Journal of Geochemical Exploration 142 (2014) 112–121

Contents lists available at ScienceDirect

Journal of Geochemical Exploration

journal homepage: www.elsevier.com/locate/jgeoexp

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

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ARTICLE INFO

Article history:
Received 1 October 2013
Accepted 29 January 2014
Available online 6 February 2014

Keywords:
Bioassays
Bone meal
Sheep manure
Urgeiriça mine

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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http://dx.doi.org/10.1016/j.gexplo.2014.01.029

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

Petri dishes. Seedlings with a seminal root length of 15 mm and shoot length of 2–4 mm were selected (15 seedlings (5 seedlings × 3 beakers) per treatment and plant) and transferred to each low-form beaker containing 50 mL of leachates from the different treatments. The plants were supported by a thin and flexible plastic net placed on each beaker, in a way that only the roots were immersed in the leachates. After seven days of growth, in the growth chamber, under the same conditions as described before for filter paper and soil tests, the same biological parameters were assessed.

2.5. Data analysis

The data were analyzed by a one way ANOVA and Tukey test ($p < 0.05$) using the statistical program SPSS v18.0 for Windows software. Bivariate Pearson correlations were used to correlate the soils and plants' characteristics ($r > 0.85$). Quality control of the analysis was made by analytical replicate samples (except the chemical characteristics of the initial soil and amendments) and laboratory standards at the Activation Laboratories and Laboratoire Algade.

3. Results and discussion

3.1. Soil and amendments' characterization

The Urgeiriça soil used in microcosm experiments presented characteristics within the range of the agriculture soils developed on alluvial materials from granitic origin (Table 1): pH moderately acid, low electrical conductivity and medium fertility as well as CEC. Concentration of nitrogen–NO_3 was higher than nitrogen–NH_4, however both inorganic forms of nitrogen represent less than 0.25% of the total nitrogen. The soil texture was loam with 38% of materials in fraction of >2 mm distributed as: ≥10 mm (1%); 8–10 mm (2%); 5–8 mm (11%); and 2–5 mm (24%).

This soil can be considered contaminated in uranium and radium because the uranium total concentration ($(6.6 \pm 1.0) \times 10^2$ mg U/kg) and the radium-226 activity ($(2.31 \pm 0.35) \times 10^3$ Bq/kg) are 28-fold and 13-fold higher than the allowed values of total uranium (23 mg U/kg soil) and radium-226 activity (185 Bq/kg soil), respectively, for agricultural use (CCME, 2007; EPA, 1998; Sumner, 1995). The soil contamination can be ascribed to the dispersion of tailing materials. Pereira et al. (2004, 2005) reported that the most important source of radionuclides contamination in Urgeiriça was the mill tailings deposit composed of precipitates from an old settling basin containing high concentrations of uranium, and sludge from treatment plant with high radium-226 activity. These materials were submitted to water erosion processes giving rise to solid dispersion.

Table 1
Characteristics of Urgeiriça soil and amendments applied to the soil.

	Initial soil	Sheep manure	Bone meal
pH (H_2O)	5.15	8.50	6.27
EC (mS/cm)	0.06	12.68	9.45
Organic C (g/kg)	17.60	349.60	624.60
N (mg/kg)			
Total	1730	18,850	76,100
NH_4	2.61	21.87	156.60
NO_3	4.29	142.49	449.50
Extractable P (mg/kg)	25.62	7.24	0.55
Extractable K (mg/kg)	54.78	1.51	0.41
CEC ($cmol_c$/kg)	10.08	Nd	Nd
$Na_{exchangeable}$ (mg/kg)	4.6	Nd	Nd
$K_{exchangeable}$ (mg/kg)	82	Nd	Nd
$Mg_{exchangeable}$ (mg/kg)	124	Nd	Nd
$Ca_{exchangeable}$ (g/kg)	1.04	Nd	Nd
Na_{total} (g/kg)	8.7	Nd	Nd
K_{total} (g/kg)	26.4	Nd	Nd
Mg_{total} (g/kg)	2.7	Nd	Nd
Ca_{total} (g/kg)	2.4	Nd	Nd

EC: electrical conductivity; CEC: cation exchangeable capacity; Nd: not determined.

The radium-226 activity in the studied soil ($(2.31 \pm 0.35) \times 10^3$ Bq/kg) was much higher than those determined in other agriculture soils sampled near the Urgeiriça mine (263–1253 Bq/kg; Carvalho et al., 2009a). The values determined for the studied soil were also much higher than the values published by Pereira et al. (2004) for soils (257–271 Bq/kg) and stream sediments (282 Bq/kg) also collected in the in Urgeiriça area. Carvalho et al. (2009a) reported the value of 1690 Bq/kg for the radium-226 activity in the materials collected from the sludge pond resulting of the acid mine water treatment in the same mine.

The soil total concentration of uranium in available and total fractions as well as the radium-226 activity in total fraction (Table 2) were higher than the published referred physical quantities for uranium (total fraction: 35–427 mg U/kg soil; available fraction: 7–14 mg U/kg soil) and radium-226 (793–1200 Bq/kg soil) in agriculture soils from the Cunha Baixa uranium mine, which belongs to the same metallogenic province (Carvalho et al., 2009b; Neves and Abreu, 2009; Neves et al., 2009). The high total concentration of uranium and radium-226 activity in the available fraction, which represent 100% and 20% of the total, respectively, indicate that this soil can pose high environmental risk.

