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Environmentally friendly oxidation processes

EFFECT OF HEAVY METALS ON THE GREEN ALGA *Scenedesmus incrassatulus*

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ABSTRACT

Green alga *Scenedesmus incrassatulus*, strain R 83, was treated by high concentrations of Cu, Cd and Pb added into the nutrition medium. Cd strongly inhibited growth – it was 60% decreased, followed by Pb – about 50% decreased, while the action of Cu appeared to be the less harmful. Cd and Pb impaired cell morphology and development, and worsen the algal culture.

It was found a progressive decrease in the amount of chlorophyll *a*, *b* and carotenoids corresponding to the duration of heavy metals action. Pb had the most pronounced negative effect on the pigment composition of *Sc. incrassatulus*. Meanwhile, Cu-treated variants remained less affected by the metal toxicity. These results were supported by the changes in malondialdehyde (MDA) content showing an enhanced lipid peroxidation under Pb and Cd pollution. Therefore, it could be suggested that Cu influenced in a different way metabolic processes in the algal cells, than Cd and Pb.

Sc. incrassatulus possessed a good adsorbing capacity for metal ions, especially for cadmium, which was absorbed in a much greater extent than lead and copper. For that reason, we assumed that the strain could be used in the treatment of wastewater, polluted by Cu, Pb and Cd.

Keywords: *Scenedesmus incrassatulus,* heavy metals, pigments, malondialdehyde.

AIMS AND BACKGROUND

A number of microalgal species are currently used for biological purification of industrially polluted environments including heavy metal contaminated areas[1–3]. It is

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considered that these species are a promising, eco-friendly and sustainable option for wastewater and soil treatment with a possible advantage of improving the economics of microalgal cultivation[4].

Aquatic plants and microorganisms are able to remove metals from water through processes of biosorption and bioaccumulation[5]. Microalgae reduce metal contamination by rapid physical absorption of the metal ions over the algal cell surfaces[6,7], followed by a slow movement of these ions into the cytoplasm[8]. Moreover, when transported inside the cell, some heavy metals are deposited in cellular inclusions. Several researchers have established that Pb, Mg, Zn, Cd, Sr, Co, Hg, Ni and Cu are sequestered in polyphosphate bodies in green algae[2,4]. Thus, the polyphosphate bodies serve as a storage pool for metals and also act as 'detoxification agents'.

Phototrophic microorganisms (cyanobacteria and algae) have been used in toxicity tests designed to environmental monitoring of heavy metal pollution. The majority of the studies, in fact, have analysed the toxic effect of metals on cyanobacterial species[9–13]. However, unicellular green algae of the genera *Chlamydomonas*, *Chlorella*, *Scenedesmus*, etc. have also the ability to extract and accumulate heavy metal ions[14–17].

Scenedesmus species are often used as model objects for exploring the accumulation of heavy metals due to their wide spreading in contaminated ponds. Awasthi and Rai[18] reported that *Scenedesmus quadricauda* could be recommended to remove toxic quantities of nickel, zinc and cadmium. According to Ajayan et al.[4], *Scenedesmus* is able to capture copper extracellularly in the extrapolymeric substances, and lead extra- and intracellularly in polyphosphate inclusions. Vanerkar et al.[2] have also revealed that *Scenedesmus obliquus* accumulated Cd and Zn by increasing the amount of phosphorus in the media. In general, it is believed that the living green algae, like *Scenedesmus*, could be used for heavy metal removal from diluted wastewater and that method could be economical and effective[17]. Still, questions remain about how to detoxify heavy metals accumulated in algal cultures.

Cu, Cd and Pb are among the most abundant environmental pollutants. Cd and Pb are nonessential but highly toxic metals to living organisms[5,16,19] and copper, at excessive concentrations, is also harmful. The ability of green alga *Scenedesmus incrassatulus* to modulate polluted environments via its metabolic activity determined the purpose of the present work – investigation of Cu^{2+}, Cd^{2+} and Pb^{2+} – influence on some physiological and biochemical parameters of *Scenedesmus incrassatulus* cells.

