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TOXICOLOGY

DIFFERENT GENOTOXICOLOGICAL RESPONSES OF MINE WATERS CONTAINING HEAVY METALS

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ABSTRACT

Two samples of the acid-mine water (AMW) containing heavy metals were collected in the year 1995 and 1998 in the former mining area of Banská Štiavnica-Šobov (Slovakia), and assayed for their genotoxic potential by the Ames assay and the DNA-topology assay. The content of toxic metal elements, determined by atomic absorption spectroscopy, was decreased in the sample of AMW collected in the year 1998 in comparison with that determined in the year 1995. The sample collected in 1995 was genotoxic after its application on bacterial strain *Salmonella typhimurium* TA97 (without metabolic activation), and TA102 (with metabolic activation). The sample collected in 1998 did not increase the frequency of his^+ revertants in the same bacterial strains. Moreover, the DNA-topology assay (which facilitates electrophoretic monitoring and densitometric quantification of changes in a plasmid DNA structure) revealed the presence of 81.3% of the slowly migrating plasmid DNA, and 18.2% of the relaxed plasmid DNA after incubation of the plasmid DNA with the sample collected in 1998, and respectively after the joint incubation of this sample with glutathione and hydrogen peroxide.

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Key Words: Heavy metals; Genotoxicity; Atomic absorption spectroscopy; Preincubation Ames assay; DNA–topology assay; Electrophoresis; Plasmid DNA

Abbreviations: AAS, atomic absorption spectroscopy; AMW, acid-mine water; pDNA, plasmid DNA; DNA form (I), supercoiled plasmid DNA; DNA form (II), relaxed plasmid DNA; sm-DNA, slowly migrating DNA; GSH, glutathione; H_2O_2, hydrogen peroxide

INTRODUCTION

Our environment is deteriorated by many anthropogenic activities. Mining dumps as products of extension mining activities are often the source of waste mining waters, containing a high amount of toxic metals.[1] Heavy metals belong to important environmental pollutants. Biological activities (e.g., physiological, biochemical, genetical etc.) of metal ions have promoted a huge number of studies on mechanisms and range of their effects.[2–4] The environmental exposure to metals has been associated with a wide range of toxic, mutagenic and carcinogenic effects.[5–8] Nowadays, monitoring such elements and looking for means of their elimination are of importance to secure the environment protection. There were studied the acid mine-water (AMW) samples, collected in different years from the former mining area of Banská Štiavnica-Šobov, as the potential source of a serious genetic risk in Slovakia. The aim of this research was to compare the potential genetic risk of samples containing toxic metal elements using two different experimental approaches: the *Salmonella typhimurium* mutagenicity assay (the Ames assay) and the DNA-topology assay.

MATERIAL AND METHODS

Metals Determination

Samples of the AMW were collected in polyethylene bottles at a dump site in the area of Banská Štiavnica-Šobov (Slovakia) in the year 1995 and 1998. After being transported to the laboratory they were kept refrigerated until analysed. Toxic metal elements were determined by atomic absorption spectroscopy (AAS) using a Perkin-Elmer spectrometer, model 1100. The measurements were performed under operating conditions suggested in data processor of the manufacturer. SO_4^{2-} ions were analysed gravimetrically by precipitation of barium sulphate.

Salmonella typhimurium Mutagenicity Assay (The Ames Assay)

The modified preincubation assay was performed.[6,8] The potential mutagenic effect of AMW was measured by histidine reversions from histidine auxotrophy to histidine prototrophy in *S. typhimurium* tester strains TA97, TA98, TA100 and TA102. The assay was conducted in the test tubes by adding 0.1 mL of the overnight bacterial culture to AMW in the appropriate concentrations, and in the case of metabolic activation 0.5 mL of S9 mixture was supplemented. The S9 fraction was prepared from Delor 103-induced rats. Tubes were incubated for 24 h at 37°C without shaking. 2.5 mL of top agar was added to each tube, the content was mixed and plated on minimal bottom agar. His^+ revertants were counted after 72 h of incubation at 37°C on Biotran III Colony Counter (New Brunswick Scientific Co.). All data were statistically analysed using the Student's *t*-test.

DNA-Topology Assay

It is the method for electrophoretically monitored DNA damaging described in detail by.[9,10] The reaction mixture (final volume 20 μL) contained 200 ng of the supercoiled plasmid DNA in buffer and the sample of AMW either alone or with GSH and H_2O_2. Plasmid DNA was treated for 30 min at 37°C. Analysis of DNA modifications were made by agarose gel electrophoresis (1.5% agarose, 60 min/60 V). The final effect was presented by the change from a supercoiled DNA form (I) to a slowly migrating (sm-DNA) and a circular relax DNA form (II). Percentage of supercoiled, relaxed and sm-DNA forms were calculated by a computer program (Uthesa, Image Tool for Windows).