As expected, chemical characteristics of the organic and inorganic materials used as amendments were considered beneficial and secure for land application (Table 1). Concentrations of organic carbon, and total and mineral nitrogen in the amendments were higher than those in the Urgeiriça soil (Table 1), contributing to an increase of the soil fertility. The presence of some meat in bone meal explains the high organic carbon concentration in this material. The C/N ratios of the amendments are lower than 25 (sheep manure: C/N = 19; bone meal: C/N = 8), which indicate the possibility of a fast organic matter decomposition causing net increase of available nitrogen as ammonium, nitrite and nitrate ions (Varennes, 2003).

The amendments contained low concentrations of the extractable phosphorus and potassium (Table 1), and high values for the EC, when compared to the soil. However, their application is considered without any environmental risk.

After the two months of incubation, the values of pH and EC in the amended soils were higher than the control (Fig. 1). The highest pH (6.56) was obtained in the soil amended only with organic matter (SM), which can be related to the high pH of this material (Table 1). However, the pH of the treatments containing bone meal (pH: 5.68–6.14) had lower values than those measured in the soil amended with sheep manure. In spite of the presence of some meat attached to the bone, it is possible that the dissolution of the bone (carbonate-substituted calcium-deficient apatite-(CaOH) ($Ca_{5-x}(PO_4,CO_3)_3(OH)$)) promotes the increase of the amended soil pH to the values observed in the present work (Hodson et al., 2001; Sneddon et al., 2008). The increase in the soil pH due to addition of sheep manure can be explained by the biological processes that convert organic nitrogen into ammonia, which originates the hydroxide ions by reaction with water (Varennes, 2003). Once ammonia is more soluble in water than the constituents of the bones, and sheep manure mineralizes faster than the organic matter associated with ground bone, it is expected that the pH of the soil amended with sheep manure attained higher values than that of the soil amended with bone meal. Moreover, the products of dissolution of the bones, which are carbonates and phosphates, have a higher buffering capacity in the pH range of the systems under study than the pair ammonium/ammonia controlling the values of the pH in the systems where they are present.

The soils amended with a single material presented lower EC (B1: 1.53 ± 0.24 mS/cm; SM: 2.48 ± 0.41 mS/cm) than soils with application of mixtures of amendments (B1 + SM: 3.36 ± 0.16 mS/cm; B2 + SM: 4.73 ± 0.21 mS/cm). The soil amended with the mixture of 20 Mg/ha of bone meal and 70 Mg/ha of sheep manure (B2 + SM) had higher EC value than the soil amended with a double amount of bone

Table 2
Total concentration of $[U]_{total}$ and activity of ^{226}Ra in the total and available fractions (soluble + exchangeable) of the Urgeiriça soil.

	Initial soil	
	Total	Available fraction*
$[U]_{total}$ (mg/kg)	$(6.6 \pm 1.0) \times 10^2$	$(6.6 \pm 1.0) \times 10^2$
^{226}Ra (Bq/kg)	$(2.31 \pm 0.35) \times 10^3$	$(4.56 \pm 0.70) \times 10^2$

* Extracted with aqueous solution of ammonium acetate.

meal and the same content of sheep manure (B1 + SM). This is a result of the increase of the solubility of the calcium phosphates from the bone meal, which is due to the slight decrease of the pH of the amended soil (Fig. 1) that promotes the hydrolysis of the dissolved carbonate and phosphate ions increasing the concentration of total ions in the solutions. Although the EC of the amended soils can be considered high, these values are in the same range than the values from agriculture soils located near other uranium mine from the same metallogenic province (Cunha Baixa: 0.3–6.4 mS/cm; Neves and Abreu, 2009; Neves et al., 2012).

3.2. Soil extractable solutions

The composition of the solutions obtained by the method DIN 38414-S4 (1984), which simulates the soil leachates, after two months of incubation, is presented in Figs. 2, 3 and 5. The values of the pH and the EC in leachates showed the same tendency than the values of pH and EC in the soils (Figs. 1 and 2). After the two months of incubation, the pH (5.93 ± 0.15) and EC (0.107 ± 0.02 mS/cm) values of the leachates from the soil used as control were lower than the corresponding values of the amended soils.

The comparison between the data of pH and EC in soils, after the two months of incubation (Fig. 1), and the corresponding values in the soil leachates (Fig. 2) show that the solutions obtained by the DIN method (DIN 38414-S4, 1984) reflect the variations that occurred in the soils following the amendments' addition. Just like in the soils, the values of the pH and EC in the soil leachates were in the range of the values measured in irrigation waters (pH: 4.2–6.2; EC: 0.4–1.8 mS/cm) used for agriculture activities in the vicinity of Urgeiriça and Cunha Baixa mines (Carvalho et al., 2009a; Neves and Abreu, 2009; Neves et al., 2012). The pH of the leachates from treatment B2 + SM was different and lower than the values from the other amended treatments (Fig. 2). The slight increase of the pH observed in the present work has the same origin than the above referred for the pH increase in the soils amended with bone meal. As in the soils, the highest value for EC was measured in the leachates from the amended soil with the mixture B2 + SM (1.72 ± 0.11 mS/cm). In the leachates from the amended soils with the mixture B1 + SM the EC was higher (1.507 ± 0.067 mS/cm) than in the leachates obtained from the treatments where bone meal or sheep manure alone were applied (B1: 0.683 ± 0.020 mS/cm; SM: 0.785 ± 0.008 mS/cm). The increase of the EC in the leachates from

