USE OF SELENASTRUM CAPRICORNUTUM GROWTH INHIBITION TEST FOR TESTING TOXICITY OF METAL IONS IN SOIL AND WATER

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ABSTRACT

The unicellular green alga Selenastrum capricornutum (Rhapidocelis subcapitata) growth inhibition assay was applied to the toxicity testing of water, suspensions of heavy-metal-polluted clinker and waste ore pile. The ISO 8692:2004 standard test procedure was followed, and growth rate inhibition indexes were determined. Despite high concentrations of zinc in water (38.47 mg/L of Zn), exceeding by approximately 400-fold the EC_{50} *value for S. capricornutum (0.1 mg Zn/L), 72-h algal growth in the soil extracts was inhibited with 50% in comparison with growth in the standard control ISO mineral medium. Cell dead of algae was observed by clinker leacheates contained up to 796 mg Cu/L. Decreasing of inhibition effect and rate of cell dead could not be explained by supplementary nitrogen, phosphorous, and carbonate leached from the waste ore pile and clinker.*

Introduction

Growth inhibition toxicity tests were usually used for determining the presence of toxic substances in environment (9). Microbial growth rate decreases with increasing toxicity (4). Bacterial growth inhibition tests could not be used in experiments with pure water, because they compete, when the nutrient medium is depleted. In such situation green algae could be used successfully for determination of the toxic and mutagenic effect of chemicals, heavy metals, pesticides or industrial wastes (10, 1).

Algae are important system, because they could accumulate and metabolize non-mutagenic substances (promutagens) till mutagenic products, different from these in high plants and animals (11).

The aim of this work was to compare the sensitivity of internationally accepted algal test for the toxicity assessment of soil and water. We determined the environmental toxicity of metal ions in drainage waters and leached solution of clinker, waste ore pile using the growth inhibition of *Selenastrum capricornutum* – ISO 8692:2004 .

Materials and Methods

Investigated materials

- Drainage waters, collected from the East side of Plant for Color Metals Processing (PCM) – Plovdiv;
- Drainage waters, collected from the West side of waste ore pile next to Agria plant -Plovdiv ;
- Clinker, collected from waste ore pile with temperature 80-90 ºC (hot pile);

Processing of the samples

Immediately after collection the samples were put on ice and transported to the lab, where they were kept frozen till the processing. They were defrosted on ice for several hours without disturbing of their composition.

Water samples were centrifuged at 2500 rpm for a 15 min, after that they were filtered through filter paper. Clinker and waste ore pile samples were mixed with bidistilled water in the proportion 1/10 for

TABLE 1

Chemical analysis of PCM-Plovdiv (ICP AES)

Sample N	Sample N 1	Sample N 2	Sample N 3
As	< 5 ppb	< 5 ppb	0.07 mg/ l ± 31.51 %
Cd	4.05 mg/ l ± 0.44 %	0.13 mg/ l ± 1.37 %	0
Cu	0.78 mg/ l ± 0.53 %	0.23 mg/ l ± 1.78 %	796
Fe	< 5 ppb	< 5 ppb	26. 58mg/ l ± 0.84 %
Hg	< 5 ppb	< 5 ppb	< 5 ppb
Mn	-	-	360
Ni	22.12 mg/ l ± 1.17 %	< 5 ppb	3 mg/ l ± 0.14 %
Pb	0.45 mg/ l ± 19.04 %	< 5 ppb	1.07 mg/ l ± 0.95 %
Zn	-	38.47 mg/ l ± 0.61 %	273

Sample 1: Drainage waters, collected from the East side of Plant for Color Metals (PCM) –Plovdiv;
Sample 2. Drainage waters, collected from the West side of waste ore pile;
Sample 3. Leached solution of clinker, collected from waste ore pile with temperature 80-90 ºC (hot pile)

30 min at 220 rpm, and than were settled for 30 min and processed as the water samples.

Test-organism *Selenastrum capricornutum* Printz - resemble to the strain *Rhapidocelis subcapitata* ATCC 22662, used in international standard ISO 8692:2004 .

Methods. The tests were conducted according the procedures in ISO 8692:2004 for growth inhibition of *Selenastrum capricornutum*. The only change was the volume of the test glasses (50 ml), but the relation volume/nutrient medium was the same (1:5) as in the standard. Non- diluted and 2-, 4-, and 8- fold diluted environmental samples were used for the cultivation of algae. Cells density till 72 h was determined every 24 h using a hemacytometer under a light microscope. Three independent determinations for every sample and dilution were processed.

Results and Discussion

Investigated waters and soil had high content of diluted heavy metals, which several times exceeded the Bulgarian State standard (bold). The results from chemical analyses were presented in **Table 1**.

