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Geno-toxicity assay of sediment and water samples from the Upper Silesia post-mining areas, Poland by means of *Allium*-test

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ABSTRACT

Genotoxic potential of two environmental compartments (water and sediment) from the Upper Silesia Coal Basin (USCB), Poland were evaluated and compared by employing root meristem cells of *Allium cepa*. The clear genotoxic effect of water and sediment sampled was shown, with an important contribution of severe types of cytogenetic abnormalities. The most biologically relevant pollutants were revealed through multivariate statistical analysis of relationships between biological effects registered and the environment contamination. Overall, results of simultaneous use of conventional monitoring methods and biological tests suggested that contemporary levels of persistent pollutants in post-mining areas of the USCB may enhance the risk both for human health and biological components of natural ecosystems.
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1. Introduction

Coal mining is an activity with high potential for environmental pollution (Silva et al., 2008). In some post-mining areas there are specific ecological problems resulting from both the naturally occurring radionuclides, their environmental behavior and derived biological effects. Post-mining areas where enhanced concentrations of natural radionuclides occur are located, for example, in the Komi Republic and Yakutia, Russia (Geras'kin et al., 2007) as well as in the Upper Silesia, Poland (Chałupnik et al., 2001). Observed there radium isotopes originate from underground formation waters associated with fossil fuels as hard coal or crude oil. Radium is moved to the liquid phase due to "alpha recoil effect" (Michalik et al., 2002) that is why in such water there are no uranium and thorium. Such waters are highly mineralized and after releasing into environment can lead to creation of sediments containing radium in concentration a few orders of magnitude exceeding the natural level. While limited in number, radioecological studies in post-mining areas suggest that deleterious effects caused by ionising radiation may be recognized in such environments (Geras'kin et al., 2007; Evseeva et al., 2009). These contaminated sites recently became ones of special concern and may represent a serious hazard to humans and wildlife, especially where run-off and erosion can lead to undesirable material leaving from post-mining landscapes influencing surrounding lands and watercourses. In case of densely populated regions like the Upper Silesia land reclamation of post-mining areas is important issue and needs well-founded information about existing hazard (Michalik, 2008).

Heavy metals and natural radionuclides are usually distributed very irregularly within ecosystem compartments. Such elements may accumulate in certain food chains and finally reach concentrations high enough to produce toxic or genotoxic effects. Radium isotopes and their progeny are α-emitters with high relative biological efficiency, and their non-uniform accumulation in living organisms results in increased doses absorbed by some critical organs (Alexakhin et al., 1990). Certainly, there is no reason to attribute all biological effects observed in these areas only to radiation exposure. An accumulation of radium in living organisms is accompanied, as a rule, by an accumulation of associated chemical elements; many of them also have strong toxicity. Our previous data (Evseeva et al., 2003; Geras'kin et al., 2005) suggest that combined exposure to low concentrations of metals and radionuclides may cause substantial biological effects.

Wastewater and sediment samples from post-mining areas are often so complex mixture of chemicals that a detailed knowledge on composition and concentrations of various components is difficult to collect even applying nowadays analytical capabilities. In addition, the use of chemical measurements to determine the impact of toxicants into the environment may mean that compounds of concern are bypassed, either because the concentrations of the

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individual chemicals do not exceed regulatory guidelines or because the toxic component of the mixture has not been measured. In addition, the assertion that individual chemicals pose no harm because they are present at low, ineffective levels may be irrelevant (Silva et al., 2002) when dealing with mixed exposures. This makes almost impossible to carry out an assessment of a hazard based on chemical or radiochemical analysis only. On the other hand, bioassays have provided meaningful parameters to assess the toxicity of complex mixtures like industrial waste (Rank and Nielsen, 1998) even without knowledge of its chemical composition. In contrast to the specific nature of the assessment based on exposure, studies of biological effects integrate the impacts of all harmful agents, including synergistic or antagonistic effects. Certainly, it will never be possible to replace direct physical–chemical measurements of pollutant concentrations entirely by a detection of effects in bioindicators. Chemical and biological control methods need to be used simultaneously, which allows an identification of the relationships between the pollutant concentrations and the biological effects that they cause. In turn, such relationships may help in an identification of the contribution from specific pollutants to the overall biological effect observed.

A standard test for environmental hazard assessment should be easy to perform and the results should be rapidly obtained and reproducible. Observation of the root tip meristem constitutes a rapid and sensitive method for environmental monitoring. This is because the root tips are often the first to be exposed to pollutants spread in soil and water. In our study, *Allium cepa* bulbs were used. A cell cycle duration and sensitivity to many widespread mutagens are both well-known for *A. cepa* cells (Antonsiewicz, 1990; Leme and Marin-Morales, 2009). Generally speaking, the *Allium*-test will give a useful estimate of the total toxicity effect resulting from treatment of onion roots with the mixture, whatever its compounds might be.