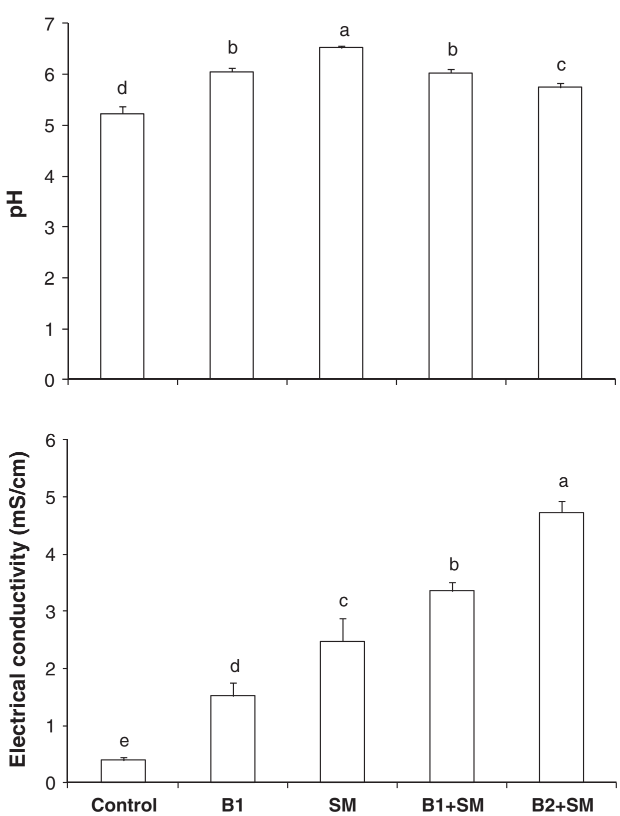

Fig. 1. Values of pH and electrical conductivity of the Urgeiriça soils, after two months of incubation from different treatments (control; soils amended with: bone meal at 40 Mg/ha (B1), sheep manure at 70 Mg/ha (SM), mixture composed of bone meal at 40 Mg/ha and sheep manure at 70 Mg/ha (B1 + SM), mixture composed of bone meal at 20 Mg/ha and sheep manure at 70 Mg/ha (B2 + SM)). Different letters indicate significant differences ($p < 0.05$).

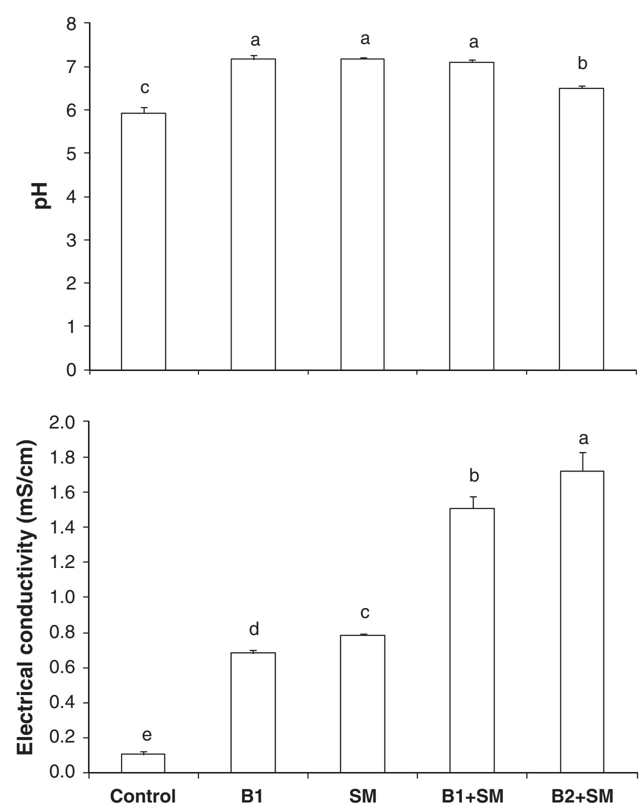

Fig. 2. Values of pH and electrical conductivity in the leachates from Urgeiriça soils, after two months of incubation, from different treatments (control; soils amended with: bone meal at 40 Mg/ha (B1), sheep manure at 70 Mg/ha (SM), mixture composed of bone meal at 40 Mg/ha and sheep manure at 70 Mg/ha (B1 + SM), mixture composed of bone meal at 20 Mg/ha and sheep manure at 70 Mg/ha (B2 + SM)) (Mean ± SD; $n = 3$). Different letters indicate significant differences ($p < 0.05$).

