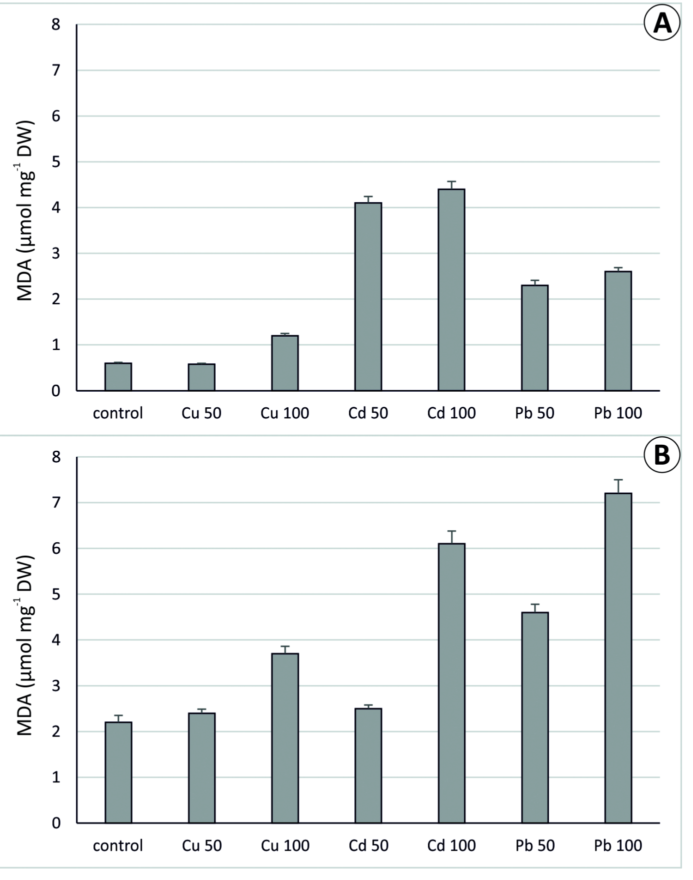

Figure 3. Changes of malondialdehyde content (MDA, µmol mg^{-1} DW) in the cells of *Arthronema africanum* – **A**, and *Coelastrella* sp. BGV– **B**, on the 10th day after treatment by Cu, Cd and Pb (each metal added in concentrations 50 µM and 100 µM).

Cu (Fig. 4B). It has been observed that the catalase activity varied differently compared to SOD. In the *A. africanum* cells, the extreme levels were measured at 100 µM Cd and 100 µM Pb, two times above the control. Copper ions had a lesser effect on the enzyme activity (Fig. 5A). Similar values were obtained for CAT activity in *Coelastrella* sp., which was most significantly increased at 100 µM Cd and 100 µM Pb, while Cu did not have such a strong effect (Fig. 5B).

Both experimental strains have been shown to accumulate large amounts of the heavy metals added to the medium. Algal cells appeared to be most willing to absorb Pb ions, followed by Cd and Cu. *Coelastrella* sp. showed a particularly high tendency to accumulate heavy metals in the biomass. The green microalgal strain expressed a much higher uptake capacity, compared to the cyanoprokaryote *A. africanum* (Table 2).

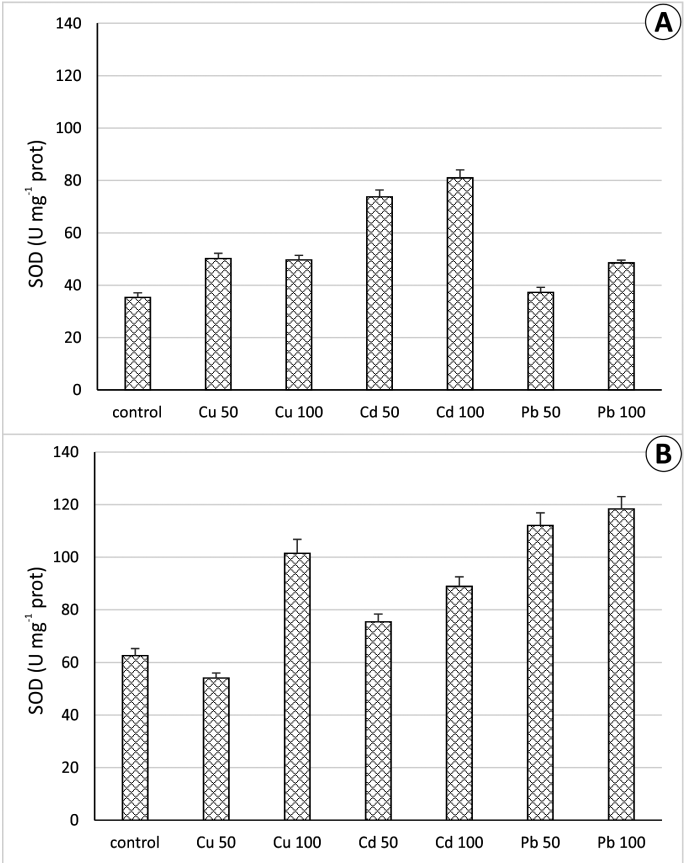

Figure 4. Changes of superoxide dismutase activity (SOD, U mg^{-1} prot.) in the cells of *Arthronema africanum* – **A** and *Coelastrella* sp. BGV – **B**, on the 10^{th} day after treatment by Cu, Cd and Pb (each metal added in concentrations 50 μM and 100 μM).