The present work is focused on: (1) evaluating and comparing the genotoxic potential of two environmental compartments (water and sediment) from the Upper Silesia Coal Basin post-mining areas, Poland by employing root meristem cells of *A. cepa*; (2) revealing the most biologically relevant pollutants through multidimensional mathematical analysis of relationships between biological effects registered and the environment contamination.

2. Materials and methods

2.1. Water and sediment sampling

Water and sediments samples were collected in April 2005 in the Upper Silesia mining areas, Poland contaminated by discharge of formation waters containing enhanced concentration of radium isotopes. Sample of waters were taken directly from underground galleries of Chwalowice (W1), Zory (W2) and Ziemowit (W3) coal mines and from inland water affected by mine effluents, the Gostynka River near the Bojszowy settling pond (W4) and the Rontok settling pond vicinity (WRef, natural control). Together with the control sample five samples of water were taken.

Water samples were collected using open water grab sampler (1.5 L capacity) and kept in 2 L polyethylene plastic bottles cleaned with metal free soap, rinsed many times with distilled water and soaked in 10% nitric acid for 24 h, finally rinsed with ultrapure water. All water samples were stored in insulated cooler containing ice and delivered on the same day to laboratory and all samples were kept at 4 °C until processing and analysis (Clesceri et al., 1998). As an additional control, distilled water (DW) was used following practice of our previous study (Evseeva et al., 2005).

Sediment samples were collected in April 2005 from settling ponds had been used by Silesia and Piast mines: the bank of the Rontok settling pond (S1) and bottom of the Bojszowy settling pond (S2 and S3). Also the sample from the underground galleries of Chwalowice mine was taken (S4). There were five sampling points at each plot, located at the four corners and the center of 5 m × 5 m squire (an 'envelope' technique). Samples collected were pooled and mixed well so that finally only one sediment sample was analyzed from every plot. As control, chernozem leached loamy soil (S0) was used, similar to our previous study (Geras'kin et al., 2005). Together with the control sample five samples of sediments and soil were taken. Between sampling points, the sampler was decontaminated by rinsing with distilled water. All samples were put in plastic bags, sealed and labeled.

2.2. Determination of physical–chemical characteristics of water and sediment samples

Basic chemical composition and contents of trace elements in the examined sediment samples were determined by X-ray fluorescence method (XRF) with the use of the Philips sequential X-ray spectrometer type PW 1404. As a source of X-rays radiation 3 kW Rh X-ray tube was used. All measurements were performed according to the analytical procedures elaborated in Department of Environmental Monitoring (Central Mining Institute) in accordance with ISO 17025 standard. All standards used for spectrometer calibration were prepared based on similar mineral matrix. The concentrations of analyzed elements in standards were found using other instrumental techniques (AAS, ICP) and classical methods. Samples for XRF measurements were ground to particle size below 0.03 mm and pressed with a binding reagent (cellulose). Measuring time depended on the content of a given element and varied in the range from 10 to 40 s. For the trace elements the background correction was applied (Stempin and Kwapuliński, 2004).

Basic component of water were determined in accordance with the Polish standards as follow:

- chlorides by Mohr's method;
- sulphates by gravimetric method;
- pH value by electrometric method.

Heavy metal concentration in the water samples were determined by plasma emission spectrometry (AES-ICP) with the use of Perkin Elmer Optima 3000 DV spectrometer (Montaser and Golightly, 1987). Mercury was determined by cold vapour method in atomic emission spectrometry technique according to the Polish standard.

2.3. Determination of naturally occurring radionuclides concentrations in water and sediment samples

Measurements of radium activity concentration in water were performed using a low-background Wallac Quantulus 1220™ liquid scintillations spectrometer that has a heavy Pb shielding, an anticoincidence guard and pulse-shape analyzer, which enables distinguishing α and β particles. In the samples, after radiochemical separation consisted on radium precipitation with added barium, three independent Ra chains, headed by ^{226}Ra, ^{228}Ra and ^{224}Ra were found. Based on their different half life and applying accordant measurement time regime one can measure activity concentration of all radium isotopes (Chałupnik and Lebecka, 1993). For a sample prepared from 1 L of water, and with two 1-h measurements, the following limits of detection (LLD), calculated according to (Currie, 1968) at confidence level of 0.95 can be achieved: ^{228}Ra = 20 Bq m^{-3}; ^{224}Ra = 20 Bq m^{-3}; ^{226}Ra = 3 Bq m^{-3}.

The radium isotopes activity concentration in sediment samples was measured by high resolution gamma spectrometer equipped

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