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Red mud a byproduct of aluminum production contains soluble vanadium that causes genotoxic and cytotoxic effects in higher plants

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HIGHLIGHTS

- Red mud, a by-product of aluminum production, causes DNA-damage in higher plants.
- We showed that this effect is caused by vanadate a known carcinogenic genotoxin.
- Vanadate is contained in high concentrations in the residue.
- Release of red mud may cause adverse effects in ecosystems and affect human health.

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ABSTRACT

Red mud (RM) is a byproduct of aluminum production; worldwide between 70 and 120 million tons is produced annually. We analyzed RM which was released in the course of the Kolontar disaster in Hungary into the environment in acute and genotoxicity experiments with plants which are widely used for environmental monitoring. We detected induction of micronuclei which reflect chromosomal damage in tetrads of *Tradescantia* and in root cells of *Allium* as well as retardation of root growth with contaminated soils and leachates. Chemical analyses showed that RM contains metals, in particular high concentrations of vanadium. Follow-up experiments indicated that vanadate causes the effects in the plants. This compound causes also in humans DNA damage and positive results were obtained in carcinogenicity studies. Since it was found also in RM from other production sites our findings indicate that its release in the environment is a global problem which should be studied in more detail.
Capsule abstract: Our findings indicate that the red mud causes genotoxic effect in plants probably due to the presence of vanadate which is contained at high concentrations in the residue.

1. Introduction

Red mud is a by-product of aluminum production with the Bayer process. Its global production is in the range between 70 and 120 million tons per year (Mayes et al., 2011b). The material consists mainly of iron-, aluminum- and titanium-oxides and hydroxides (Burke et al., 2012; Mayes et al., 2011b). Chemical analyses showed that it also contains radionuclides (e.g. ^{226}RA, ^{230}Th and ^{40}K), as well as heavy metals including As, Cr, Co, Cd, Ni and V (Mayes et al., 2011a; Ruyters et al., 2011).

On Oct 4th 2010, approximately 1 million m^3 of the residue was released into the environment from the aluminum plant Ajkai Timfoldgyar Zrt in Western Hungary. According to the Hungarian Ministry of Interior the "Kolontar disaster" is the biggest environmental catastrophe which ever happened in this country (Ádám et al., 2011). Hundreds of houses were destroyed, 265 individuals were injured and ten died (Gundy et al., 2013).

After the accident, attempts were made to investigate the impact of the release of the material into the environment and to assess the health consequences in humans. Ecotoxicological studies were conducted with different plant species and bacteria concerning toxic effects (Klebercz et al., 2012; Ruyters et al., 2011); the motility and the concentrations of toxic trace elements were studied in physico-chemical measurements (Burke et al., 2012). Furthermore, studies were conducted to

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assess the consequences of inhalation of dust particles in humans and rodents (Czovek et al., 2012; Gelencser et al., 2011).

Radionuclides as well as certain heavy metals (found in red mud) cause damage of the genetic material (Knasmuller et al., 1998; Minouflet et al., 2005) which may lead to destabilization of ecosystems (Sarkar et al., 2006; Zvereva et al., 2008) and also cause adverse effects in humans such as cancer, aging, infertility and birth defects in the offspring (Aitken and De Iuliis, 2007; Assem and Levy, 2009). Therefore it is of particular interest if the release of this material into the environment induces chromosomal damage. This question has been addressed in a human study (Gundy et al., 2013) and in bacterial tests (Gelencser et al., 2011), but no firm conclusions can be drawn from these studies (for details see discussion).

The primary aim of the present study was the investigation of the genotoxic properties of red mud in two plant bioassays, namely in the micronucleus (MN) test with tetrads of *Tradescantia* (Trad-MN assay) and with root tip cells of *Allium cepa* (A-MN assay). The experiments were conducted with tetrads as they reflect damage in meiotic cells. Root tip cells were used as they enable the detection of effects in mitotic cells. It was postulated that differences exist in regard to sensitivity of these cell types towards DNA reactive compounds (Rodrigues et al., 1997). MNi are formed as a consequence of chromosomal breakage or aneuploidy and can be monitored in a variety of organisms (Heddle et al., 2011). In addition, acute toxic effects were studied in root cells of *A. cepa* by measuring the impact of the material on the root growth and by calculating the division rates of the cells. These bioassays are at present the most widely used genotoxicity tests with higher plants and have been employed in more than 300 investigations for the detection of DNA damaging properties of chemicals and complex mixtures (for reviews see Leme and Marin-Morales, 2009; Misik et al., 2011). We used these test systems since they provide information on environmental effects and it is known that they are, in contrast to other genotoxicity assays with bacterial indicators and mammalian cells (which are also used for environmental monitoring), highly sensitive towards radiation (Ma and Davies, 2009; Misik et al., 2011) and heavy metals (Knasmuller et al., 1998; Majer et al., 2002; Steinkellner et al., 1998).

