Environ Geochem Health (2014) 36:285–301
DOI 10.1007/s10653-013-9563-6

ORIGINAL PAPER

Uranium mining in Portugal: a review of the environmental legacies of the largest mines and environmental and human health impacts

R. Pereira · S. Barbosa · F. P. Carvalho

Received: 25 November 2012 / Accepted: 17 July 2013 / Published online: 13 September 2013

Abstract The history of uranium mining in Portugal during almost one century has followed international demand peaks of both radium and uranium, which in turn were driven by medical, military, and civil applications. Nowadays, following price drop in the 1980s, mining activities decreased and ceased in 2001. The current challenge is to deal with environmental legacies left by old uranium mines, mainly located in Viseu and Guarda districts. In 2001, based on several radiological surveys carried out, the Portuguese government assumed the remediation costs of abandoned mine areas for environmental safety and public health protection. Detailed environmental and public health risk assessments were performed under the scope of studies both requested by the government and by funded research projects. It was found that the existing risks, due to radiological and chemical exposures to metals and radionuclide's, were particularly high at the old milling facilities and mines where in situ and heap leaching of low-grade ore occurred. The different studies, involving both humans and non-human species from different trophic levels, demonstrated the existence of effects at different levels of biological organization (molecular, cellular, tissues, individuals, and populations) and on ecosystem services. To mitigate the risks, the environmental rehabilitation works at the Urgeiriça mine complex are almost complete, while at Cunha Baixa mine, they are presently in progress. These works and environmental improvements achieved and expected are described herein.

Keywords Portuguese uranium mines · History · Human health effects · Ecotoxicological effects · Ecological effects · Remediation works

Introduction

The uranium market, throughout the history, has shown several demand peaks, motivated by new scientific discovers and applications. In the beginning

---

S. Barbosa is in representation of all the ATA team of EDM-Empresa de Desenvolvimento Mineiro, S.A., Lisboa, Portugal.

R. Pereira (✉)
Departamento de Biologia, Faculdade de Ciências,
Universidade do Porto, Rua do Campo Alegre,
4169-007 Porto, Portugal
e-mail: ruthp@ua.pt; ruth.pereira@fc.up.pt

R. Pereira
Centro de Estudos do Ambiente e do Mar (CESAM),
Universidade de Aveiro, Aveiro, Portugal

S. Barbosa
Empresa de Desenvolvimento Mineiro (EDM), S.A.,
Lisbon, Portugal

F. P. Carvalho
Instituto Tecnológico e Nuclear, Instituto Superior
Técnico (ITN/IST), Universidade Técnica de Lisboa,
Lisbon, Portugal

of the twentieth century, after Peter and Marie Curie have discovered radium, one of the daughter radionuclides of the uranium decay series and its potential application in cancer treatment, the demand for uranium ore, as well as its market price increased exponentially. In the early twentieth century, most radium was supplied by uranium mines in Europe. With new radioactive ore deposits discovered in United States, Australia, the Belgian Congo, and Canada, the production of radium increased and its price became affordable for cancer treatment in hospitals worldwide (Carvalho 2011). Following, the German chemist Otto Hahn has successfully demonstrated the first nuclear fission, in 1938, and especially after the Second World War, a new demand peak for uranium (U) was driven by military applications. This second peak, to fulfill the needs of opposite potencies for building up its nuclear arsenal, lasted till the end of Cold War, in 1991. The uranium demand was also strengthened when the first commercial nuclear power plant for electricity generation—Calder Halll—came into operation in 1956, in Windscale, England. The oil crisis in 1973, created by the oil embargo of the Organization of Petroleum Exporting Countries (OPEC), has lead countries like France and Japan to make large investments in nuclear energy production. Nevertheless, the Chernobyl accident, in 1986, has caused a long and stable low uranium price period. Between the 80s and 2000, due to a slowdown in the growth rate of the nuclear installed capacity and, after the Cold War, the consumption of uranium has decelerated (Kahouli 2011). Nowadays, while we are dealing with the legacies of past uranium mining, the world is facing a new peak in uranium demand mainly caused by both energy requirements of economic development and concerns with global warming and subsequent climate changes. In the next 50 years, the nuclear power is expected to remain an important part of the energy mix worldwide (IAAE 2001). In this scenario, developed countries have renewed their interest on the development of nuclear power plants, to diminish their dependency from fossil fuels and to maintain their economic development without increasing greenhouse gas emissions. In fact, although uranium mining, transport, and enrichment are responsible for carbon dioxide (CO_2) emissions, the nuclear power plants have no emissions becoming, from this viewpoint, much more competitive than fossil fuel energy sources (Kleiner 2008). A question that stills persist is if uranium resources will sustain the requirement of these power plants, and if the mining and the extended treatment of low-grade ores, *per si*, will not increase substantially CO_2 emissions (Kleiner 2008). This subject is particularly meaningful since in the future, an increase in primary uranium supply (mining) is expected to occur to fulfill uranium demand (IAEA 2001).