EXPERIMENTAL

The green alga *Scenedesmus incrassatulus,* strain R 83, isolated from Rupite region, Southwest Bulgaria, was maintained as a batch culture at the Algal Culture Collection, section 'Experimental Algology', IFRG, BAS. Algal suspension was intensively cultivated on the Setlik nutrition medium[20] (modified by Georgiev et al.[21]), in 200 ml vessels, at 28°C under continuous illumination by luminescent lamps, at light intensity 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 culture was 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 0.7 mg ml^{-1} DW (dry weight).

Heavy metals treatment was performed by adding into the nutrition medium 50 and 100 μM of Cu^{2+}, Cd^{2+} and Pb^{2+} (added as $CuSO_4$, $CdCl_2$ and $(CH_3COO)_2Pb$). Growth and physiological changes of the algal cultures were determined on the 3rd, 5th and 7th day from the start of the treatment.

Scenedesmus incrassatulus growth rate was measured by the increase of dry weight. Pigment content was determined spectrophotometrically after methanol extraction and calculated according to McKinney[22]. Malone dialdehyde (MDA) content was determined according to Dhindsa et al.[23], using the extinction coefficient of 155 mM^{-1} cm^{-1}. The content of Cu, Cd and Pb in dry algal biomass was analysed by an atomic absorption spectrophotometer Perkin-Elmer.

The experimental data were averaged of triplicate measurements. The significance of differences between control and each treatment was determined using Student *t*-test, $p \leq 0.05$.

RESULTS AND DISCUSSION

Responses of algal cells to metal exposure are typically measured in terms of cell density, biomass, growth rate, and chlorophyll content or chlorophyll absorbance. The strongest toxic effect of heavy metal ions that occurred was found in the cultures of *Sc. incrassatulus* polluted by cadmium (Fig. 1). Pb^{2+}-treatment caused lower levels of toxicity, followed by Cu^{2+}, which appeared to be the least harmful. The highest reduction of algal dry weight was measured after 100 μM Cd was added to the medium – growth rate was more than 60% reduced as compared to control. A similar inhibition of growth was also observed after applying of 100 μM Pb – about 50% below the control. However, algal cells exposed to Cu (50 and 100 μM), did not significantly reduce their growth rate until 72nd hour of treatment, when the dry weight of biomass remained close to the control samples.

Studying the effect of cadmium on the algae several researchers found such a severe growth inhibition, as we did[3,15,24], etc. In addition to the decrease of growth, high cadmium concentrations disturb photosynthesis and reduce cell size[19]. The excess of Pb, in turn, causes stunted growth, chlorosis, inhibits photosynthesis, mineral nutrition and water balance[25,26]. Cadmium and lead are considered to affect growth by different mechanisms than Cu – they can form stable complexes with some structural proteins and enzymes, and thus negatively influence cellular metabolism. High Cu concentration could affect the normal growth and development of the plant cells by the ability of copper to displace other metal ions from physiologically important centers. Cu causes a decrease of chlorophyll *a*, photosynthetic O_2 evolution, cell division rates, as well as growing numbers of deformed and broken cells[27,28].

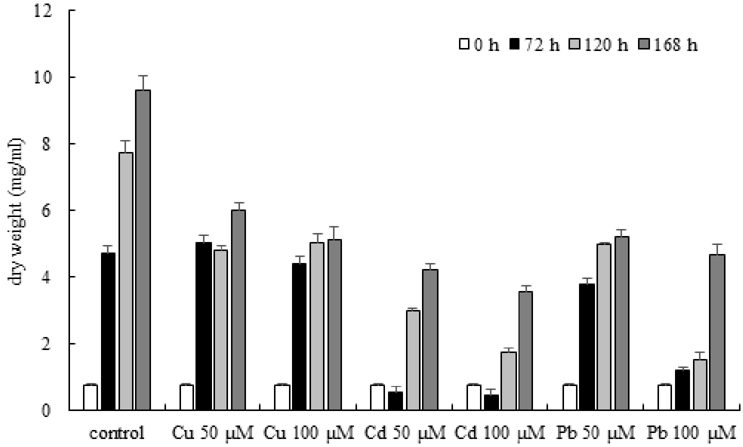

Fig. 1. Growth rate (dry weight, mg/ml) of *Scenedesmus incrassatulus* after Cu, Cd and Pb treatment

