UDC: 579.871:631.427:631.453
original scientific paper

Acta Agriculturae Serbica, Vol. XII, 23 (2007) 3-9

Acta Agriculturae Serbica
FACULTY OF AGRONOMY • ČAČAK •

Using *Arthrobacter globiformis* Bioassay for Assessment of Bioavailability and Toxicity of Soil and Sediments

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Abstract: We used the bacterial test on the cells called *Arthrobacter globiformis* in order to detect the level of toxins in the sediment of the Lake Skadar. We kept an eye on the influence of the sediments on the activity of the whole of bacterial dehydrogenase (DNA) by the process of the reduction of the oxidoreduction color of the resasurine into the resofurine. We used the organic fraction of the sediments (the extracts of sediments) in combination with the native sediments. The sediments of the lake indicated lower or higher level of the toxin depending on the locality and the presence of certain substances that have toxic effects which are biologically easily accessible to the living organisms. The highest level of toxins indicated the sediments of Radus and the sediments of the estuary of the river Moraca into both of the systems.

Keywords: bioassays, sediment, toxicity, *A.globiformis*, Skadar Lake.

Acknowledge: We acknowledge the active support of German Rector's Conference (HRK) for foundation this research and authors would also like to thank dr Henner Hollert from University of Heidelberg for supplying and helping the bioassay.

Introduction

Toxicological experiments usually determine specific endpoints out of a huge range of possible effects. Several bioassays are necessary for a comprehensive assessment of the eco-toxicological hazard of sediment samples (Neumann-Hensel *et al.*, 2000; Hollert *et al.*, 2002). A battery of bioassays for soil and sediment quality assessment was recommended by Ahlf and co-workers (2005), covering the main routes of exposure, different eco-toxicological endpoints – and therefore several levels affected by toxic contamination (i.e. molecular mechanisms, cells, whole organism) – and various model species representing distinct organisms groups in their habitats. The bioassays used in the current study were selected with respect to assess of toxicity effectiveness *in vitro* of sediment samples.

Materials and Methods

Sediment samples were collected from different locations of the Lake Skadar: Radus (T_1), Middle lake (T_2) and Mouth river Moraca (T_3) (Fig 1.)