Discussion

A detailed knowledge of heavy metals pollutants' action is necessary for the successful application of microalgae in the process of phytoremediation. Therefore, a screening of promising, fast-growing strains, able to absorb heavy metals with higher affinity, is required. In our experiments, it was found that treatment with Cd and Pb strongly inhibited the growth of *Arthronema africanum* and *Coelastrella* sp. BGV, more essentially that one of *A. africanum*. (Figs 1, 2). Cd and Pb, applied at 100 μM concentration, affected the morphology and worsened the condition of the algal cultures.

It became clear that the effect of Cu was less pronounced and in the initial stages of the experiment 50 μM Cu had a certain stimulating effect on growth, especially

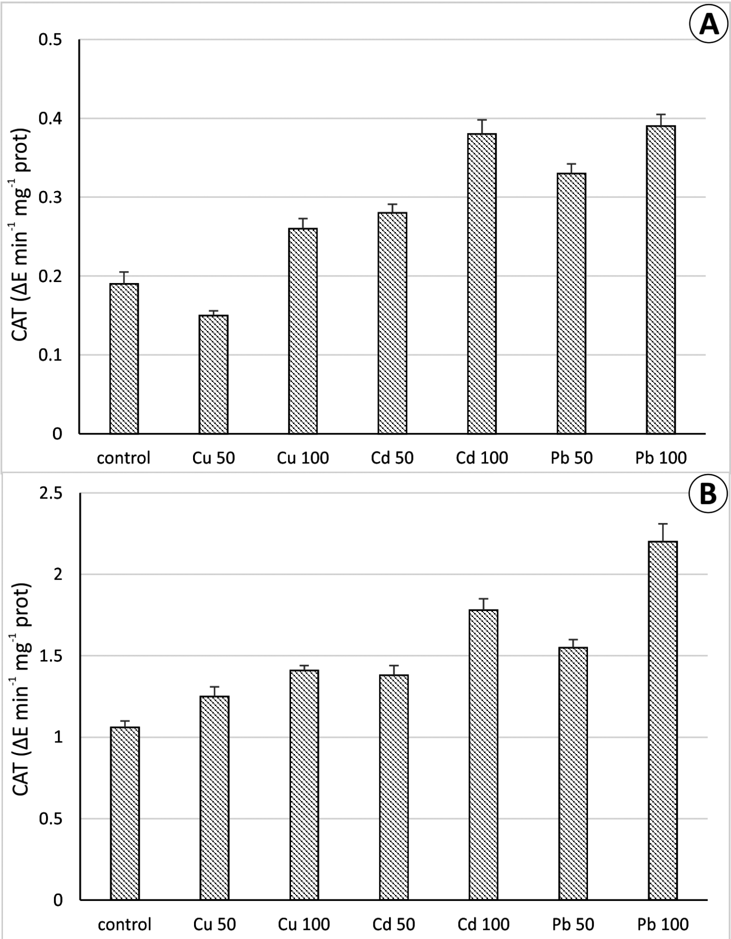

Figure 5. Changes of catalase activity (CAT, ΔE min^{-1} mg^{-1} prot.) in the cells of *Arthronema africanum* – **A** and *Coelastrella* sp. BGV – **B**, on the 10^{th} day after treatment by Cu, Cd and Pb (each metal added in concentrations 50 μM and 100 μM).

Table 2. Accumulation of Cu, Cd and Pb (mg kg^{-1} DW) in the biomass of *Arthonema africanum* and *Coelastrella* sp. BGV, 10^{th} day.