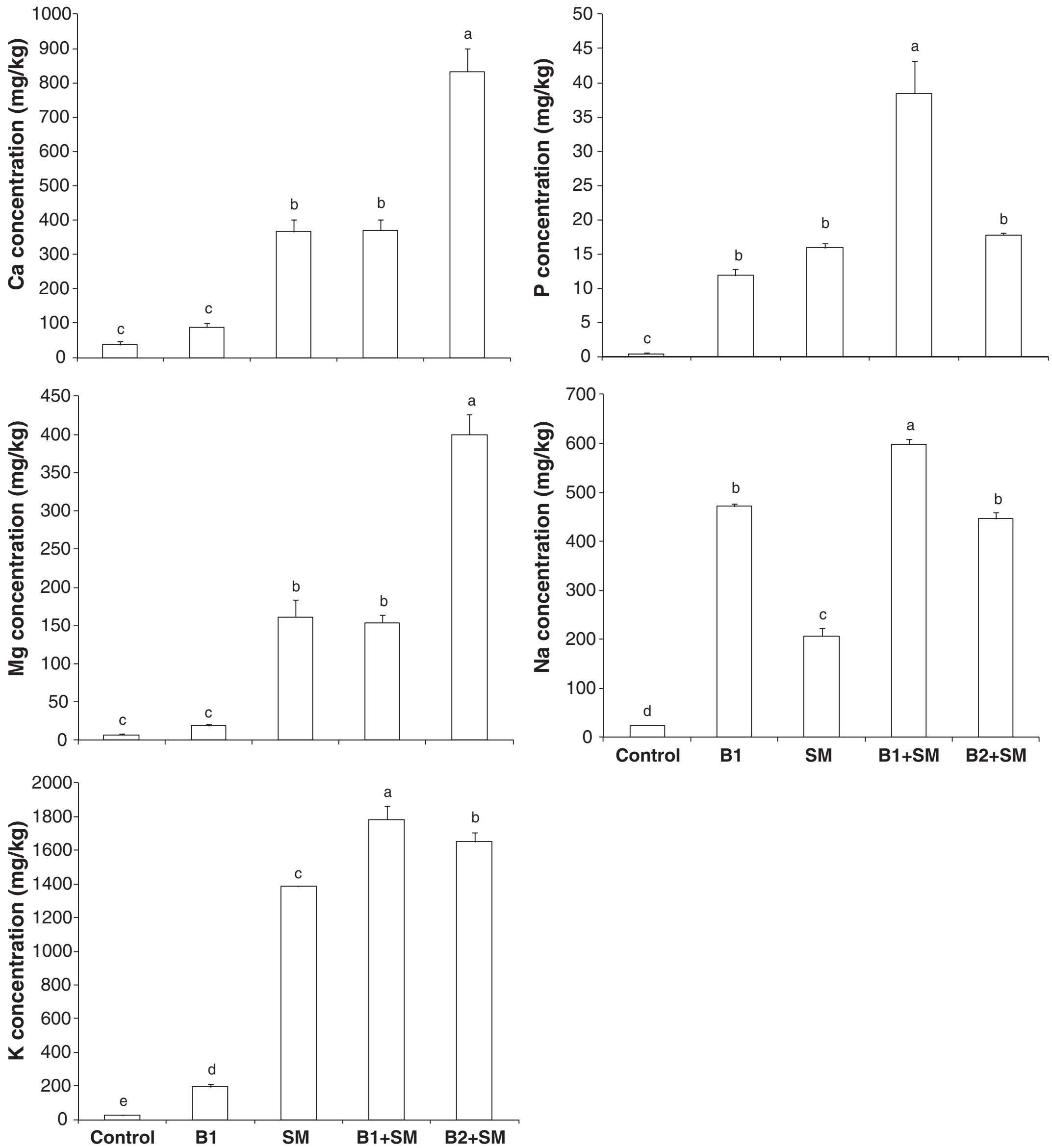

Fig. 3. Concentrations of Ca, P, Mg, Na and K in the leachates from different Urgeiriça soil treatments (control; soils amended with: bone meal at 40 Mg/ha (B1), sheep manure at 70 Mg/ha (SM), mixture composed of bone meal at 40 Mg/ha and sheep manure at 70 Mg/ha (B1 + SM), mixture composed of bone meal at 20 Mg/ha and sheep manure at 70 Mg/ha (B2 + SM)) (Mean ± SD; $n = 3$). Different letters indicate significant differences ($p < 0.05$).

the amended soils is obviously related to the increase of the concentrations of the dissolved ions in solution.

The variation of the total concentrations of the main ions (calcium, magnesium, potassium, sodium and phosphates) contributing to the EC of the leachates extracted after two months of incubation, from the control and amended soils, is represented in Fig. 3. The total concentrations of calcium and magnesium in the leachates from the amended soil with the mixture B2 + SM ($[Ca]_{total} = 834 \pm 66$ mg/kg; $[Mg]_{total} = 400 \pm 26$ mg/kg) are 2.3- and 2.6-fold higher than the total concentrations of calcium and magnesium in the leachates from the amended soils B1 + MS, respectively. On the other hand, the total concentration of phosphates in the leachates from the amended soils B2 + SM is 2.2-fold lower than the total concentration of phosphate in the leachates from the amended soil B1 + SM ($[phosphate]_{total} = 38.4 \pm 4.8$ mg P/kg). Calcium and phosphate are the main components of the mineral phase of the bone meal and the difference in the total concentrations of calcium and phosphate in the leachates from B1 + SM and B2 + SM can be explained by the formation of other solid phases containing lower calcium/phosphate ratio than the apatite of the bone due to the pH decrease. For solutions containing calcium and phosphates total concentrations in the range of the present work it is possible a change in the calcium phosphate solid phases for pH lower than six (Magalhães et al., 2006). Under these conditions, brushite ($CaHPO_4 \cdot 2H_2O$) is the most stable calcium phosphate for pH lower than six. However, the change from apatite-(CaOH) into brushite can be made through an intermediate less stable solid phase (OCP($Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$)), which is represented by the chemical equation:

$$2\ Ca_5(PO_4)_3(OH)(s) + 4H^+(aq) + 3\ H_2O(l) \rightarrow Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O(s) + 2\ Ca^{2+}(aq).$$

Thus, it is possible to explain the lower concentration of total phosphates in B2 + SM when compared to B1 + SM and the higher total concentration of calcium, as a result of the lower pH (5.75 ± 0.08) in the amended soil B2 + SM. The amended soil pH will be kept around the referred value due to the possible calcium to hydrogen ions exchange on the exchangeable complex of the amended soil organic matter, promoted by the calcium concentration increase in the soil solution.