In proliferating algal cultures, the growth of protozoa is obviously strongly inhibited by algae. However, we observed that the increased levels of heavy metals caused a rapid development of unicellular eukaryotic organisms, especially in Cd- and Pb-treated variants. 100 µM Cd forced *Sc. incrassatulus* to form rounded autospores with slow development, as well as multiple agglutinates in the medium. Moreover, nearly 30% of the biomass belonged to protozoa. When Pb ions were added to the medium, about 20% of the cells were rounded in shape, with impaired morphology. There were very few single cells – mostly agglutinates of 2–4 cells, and almost 10% of the biomass consisted of protozoan organisms (data not shown). Furthermore, depending on the type of heavy metal treatment, different morphological changes were observed in *Sc. incrassatulus* cells. For example, when treated with divalent ions, such as Cu^{2+} and Cd^{2+}, the unicellular forms were predominantly observed, while Cr^{6+} determined the appearance of irregularly shaped prisms and grouped cells[29].

Studying the changes in *Sc. incrassatulus* pigment composition, we found a strong decrease in the amount of chlorophyll *a* in all heavy metal polluted variants (Fig. 2). Both, Cd and Pb severely reduced chlorophyll *a* content – the lowest level was measured at 100 Pb µM – 88% decrease as compared to the control. Cu, in fact, caused a significantly lower reduction in chlorophyll *a* (nearly 45%). Changes in the amounts of chlorophyll *b* in the biomass followed an equal trend to that one of chlorophyll *a*. The decrease of chlorophyll *b* content was most pronounced in Cd and Pb treated suspensions – 84% at 100 µM Cd and 87% at 100 µM Pb (Fig. 3).

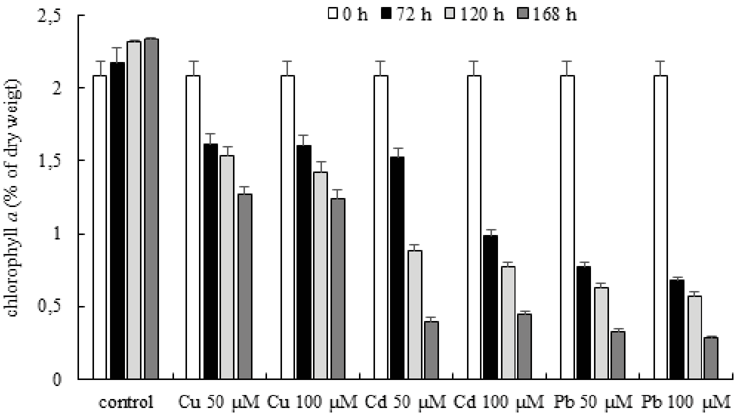

Fig. 2. Changes of chlorophyll *a* content (% of dry weight) in *Scenedesmus incrassatulus* cells, treated by Cu, Cd and Pb

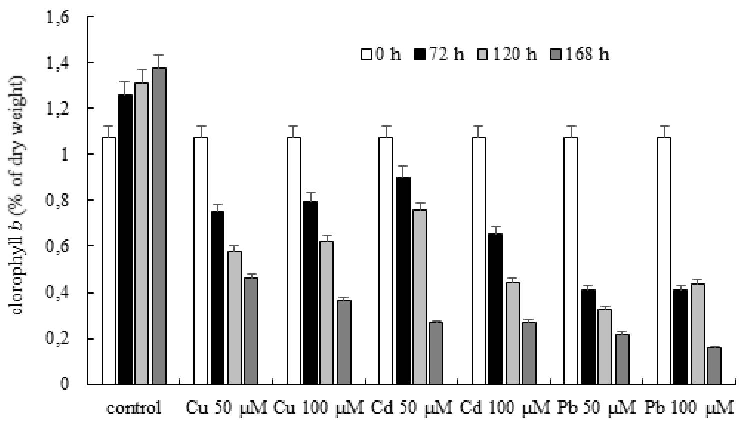

Fig. 3. Changes of chlorophyll *b* content (% of dry weight) in *Scenedesmus incrassatulus* cells, treated by Cu, Cd and Pb