In Portugal, uranium exploration lasted for almost one century, after the first deposit discovery in 1907, in Barracão, a village near Guarda city (Center of Portugal) (Lierre and Pio Leite 1934). The different nature of uranium ore beds, within the uranium metallogenetic province of Central Iberia determined different exploration methods varying from open pit to underground mining, usually followed by in situ and/or heap leaching of low-grade ore with sulfuric acid (Nero et al. 2003). Within the national territory, several ore beds were explored by a French Society (L' Urane, Urban, Feige et Companie, later named Société Urane-Radium), mainly in Viseu and Guarda districts in the Center of Portugal (Lierre and Pio Leite 1934). By then, the element of interest was radium, while uranium was a by-product. Ten grams of radium were obtained in these two decades. The Urgeiriça deposit (near Viseu city), one of the largest in Europe, was already explored in this period. In 1929, the "Companhia Portuguesa de Rádio (CPR), a Portuguese–British" consortium was created and become responsible by the exploration of several uranium deposits, in the national territory, including the Urgeiriça deposit. More 40 g of radium were extracted by CPR, which was exported as radium-barium sulfate to the United Kingdom. Since 1945, the uranium was the element of interest and its exploration was carried out mainly in Urgeiriça and Quinta do Bispo (near Viseu city), but also in other 16 small mines. In 1954, the Portuguese government launched a national organization responsible for nuclear energy, named Junta de Energia Nuclear (JEN). JEN replaced CPR in uranium mining and was in charge of a systematic uranium prospection within the national territory and in several African territories (Oliveira 2002). After 1978, a new company—Empresa Nacional de Urânio (ENU)—has entrusted the exploration and chemical treatment of radioactive ore and operated till 2001. To this State owned company was committed also the responsibility of keeping old mine sites under surveillance and radiological control, according to obligations imposed by legal regulations (Decree no 34/92, 4 of December) and did so until the close out of the last mine and milling facilities in Urgeiriça (Carvalho 2007a, b, c).

The largest uranium mine complexes and milling facilities

As mentioned previously, uranium mining activity was particularly meaningful in the center of Portugal (Fig. 1). The Urgeiriça mine complex, the most important one, was located in the village of Canas de Senhorim (near Viseu), a settlement with about 5,000 inhabitants. During its activity, this mine had six wells following the mineral vein, including the ventilation well. At this mine, the ore was explored till 500 m in depth and 1,600 m horizontally. The exploration was carried out through 19 floors separated by a distance of 40 m, between each two floors. Between 1950 and 1951, the CPR company built a chemical treatment plant in this complex, which occupied a surface area of 4.5 ha and was the first prepared for the production of 125 tons of U_3O_8, later improved for the production of 200 ton. Between 1970 and 1991, underground exploration was carried out until 1973 followed by heap leaching of low-grade ore. For this procedure, the ore was crushed, heaped, and sprayed with sulfuric acid, which percolated through the heap, leaching out uranium. The resulting leaching solution, also called "*pregnant liquor*" was then pumped and passed through a fluidized bed ion exchange resin columns (Cordeiro Santos et al. 1983). Afterward, the resins were sent to the chemical treatment plant for the recovery of uranium. About 4,400 tons of uranium oxide was produced at the chemical treatment plant of the Urgeiriça mine.