RESULTS

The results of the toxic metal chemical analyses made by AAS in AMW collected in the year 1995 and 1998 are listed in Table 1. Despite of that the concentrations of toxic elements were decreased in the sample collected in the year 1998, most of them still exceeded limits given by Slovak Technical Norm for Drinking Water. Both samples were toxic, and they had to be diluted. This was the reason why we tried to find such dilutions and concentrations in which the mutagenic potential of both samples would not be overlapped by toxicity after their application on bacterial *S. typhimurium* strains. As the sample of AMW collected in the year 1995 was more toxic it had to be more diluted (1000-fold) than sample collected in the year 1998 (100-fold dilution). Of four bacterial strains used, AMW collected in the year 1995 was mutagenic in two of them (TA97 and TA102). Dose-dependent increase of his^+

Table 1. Concentrations of Toxic Metals (mg/L) in Samples of AMW from Banská Štiavnica-Šobov (Slovakia), and the Respective Limits and pH Values

Components of AMW	Limits	Sample of AMW Collected in 1995	Sample of AMW Collected in 1998
Al	3	1119	744
Cu	1	7.5	1
Fe^{3+}	15	3254	1350
Mn	0.3	84	75
Zn	2	10.2	2
Mg	100	–	416
SO_4^{2-}	250	17 810	10 000
pH	6–8	2.26	2.26

Table 2. Comparison of the Potential Mutagenic Effect of Two Samples of AMW Using the Modified Preincubation Ames Assay

Bacterial Strain *Salmonella typhimurium* TA 97 (−S9)			
Sample of AMW Collected in 1995		Sample of AMW Collected in 1998	
μL/Plate	His^+Revertants/Plate	μL/Plate	His^+ Revertants/Plate
Control (0)	126 ± 9.1	Control (0)	155 ± 15.3
100	148 ± 20.2	25	159 ± 17.1
250	170 ± 22.2*	50	158 ± 17.3
500	255 ± 19.7*	75	159 ± 12.6
750	348 ± 22.6*	100	168 ± 15.1
850	475 ± 23.9*	150	166 ± 13.5

Values represent the means of triplicate plates ± standard deviation for three consecutive experiments.
*Significant difference at $p < 0.05$ in comparison with the control.

revertants in the presence of sample collected in 1995 was demonstrated (Tables 2 and 3). Since the AMW sample collected in 1995 was genotoxic in bacterial strain *S. typhimurium* TA97 without metabolic activation (−S9) and TA102 with metabolic activation (+S9), the sample collected in 1998 was also applied on the same bacterial strains. It is obvious from comparison of the potential mutagenic effect of two samples that the sample collected in the year 1998 was not genotoxic (Tables 2 and 3). This conclusion resulted from comparison of his^+ revertant frequences induced in two bacterial *S. typhimurium* strains (TA97, TA102) by different concentrations of samples collected in the year 1995 and 1998 with those in corresponding controls. Statistical evaluation of data presented in Tables 2 and 3 proved the significant difference between the rates of his^+ revertants induced by AMW

Table 3. Comparison of the Potential Mutagenic Effect Two Samples of AMW Using the Modified Preincubation Ames Assay

Bacterial Strain *Salmonella typhimurium* TA 102 (+S9)			
Sample of AMW Collected in 1995		Sample of AMW Collected in 1998	
μL/Plate	His^+ Revertants/Plate	μL/Plate	His^+ Revertants/Plate
Control 0)	261 ± 17.3	Control (0)	256 ± 21.4
100	266 ± 15.1	25	254 ± 17.2
250	389 ± 22.1*	50	261 ± 21.2
500	445 ± 18.5*	75	271 ± 23.4
750	649 ± 25.2*	100	243 ± 16.9
850	715 ± 37.4*	150	269 ± 22.6

Values represents the means of triplicate plates ± standard deviation for three consecutive experiments.
*Significant difference at $p < 0.05$ in comparison with the control.

collected in 1995 and the spontaneous frequency of his^+ revertants in the relevant controls. But, no significant difference was found between the frequency of his^+ revertants induced by AMW collected in 1998 and the spontaneous frequency of revertants in the corresponding controls.

Electrophoretic monitoring and densitometric quantification of changes induced in the structure of a plasmid DNA by sample collected in 1998 are presented in Fig. 1 and Table 4. Data in Table 4 and Fig. 1 illustrate that only about 8% of DNA form (II) was found in the case when a control plasmid DNA alone or in the presence of glutathione and hydrogen peroxide were evaluated. However, after incubating plasmid DNA with the sample of AMW collected in 1998, 81.3% of slowly migrating DNA or aggregated DNA was detected. But, in the presence of glutathione, hydrogen peroxide and AMW the cleavage of the supercoiled DNA form (I) to18.2% of the circular relaxed DNA form (II) was exhibited.

DISCUSSION

For the assessment of the toxic metal element concentrations in both samples of AMW, AAS as the main analytical technique was used owing to its simplicity, precision and accuracy. Its convenience for the metal element determination was recently proved.[11–13] However, as it follows from Table 1, the content of some toxic metal elements (e.g., Fe, Al), was relatively high even in the sample collected in the year 1998.