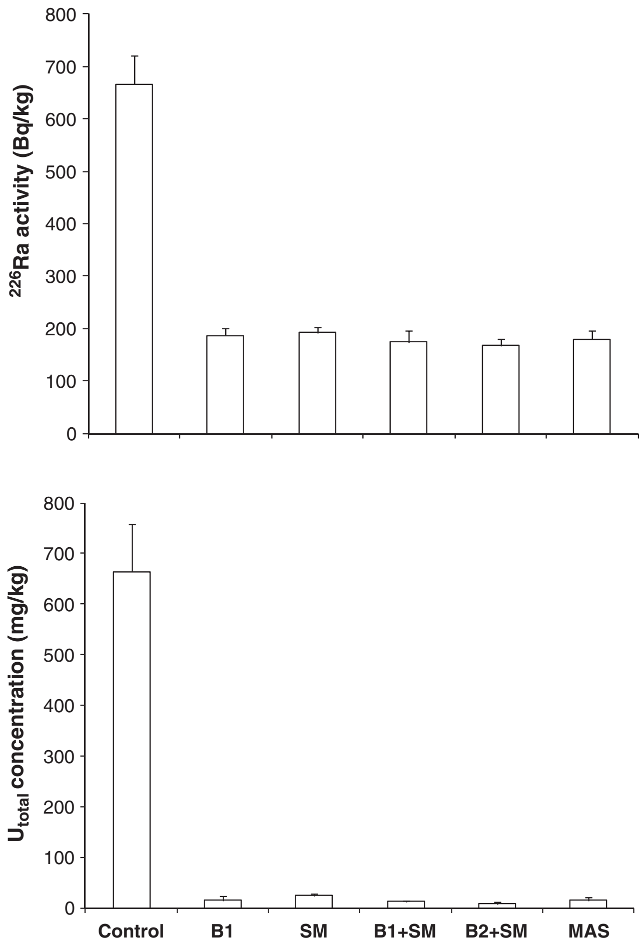

Fig. 4. Concentrations of $[U]_{total}$ and activity of ^{226}Ra in the available fraction (extracted with ammonium acetate) from different Urgeiriça soil treatments (control; soils amended with: bone meal at 40 Mg/ha (B1), sheep manure at 70 Mg/ha (SM), mixture composed of bone meal at 40 Mg/ha and sheep manure at 70 Mg/ha (B1 + SM), mixture composed of bone meal at 20 Mg/ha and sheep manure at 70 Mg/ha (B2 + SM)); mean values of amended soils (MAS) (Mean ± SD; $n = 3$ for all treatments, except MAS (mean of the all treatments (B1, SM, B1 + SM, B2 + SM), $n = 12$)).

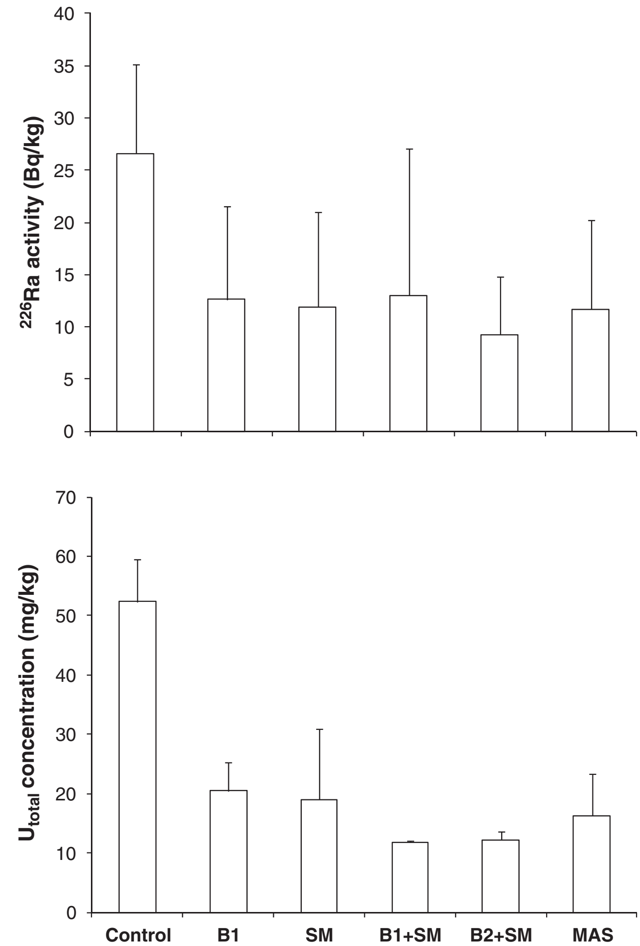

Fig. 5. Concentrations of $[U]_{total}$ and activity of ^{226}Ra in the leachates from different Urgeiriça soil treatments (control; soils amended with: bone meal at 40 Mg/ha (B1), sheep manure at 70 Mg/ha (SM), mixture composed of bone meal at 40 Mg/ha and sheep manure at 70 Mg/ha (B1 + SM), mixture composed of bone meal at 20 Mg/ha and sheep manure at 70 Mg/ha (B2 + SM)); mean of amended soils (MAS) (Mean ± SD; $n = 3$ for all treatments except MAS (mean of the four treatments (B1, SM, B1 + SM, B2 + SM), $n = 12$)).

The magnesium increase in the leachates from the amended soils can be ascribed, mainly, to the sheep manure (Fig. 3). However, the 2.5-fold increase of the total concentration of magnesium in the leachate from B2 + SM in relation to the SM and B1 + SM leachates total concentration of the magnesium can be explained by the above described solid phase change occurring in the B2 + SM. The bones contain around 1% (*m*/*m*) of magnesium (Beighle et al., 1994) and the above described solid phase change that can occur with calcium phosphates can liberate magnesium to the aqueous solutions.

The increase of the potassium concentrations in the leachates from SM, B1 + SM and B2 + SM is mainly due to the sheep manure, whereas the high sodium concentrations in the leachates from the amended soils can be ascribed to the bone meal (Fig. 3).

The total concentration of uranium and the activity of radium-226 in the ammonium acetate extracting solutions from all treatments (control and amended soils), representing the soil available fraction (soluble + exchange fractions), after the two months of incubation, are shown in Fig. 4. The amendments' addition, independently of the type of amendment, decreases the total uranium available fraction concentration of 98 ± 1% in relation to the control, while the activity of radium-226 was reduced of 75 ± 2%.