Variant	**Cu** (mg kg^{-1} DW)	**Cd** (mg kg^{-1} DW)	**Pb** (mg kg^{-1} DW)
		Arthronema africanum	
control	49.1	129.3	36.1
Cu 100 μM	349.8	106.8	16.9
Cd 100 μM	38.8	3258.3	13.7
Pb 100 μM	37.3	145.7	6431.3
		***Coelastrella* sp. BGV**	
control	66.7	69.5	56.0
Cu 100 μM	1285.2	12.0	99.0
Cd 100 μM	59.5	5069.9	33.8
Pb 100 μM	75.5	29.5	12230.2

of *Coelastrella* sp. BGV (Fig. 2). The protein content of both strains was the least affected by heavy metal treatment compared to the other parameters we investigated (Table 1). It was reported (Marinova et al. 2018; Pham et al. 2020) that Cd and Pb strongly inhibited growth of green alga *Scenedesmus* sp. but the strain could efficiently remove Cd, Pb and Cu at low concentrations. The pigment composition was strongly influenced by Cd and Pb, which had a pronounced inhibitory effect on the chlorophyll content of both strains. The decrease of chlorophyll content might be a result of distortion of its structure or inhibition of pigment biosynthesis (Shen et al. 2021). However, a significant increase of carotenoids was observed, particularly well manifested in *Coelastrella* sp. BGV, which indicated their antioxidant role in stressful conditions provoked by adding Cd and Pb into the medium (Table 1). It was reported (Shen et al. 2021) that Cd ions were highly toxic to the cyanoprokaryote *Synechocystis* sp., by inhibiting its growth and pigment biosynthesis. Furthermore, the authors found a reduced carotenoid content under Cd treatment, in contrast to our study, in which the strong enhancement of carotenoids could be considered a protective reaction against oxidative stress caused by Cd and especially by Pb.

Heavy metals can damage membrane molecules, disturbing the homeostasis under the enhanced generation of reactive oxygen species (ROS) and increased lipid peroxidation (Ajayan and Selvaraju 2012). The level of MDA in the biomass of *Coelastrella* sp. increased significantly after application of 100 µM Cu and 100 µM Pb and that effect was particularly pronounced after Pb treatment. The stress response of *A. africanum* was much more intense and different – the largest increase in MDA levels was measured after Cd treatment followed by Pb (Fig. 3A, B).

Microalgae develop various antioxidant mechanisms – enzymatic and non-enzymatic, to alleviate oxidative damage caused by heavy metal stress (Zhang et al. 2019). Heavy metals treatment significantly increased SOD activity in both experimental strains. The activity of SOD of *A. africanum* was two times enhanced under Cd stress, while in *Coelastrella* sp. a similar increase in SOD was observed after the addition of Pb (Fig. 4A, B). Cd and Pb also led to a significant increase of CAT activity, but these changes did not strictly correspond to those of SOD and MDA levels (Fig. 5A, B). In *Coelastrella* sp., the most significant increase in CAT was observed after the addition of Pb in the medium, while in the cells of *A. africanum* Pb and Cd caused a similar increase of the activity. It is obvious that the enzymatic antioxidant protection of *A. africanum*, as a part of the anti-stress response, was not sufficient to overcome heavy metal toxicity, which was manifested by the severely inhibited growth of the strain. It is likely that in both strains the elimination of ROS involved certain additional protective mechanisms in addition to antioxidant enzymes, which should be studied in more detail.

The results concerning physiological and biochemical changes in *A. africanum* and *Coelastrella* sp. suggested that Cu influences the metabolic processes in the algal cells by a different mechanism, compared to Pb and Cd. That understanding was confirmed by the way both strains accumulated heavy metals from the nutrition medium. Both microalga showed a high absorption capacity for metal ions, especially for Pb, which accumulated in the biomass in much greater concentrations than copper and cadmium. The degree of TM uptake was performed as follows Pb>Cd>Cu. (Table 2). *Coelastrella* sp., in particular, had a significantly higher ability for metal ion absorption, which did not affect her growth so strongly as *A. africanum*. Similar results were obtained in *Sc. incrassatulus* treated by 50 μM and 100 μM Cu, Cd and Pb (Goswami et al. 2014; Marinova et al. 2018). The green microalga manifested a very high absorption capacity, accumulating predominantly Cd from the environment. For that reason, we assumed that the investigated strains, and especially *Coelastrella* sp., could find real application in the treatment of water bodies contaminated with Cu, Pb and Cd.

Conclusion

The obtained results supported the understanding of microalgae as very reliable for the purposes of phytoremediation. In addition to the differences in their uptake capacity, it is possible that one definite heavy metal ion can interact specifically with a particular algal strain. Cd and Pb proved to be the most toxic for growth and pigment biosynthesis and provoked the strongest antioxidant response and enhanced MDA levels and activity of antioxidant enzymes in both investigated strains. The cyanoprokaryote *A. africanum* was much more sensitive to heavy metal stress, in contrast to *Coelastrella* sp. BGV, which showed a significantly higher absorption potential. We believe that after further research we could suggest *Coelastrella* sp. BGV as a suitable species for non-toxic and effective heavy metals' removal.

In conclusion, this study could contribute to expanding knowledge about the mechanisms of heavy metal stress in microalgae, as well as their future application for the needs of phytoremediation.

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