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# Investigation of genotoxicity in river sediments

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**Abstract**

The purpose of the present study was to develop a useful screening method to assess genotoxic effect of polluted bottom sediments from the water reservoir Ružin No.I. The Hornád and Hnilec Rivers drained a former mining area, have been polluted in the long-term by heavy metals (Cu, As, Sb, Hg), which significantly contributed to environmental degradation. Genotoxicity of bottom sediment was evaluated by test SOS-ChromoPadTM 3.0 for solid samples without extraction. The mentioned test represents simple, quick and direct sediment phase toxicity testing procedure. In this test bacterial strain Escherichia coli K12 PQ37 was used. The results of SOS-ChromoPadTM 3.0 showed that sample Hornád has low potential genotoxic effect on the environment. It was determined on the basis of slight blue colouration of chromogenic paper at the point of sediment application. The sample Hnilec was negative. This test allows significantly reduce the time for obtaining information about sediments genotoxicity and accept necessary security proceeding in time.



## Introduction

Heavy metals in mine wastes can considerably influence surrounding surface waters, sediments and human health. To estimate environmental impact, heavy metal concentrations in sediments can be utilized because they are indicators of contamination and change negligibly with time. In the contaminated sediments of the water reservoir Ružín No.I (Eastern Slovakia) there are mainly heavy metals (Cu, As, Sb, Hg), which origin are in the geological environment of the area and are connected with the complex treatment of ore. The River Hornád and Hnilec dewater the area of old mining load after extraction and treatment of sulfitic ore connected with abandoned mines (Rudňany, Smolník and Krompachy) (Šestinová *et al*. 2015).

Heavy metals can affect organisms in different ways. Many of them are toxic, and some of them may cause loss of genomic stability by altering the balance in chromatin metabolism. They are classified as genotoxic. Genotoxic effects of heavy metals are very important as organisms health is based on genome (Factori *et al*. 2014; Marple and Hasty 2004). A result of genetic damage material of an organism can thus show up for a long time, sometimes in the next generation (Guecheva *et al*. 2001; Huang and Wai 2004; Malachová *et al*. 2014). Many of these metals have genotoxic effects that act directly or indirectly on organisms (mutagens). Action of matter with mutagenic effects leads to damage of genetic information stored in nucleic acids. In cells non-lethal genetic changes are formed, that are of permanent character. These can be reflected into defective offspring of cell. In some cases, the process of cell

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replication as a result of damaged information can completely overthrow cells transformation and develop, for example, carcinogenicity. For testing genotoxicity organisms or community of organisms with specific laboratory sets such as *Salmonella typhimurium* (e.g. ChromoPlate kit) or *Escherichia coli* (e.g. SOS-ChromoTest) are most commonly used (Ghani 2010; Feng *et al.* 2012). The genotoxicity tests have been developed for the detection of chemical mutagens and carcinogens in environmental samples. In addition to assessing the genotoxic potential of test samples they allow for direct determination of the toxic effects of wastewater and sediment samples (Dutka *et al.* 1995; Vojtková and Janáková 2011; Zotina *et al.* 2015; Malachová *et al.* 2014). The mentioned tests are commercially available in the form of sets involving concentrated solutions, reference materials and test organisms in the form of latent eggs, which allow to detect a genotoxic material even in non-laboratory conditions. Because of the rapid indicative determination of acute toxicity in monitored sediments, the SOS-ChromoPad$^{TM}$ test was selected and used of the available short term indicative genotoxicity tests,. The SOS-ChromoPad is a rapid bacterial-based colorimetric bioassay kit for the determination of toxicity of sediments, suspended sediments, soils and solid wastes. It is sensitive to a wide spectrum of toxic substances such as heavy metals, and organic and inorganic pollutants, and may be used to detect the presence of toxicants directly without solvent extraction. In the present study, the screening method, genotoxicity of sediments collected from the water reservoir Ružín No. I (Hornád and Hnilec Rivers) was used to evaluate the potential genotoxic risks on the given aquatic environment.