When analysed the amount of carotenoids, we observed a similar tendency to decline in all experimental variants (Fig. 4). Their content progressively decreased with time of exposure and reached the lowest values on the 7th day of treatment. Moreover, the decrease of carotenoids was relatively independent of the heavy metals concentrations that were used – 50 or 100 µM. The only exception was 50 µM Cd, wherein carotenoids responded by a relatively lower decrease at the beginning of the period – they were 32% decreased. Thus, there was no reason to believe that carotenoids possessed any protective role against heavy metal stress developed in *Sc. incrassatulus*.

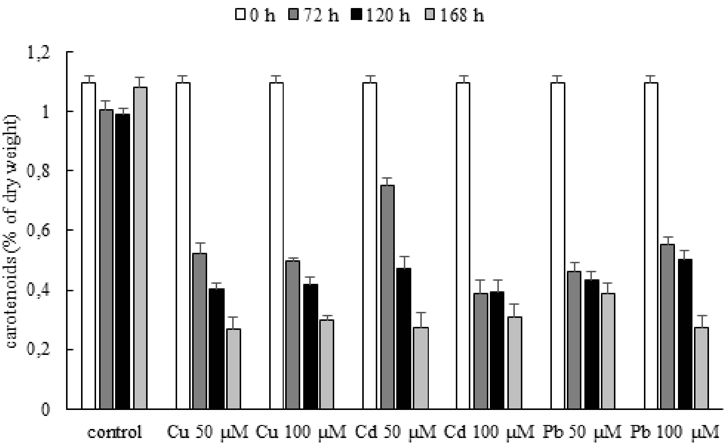

Fig. 4. Changes of carotenoid content (% of dry weight) in *Scenedesmus incrassatulus* cells, treated by Cu, Cd and Pb

In general, Pb had the strongest negative effect on the pigment composition of *Sc. incrassatulus*, followed by Cd. This may be attributed to the damage of algal photosystems by the excessive amounts of heavy metals, especially Cd and Pb, resulting in a reduction of photosynthetic pigments, particularly chlorophyll *a*[15,24,26]. One more reason was recognised as blocking of photosynthesis by the replacement of Mg^{2+} ion in the chlorophyll molecule with some metal ions – Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+} or Ni^{2+} (Ref. 30). A nearly proportional relationship between metal toxicity and the level of substitution of Mg in the chlorophyll ring has been established.

Copper, cadmium and lead damage cell membranes by binding to the sulfhydryl groups of the membrane proteins, resulting in peroxidation of the lipids[31]. Changes of malondialdehyde amounts are often used as a routine method to determine the degree of lipid peroxidation in the plant cells, and increased MDA content have been frequently registered as a result of heavy metal toxicity[32,33]. In the course of the experimental work, we have also found severely elevated levels of MDA in Cd- and Pb-polluted *Scenedesmus* cultures (Fig. 5). The highest values were recorded at 50 µM and 100 µM Pb in the medium (2–2.5 times higher than the control, respectively). 100 µM Cd caused 70% increase of MDA, while Cu-treated variants remained close to the control. The only exception was at 50 µM Cd, where MDA was even reduced – it was 30% below the control value.