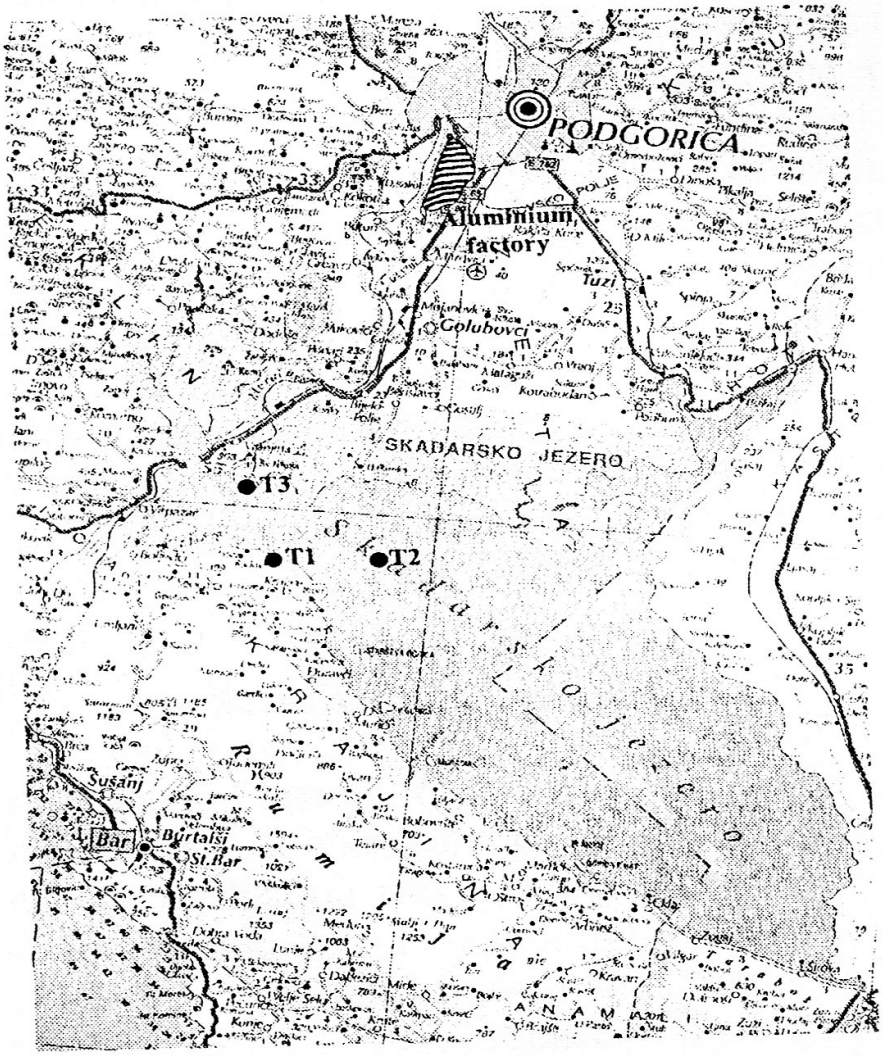

Fig.1. Outline map of Lake Skadar showing its geographical location and the locations of the various sampling sites.

The sediments were taken from the surface (0-5 cm) by the Van Veen's mechanical shovel and they were held in the freezer after the homogenization at the -20 Celsius degrees (Hollert *et al.*, 2002). The samples of the sediments were drained in the lyophilisator at the -20 Celsius degrees. The samples were kept in the bottles made from polyethylene in the darkness at the -4 Celsius degrees (Kosmehl *et al.*, 2004).

We added 20g of the drained sediments with 400ml of acetone, and then isolated the samples of the sediments within six cycles in the course of 24 hours (Kosmehl *et al.*, 2004; Hollert *et al.*, 2000; Hollert and Braunbeck, 1997). The obtained extracts were reduced in the rotor evaporator at 400 mbar, 36-38 Celsius degrees, and after that the extracts were concentrated in the flowing nitrogen (Hollert *et al.*, 2000). The acetone was replaced by 1 ml of the dimethylsulphoxide (DMSO). The extracts of the sediments were being kept at -20 Celsius degrees for the bio testing.

Bioassay with A. globiformis

The test on the bacterial cells A.globiformis consists of the measuring of the cumulative bacterial dehydrogenase activity (DHA) with the presence of the sediments (Liβ and Ahlf, 1997; Brouwer et al., 1990; Rőnnpagel et al., 1995) by means of the reduction of the color of the oxy reduction of resasurine (C6 H12 NO3 Na) (Liu, 1986). This reduction is compared to the non contaminated control and the level of the inhibition of the cumulative bacterial de- hydrogenase activity is proportional with the level of the toxins. The test is valid if the reduction of the resasurine in the non contaminated control figures out at 70% in relation to the positive control, which contains 4- nitro phenol. This procedure was carried out according to the procedure by means of Rőnnpagel and co- workers (1995).

The analyses of the level of the toxins were completed in the Laboratory for Aquatic Toxicology at the Institute for Zoology in Heidelberg, Germany.

Results and Discussion

We used the bacterial test on *A. globiformis* in order to detect the level of the toxins in the sediments. We kept an eye on the influence of the activity of the bacterial DHA. The evaluation was completed by means of Liβ and Ahlf (1997). According to the authors the inhibition of the cumulative bacterial dehydrogenase is commensurate with the level of the toxins in the sediments.

The results of the testing of the influence of the toxins can be seen at the figure no.2. The significant inhibition of the bacterial DHA can be seen through the results given at the figure no 2. The inhibited effect of the 0.125% of the sediments on the activity of the bacterial DHA can be observed in the dilution process. At the locality Radus (T1) the percentage of the inhibition is 28. 39% (fig.2). The percentage of the inhibition of DHA is rising with the increase of the tested concentration.

As it can be seen at the figure no.2, the extracts of the sediments taken from the middle of the lake indicate the smaller variations of the percentage of the inhibition of the bacterial DHA within various concentrations (the percentage

of the varied from 17.6%; 18.1% to 20.5%). At the highest level of the concentration consisting of 100% of the extracts of the sediments, the percentage of the inhibition of the bacterial DHA comes to 47.2 % at Radus (T1); 40.9 % at the estuary of the river Moraca, and 20.5% at the middle of the lake (T2). The obtained results showed that the sediments at the locality Radus are toxic.