The total concentration of uranium and the activity of radium-226 in the leachates (DIN method) from all treatments (control and amended soils), after the two months of incubation, are represented in Fig. 5. The activity of radium-226 in these leachates was much lower than the activity of radium-226 in the corresponding ammonium acetate extracting solutions (Table 3). The total concentration of uranium in the leachates from control was also much lower than its concentration in the ammonium acetate extracting solutions, but the total concentration of uranium in both extracting solutions (leachate (DIN method) and ammonium acetate (soil available fraction)) from the amended soils had similar values (Table 3). The much higher values obtained for the activity of radium-226 in the ammonium acetate aqueous solutions when compared to the extractions with water (DIN method) show that this element must be mainly in the exchangeable complex of the soil. The amendments' addition to the soil increase the number of exchangeable sites, where radium was preferentially adsorbed, decreasing the concentration of radium-226 in the water extracted solutions.

The activity of radium-226 in the leachates from the control (1.38 ± 0.45 Bq/L) was higher than the values (26.3 ± 1.6 and 153 ± 8 mBq/L) reported by Carvalho et al. (2009a) in waters from wells sampled near the Urgeiriça mine and used for irrigation of kitchen gardens.

The total concentrations of uranium in the leachates obtained from the control were higher (2.7 mg U/L) than the values in the contaminated irrigation waters (0.94–1.04 mg U/L) from Cunha Baixa uranium mine area (Neves and Abreu, 2009). Nevertheless, the values of the total concentration of uranium in the leachates from the amended soils (0.76 mg U/L) were lower than the above referred values from

Table 3
Concentration of $[U]_{total}$ and activity of ^{226}Ra in the soil available fraction and leachates from control and amended soils after the two months of incubation (Mean ± SD; *n* = 3 and 12 for control and amended, respectively).

	Available fraction*		Leachates**	
	Control	Amended	Control	Amended
$[U]_{total}$ (mg/kg)	573 ± 85	11.6 ± 6.1	45 ± 12	12.8 ± 6.9
^{226}Ra (Bq/kg)	575 ± 45	141 ± 14	22.9 ± 7.5	9.2 ± 6.7

* Extracted with aqueous solution of ammonium acetate.
** Extracted by DIN method (1984).

Cunha Baixa, but above the limit value for irrigation purposes (100 μg U/L) according to ANZECC (2000), although the amendments had decreased significantly the total concentrations of this element in the leachates.

The fact that the total concentrations of uranium in both leaching aqueous solutions from the amended soils are low and similar shows that uranium ions were immobilized by the used amendments being bind to the solid phases by strong specific bonds.

3.3. Plants' bioassays

The plant response to the toxicity of the contaminated soils and their soil leachates depends on its biological characteristics and sensitivity to the chemical elements, so it is essential to evaluate several endpoints for each organism (Sheppard et al., 2005). The results from the ecotoxicity tests for each plant species are presented in Figs. 6 and 7.

A negative effect of the soil matrix was observed for lettuce seeds germination for the amended treatments (except for sheep manure treatment) being germination inhibited in all treatments containing bone meal, while for maize this effect was not observed and the germination rate was not different among the amended treatments and control. The lack of lettuce germination in the treatments amended with bone meal is probably due to a physical effect of the matrix, as the bone meal addition to the soil creates a jelly-like material (effect of some meat on the bone meal) that covered the small lettuce seeds not allowing them to germinate. Whereas in control and the treatment amended with sheep manure the lettuce germination was higher than 60%, except in one amended replicate where the EC reached the highest value (≈3 mS/cm). The different sensitivity of seeds' germination between both species and among treatments in soil test can also be related to the soil EC after two months of incubation (EC (μS/cm): control = 398; B1 = 1525; SM = 2485; B1 + SM = 3360; B2 + SM = 4731), because lettuce shows a low salt tolerance (less than 3000 μS/cm; Santos (1996)). In fact, a negative influence of high EC values in lettuce germination was observed ($r \approx -0.99$).

The seeds' germination rate on filter paper test was great in lettuce than in maize, but no differences were observed among control and treatments for both plant species. Germination of the maize seeds was not also significantly different among treatments and test matrices (filter paper and soil).

The concentration of uranium and radium in the soil available fraction and in the leachate solution (DIN method) did not influence the seeds' germination of both species. This fact can be a consequence of the barrier function from the seed coats that can protect the growth of the embryonic as was already stated by Salvatore et al. (2008).

Comparing the ecotoxicity tests for each plant species, a clear distinction among tests was not verified, however in one biological parameter from each plant species there seemed to be a slight tendency. The lettuce roots in the filter paper test reached the highest elongations independently of the treatment (Fig. 6). However, for the maize, the hydroponic test presented aerial part elongations higher than those in filter paper test (Fig. 7) probably, due to the better plant-solution contact that can increase the nutrients uptake.