## Experimental

*Material for sediment genotoxicity testing*

The sediment samples were taken in two areas from the water reservoir Ružín No.I, in the year 2011–2012. The sediments from the locality Hornád and Hnilec branch were collected into plastic bottles by a multisampler sample device (at a depth of 50 cm). For the physicochemical and bioassays, the sample was dried at laboratory temperature, then quartered and sieved into fraction of 63 μm. The total extractable metal concentrations in the sediments were determined after mineralization with a mixture of acids (HCl/$HNO_3$/HF) in a microwave pressure digestion system (MWS-3, GER). The metal concentrations in the sediments were determined by atomic absorption spectrometry (VARIAN, AUS). CHNS analysis was performed by an elementary analyzer (Vario MACRO cube GER) using a thermal conductivity detector (Findorakova *et al.* 2017). The certified reference material LGC6187 Control was river sediment (Elbe). The reference sample was systematically used to control the analytical precision. Incubator-drier Memmert 100-800 (incubation at 37°C) was used for acute genotoxicity study. The chemicals and media of the SOS-ChromoPad$^{TM}$3.0 test contained: a nutrient medium for bacterial growth, lyophilized bacteria *Escherichia coli* K12 PQ37 (non-pathogenic strain), a Petri dish with blue chromogenic substrate, 4NQO (4-Nitro-Quinoline-oxide-for positive control), disposable plastic pipette, test tubes, plastic bags for incubation and ampicillin. Sample (0.1 g) treatment before determining genotoxicity was performed by the following procedures: SOS-ChromoPad$^{TM}$ ver. 3.0-fresh-solid. Sediment samples without extraction were used. Three replicates were performed for each sample set.

*Preparation of bacterial strain* Escherichia coli *K12 PQ 37*

For sediments genotoxicity testing, bacterial strain *Escherichia coli* K 12 PQ37 in test SOS-ChromoPad$^{TM}$ was used. Lyophilized bacteria *Escherichia coli K12 PQ37* were 24 hours before each test mixed with 10 ml of culture medium and incubated in a thermostat for 16-18 hours at temperature 37°C. On the next day, the concentration of bacteria was measured by UV-Vis Spectrophotometer HELIOS-Y at a wavelength of 620 nm. Required bacteria concentration corresponded to the absorbance value of 0.07. The principle of the test SOS-ChromoPad$^{TM}$ ver. 3.0 is that genotoxic agents cause DNA damage and immediately thereafter, the cell tries to repair this damage with activation reparation system, which is called SOS. The result of SOS reparations affects

**Table 1.** Metal concentration in the sediment from the localities in Hornád, Hnilec and Control (average ± standard deviation).

| Sample | Cu | As | Sb | Hg |
|---|---|---|---|---|
| | (mg/kg) dry weight | | | |
| 1/2011 | 333±2.7 | 75±6.1 | 62±2.8 | 5.9±0.2 |
| 1/2012 | 198±3.4 | 39±2.7 | 27±3.6 | 6.5±0.4 |
| 2/2011 | 354±2.8 | 56±3.4 | 59±3.7 | 1.8±0.2 |
| 2/2012 | 359±5.1 | 49±4.3 | 32±5.7 | 1.9±0.5 |
| Control | 82±2.7 | 25±1.9 | 14±2.5 | 1.6±0.3 |
| | Limiting values (mg/kg) dry weight* | | | |
| TV | 36 | 29 | 3 | 0.3 |
| MPC | 73 | 55 | 15 | 10 |
| IV | 190 | 55 | - | 10 |

(1/2011-2012 Hornád and 2/2011-2012 Hnilec) *Norm No. 549/1998-2: TV-Target Value (Negligible Risk), MPC –Maximum Permissible Concentration (Max. Tolerable Risk), IV-Intervention Value (Serious Risk), Control (certified reference material)