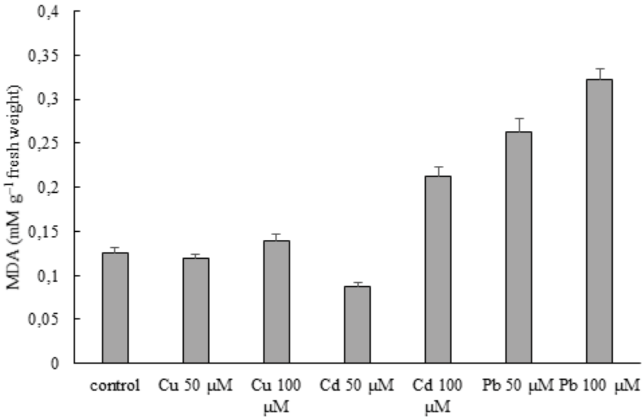

Fig. 5. Influence of Cu, Cd and Pb on malondialdehyde levels (mM g^{-1} fresh weight) in *Scenedesmus incrassatulus* cells

The enhanced production of malondialdehyde measured in the Pb- and Cd-treated variants (Fig. 5), which was accompanied by the chlorophyll degradation (Figs 2 and 3), was a serious evidence of lipid peroxidation and membrane damages in *Sc. incrassatulus* cells. It could be therefore suggested, that lead and cadmium largely transform cell membranes and membrane transport, on the one hand, blocking specifically a number of transport proteins[34] and on the other hand, by the enhancement of membrane lipid peroxidation. Cd, itself, is well-known as a factor damaging phospholipid structures of chloroplasts and mitochondrial membranes – the main target organelles for cadmium poisoning[35], while Pb changes plant hormonal status and affects membrane structure and permeability[36]. The toxicity of Cu, which is often studied as a pro-oxidant, may include a development of oxidative stress[24,31]. Like many other metal ions, Cu^{2+} accelerates the formation of oxyradicals, which in turn enhances lipid peroxidation causing membrane disintegration and failure of osmoregulation.

Our results demonstrated the affinity of *Scenedesmus incrassatulus* for Cu^{2+}, Cd^{2+} and Pb^{2+}-uptake (Table 1). We found that the capacity of *Scenedesmus* for biosorption of both, Cu and Pb, was almost the same, while Cd was accumulated in a much higher extent. However, taking into account its impact on growth and pigment content, Cu appeared to be not as toxic as Pb. Therefore, it could be considered that *Sc. incrassatulus* have developed some mechanisms to eliminate copper and/or to prevent its involvement into the cell metabolism[24]. In fact, it was established that algae may exclude a larger part of the added Cu from their cytoplasm by adsorbing to cell walls[37]. Moreover, some algal strains synthesised high levels of phytochelatins and thiol-containing peptides, in response to the copper stress[28]. It was also reported a higher excretion of extracellular polymeric substances to surround the cells of *Scenedesmus*, which could explain the external uptake of both, copper and lead[38]. Besides, some microorganisms, including *Scenedesmus,* have the capacity to accumulate Pb in polyphosphate granules[39]. *Scenedesmus* was also found to capture copper externally in the extrapolymeric substances and lead extra- and intracellularly in polyphosphate inclusions[1].

Table 1. Accumulation of Cu, Cd and Pb (mg g^{-1} DW) in *Scenedesmus incrassatulus*

Heavy metals	Variants	Accumulation of heavy metals (mg g^{-1} DW)
Cu	control	0.041± 0.002
	50 µM	0.115 ± 0.005
	100 µM	0.188 ± 0.007
Cd	control	–
	50 µM	0.606 ± 0.028
	100 µM	0.799 ± 0.036
Pb	control	–
	50 µM	0.159 ± 0.006
	100 µM	0.193 ± 0.009

On the other hand, it should be taken into account that the rate of heavy metals absorption by plants is highly pH-dependent[29]. Some of the appropriate culture media could affect the removal rate for some bivalent metal ions, and respectively, the efficiency of microalgal wastewater treatment. For practical microalgal application in bioremediation of heavy-metal polluted areas, a further study is necessary to ascertain the influence of higher pH values on the efficiencies in the heavy metals absorption/adsorption. In the current experiments, the pH value was maintained around 7.0. *Sc. incrassatulus*, grown under these conditions, in fact, cannot be considered as a strain that strongly accumulates heavy metals, unlike some other microalgae. This conclusion follows from the results of our previous studies[40–42], in which we have found that some cyanobacteria and green algae accumulated heavy metals in their biomass at much higher concentrations than *Sc. incrassatulus* did.