The growth inhibition of *Selenastrum capricornutum* exposed to different concentrations of drainage waters from the East side of PCM – Plovdiv were measured as survived cells/ml. Significant inhibition of growth occurred at concentration of 2- and 4- fold diluted samples. This inhibition was observed during the first 24 h of cultivation and was maintained until the end of the 72 h exposure period. On the other hand, significant inhibition of growth was demonstrated at the 8-time diluted sample after 24 h of cultivation. Results are in congruence with chemical analyses, revealed 4 mg/l Cd in the water. This content is 80-fold more the value that cause reduction of growth and 172-fold EC_{50} value for *S. capricornutum* (5). These results show that environmental water must be diluted more than 8 times to be harmless for the environment (**Fig. 1**).

The results from mathematical calculations (**Table 2**) were in congruence with the graphics. Growth rates of algae with 2- and 4-fold diluted sample were negative because of the cell dead. Inhibition could not be accounted for the same reason. The growth inhibition indexes were significant (more than 100%) in all tests variants. One exception was the algal growth for the first 24h in the most diluted sample (8fold) where it was comparable or better than growth in the standard control ISO mineral medium.

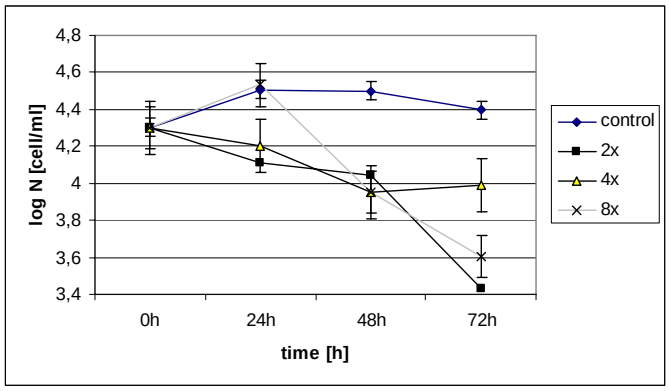

Fig. 1. Growth inhibition of *Selenastrum capricornutum* by drainage waters from the East side of PCM – Plovdiv measured as log cells/ml.

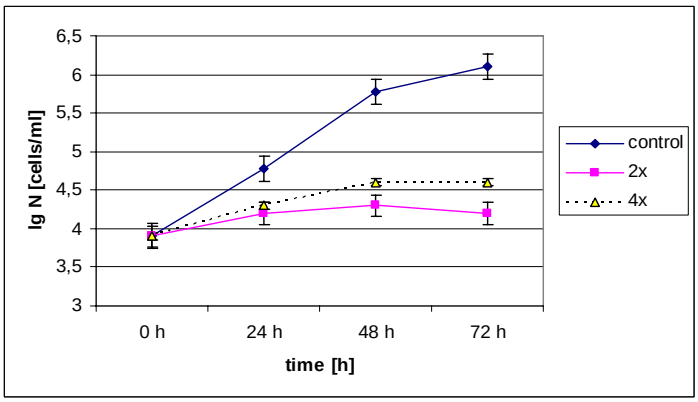

Fig. 2. Growth inhibition of *Selenastrum capricornutum* by drainage waters from the West side of PCM-Plovdiv.

TABLE 2

Values of growth rate (μ) and generation time (td) of *Selenastrum capricornutum*, influenced by drainage waters from East side of PCM – Plovdiv

Dilution	Specific growth rate μ for 0-24h, [h^{-1}]
Control	0.0085
2x	-0.0078
4x	-0.004
8x	0.0096

The growth inhibition of *Selenastrum capricornutum* by drainage waters from the West side of PCM – Plovdiv showed less toxicity of second sample than the first tested sample from East side (**Fig. 2**). There was no concentration dependence during the period of exposure. The growth inhibition was demonstrated after 24th hours of cultivation. The growth of *Selenastrum capricornutum* was reduced with around 12% till the 48 h, but showed significant depression at the 72nd h. The Zn toxicity obviously is not as harmful to algae as cadmium and nickel content in the first tested sample – only three times more than IC 50 12.5mg/L (12).

The direct counting of *Selenastrum capricornutum* cells has shown the toxicity of the second sample. The specific growth rate and generation time of algae have proven the same (**Table 3**). Inhibition is

TABLE 3

Values of growth rate (μ) and generation time (td) of *Selenastrum capricornutum*, influenced by drainage waters from the West side of PCM – Plovdiv

Dilution	Specific growth rate μ, [h^{-1}]			Generation time $t_{d\ 24\text{-}0h}$, [h]
	24 h	48 h	72 h	
Control	0.03625	0.04167	0.01375	8.28
2x	0.0125	0.00417	-0.0042	24
4x	0.017	0.0125	0	18

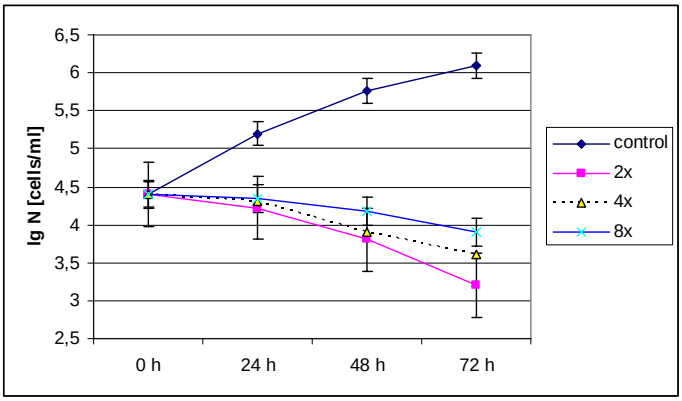

Fig. 3. Growth inhibition of *Selenastrum capricornutum* by drainage waters from the hot pile of PCM-Plovdiv.