**Table 2.** Results of elemental CHNS in sediments from Hornád and Hnilec.

| Sample | C | H | N | S |
|---|---|---|---|---|
| | (%) | | | |
| 1/2011 | 40.80 | 7.59 | 9.32 | 0.75 |
| 1/2012 | 39.25 | 6.85 | 8.71 | 0.65 |
| 2/2011 | 38.93 | 6.46 | 8.14 | 0.63 |
| 2/2012 | 37.31 | 6.15 | 8.06 | 0.61 |

replacement of cell, it leads to permanent changes of the cell genetic structure, which may lead to genetically portable mutation or carcinogenic cell transformation. Induction of SOS functions is evaluated using the *sfiA* gene (Junior *et al.* 2007).

*Working scheme of SOS-ChromoPad™ ver. 3.0*

*Preparation of test tubes for each sample:* The bacterial suspension and sediment in the tube 1 by mixing 1 mL active bacterial culture with 0.1 g weighed portion of sediment was prepared in the shaking incubator. Dilution of prepared suspension in tubes 2-5 of concentrations were: 0.5n (50%) – 0.25n (25%) – 0.125n (12.5%) – 0.0625n (6.25%) – 0.0313n (3.13%); 6 – negative control (only bacteria/0.5 mL), 7 – positive blue control (4NQO/0.050 mL). Incubation of the tubes was in a thermostat during 4 hours at temperature 37°C. Then 0.020 mL of a bacterial sludge in the form of dots on a Petri dish with blue chromogenic substrate was applied. Arrangement of dots was as follows: in the middle was positioned a positive control, a negative control and diluted samples were on the edges (7 dots on chromogenic plate). Incubation of the Petri dishes with active bacterial culture was in a thermostat for 20 hours at 37°C. After incubation, a wash of the sediment particulate from the surface of chromogenic paper was performed. Evaluation was realized by photographic documenting (Fig. 1) and visual evaluating of the intensity of blue colour dots versus positive indicator (dark blue) using a colour-index. The scale is based on 5 colour tones. White colour corresponds to colour-index 0 (non-genotoxic) and the blue colour corresponds to the colour-index 5 (the genotoxic sample). Finally, the minimum effective concentration in a volume of the sample was determined.

## Results and Discussion

The pH of sediment samples (1/2011-2012 and 2/2011-2012) was in range 7.25 – 7.59, indicating a slight alkaline nature. Organic matter content of sediments ranged from 8.1 to 14.2 % (Brázová *et al.* 2015; Šestinová *et al.* 2015). Table 1 summarizes the results of chemical analyses of Cu, As, Sb and Hg in the sediments compared to limiting values for contamination with copper and arsenic according to law No. 549/1998-2 of the Methodological Instruction of the Ministry

