Arch. Biol. Sci., Belgrade, 65 (3), 1189-1194, 2013 DOI:10.2298/ABS1303189P

ASSESSMENT OF THE MUTAGENIC POTENTIAL OF SKADAR LAKE SEDIMENTS USING THE *SALMONELLA* MICROSOMAL ASSAY

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Abstract - Organic extracts of sediments from Skadar Lake (National park and protected RAMSAR site) were investigated for their mutagenic potential using *Salmonella typhimurium* TA98 and TA100 strains in the presence and absence of metabolic activation. Five different concentrations of sediment extracts from five different sampling sites were assayed, and mutagenic results were obtained for both strains. These studies present a part of the EULIMNOS project and included a battery of bioassay testing and *in situ* investigations of microbial community structures of Skadar Lake sediments. The obtained results show mutagenic risk potential in five collected samples in the absence and presence of metabolic activation. Additional studies are required in order to identify and quantify the chemical compounds responsible for the mutagenic activity present in Skadar Lake sediments.

Key words: Skadar Lake, sediment extracts, mutagenic activity, *Salmonella typhimurium* TA98 and TA100.

INTRODUCTION

Current national monitoring of freshwater in Montenegro includes the determination of basic variables (temperature, pH, conductivity, and dissolved oxygen), organic pollution indicators (total organic carbon, biochemical oxygen demand, and chemical oxygen demand), eutrophication indicators (nitrogen and phosphorus) and major specific ions (Ca, Mg, Na, K, etc.). Moreover, the monitoring program includes the determination of heavy metals and pathogens according to European Community legislation. Since physicochemical analyses do not provide enough information about the biological effects of pollutants, we are studying the possibilities of including biological tests in water and sediment monitoring, especially in places that are important tourist bathing zones, and sites important for fish growth and catching.

Chemical compounds of anthropogenic origin provide a significant contribution to the contamination of freshwater, in both water column and sediments, resulting in a potential ecotoxicological risk (Vargas et al., 2001). It is known that chemical analysis does not evaluate bioavailability and biological effects (such as genotoxic, mutagenic, dioxin-like and estrogens-like activity, etc.) of chemicals nor their possible synergistic or antagonistic interactions in a complex environmental sample. Indeed the mixture's mutagenic potential could be different from the sum of the effects of the components (Ozhan et al., 2008). Therefore, short term bioassays coupled with chemical analysis is a valuable technique for screening

toxic components in environmental samples (Lah et al., 2005). A detection of compounds with mutagenic potential in environments is especially important because of their capability of inducing mutation processes and thereby DNA damage in germ lines of living organisms (Kutlu et al., 2004; Kataoka et al., 2000), leading to negative genetic changes in future generations (Boyacioglu et.al., 2008). Aquatic mutagenic studies are of great interest, especially because epidemiological investigations have revealed a link between mutagenic water intake and a rise in cancer cases. Aquatic organisms such as fish accumulate pollutants directly from contaminated water and sediments or indirectly through the ingestion of contaminated aquatic organisms. Genotoxic pollutants may lead to the contamination not only of the aquatic organisms themselves, but also of the entire ecosystem and, finally, of humans via the food chain (Matsumoto et al., 2006).

The *Salmonella* mutagenicity assay which was specially designed to detect chemically induced mutagenesis, is widely used to evaluate the mutagenicity of complex mixtures in the air, in rivers, lakes, industrial effluents and drinking waters (Mamber et al., 1993; Park et al., 2000; Černá et al., 1998).

The purpose of this study was to determine and compare the mutagenic potential of organic sediment extracts in the waters of Skadar Lake. Sediment samples were collected from five points in the Lake, selected on the basis of monitoring zones exposed to high agricultural, industrial and touristic activities.

MATERIALS AND METHODS

Sediment sampling

Samples were collected during summer 2005 from five locations in Skadar Lake (Fig. 1): T1 – sampling point "Radus": waters from a strong underground spring and important fish hatching zone); T2 – sampling point "Mid. lake": the zone distant from the river confluence and potential pollutant discharges; T3 – sampling point "Riv. Morača": mouth of the river Morača, a potential major source of lake pollution

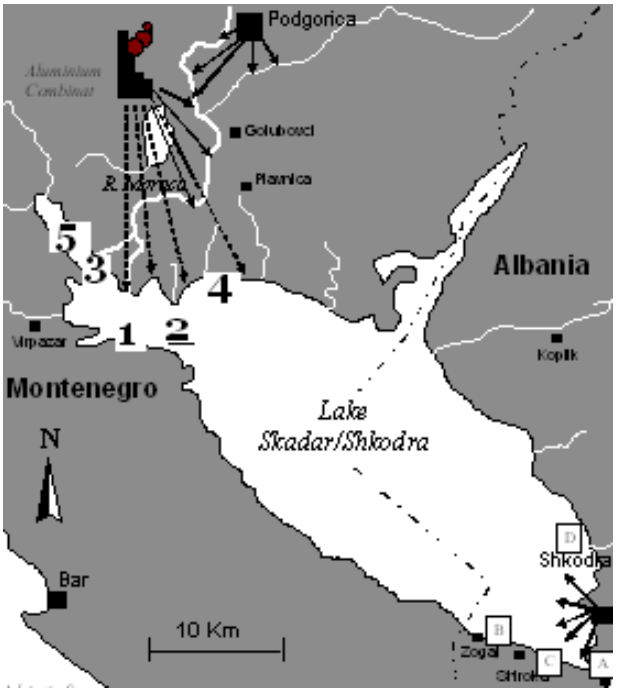

Fig 1 Outline map of Lake Shkodra/Skadar showing its geographical location and the locations of the various sampling sites (numbers 1 to 5) used in this (Source: Kostanjsek et al, 2005)

