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Ecotoxicity evaluation of an amended soil contaminated with uranium and radium using sensitive plants

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ABSTRACT

As a result of former uranium mining at Urgeiriça (central-northern Portugal), the studied adjacent agriculture soils (Fluvisols) had high total concentration of uranium (~660 mg/kg) and high radium-226 activity (~2310 Bq/kg). The environmental risk of these soils is also related to the high available concentrations (soluble + exchangeable fraction extracted with ammonium acetate) of uranium$_{total}$ and radium-226, which represent 100% and 20% of their total concentrations, respectively. The objective of this work was to evaluate the effect of different amendments (sheep manure and bone meal) in the toxicity reduction from agricultural soils contaminated with uranium and radium, by bioassays using two sensitive plants (*Lactuca sativa* L. and *Zea mays* L.). Pot experiments (microcosm experiments), under controlled conditions, were undertaken during two months of incubation at 70% of the soil water-holding capacity. Bone meal at 40 Mg/ha, sheep manure at 70 Mg/ha, and two mixtures of bone meal and sheep manure (40 Mg/ha + 70 Mg/ha and 20 Mg/ha + 70 Mg/ha, respectively) were used as amendments. The amendments' application, independently of their type and concentration, reduced drastically the radionuclides concentrations in the soil available fraction and in the soil leachates. Bioassays using the two above plant species, in different matrices (filter test, soil test and hydroponic test), showed that the soil from Urgeiriça did not have any ecotoxic effect from the radionuclides.

1. Introduction

In Portugal, radium and uranium mining began in 1909, being the extraction of radium the main activity until 1944, when the uranium production became the main goal of the mining exploitation. It was an important economic activity, which ceased around 2001. The exploitation was dispersed for a large number of small mining sites, with the majority of the uranium ore treatment centralized at the Urgeiriça mine. These abandoned mining areas are often located near villages and in agriculture areas raising the potential risk of soil radionuclides contamination and their transfer into the food chain (Carvalho et al., 2009a).

Some soils used for agriculture, located in the mine areas, had significant radionuclides contents (Carvalho et al., 2009a,b; Neves and Abreu, 2009) being their rehabilitation essential in order to minimize the environmental and health risks. Several methods for rehabilitation of soils contaminated with radionuclides are known but only few are sustainable under large-scale conditions. In situ bioremediation methodologies have been proposed (Abreu and Magalhães, 2009, and references therein) to substitute environmental disruptive and very expensive conventional engineering type remediation technologies of soils contaminated with radionuclides (Gavrilescu et al., 2009). The phytoremediation, with or without amendments' application, can be a successful and cost effective process.

Although soil total concentrations of elements have been used as guidelines to establish a soil contamination degree, Adriano (2001) and Kabata-Pendias (2004) reported that only the chemical elements in soil solution and/or exchangeable positions are available and can affect the organisms. The presence of contaminants in waters or in soils available fraction can be detected by the responses of the organism using bioassays. Bioassays can be used to evaluate potential environmental risks (Antunes et al., 2007a,b; Gopalan, 1999; Pereira et al., 2009), however several parameters (physical, chemical and biological) shall also be taken into consideration together with the bioassay results. Vascular plant bioassays present some advantages to assess contaminants' toxicity of the soils (direct bioassays) or leachates (indirect bioassays), through the evaluation of a large number of sensitive plant parameters (Ferrari et al., 1999; van Gestel et al., 2001). The indirect exposure bioassays are used to make a screening of the potential toxicity of sediments and soils as source of contaminants spread for adjacent

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areas through the generated leachates (van Gestel et al., 2001). Direct exposure tests can be used to evaluate the toxicity of the soil matrix itself.

Although some studies reported the evaluation of environmental risks, based on the geochemical characterization, ecotoxicological assays and health evaluation, for Cunha Baixa uranium mine (Antunes et al., 2007a,b; Neves and Abreu, 2009; Neves et al., 2009, 2012; Pereira et al., 2009), the same information for Urgeiriça mine is scarce (Carvalho et al., 2009a; Pereira et al., 2004). As far as we know, there is no information concerning the combined assessment of the chemical characteristics and ecotoxicity of agriculture soils contaminated with radionuclides from Urgeiriça mine following amendments' application. The objective of this work was to evaluate the effect of two amendments (sheep manure and bone meal) and their mixtures, in the toxicity decrease of an agricultural soil contaminated with radium and uranium from Urgeiriça mine area, through bioassays using two sensitive plants (*Lactuca sativa* L. and *Zea mays* L.).

2. Materials and methods

2.1. Study area

Urgeiriça mining area is located near Canas de Senhorim (Viseu district) in the Portuguese Central Iberian Geomorphotectonic Zone, southwest sector and part of the Douro–Beiras sector corresponding to older Proterozoic formations up to the Carboniferous (Godinho et al., 2010). The uranium mineralization occurs in siliceous-iron type veins as pitchblende, associated with pyrite and galena, intruded into a NE–SW fault that cuts a porphyritic medium to coarse grained Hercynian biotite granite (Pereira et al., 2005).

Urgeiriça mine was the most important uranium exploitation and ore processing in Portugal. Extraction of radioactive ores occurred between 1913 and 1992 being the ore processed chemically also in the region until 2001 (Carvalho et al., 2009a). Between 1913 and 1944 the exploitation was directed for radium while afterward only uranium was recovered (Pereira et al., 2004).