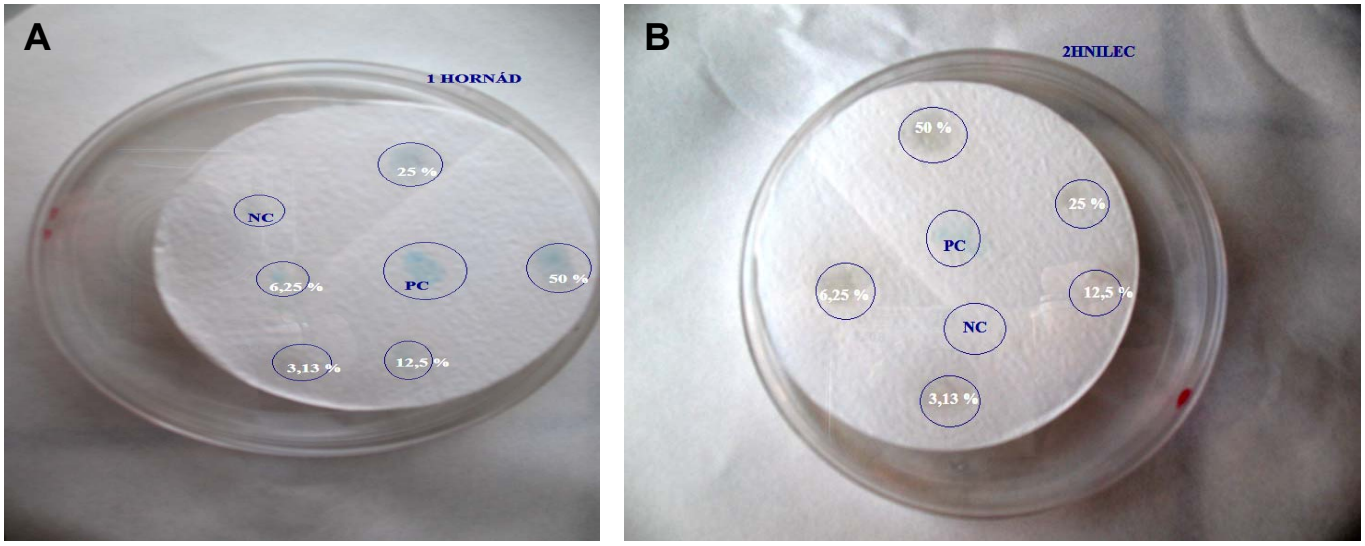


**Fig. 1.** The colour evaluation of the samples from Hornád and Hnilec at SOS-ChromoPad plate. Values indicated for the circled areas of sample application indicate the sample dilution (in %) (NC – negative control-without sediment and PC – positive control with the genotoxic standard 4NQO).

of Environment of the Slovak Republic for Assessment of Risks from Pollution of Sediments of Streams and Water Reservoirs. The present data was compared with metal concentrations in other sediments reported for similar mining areas in Krompachy, during the last decade (Findoráková *et al.* 2017).

From Table 1-2 it is obvious that the sample Hornád is a slightly more contaminated and has higher values of CHNS as the sample Hnilec.

SOS-ChromoPad test has great advantage that the sediments are applied in solid state. The endpoint of the bioassay is an easy to interpret colour reaction. The results corresponding to two parallel tests of sediments are visual–colour evaluated according to a simple colour-index (Fig. 1). Dots on chromogenic paper enable rapid detection of genotoxicity or DNA damage in sediments compared to negative and positive signal light. The basic of scores is the intensity of the blue colour of serially diluted (%) positive sample comparing with samples. Principally, toxic materials interfere with recovery process and thus with synthesis of the enzyme and colour reaction. Fig. 1 and Table 3 show colour evaluation of the environmental samples assayed at plates SOS-ChromoPad. From the Fig. 1A it is evident that the sample Hornád is non-toxic at 3.13% dilution. Concentrations of responsible sediment components exceeding this dilution value bear potentially genotoxic effect(s) on the environment. In contrast, the The Fig. 1B shows the results of the sediment Hnilec that generated no blue colour at chromogenic paper at point of application. It confirms that the sample Hnilec is negative and can be considered as non-toxic to environment according to this test.

In this study we used three technical replicates of each sample for evaluation of genotoxicity (Table 3). The colour reactions of the individual samples at chromogenic pad were reproducibly similar in all three repetitions. In some cases, however, the visual–colour evaluation in the SOS-ChromoPad test was difficult due to appraisal colour reaction of sediments and their corresponding appraisal genotoxic effect (Table 3). According to Rönnpagel *et al.* (1995) the effects of sediment or soil matrices on the bioavailability of compounds can contribute to such difficulties to screen toxicity of solid-associated contaminants. The majority of bioassays for testing toxicity of soils and sediments have been performed on water or solvent extracts (Rönnpagel *et al.* 1995).