with industrial waters mainly from an aluminum factory and municipal wastewaters of Podgorica city; T4 – sampling point "Plavnica": potential impact of agricultural area mainly the northern part of the lake; T5 – sampling point "Kamenovo**":** positioned upstream toward River Crnojevica and before inflow of the Morača River into the Lake)

Extraction of sediments

The sediments were taken from the surface (0-5 cm) by an Ekman sampler. They were kept in a cool place no more than 24 h and drained in a *lyophilization apparatus* (beta 1-8 K; Martin Christ, Germany) at -20°C. From each sample 20 g of the drained sediments were added to acetone, and an organic fraction was extracted in six cycles by the Soxhlet extraction procedure (Kosmehl et al., 2004; Hollert et al., 2000; Hollert and Braunbeck, 1997). The obtained extracts were reduced in a rotor evaporator at 400 mbar, 36-38°C, and after that the extracts were

concentrated by flowing nitrogen gas (Hollert et al., 2000). The resulting concentration of extracts was 20 g dry sediment-equivalent/1 ml of dimethyl sulfoxide (DMSO) solvent. The extraction process of the sediments and measurement of their mutagenic potential were completed in the Laboratory for Aquatic Toxicology at the Institute for Zoology and Laboratory of Hygiene Institute, University of Heidelberg, Germany.

Testing by the Ames Salmonella typhimurium assay

Mutagenicity of the sediment samples was assessed using a *Salmonella* plate incorporation assay according to the standard procedures described by Maron and Ames (1983) with and without exogenous metabolic activation (S9 induction by β-naphthoflavone/phenobarbital). Tester strain TA98 (*hisD3052 rfa* Δ*uvrB* Δ*lacZ)* was used to determine frame-shift mutations, and strain T100 (*hisG46 rfa* Δ*uvrB* Δ*lacZ*) to determine base pair substitution mutations. Samples diluted in DMSO where tested at concentrations 62.5, 125, 500, 1000 and 2000 mg/plate, with and without S9-induced metabolic activation. Nitropyrene, 2-amino fluorine (2AF) and 4-nitroquinoline-N-oxide (4NQNO) (all from Sigma-Aldrich) were used as positive controls and the DMSO was used as a negative control. After 48 h incubation on agar plates at 37°C, counting of bacterial colonies was performed.

Evaluation and interpretation of the results

In the screening assays using a single dose, the sample was considered to be mutagenic when the number of revertant colonies in the test plates exceeded the number of revertants in the solvent (negative) control. Revertant colonies were counted and induction factors were calculated dividing the number of revertants of the extract concentration by the mean of the revertants number of the negative control. Depending on the variance of replica and control groups, the mutagenic activity was assumed if the induction factor (IF) was higher than 1.5.

RESULTS AND DISCUSSION

In this work, mutagenicity of the samples was assessed by the *Salmonella* plate incorporation assay according to the standard protocol of Maron and Ames (1983). The results are presented in Tables 1-4. The highest concentration of sediments tested in the assay was 2000 mg/plate, the lowest was 62.5 mg/plate. Depending on the variance of replica and control groups, a significant mutagenic activity was assumed if the induction factor was higher than 1.5.

Tables 1 and 2 show the results of the mutagenicity of sediment samples in the test system with the strain TA98 (with and without metabolic activation). The assay with strain TA98 without S9 supplementation revealed a statistically significant increase in mutagenicity only in the surface sediment samples from the mouth of the river Morača. The mutagenic potential of sediments was observed at concentration 500 mg/plate and the induction factor was 1.6. The maximum number revertant *his*$^-$ → His$^+$ was observed at the maximum concentration (2000 mg/plate) where the induction factor was 2.2. This result shows that components that may have potential mutagenic effects are present in sediments.

After exogenous S9 bioactivation, other samples showed more mutagenic activity, except samples from the middle of the Lake. The most mutagenic extracts were samples from Radus with an induction factor of 2.4.

For some substances that are responsible for the induction of reverse mutation in a system with TA98 cells, essential metabolic processes revealed a mutagenic effect. These substances can be different types of polycyclic hydrocarbons such as benzopyrene, dibenzopyrene, benzanthracene and others, which have been detected in previous investigations into the sediments of Lake Skadar. These substances show maximum mutagenicity only in the presence of the activation of metabolic systems tested on different organisms (Phillipson and Loannides, 1989).