Fig. 1 Geographical location of the two main Portuguese districts in terms of radioactive ore exploration (Pereira and Neves 2012)

The wastewaters and the fine grain ore from the chemical treatment plant were then pumped and stored in a dam (Barragem Velha) for solid material's settling. The liquid drainage was transferred to a water pond (Barragem Nova) where a pH neutralization treatment was carried out with addition of both lime and barium chloride. This generated sludge contained radionuclides precipitated from wastewater where concentrations of uranium, radium, and lead attained 50 kBq kg^{-1} (Carvalho et al. 2007a, 2011). The neutralized water was released into a local stream (Ribeira da Pantanha), and the sludge was dumped in evaporation ponds near the Barragem Nova dam. This procedure contributed over decades for the contamination of the stream, a tributary to the Mondego River, one of the main Portuguese rivers. High concentrations of ^{238}U and of $^{238}U/^{232}Th$ ratios were recorded both at the milling tailings, stored in the Urgeiriça mine complex and in the sediments of the stream (Carvalho et al. 2007b).

At the end of the 1990s decade, when the mining and milling activity has stopped, about 80,000 m^3 of tails were dumped, near the main well of the mine, occupying an area of 1.5 ha. A warehouse was built on the top of these wastes to store uranium (yellow cake) before being exported. Two additional waste piles were also found in the area, near other extraction wells, as well as low-grade ore brought from other mines.

The Cunha Baixa uranium mine is located in a small village, with the same name, in the municipality of Mangualde (near Viseu) with an area of about 16 km^2 and 884 inhabitants. The mine surrounded by a pine tree forest and small agricultural fields, explored by local inhabitants, was explored between 1967 and 1991, through both, underground and open pit methods. The underground works were performed between 1970 and 1987, attaining a depth of about 150 m. In 1971, the open pit mining started at two different sites (CAI and CAII) and lasted till 1991. A total amount of about 0.5 Mtons of ore were extracted from this mine, by both methods, corresponding to a production of about 901 tons of U_3O_8 (Nero et al. 2005). In the last years of activity, in situ leaching was carried out in underground tunnels, as well as heap leaching of low-grade ore at CAI. The liquors were pumped from the first floor of the mine, the uranium recovered, by the method previously described, and the resins sent to the Urgeiriça chemical treatment plant. About 76 tons of uranium was produced by heap leaching at this mine, by treating 400,000 tons of low-grade ore from other mines in the region.

Even after the activity has ceased, the production of an acidic mine drainage has persisted contaminating groundwater and surfacing with the uprising of the water table (Fig. 2). This effluent water with a pH of about 3 was also characterized by high concentrations of ^{238}U and ^{226}Ra (2,200 and 84 mBq L^{-1}, respectively), as well as of other metals like beryllium, zinc, manganese, iron, and cobalt (Antunes et al. 2007a; Carvalho et al. 2009). To mitigate the problem, first ENU and then EXMIN (a company incorporated in the EDM holding) were responsible by the treatment of this effluent, which was carried out in a local treatment plant, including again the water pH neutralization and precipitation of radionuclides (Fig. 3). The treated effluent was then discharged into a small stream, passing through the village, which joins the Castelo River, also a tributary of the Mondego River. The sludge from the effluent treatment pond, rich in metals and radionuclides, was spread in the area at different sites (Figs. 4, 5). The Quinta do Bispo uranium mine is located 2 km west of the Cunha Baixa mine, also in the drainage basin of the Castelo River (Fig. 6). This ore bed was discovered in 1957 and the exploration by open pit started in 1979 and lasted for 8 years. This exploration occupied a surface area of 158,000 m^2 and attained a depth of 75 m. About 460,000 tons of ore were extracted from this mine, which was first sent to Urgeiriça for treatment by heap leaching. Between 1987 and 1992, a significant concentration of uranium was found in the water accumulated in the open pit, justifying the local installation of facilities for ionic exchange. After 1992, the poor ore was treated in situ, by heap leaching with acidic waters, within the open pit. For that purpose, the water was pumped from the pit, its bottom was sealed with clay, and wells were constructed to collect the liquors. The liquors were locally treated, and the acidic waters, recycled from the ionic exchange resins, were sent back to the pit, through a closed pipe system. After the activity has finished, the treatment of the effluent water was minimal, and it was released in the local stream with a low pH and high concentrations of radionuclides. The discharges from both mines, Quinta do Bispo and Cunha Baixa, were thus the source of radioactive