This study builds on our previous article, in which genotoxicity of sediment extracts (Soxchlet extraction) from EBPI (CAN) has been described, while the SOS-ChromoTestTM ver. 6.4 for liquid samples was used (Šestinová and Findoráková 2017). The two tests – SOS-ChromoPad and ChromoTest – were performed in parallel and the results were compared. Test results of sediment samples obtained using SOS-chromotest pointed on genotoxicity (blue staining)

**Table 3.** Results of visual evaluation of three parallel tests of sediments according to colour-index.

| **Colour** | **NC** | **PC** | **1** /50 | **2** /25 | **3** /12.5 | **4** /6.25 | **5** /3.13 |
|---|---|---|---|---|---|---|---|
| **Index** | ○ | ● | ● | ● | ● | ● | ○ |
| I. Replicate | NC | PC | 50 % | 25 % | 12.5 % | 6.25 % | 3.13 % |
| **Hornád** | **W** | **B+++** | **B++** | **B+** | **B+** | **B** | **W** |
| I. Replicate | NC | PC | 50 % | 25 % | 12.5 % | 6.25 % | 3.13 % |
| **Hnilec** | **W** | **B+++** | **W** | **W** | **W** | **W** | **W** |
| II. Replicate | NC | PC | 50 % | 25 % | 12.5 % | 6.25 % | 3.13 % |
| **Hornád** | **W** | **B+++** | **B++** | **B+** | **B+** | **W** | **W** |
| II. Replicate | NC | PC | 50 % | 25 % | 12.5 % | 6.25 % | 3.13 % |
| **Hnilec** | **W** | **B+++** | **B** | **W** | **W** | **W** | **W** |
| III. Replicate | NC | PC | 50 % | 25 % | 12.5 % | 6.25 % | 3.13 % |
| **Hornád** | **W** | **B+++** | **B+** | **B+** | **W** | **W** | **W** |
| III. Replicate | NC | PC | 50 % | 25 % | 12.5 % | 6.25 % | 3.13 % |
| **Hnilec** | **W** | **B+++** | **W** | **W** | **W** | **W** | **W** |

**W** – white colour, **B** – blue colour, **+** intensity colour**, NC** – negative control, without sediment, **PC** – positive control (4NQO)

only for the positive standard (4NQO), while the sediment samples yielded yellow coloration. This indicates to low genotoxic potential of all the extracted leachate of the studied samples. Subsequently, corresponding instrumentation absorbance values of the samples were measured and the inducing activity (Ic) of each extract dilution was calculated (using EVP-extractable organic fraction and EOP-extractable aqueous fraction). The obtained results confirmed that the EPO of the samples Hornád does possess a potential genotoxic effects. Only a single sample dilution (12.5%) had a value of Ic-1.66, exceeding the critical value of Ic-1.5. Nevertheless, the total Ic value was closely below the critical value suggesting that neither sediment sample represents a risk in terms of genotoxicity. Since no monitored metals were found in the sediment samples and effective substances could be extracted in EOP, the effective compound might be a chemical substance in an organic form, with the ability to immobilize ions of toxic metals. Such substances can include e.g. humic acids. It was also demonstrated by higher Ic values for sample Hornád in the EOP. According to Šestinová and Findoráková (2017) the results of yield in the EOP for individual metals were lower than yields in EVP (especially for Cu and Sb in samples Hornád, Hnilec), on the contrary was determined their Ic value in EOP greater than in EVP. On the basis of above mentioned results of sediment samples, we can assume for sample Hornád in EOP potential genotoxic effect. Significant genotoxic effect has not been established for sample Hnilec.

## Conclusions

The ecological impact of genotoxic sediments on aquatic biota is difficult to estimate, since depends on a wide range of environmental and biological factors. A result of genetic damage material of an organism can thus show up for a long time, sometimes in the next generation. The purpose of this study was to describe SOS-ChromoPad$^{TM}$ ver. 3.0 test for testing of bottom sediments from the water reservoir Ružín No.I (in the area Hornád and Hnilec Rivers) for genotoxicity directly without extraction. The results of acute genotoxicity were for sample Hornád low potential genotoxic and for Hnilec sample non-toxic in used SOS-ChromoPad test. The test SOS-ChromoPad is usefulness because is easy to perform, faster, require minimal amount of sample, environmentally friendly but is not so precise in the determination. Obtained information from this study can be useful for hazard identification and risk assessment of sediment-associated contaminants.

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