Environmental agents (including toxic metals) implicate potential genetic risk for man because they may be genotoxic, with a potential to induce mutations either in germinal or somatic tissues. The detection of

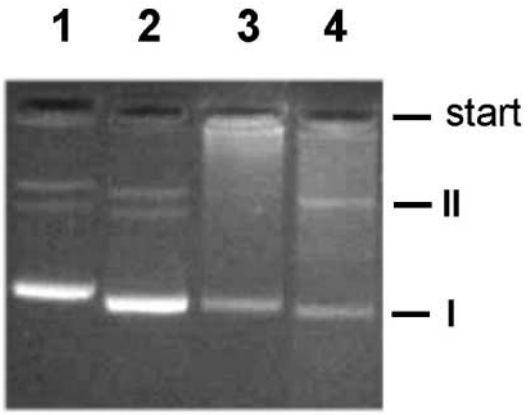

Figure 1. The effect the acid-mine water sample collected in 1998 on aggregation and single-strand DNA break formation in a plasmid DNA. Samples contained pDNA (control, lane 1) and GSH + H_2O_2 (lane 2) are without significant effect on DNA (only 8% of DNA form II). Aggregation of the supercoiled pDNA was detected after DNA incubation with AMW (lane 3) and the cleavage of supercoiled DNA to relaxed form (II) was observed after the joint incubation of pDNA with AMW and GSH + H_2O_2 (lane 4).

Table 4. Densitometric Quantification of Topological DNA Forms

	Topological Forms of DNA (%)		
Experimental Variants	Form	Form(II)	Aggregated or sm-DNA
Control pDNA	83.7	8.7	7.5
pDNA, GSH + H_2O_2	89.3	8.4	2.3
pDNA, AMW	18.7	0.0	81.3
pDNA, AMW + GSH + H_2O_2	38.2	18.2	43.5

pDNA, GSH + H_2O_2 = plasmid DNA treated with 50 μM glutathione and 50 μM H_2O_2.
pDNA, AMW = plasmid DNA treated with acid-mine water collected in 1998.

mutagens and determination of the types of mutation induced are of importance to understanding of etiology of cancer and other degenerative disease that involve mutations.[14]

It is known that the genotoxic potential of heavy metals applied on bacterial *S. typhimurium* strains is dependent on their concentrations. When the heavy metal concentration is very low, it is not possible to reveal their mutagenicity.[15]

Standardized tests for determining the mutagenic and, in broader sense, the genotoxic properties of agents are an integral part of current safety strategies contributing to the environment protection.[16] The bacterial

S. typhimurium mutagenicity assay is a standardized test for histidine auxotrophy to histidine prototrophy; $his^- \rightarrow his^+$ assessment.[17] Relatively low sensitivity of the plate incorporation Ames assay for the metal element mutagenicity detection has been generally attributed to their low bioavailability to the bacterial cells, and only the modified preincubation assay facilitates to detect their genotoxic potential.[6,15,18] The DNA-topology assay enables electrophoretic monitoring of changes induced by environmental agents in the plasmid DNA structure on the basis of different migration of the topological isomers of the control DNA and DNA modified in the particular experiment. Due to the single- and double-strand DNA breaks induction the treated supercoiled DNA form (I) is converted to the relaxed circular DNA form (II) or the linear DNA form (III).[9,10,19] Our experiments proved that this new genotoxicological assay is sensitive enough to monitor changes induced by toxic metal elements in a plasmid DNA. Phenomenon of aggregation or slowly migrating DNA (sm-DNA) (the 3rd category in Table 4, or Fig. 1, lane 3) was also described by Stellwagen.[20,21] However, its biological value is still unknown.

It was reported that ferric citrate caused a dose- and time-dependent induction of single- and double-strand breaks in the supercoiled plasmid DNA.[22] A direct interaction of aluminium with DNA in vitro was found.[23] These findings support our suggestion that iron and aluminium may mainly contribute to changes induced in the plasmid DNA topology.

CONCLUSION

Two samples of the AMW collected in the former mining area of Banská Štiavnica-Šobov (Slovakia) in the year 1995 and 1998 differed in the content of toxic metal elements determined by AAS and in their genotoxicological potential evaluated by the modified *S. typhimurium* mutagenicity assay and the DNA-topology assay. Whiles, the sample collected in 1995 exhibited the mutagenic effect after its application on bacterial *S. typhimurium* strains TA97 and TA102, the sample collected in 1998 was not genotoxic. But, this sample changed migration of the treated plasmid DNA what was documented by the presence of 81.3% of sm-DNA. The authors of this article believe that results obtained might contribute to the positive decision of the Slovak Ministry of Environment to build a cleaning plant in the former mining area of Banská Štiavnica-Šobov (Slovakia).

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