Fig. 2 Cunha Baixa uranium mine, aspect of the effluent pond (Pereira et al. 2012)

Fig. 3 Cunha baixa uranium mine: aspects of the effluent treatment pond (Pereira et al. 2012)

Figs. 4 and 5 Cunha Baixa uranium mine: aspects of the sludge removed from the effluent treatment pond and deposition sites (Pereira et al. 2012)

Fig. 6 Quinta do Bispo uranium mine: aspect of the open pit filled with effluent

contamination of Castelo River. Uranium concentrations measured many years after cessation of uranium mining were still enhanced by a factor of 4 in particulate matter carried out by the river and by a factor of 14 in the soluble phase. Concentrations of radionuclides, such as ^{226}Ra, ^{210}Po, ^{230}Th, ^{210}Pb, and ^{232}Th in the particulate matter, were also increased by the confluence of streams passing near both mines with the Castelo River (Carvalho et al. 2009a).These uranium mines, located in the center of Portugal, were the sources of radioactive discharges into several main rivers, especially the Mondego river, but also Vouga, Dão and Távora rivers. High levels of radioactivity and high concentrations of radium were recorded in the sediments and water of these rivers (Carvalho et al. 2007b, 2009a, b, c).

Impacts on human health and ecosystems

During almost one century of radioactive ore exploration, about 13 millions of tons of radioactive wastes were left in mining sites (Nero et al. 2003), including (1) mine tails; (2) low-grade ore, and (3) metal's and radionuclide's rich sludge. Such sludge was kept within mining areas contributing for the contamination of soils and introduction of contaminants in the terrestrial compartment and food chain (Carvalho et al. 2007a; Pereira et al. 2008).

After several radiological surveys carried out in sixty old mine sites by Carvalho et al. (2007a), it was possible to perceive that the majority of these sites, within the national territory, do not have high radioactive materials stocked, since they were dedicated to open pit extraction, being the ore transported to big complexes like the ones in Barracão (near Guarda) and Urgeiriça for the chemical extraction of radium and uranium, respectively. In some other mines, such as Quinta do Bispo and Cunha Baixa and Bica (near Sabugal), low-grade ore was also received for heap leaching with sulfuric acid generating mill tailings. The situation was particularly concerning in the bigger mine complexes, where waste heaps displaying beta–gamma radiation dose rates, up to 20 μSv h^{-1}, were found. When compared with radiation background of nearby reference areas (0.1–0.2 μSv h^{-1}), these levels were particularly worrisome due to the proximity of human populations. The spread of radionuclide's rich sludge, in these areas, also contributed for their hazard. The leaching of sludge and waste piles by rainwater was in fact the main source of radionuclides (especially ^{226}Ra), recorded in local surface streams converging to the Mondego River (Carvalho et al. 2007a, b).

The recognition of the risks posed by old mines lead the Portuguese government to assume, through the publication of the law by decree no 198/A, 2001 (ME 2001), that the rehabilitation of these areas was a question of public interest. The EDM company (Empresa de Desenvolvimento Mineiro, SA), a State holding company for the mine sector, was mandated for rehabilitation and monitorization works. A new dispatch, in 2002, classified the uranium mines previously described as prior areas for intervention (ME and MAOT 2002).