The assay with strain TA100 without S9 Table 3-4 revealed much lower levels of base substitution

Table 1 Mutagenicity testing of sediment extract from the Skadar Lake in *Salmonella*/microsome TA98 (in the presence and absence of metabolic activation)

	Number of revertants/plate (mean ±SE)[a]									
	Radus (T1)		Mid. lake (T2)		Riv. Morača (T3)		Plavnica (T4)		Kamenovo (T5)	
Concentration mg/plate	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
NC	20±8	56±5	20±8	56±5	20±8	56±5	20±8	56±5	20±8	56±5
62.5	21±4	76±7	22±7	64±4	28±4	86±3	24±5	70±7	25±4	75±6
125	23±5	87±3	24±3	68±7	29±7	89±6	22±6	77±5	24±6	80±9
500	25±9	90±6	26±5	73±6	32±9	94±5	24±3	83±6	21±8	92±5
1000	26±10	95±9	26±11	75±5	35±9	99±9	29±9	92±8	23±7	101±7
2000	26±14	133±11	28±9	78±9	44±10	109±7	25±12	83±9	26±11	112±9

[a] Data are from two independent experiments each performed in duplicates
NC negative control: DMSO

Table 2 Induction factor for mutagenicity of extract sediments in strain *S. typhimurium* TA98 in the presence and absence of (S9) supplementation

	Induction factor (IF)									
	Radus (T1)		Mid. lake (T2)		Riv. Morača (T3)		Plavnica (T4)		Kamenovo (T5))	
Concentration mg/plate	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
IF[a]										
62.5	1.1	1.4	1.1	1.1	1.4	1.5	1.2	1.3	1.3	1.3
125	1.1	1.5	1.1	1.2	1.4	1.6	1.1	1.4	1.2	1.4
500	1.3	1.6	1.3	1.3	1.6	1.7	1.2	1.5	1.1	1.6
1000	1.3	1.7	1.3	1.3	1.8	1.8	1.4	1.6	1.2	1.8
2000	1.3	2.4	1.4	1.4	2.2	1.9	1.3	1.5	1.3	2.0

Table 3 Mutagenicity testing of sediment extract from the Skadar Lake in *Salmonella*/microsome TA100 (in the presence and absence of metabolic activation)

	Number of revertants/plate (mean ±SE)[a]									
	Radus		Mid. lake		Riv. Morača		Plavnica		Kamenovo	
Concentration mg/plate	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
NC	142±13	136±23	142±13	136±23	142±13	136±23	142±13	136±23	142±13	136±23
62.5	131±23	133±12	142±16	132±17	132±9	133±21	153±17	160±21	111±11	146±22
125	141±14	147±20	143±12	134±19	134±11	130±12	150±12	171±22	80±25	162±21
500	175±19	158±12	150±13	148±21	133±19	159±19	168±22	190±21	116±19	173±19
1000	190±10	176±19	106±12	153±20	78±23	165±22	200±19	200±19	156±22	185±21
2000	195±29	274±25	139±15	153±25	223±20	172±20	260±28	211±27	213±18	295±23

[a] Data are from two independent experiments each performed in duplicates NC negative control: DMSO;

Table 4 Induction factor for mutagenicity of extract sediments in strain *S. typhimurium* TA100 in the presence and absence of (S9) supplementation

	Induction factor (IF)									
	Radus (T1)		Mid. lake (T2)		Riv. Morača (T3)		Plavnica (T4)		Kamenovo (T5)	
Concentration mg/plate	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
IF										
62.5	0.9	1.0	1.0	1.0	1.0	1.0	1.1	1.2	0.8	1.1
125	1.0	1.1	1.0	1.0	0.9	1.0	1.1	1.3	0.6	1.2
500	1.2	1.2	1.1	1.1	0.9	1.2	1.2	1.4	0.8	1.3
1000	1.3	1.3	0.7	1.1	0.5	1.2	1.4	1.5	1.2	1.4
2000	1.4	2.0	1.0	1.1	1.6	1.3	1.8	1.6	1.5	1.4

mutagens. The maximum induction factor was 1.8 for the surface sample from Plavnica. The significant increase in the number of $his^- \rightarrow His^+$ revertants was observed in test system TA100 with metabolic activation. The highest induction factor was 2.0 at the maximum concentration (2000 mg/plate) in the Radus sampling site. The different sensitivities of TA98 and TA100 on the sediment extracts point to the possible diversity of potential mutagens and genotoxic substances in the different samples.

CONCLUSIONS

A positive mutagenic potential of sediment extracts was obtained for both TA98 and TA100 *Salmonella* strains. This shows a larger or smaller extent of sediment contamination depending on the sampling point and its proximity to a source of organic pollution. Mutagenic tests have helped to detect the presence of potential mutagenic components that can have a considerable and adverse impact on the quality of this unique water ecosystem. The Ames' test is a fast and sensitive screening test in combination with chemical analysis. In our opinion, it can be a valuable source of information for quantitative and even qualitative risk assessment, which is self-recommending for its inclusion in regular pollution assessment in aquatic systems.

Acknowledgments: We thank the German Rectors' Conference for the financial support of research project EULIMNOS within which these studies were conducted.

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