TABLE 4

Growth inhibition index [I in %] for *Selenastrum capricornutum*

Time period [h]	Dilution	
	2 x	4 x
0-24	65.5	54.0
24-48	90	70

demonstrated after 24h with an index of about 12 % for every period of 24-hours till the 72h of exposure (**Table 4**).

The growth inhibition test with *Selenastrum capricornutum* by leaching waters from the hot pile of PCM – Plovdiv is presented at **Fig. 3**. Significant inhibition of the growth occurred at all dilutions of the sample. This inhibition was observed during the first 24 h of cultivation and was maintained until the end of the 72 h exposure period.

It was impossible to calculate any values of growth rate (μ) and generation times (td) of *Selenastrum capricornutum*, influenced by elutriate waters from hot pile of PCM – Plovdiv. Leached solution from clinker was so toxic, that almost all algae died in tested dilutions of sample, but not for first 24h. The complex toxicity caused by copper (16000-fold exceeded the EC50), Zink (22-fold over the IC 50) and other metal ions, could have been decreased by some substances, diluted in leached water from clinker to some extent (13).

Heavy metal toxicity was not immediately demonstrated in higher dilutions for first 24h. Growth inhibition indexes decreased from the 48th h till 72nd h of exposition.

Conclusions

Selenastrum capricornutum growth inhibition test is suitable for determination of heavy metal toxicity of polluted clinker and water. The green algae have shown different sensitivity to one metal ion and com-

plex metal pollution. The growth rate, generation time and inhibition indexes are reliable indicators of pollution. Despite high concentrations of zinc in water (38.47 mg/L of Zn), exceeding by approximately 400-fold the EC_{50} value for S. capricornutum (0.1 mg Zn/L), 72-h algal growth in the soil extracts was inhibited with 50% in comparison with growth in the standard control ISO mineral medium. Cell dead of algae was observed by clinker leached solutions contained up to 796 mg Cu/L. Decreasing of inhibition effect and rate of cell dead could not be explained by supplementary nitrogen, phosphorous, and carbonate leached from the waste ore pile and clinker.

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REFERENCES

1. **Adachi A., Matsushita K., Yata Y., Okano T.** (2004) Journal of Health Science, **50 (6)**, 613- 618.

2. **Aruoja V., Kurvet I., Dubourguier H.-C., Kahru A.** (2004) Wiley Periodicals, Inc. Environ Toxicol., **19,** 396-402.

3. **Boullemant A.**, **Campbell P.G.C.** (2002) Effects of aquatic humic substances on the bioavailability of lipophilic metal complexes to phytoplankton, http://www.uoguelph.ca/cntc/ann_symp/2002/poster-abs-metals.shtml

4. **Gabrielson J., Hart M., Jarelöv A., Kühn I., McKenzie D., Möllby R.** (2002) J. Microbiol. Methods, 50, Issue 1.

5. **EPA contract N68**-C-98-134 (2000) Update of ambient water quality criteria for cadmium. Grate lakes environmental centre, Traverse City, Michigan 49686 http://www.wca-infonet.org/cds_upload/1069775852572_un2.pdf

6. **Ivanova I., Doycheva L., Nenkov K., Ilieva S.** (2002) Soil Science, Agrochemistry and Ecology**,** 28(1-3), 61-63.

7. **Ivanova I.A., Krumova T., Stoyanov J., Pesheva M.** (2001) Bioremediation extent assessment of crude oil-polluted soil using microbial and high plant tests. First European Bioremediation Conference, Chania, Crete, 1-7 July 2001, 35-39.

8. International standard for toxicity assessment of surface, soil and waste waters on *Pseudomonas putida* (ISO 10712 :1995).

9. **Pardos M., Benninghoff C., Thomas R.L.** (1998) Journal of Applied Phycology, **10(2),** 145-151.

10. **Peterson S., Stauber L.** (1996) Bull. Environm. Contam. Toxicol**., 56,** 750-757.

11. **Miadoková E., Vlčková V., Podstavková S., Slaninová M., Vlček D. (**1998) Environmental and Molecular Mutagenesis, **31(4),** 383-389.

12. http://www.nch.se/se/pdf/ncvblad/HY%20ZINK%20EXTRA%20(AE).pdf

13. http://www.epa.gov/waterscience/criteria/copper/pdf/table-4a-freshwater-plant-cdm.pdf