In the filter paper bioassay using lettuce, no significant differences were observed among the elongations of the aerial part and roots from all the treatments. Although the plants from treatment B2 + SM

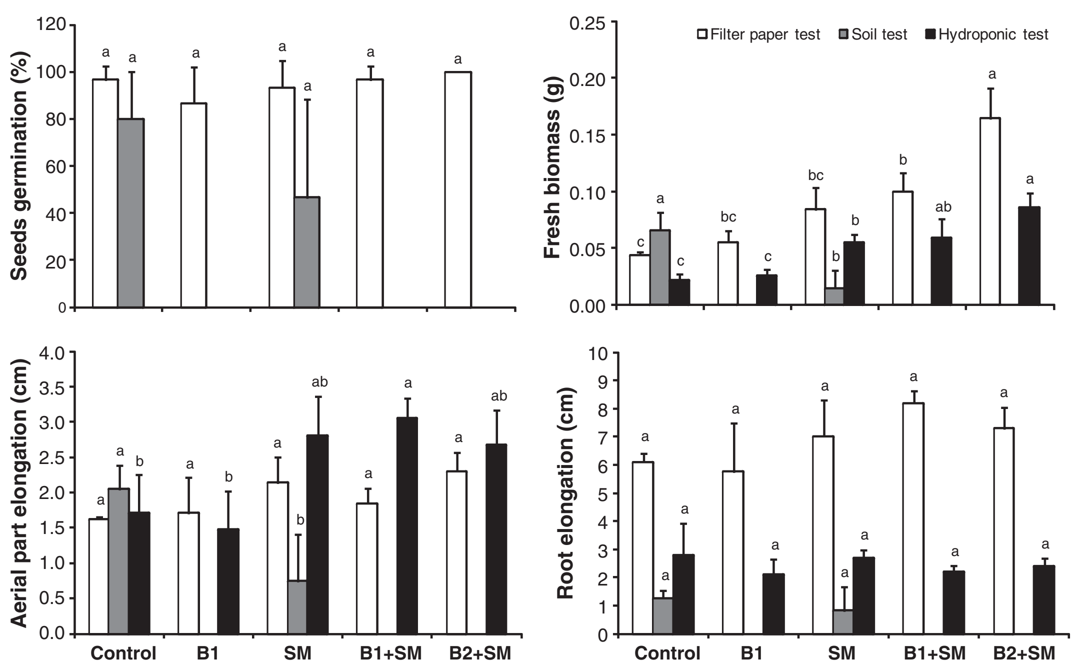

Fig. 6. Biological parameters obtained in the bioassays with *Lactuca sativa* from the different treatments (Mean ± SD; *n* = 3). Different letters indicate significant differences among treatments in the same matrix ($p < 0.05$).

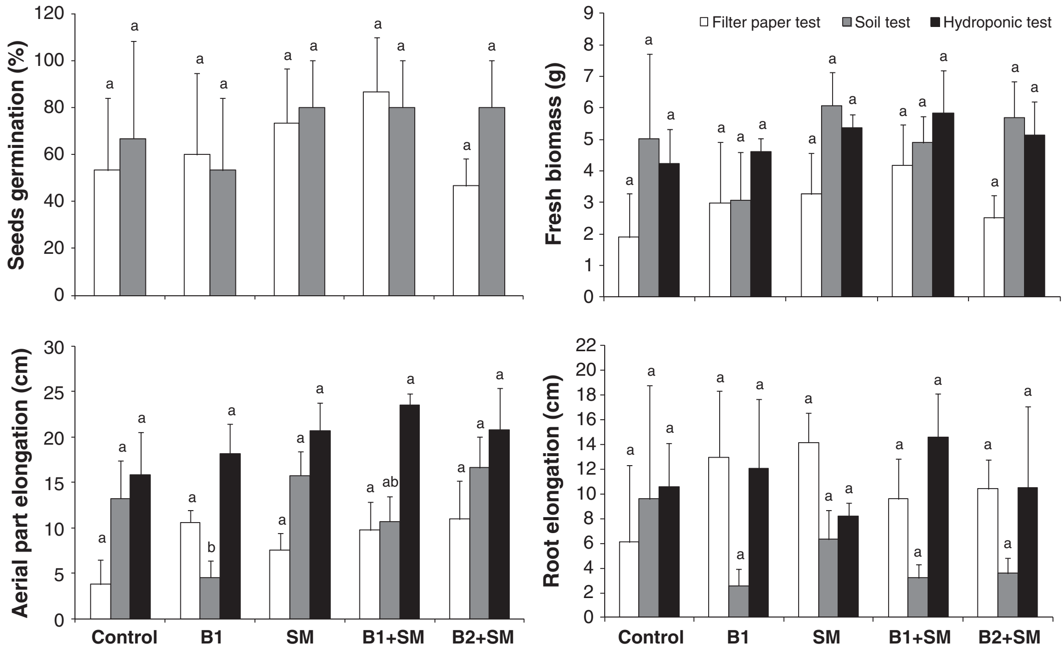

Fig. 7. Biological parameters obtained in the bioassays with *Zea mays* from the different treatments (Mean ± SD; $n = 3$). Different letters indicate significant differences among treatments in the same matrix ($p < 0.05$).

have shown the highest fresh biomass value (0.16 g), the other amended treatments also presented higher fresh biomass (0.06–0.10 g) than the control (0.04 g). The high values of fresh biomasses in amended treatments can be associated to the increase (between 4 and 10%) of water accumulated in lettuce tissues.

In general, the biological characteristics of lettuces growing on soils amended with sheep manure were significantly different than those in control; in soil bioassay, no differences between the root elongations of lettuces from control and amended soil were observed. However, for aerial part and fresh biomass control plants presented the highest values. These results showed that the uranium and radium-226 concentrations in the available fraction, at least until 664.34 mg U/kg and 666.93 Bq ^{226}Ra/kg allowed the development of the lettuces (aerial part: $r \approx 0.88$ for both chemical elements; fresh biomass: $r = 0.91$ for uranium and $r = 0.93$ for radium-226). These data show that the lettuces can grow, without any signs of toxicity and yield decrease, on soils presenting available concentrations of uranium and radium-226 much higher than those measured in agriculture soils from Cunha Baixa and Urgeiriça mines (Carvalho et al., 2009b; Neves and Abreu, 2009).