CONCLUSIONS

The results showed that Cd and Pb are highly toxic to *Sc. incrassatulus* – they severely inhibited growth, reduced pigment content and increased lipid peroxidation. Copper did not inhibit growth and chlorophyll biosynthesis in the algal strain so strongly and did not enhance MDA production, although it accumulated in the biomass at concentrations commensurate with those of lead. Thus, on the basis of these arguments, we could suggest that *Scenedesmus* had the ability to isolate copper from the cellular metabolism and also, that the strain had different mechanisms of incorporating Cu from those of Cd and Pb.

The accumulation of large amounts of metal ions, especially Cd, in the algal mass, that we registered, could be a reason to determine a broad practical application of *Sc. incrassatulus* in heavy metals contaminated areas (soils or wastewater). In general, like some other microalgae, *Sc. incrassatulus* could play an important role in biorepairing some areas polluted by metals, as a result of the ability to tolerate or resist high concentrations of metals and to bioaccumulate them, extra- and intracellularly.

REFERENCES

1. A. BURGOS, J. MALDONADO, A. DE LOS RIOS, A. SOLE, I. ESTEVE: Effect of Copper and Lead on Two Consortia of Phototrophic Microorganisms and their Capacity to Sequester Metals. Aquat Toxicol, **140–141**, 32 (2013).
2. A. P. VANERKAR, A. B. FULKE, S. K. LOKHANDE, M. D. GIRIPUNJE, S. SATYANARAYAN: Recycling and Treatment of Herbal Pharmaceutical Wastewater Using *Scenedesmus quadricauda*. Current Science, **108** (5), 979 (2015).
3. T. BATSALOVA, I. TENEVA, D. BELKINOVA, P. STOYANOV, S. RUSINOVA-VIDEVA, B. DZHAMBAZOV: Assessment of Cadmium, Nickel and Lead Toxicity by Using Green Algae *Scenedesmus incrassatulus* and Human Cell Lines: Potential *in vitro* Test-Systems for Monitoring of Heavy Metal Pollution. Open Journal (TFMOJ), **2** (2), 63 (2017).
4. K. V. AJAYAN, M. SELVARAJU, P. UNNIKANNAN, P. SRUTHI: Phycoremediation of Tannery Wastewater using Microalgae *Scenedesmus* species. Int J Phytoremediation, **17** (10), 907 (2015).
5. P. A. TERRY, W. STONE: Biosorption of Cadmium and Copper Contaminated Water by *Scenedesmus abundans*. Chemosphere, **47**, 249 (2002).
6. J. B. K. PARK, R. J. CRAGGS, A. N. SHILTON: Wastewater Treatment High Rate Algal Ponds for Biofuel Production. Bioresour Technol, **102**, 35 (2011).
7. H. WANG, W. ZHANG, L. CHEN, J. WANG, T. LIU: The contamination and control of biological pollutants in mass cultivation of microalgae. Bioresour Technol, **128,** 745 (2013).
8. H. H. OMAR: Bioremoval of Zinc Ions by *Scenedesmus obliquus* and *Scenedesmus quadricauda* and Its Effect on Growth and Metabolism. Int Biodeter Biodegr, **50**, 95 (2002).
9. R. de PHILIPPIS, R. PAPERI, C. SILI, M. VINCENZINI: Assessment of the Metal Removal Capability of Two Capsulated Cyanobacteria *Cyanospira capsulata* and *Nostoc* PCC7936. J Appl Phycol **15**, 155 (2003).
10. L. Y. HENG, K. JUSOH, C. H. LING, M. IDRIS: Toxicity of Single and Combinations of Lead and Cadmium to the Cyanobacteria *Anabaena flos-aquae*. Bulletin Environ Contam, **72**, 373 (2004).
11. R. GONG, Y. DING, H. LIU, Q. CHEN, Z. LIU: Lead Biosorption and Desorption by Intact and Pre-treated *Spirulina maxima* Biomass. Chemosphere, **58**, 125 (2005).
12. C. SOLISIO, A. LODI, P. TORRE, A. CONVERTI, M. DEL BORGHI: Copper Removal by Dry and Re-hydrated Biomass of *Spirulina platensis*. Bioresour Technol, **97**, 1756 (2006).
13. W. L. SHING, L. HENG, S. SURIF: The Fluorometric Response of Cyanobacteria to Short Exposure of Heavy Metal. Adv Environ Biol, **6,** 103 (2012).
14. H. DILOV: Microalgae – Mass Cultivation and Application. BAS, Sofia, 1985 p. 16.
15. H. P. CARR, F. A. CARINO, M. S. YANG, M. H. WONG: Characterization of Cadmium-binding Capacity of *Chlorella vulgaris*. Bull Environ Contam Toxicol, **60**, 433 (1998).
16. H. H. ABDEL GHAFAR, A. M. ABDEL-ATY, N. S. AMMAR, M. A. EMBABY: Lead Biosorption from Aqueous Solution by Raw and Chemically Modified Green Fresh Water Algae *Scenedesmus obliquus*. Desalin Water Treat, **52**, 7906 (2014).
17. B. KIZILKAYA, G. TURKER, R. AKGUL, F., DOGAN: Comparative Study of Biosorption of Heavy Metals Using Living Green Algae *Scenedesmus quadricauda* and *Neochloris pseudoalveolaris*: Equilibrium and Kinetics. J Dispersion Sci Technol, **33**, 410 (2012).
18. M. AWASTHI, C. RAI: Toxicity of Nickel, Zinc and Cadmium toNnitrate Uptake in Free and Immobilized Cells of *Scenedesmus quadricauda*. Ecotoxicol Environ Safety, **61**, 268 (2005).