A large amount of contaminated wastes, that promoted the dispersion of the trace and radioactive elements to adjacent areas, was left in the Urgeiriça area (Machado, 1998; Pereira et al., 2005).

2.2. Microcosm soil experiments

A composite soil sample (Fluvisol; IUSS Working Group WRB, 2007) collected in 2009, within Urgeiriça mine area, was used in microcosm experiments (pot experiments) after amendments' application, under controlled conditions. The used amendments were bone meal at 40 Mg/ha (B1), sheep manure at 70 Mg/ha (SM), mixtures of bone meal at 40 Mg/ha and sheep manure at 70 Mg/ha (B1 + SM), and of bone meal at 20 Mg/ha and sheep manure at 70 Mg/ha (B2 + SM). The sheep manure was selected because it is usually used in the region by local farmers as fertilizer. The bone meal contains a mixture of bone (carbonate–hydroxyapatite) with meat, which is frequently used in organic farming as a source of phosphate. This amendment has been used for uranium immobilization in contaminated sediments and waters (Arey et al., 1999; Fuller et al., 2003). Both amendments present physical and chemical characteristics adequate for soils remediation, and can be easily obtained in large quantities with cost-effective.

The soil and amendments were air-dried, mixed manually and potted. Microcosm experiments were carried out in pots containing around 750 g of soil (fraction < 5 mm). Five treatments (each one in triplicate) were performed: a control and four soils amended with B1, SM, B1 + SM and B2 + SM. All soil treatments were incubated at 70% of water-holding capacity in greenhouse under controlled conditions for two months.

2.3. Soils characterization

Initial soil and soil samples from the different experiments were air-dried, homogenized and sieved. The initial soil (fraction < 2 mm) and amendments were characterized for (Póvoas and Barral, 1992): pH and electric conductivity (EC) in water suspension (1:2.5, *m*/*V*); extractable potassium and phosphorus (Egner–Riehm method); total nitrogen (Kjeldahl method); organic carbon (Strohlein method); and cation exchange capacity (CEC) by ammonium acetate. Concentrations of nitric and ammoniacal nitrogen were also determined (Mulvaney, 1996). In the initial soil (fraction < 2 mm), total concentration of uranium ($[U]_{total}$) was determined using ICP-MS, after acid digestion (perchloric acid + nitric acid + hydrochloric acid + hydrofluoric acid), and radium-226 activity was determined by gamma spectrometry in international certified laboratories (ISO/IEC 17025, Activation Laboratories, 2012; NFM60790-6, Laboratoire Algade, 2012).

Uranium and radium-226 were also analyzed in two extractable solutions that simulated: soil leachates (DIN 38414-S4, 1984) and soil available fraction (soluble + exchange fractions). The soil leaching was carried out using distilled water (1:10, *m*/*V*) in a rotatory shaker during 24 h at room temperature. Then, these leachates were vacuum filtrated (<0.45 μm), and the pH and EC were measured. The soil available fraction was extracted with 1 mol/L aqueous solution of ammonium acetate (Kabata-Pendias, 2004; Schollenberger and Simon, 1945) for 16 h of shaking. The obtained aqueous solutions were stored at 4 °C until analyses. The total concentration of uranium and the activity of radium-226 were determined in extractable soil solutions (leachates and available fractions) by liquid scintillation spectrometry (QUANTULUS 1220 Perkin Elmer). In leachates the concentrations of calcium, magnesium, potassium, and sodium were also determined by atomic absorption spectroscopy (AAnalyst 300 Perkin Elmer), and phosphorous as phosphate by colorimetry (Murphy and Riley method, Póvoas and Barral, 1992).

2.4. Bioassays

Ecotoxicological evaluation of the soils and the soil leachates from the five treatments (each one in triplicate) were performed using two plant species: Lactuca sativa L. var. *crispa* L. cv. Great Lakes 118 (dicot species) and Zea *mays* L. var. regional (monocot species). The selection of both plant species (a dicotyledonous and a monocotyledonous) was based on ISO recommendations (ISO, 11269–2, 1995). The toxicity effects on plants of each soil treatment and their leachates were evaluated through the germination rate, aerial part elongation and fresh biomass production (OECD 208, 2006) as well as root elongation of both plant species.

The bioassays were carried out using the following substrata: filter paper (filter paper test), soil (soil test), and leachates solution (hydroponic test). For the filter paper tests three layers of filter paper (140 mm Whatman No. 1 filter) were put on the bottom of each tall-form glass beaker, and moistened with 5 mL of leachate from each treatment (filter paper test; Salvatore et al., 2008). The soil tests were made with 15 g of each soil samples (control and treatments, fraction < 5 mm) that were put in each tall-form glass beaker and moistened at 70% of water-holding capacity (soil test; Martí et al., 2007). Seeds of each species (15 seeds (5 seeds × 3 beakers) per treatment and bioassay) were germinated in a growth chamber under controlled conditions (25 ± 1 °C; 16 h light/8 h darkness). The criterion of germination was the emergence of a radicle through the seed coat. After 50% radicle emergence in control, seedlings were left growing, under the same controlled conditions, for seven days. The filter papers and soils were kept moist during the germination and growth time, and the above described biological parameters were evaluated after germination and growth.

For the hydroponic tests, seeds of both species were previously germinated in the dark at 25 °C on water-moistened filter paper in