In the hydroponic bioassay a clear difference was not observed in the aerial part elongation of the lettuces growing in the leachates collected from the different treatments. The plants that grew in the leachates from treatments with application of sheep manure (SM, B1 + SM and B2 + SM) presented similar aerial part elongations (around 3 cm), which can be related to the phosphorous concentration in the leachate solution ($r = 0.99$). However, only the aerial part elongation of the lettuces that grew in the leachates from treatment B1 + SM was significantly different from the aerial part elongation of the lettuces growing in the leachates from treatment B1 and control (Fig. 6). As a consequence of the small aerial part elongation in hydroponic bioassay, the lettuces from treatment B1 and control presented smaller fresh biomasses, compared to the fresh biomasses from the other treatments. The fresh biomass of lettuces growing in leachates from the treatments SM, B1 + SM and B2 + SM show a significant difference from the fresh biomass of lettuces growing in leachates from treatment B1and control (Fig. 6). The fresh biomass of lettuces that grew in leachates from treatment B2 + SM was significantly different from the fresh biomass of lettuces growing in leachates from treatment SM, however both were similar to the fresh biomass of plants of treatment B1 + SM. The aerial part elongations and the fresh biomasses of the lettuces that grew in the leachates from all the treatments can be related to the potassium concentrations in the leachates ($r = 0.81$ and 0.84, respectively).

In the three bioassay matrix experiments done with maize no significant differences were observed in all the studied parameters. The only exception was in the aerial part elongations of the maize that grew on soils where the application of 40 Mg/ha of bone meal (treatments B1 and B1 + SM) seemed to have a negative influence in the plant development. In these treatments, the maize plants presented the smallest aerial part elongation (4.53 cm), compared to plants growing in soils from control and treatments SM and B2 + SM (13.19–16.62 cm depending on treatment).

Some biological parameters of the lettuce translate its higher sensitivity than that of maize to the soil characteristics. However the differences did not correlate to the radionuclides concentrations in the available fraction from the soils and in leachates. In bioassays using Cunha Baixa soil samples, Pereira et al. (2009) reported that the lettuce was the most sensitive species, compared to maize, probably due to its capacity to accumulate high metal concentrations, namely uranium, in leaves. However, lettuces growing on agriculture soils near of the Urgeiriça mine concentrated more radium-226 than the uranium isotopes (^{234}U, ^{235}U and ^{238}U) (Carvalho et al., 2009a). This tendency was also verified in potato tubers cultivated in kitchen garden plots from Cunha Baixa uranium mine (Carvalho et al., 2009b). The low sensitivity of both species used in this study, evaluated by the biological parameters, can be associated to the low contact time span of the seedlings with the contaminated matrices.

Although root growth is known to be more sensitive than germination to the toxicity of trace elements (Araújo and Monteiro, 2005; Martí et al., 2007; Salvatore et al., 2008), the radionuclides concentrations in the leachates and in the available fraction of the soil did not induce the roots growth inhibition of lettuce or maize plants. Nevertheless, the aerial part elongations of both species growing in hydroponic and soil systems demonstrated more sensitivity to the soil characteristics, although the observed differences could not be directly ascribed to the chemical composition of the matrices. In the paper filter bioassay, the leachate volume cannot be enough for a total and homogeneous root exposition to chemical elements during growth plant. Thus, as was also reported by Salvatore et al. (2008) in bioassays to evaluate the toxicity of cadmium, copper, lead and nickel for several vegetables, the root elongations cannot be a direct response of leachates toxicity.

Bioassays using aqueous solutions (leachates or soil water extracts) can predict the risks of contaminants in surface and groundwaters, but they did not include the interactions and exchange reactions that occur in the whole soil (Leitgib et al., 2007; van Gestel et al., 2001). Thus, it is useful for an ecotoxicological approach including both bioassay types: direct (with whole soil) and indirect (with soil leachates).

4. Conclusions

The agriculture soils from Urgeiriça presented high total concentration of uranium and high activity of radium-226 both for total and available (extracted with ammonium acetate) fractions. Independently of the amendments' type used for the soil rehabilitation, their application reduced the concentration of the radionuclides in the soil available fraction and leachates being considered a promising action for the soil containment of uranium and radium. Compared with the control, the amendments' (sheep manure and bone meal, single or mixed) addition to the Urgeiriça soil, independently of the type and concentration of the amendments, leads to a decrease in the total uranium available fraction concentration and the activity of radium-226 of 98 ± 1% and 75 ± 2%, respectively. In addition, the availability of nutrients in amended treatments can be an advantage in the rehabilitation process of the soils and their use for agriculture purposes.

Some biological parameters in lettuce bioassays showed more sensitivity than those in the maize however the differences were not related to the radionuclides concentrations in the soil available fraction and soil leachates. The bioassays did not demonstrate any ecotoxicity effect regarding the Urgeiriça soil, at least using lettuce and maize, two species recommended as sensitive to hazardous chemical elements. In addition these species are largely cultivated in the kitchen gardens of the inhabitants of the mine district. This can also indicate that these plant species are not good indicators of soil contamination with uranium and radium. Other bioassays using distinct organisms should be considered in a future work.

Acknowledgments

The authors would like to thank to the Portuguese Foundation for Science and Technology (FCT) for financial research support of UIQA — Unidade de Investigação Química Ambiental (Program Pest — PEst-OE/AGR/UI0528/2011), CICECO — Centre for Research in Ceramics and Composite Materials (Program Pest — PEst-C/CTM/LA0011/2013), and PhD grant (SFRH/BD/80198/2011). Thanks are also due to Prof. Alcides Pereira — Universidade de Coimbra, EDM, Eng. Miguel Fornelos — Florecha, and Dr. António Campos — Ambicanas, for samples supply and radium and uranium analysis.

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