19. G. F. LEBORANS, A. NOVILLO: Toxicity and Bioaccumulation of Cadmium in *Olisthodiscus luteus*. Water Resources, **30** (1), 57 (1996).
20. I. ŠETLIK: Contamination of Algal Cultures by Heterotrophic Microorganisms and Its Prevention. Annual Report of the Algology for the Year 1966, Trebon, CSAV, Institute of Microbiology, 1967, p. 89.
21. D. GEORGIEV, H. DILOV, S. AVRAMOVA: Millieu nutritif tamponne et méthode de culture intensive des microalgues vertes. Hydrobiology, **7**, 14 (1978).
22. G. MCKINNEY: Absorption of Light by Chlorophyll Solution. J Biol Chem, **140** (2), 315 (1944).
23. R. S. DHINDSA, P. PLUMB-DHINDSA, T. A. THORPE: Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase. J Exp Bot, **32**, 93 (1981).
24. M. MOHAMMED, B. MARKERT: Toxicity of Heavy Metals on *Scenedesmus quadricauda* (Turp.) de Brébisson in Batch Cultures. ESPR – Environ Sci & Pollut Res, **13** (2), 98 (2006).
25. J. MALDONADO, A. SOLE, Z. PUYEN, I. ESTEVE: Selection of Bioindicators to Detect Lead Pollution in Ebro Delta Microbial Mats, Using High-resolution Microscopic Techniques. Aquat Toxicol, **104**, 135 (2011).
26. L. DAO, J. BEARDALL: Effects of Lead on Two Green Microalgae *Chlorella* and *Scenedesmus*: Photosystem II Activity and Heterogeneity. Algal Research, **16**, 150 (2016).
27. J. W. RIJSTENBIL, J. DERKSEN, L. GERRINGA, T. POORTVLIET, A. SANDEE, M. VANDE-BERG, J. van DRIE, J. WIJNHOLDS: Oxidative Stress Induced by Copper: Defense and Damage in the Marine Planktonic Diatom *Ditylum brightwellii* Grown in Continuos Culture with High and Low Zinc Levels. Mar Biol, **119**, 583 (1994a).
28. I. YRUELA: Copper in Plants. Braz J Plant Physiol, 17 (1), 45 (2005).
29. J. PENA-CASTRO, F. MARTINEZ-JERONIMO, F. ESPARZA-GARCIA, R. CANIZARES-VIL-LANUEVA: Heavy Metals Removal by the Microalga *Scenedesmus Incrassatulus i*n Continuous Cultures. Bioresour Technol, **94**, 219 (2004).
30. G. KOWALEWSKA, L. FALKOWWSKI, S. K. HOFFERMANN, L. S. SZCZEPARIAK: Replacement of Magnesium by Copper in the Chlorophyll Porphyrin Ring of Planktonic Algae. Acta Physiol Plant (Poland), **9** (2), 43 (1987).
31. B. N. TRIPATHI, S. K. MEHTA, ANSHU AMAR, J. P. GAUR: Oxidative Stress in *Scenedesmus* sp. during Short- and Long-term Exposure to Cu^{2+} and Zn^{2+}. Chemosphere, **62**, 538 (2006).