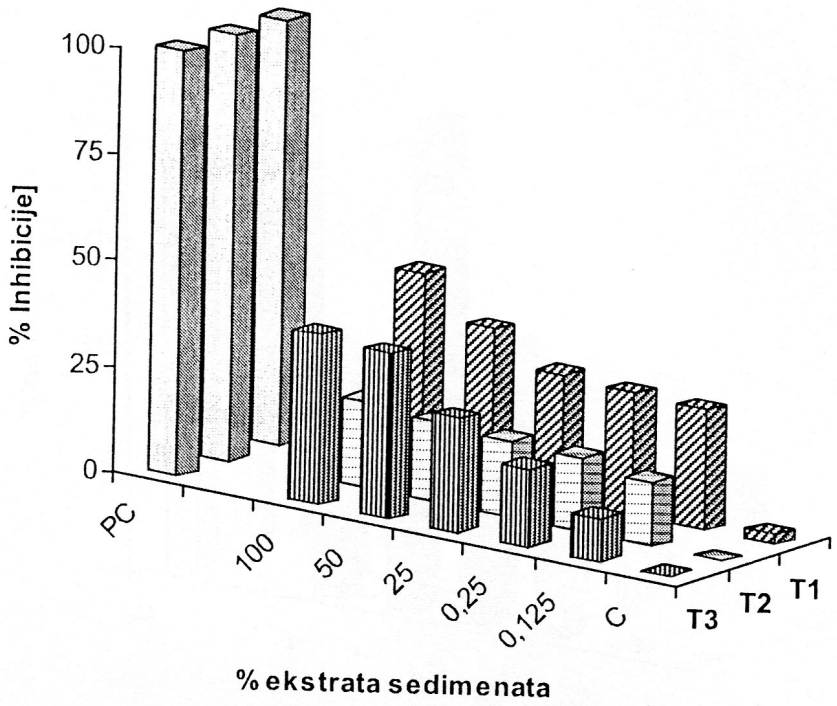

Fig. 2. Inhibition of microbial dehydrogenase synthesis according to the concentration of extracts sediments (DHA test) applied in Arthrobacter globiformis microbial culture .Three investigated sites were compared: T_1 Raduš, T_2 Middle lake, T_3 Mouth River Moraca (*C- controle; PC- positive controle)*

At the figure no3 we showed the level of toxins within all the sediments (the native ones) at the bacterial test *A. globiformis*. The figure clearly indicates the influence of the sediments on the inhibition of the bacterial DHA, registered in all the samples of the localities that have been researched. The results showed that there is the same correlation of the level of the toxins between the localities as it is in the test with the extracts of the sediments (Fig.2), but the percentage of the inhibition of the bacterial DHA was much higher. The maximal concentration of 2g of the sediment show the inhibition of 100% of the bacterial DHA at Radus (T1); at the estuary of the river Moraca (T3) the inhibition was 57% and in middle of the lake (T2) the level of the inhibition was 32,5%. Both of the tests (with the extracts and with the complete sediments) showed that the sediments of the lake were toxic and biologically available.

Most researches (Liβ and Ahlf, 1997) use strictly the extracts of the sediments and subterranean water for the procedure because it would simplify the whole procedure. But, we have to be mindful of the fact that the extracts (those which contain only the organic fraction) cannot indicate the level of

toxins in the sediments, because they don't have the non organic components which can be found in the natural sediments. We also have to be mindful of the fact that the subterranean water doesn't contain the distribution of the substances between the water phases.