We investigated the acute cytotoxic and genotoxic activities of red mud and of soils which were contaminated with the material from fields and gardens which were used for cultivation of crops and vegetables. Furthermore, we studied also the effects of waters leached from red mud and affected soils. In additional experiments, attempts were made to identify the compound(s) which cause genotoxic effects. We determined the concentrations of trace elements in solid samples and in leachates and conducted additional Trad-MN assays with sodium metavanadate ($NaVO_3$) since vanadium was found to be the most abundant heavy metal in the samples.

2. Materials and methods

2.1. Sampling

Soil samples were collected from the Torna catchment on December 15th 2010. Table 1 contains a description of the sampling sites and specifications of their GPS locations. From each site, samples were collected from the 0–5; 5–15 and 15–25 cm horizons to assess if potentially toxic constituents in red mud contaminated the soils.

Total organic carbon (TOC) was determined in soil samples using a LECO SC-144DR elemental analyzer after removal of the inorganic carbon fraction using 20% HCl.

2.2. Preparation of the leachates

Soils were used as sampled (field moist). 50 g of each sample was suspended in 100 mL of deionized water in a 250 mL glass beaker for 2 h using a magnetic stirrer. The extracts were filtered with filter paper before they were tested in stem absorption experiments. The pH values of the leachates were measured with a pH Meter 526 (WTW, Weilheim, Germany) and are listed in Table 1.

2.3. Measurements of the trace elements in the soil samples and leachates

The detection of the trace elements is described in detail in a recent paper of Renforth et al. (2012). Prior to analysis by XRF, the samples were prepared as follows. For major element analysis, samples were prepared as fused beads (after loss on ignition at 1050 °C) with lithium metaborate/tetraborate flux (Johnson Matthey Spectroflux JM100B) (0.6 g sample; 3 g Flux). For minor/trace element analysis approximately 10 g of dried sample was prepared as a pressed pellet using ~10–20 drops of 6.6% w/v polyvinyl alcohol in a 1:6 mix of methanol and distilled deionized water as a binder (Moviol 88 solution). Elemental analysis of the soil composition was achieved using a PANalytical Axios Advanced X-ray Fluorescence (XRF) spectrometer (data corrected for loss on ignition; % weight loss after furnace treatment at 1050 °C). The aqueous phase produced during water extractions was separated from solids by centrifugation (10 min; 2000 *g*) followed by membrane filtration (0.45 μm); the filtered samples were then acidified by addition of 2% v/v HNO_3. Aqueous elemental concentrations were then determined using a PerkinElmer Optima 5300 DV ion-coupled plasma, optical emission spectrometer (ICP-OES).

2.4. Tradescantia micronucleus assays

Tradescantia clone #4430 was cultivated according to the protocol of Ma et al. (1994). For stem absorption experiments, 15 young inflorescences were treated in each group. The stems were cut to a length of 10–15 cm, transferred to plastic breakers (250 mL) and exposed to aqueous leachates (soil:water — 1:2) of the contaminated soils for 24 h. Subsequently, they were transferred to water for a 24 h recovery period. The flower buds were then collected and fixed in acetic acid and ethanol (1/3, v/v). After 24 h, they were transferred to 70% ethanol. Maleic hydrazide (20 mg/L; MH, Sigma-Aldrich, SL, US) was used as a positive control.

The protocol for root absorption assays is described in an article of Steinkellner et al. (1998). The soil which was used in the control cultures was biological, pesticide free (from Composana, Wien, Austria). Intact plants were removed from hydroponic culture, subsequently the roots were rinsed and individual plants with at least 15 inflorescences placed into plastic pots (250 mL, diameter 12 cm) which were filled with the different soil samples. The plants were exposed under standard conditions for 48 h (Ma et al., 1994). Subsequently, 15 inflorescences were collected from each soil sample in all experiments and fixed in a solution of acetic acid and ethanol (1/3, v/v). After 24 h, they were transferred to 70% ethanol.

To find out if vanadium accounts for the effects which were found in the soils and in the leachates, additional experiments were carried out with sodium metavanadate. The salt was dissolved in 0.1 M NaOH (pH 13) at 1000 ppm (colorless solution) since earlier findings indicate that vanadium is present as V^{5+} in the soil (Burke et al., 2012). The stock solution was diluted with tap water and different concentrations (0.5–10.0 ppm) were used in experiments with *Tradescantia*.

Slides were prepared and evaluated as described in the protocol of Ma et al. (1994). The tetrads were stained with a 2% acetocarmine solution. Early tetrads were analyzed under 400-fold magnification. For each experimental point a minimum of five buds with early tetrads was evaluated (300 tetrads were evaluated per slide, 1500 per sample).

2.5. Micronucleus assay with A. cepa

The experiments were carried out according to the standard protocol published by Ma et al. (1995) with slight modifications. Young onion bulbs (diameter 12–21 mm, Schneeball weiss, Austrosaat, Wien, Austria) were placed in 13 mL glass tubes filled with tap water for 24