32. T. DEMIRAL, I. TÜRKAN: Comparative Lipid Peroxidation, Antioxidant Defense Systems and Proline Content in Roots of Two Rice Cultivars Differing in Salt Tolerance. Environ Exp Bot, **53**, 247 (2005).
33. S. K. YADAV: Heavy Metals Toxicity in Plants: an Overview on the Role of Glutathione and Phytochelatins in Heavy Metal Stress Tolerance of Plants. S Afr J Bot, **76**, 167 (2010).
34. S. TOPPERWIEN, R. BEHRA, L. SIGG: Competition among Zinc, Manganese, and Cadmium Uptake in the Freshwater Alga *Scenedesmus vacuolatus*. Environ Toxicol Chem, **26(3)**, 483 (2007).
35. M. E. LAMAS, M. S. SOARES, M. M. OLIVERA: Effect of Cadmium on *Euglena gracilis* Membrane Lipids. BJMBR, **29**, 941 (1996).
36. P. SHARMA, R. DUBEY: Lead Toxicity in Plants. Braz J Plant Physiol, **17** (1), 35 (2005).
37. J. W. RIJSTENBIL, F. DEHAIRS, R. EHRLICH, J. A. WIJNHOLDS: Effect of the Nitrogen Status on Copper Accumulation and Pools of Metal-binding Peptides in the Planktonic Diatom *Thalassiosira pseudonana*. Aquat Toxicol, **42**, 187 (1998a).
38. S. PEREIRA, E. MICHELETTI, A. ZILLE, A. SANTOS, P. MORADAS-FERREIRA, P. TAMAGNINI, R. de PHILIPPIS: Using Extracellular Polymeric Substances (EPS)-producing Cyanobacteria for the Bioremediation of Heavy Metals: Do Cations Compete for the EPS Functional Groups and also Accumulate Inside the Cell? Microbiology, **157**, 451 (2011).

39. J. MALDONADO, A. de lOS RIOS, I. ESTEVE, C. ASCASO, Z. PUYEN, C. BRAMBILLA, A. SOLE: Sequestration and *in vivo* Effect of Lead on DE2009 Microalga, Using High-resolution Microscopic Techniques. J Hazard Mater, **183**, 44 (2010).
40. G. CHANEVA, A. UZUNOVA, S. FURNADZHIEVA: Cadmium Toxicity in Coincubation System *Pisum sativum*/*Plectonema boryanum*. J Environ Prot Ecol, **6** (2), 271 (2005).
41. G. CHANEVA, A. UZUNOVA, V. KAPCHINA, S. CHANKOVA: Heavy Metal Stress in Coincubation System *Chlamydomonas reinhardtii/Pisum sativum*. J Environ Prot Ecol, **9** (2), 291 (2008).
42. G. CHANEVA, D. PETROVA, A. UZUNOVA: Changes in Antioxidative Enzymes of a Cyanobacterium Grown under Heavy Metal Stress. B&BE (Special Issue), **23,** 554 (2009).

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