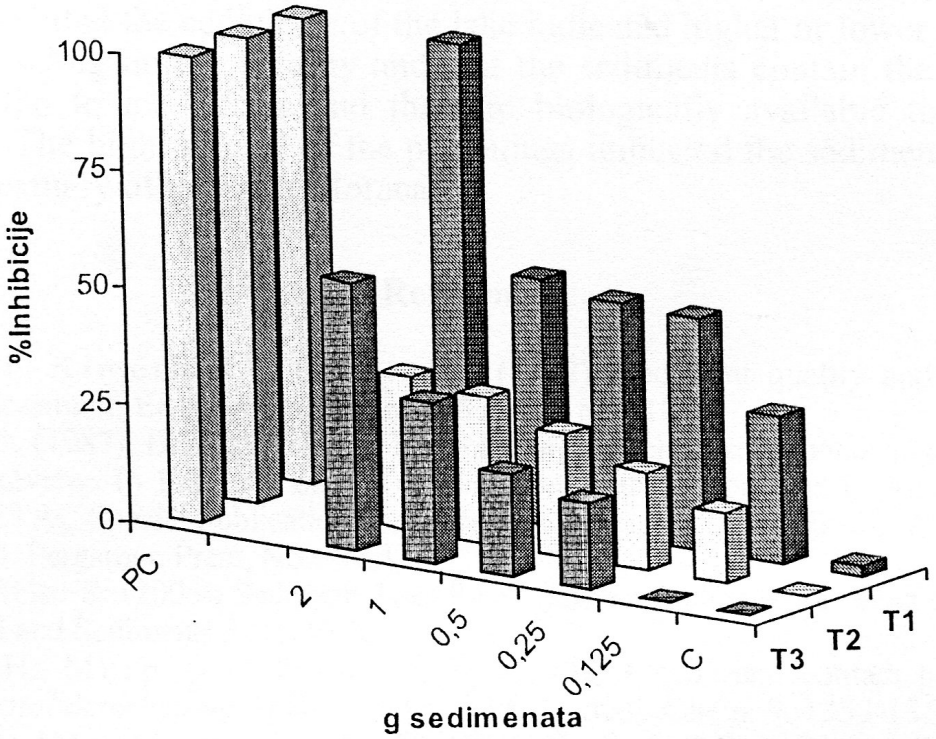

Fig. 3. Inhibition of microbial dehydrogenase synthesis according to the concentration of sediment solid phase (DHA contact test) applied in Arthrobacter globiformis microbial culture. Three investigated sites were compared: T_1 Raduš, T_2 Middle lake, T_3 Mouth River Moraca (C- controle; PC- positive controle)

We have to be mindful of the fact that the underground water doesn't contain the distribution of the substances between the water phase and solid phase of the sediments because the sediments contain only the melting components (Lib and Ahlf, 1997; Adams *et al.*, 1992; Adams, 1987), so we decided to use the solid phase (containing the whole of the sediments) and the water phase (the extracts of the organic fraction).

The relation between the concentration of the extracts of the sediments and the bacterial response of *A. globiformis* and the inhibition were dependent on the dose.

The test system of *A. globiformis* with the solid phase of the sediments (which is called contact test) indicated higher level of the sensitivity in comparison with the extracts of the sediments. We have to be aware of the fact that all the solid components of the sediments are present in the solid phase and that the toxic effects can be the result of the activities of the non organic substances.

The results obtained from the bio testing of the level of the toxins in the sediments have been confirmed in the acute cytotoxic test done on the cultures of

the fish cells RTL-W1 (hepathocyts) and the embryonic tests *Danio rerio*, which is completed on the embryos of the little zebrafish (Perovic *et al.*, 2004).

Conclusions

The inhibiting effect of the extracts of the sediments on the activity of the total bacterial de- hydrogenase has been detected at all the localities. It can be concluded that the sediments of the lake indicated higher or lower level of the toxins depending on the locality and that the sediments contain the substances that have the toxics effects and that are biologically available to the living organisms. The highest level of the poisonings indicated the sediments of Radus and of the estuary of the river Moraca.

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PRIMJENA BIOTESTA NA *Arthrobacter globiformis* ZA PROCJENU BIOLOŠKE DOSTUPNOSTI I TOKSIČNOSTI ZEMLJIŠTA I SEDIMENATA

- originalni naučni rad -

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Rezime

Bakterijski test na *Arthrobacter globiformis*, koristili smo za detekciju toksičnosti sedimenata Skadarskog jezera. U testu smo pratili uticaj sedimenata na aktivnost ukupne bakterijske dehidrogenaze (DHA) preko redukcije oksido-redukcione boje resazurina u resofurin. Za testiranje toksičnosti koristili smo organsku frakciju sedimenata (ekstrakte sedimenata) i čvrstu fazu (nativne sedimente). Sedimenti jezera su pokazali manju ili veću toksičnost zavisno od lokaliteta. U njima su bile prisutne supstance, koje kumulativno ispoljavaju toksični efekat i koje su biološki dostupne živim organizmima. Najveću toksičnost pokazali su sedimenti Raduša i ušća rijeke Morače u oba test sistema.