In the same year, and taking into consideration emerging concerns of local populations, caused by the death of former uranium mine workers by cancer, the parliament approved a recommendation for the government, suggesting the adoption of measures to prevent public access to uranium mines, to monitor the quality of underground waters and mine soils, and to submit local populations to an epidemiological study aimed in evaluating the health risks (AR 2001). Based on this recommendation, three public institutes [Instituto Superior de Saúde Dr. Ricardo Jorge (INSA), Instituto Tecnológico e Nuclear (ITN) and Instituto Nacional de Engenharia Tecnologia e Inovação (INETI)], the regional health authority and the Hospital of Viseu city, carried out a study aimed at assessing public health problems in human populations living near uranium mines. The population of Canas de Senhorim, living near the Urgeiriça mine, was targeted by this study and compared with non-exposed populations. Hair samples were collected to assess individual's exposures to internal radiation, through the analysis of ^{210}Po and ^{210}Pb. Additionally, and aimed at assessing exposure–effect relationships, the same individuals were sampled for peripheral blood to assess hematological parameters, thyroid hormones (thyroxin—T4 and thyroid stimulating hormones—TSH) and male sexual hormones (testosterone, follicle stimulating hormone FSH, and inhibin B) in the serum, and the frequency of chromosome abnormalities in lymphocytes. According to the results of this study, published in a two Reports, MINURAR I and MINURAR II (Falcão et al. 2005a, b, 2007; Carvalho et al. 2007c), the rate of ambient gamma radiation doses, the concentration of radionuclides in agricultural field soils, vegetables, water for human consumption and in atmospheric dusts measured in that county were similar to those recorded in areas inhabited by control groups. Further, the study concluded that the mining and milling wastes were mostly concentrated in the mining complex, being little dispersed in the surrounding environment. Radionuclide concentrations were only higher in some agricultural plots due to surface runoff and mixture of tailings materials with soils. It was also found that the main radionuclide transfer pathway was surface runoff and percolation of water trough mining wastes. Hence, main exposure sources for human populations were water from wells and small fountains. Dust transport by wind and exhalation of radon emitted from tailings were additional pathways identified (Falcão et al. 2005a, b). The exposure to gamma radiation was also slightly higher for the population of Canas de Senhorim than for the control groups, as well as the concentration of radon recorded in the outdoor air, in some points of the village located at the south of the mine complex (Falcão et al. 2005a, b). The levels of ^{210}Po in hair samples showed that individuals living near the Urgeiriça mine accumulated in the organism, probably in the bones, radionuclides precursors of ^{210}Po (e.g., ^{226}Ra and ^{210}Pb). Concomitantly, a slightly higher frequency of chromosomal abnormalities was recorded in the exposed group, as well as signs of impaired thyroid and reproduction functions (Falcão et al. 2007; Carvalho et al. 2007c). The hematological parameters were also compromised in the inhabitants of Canas de Senhorim, in particularly, the creatinine levels in serum, which were higher in this group, suggesting that the renal function of the individuals could have been diminished.

Phytotoxic assays with *Zea mays* (maize) and *Lactuca sativa* (lettuce), performed to evaluate soils from Cunha Baixa uranium mine area, have shown the ability of these species to accumulate high concentrations of metals. High biological accumulation factors (BAFs) were recorded for lettuce, namely for Co (16.5), Cu (28.5), Ba (7.3), Sr (7.2), and Ni (6.1) (Pereira et al. 2009), suggesting that this species and probably other vegetables and fruits growing in agricultural field soils, near the mine, could be an exposure pathway of humans. This finding was later confirmed by Neves et al. (2012a, b), who found high concentrations of uranium in leaf vegetables like lettuce (5,373 μg kg^{-1}), cabbage (255 μgkg^{-1}), and potato with peel (589 μg kg^{-1}). This last foodstuff also displayed high concentrations of aluminum and manganese when growing in fields in the vicinity of the Cunha Baixa mine and irrigated with water from private wells, with a pH range 4.2–6.2 and with average concentrations of uranium, aluminum, and manganese of 1,035, 7,750 and 4,520 μg L^{-1}, respectively. However, and based on metal concentrations of the edible parts of the plants analyzed, no lifetime risks, for non-cancer effects, were recorded for local inhabitants (children included). Accumulation of uranium family radionuclides by lettuce and potatoes was further investigated. Near the mine site, the contribution of irrigation water was found more relevant than that from the soil, especially for radium taken up by plants (Carvalho et al. 2009c). High concentrations of uranium and of ^{238}U series radionuclides, mainly ^{226}Ra, were also determined in the leaves of lettuces growing in house gardens near the Urgeiriça mine and irrigated with water from Ribeira da Pantanha, which received the discharges from the mine wastewater treatment plant (Carvalho et al. 2009b). The radionuclides such as uranium, radium, radon, polonium, and thorium primarily emit alpha particles which loose energy rapidly with distance having a low penetrating power (ASTDR 2011). However, they are of the highest concern after ingestion and/or inhalation, since once in the body they can release directly alpha particles in the cells, damaging their components. Other studies, carried out in areas with naturally occurring uranium geochemical anomalies, within the Portuguese Iberian Massif, have also observed the bioaccumulation of ^{226}Ra in broadleaf plants (cabbage maximum values: 720 Bq kg^{-1}) but have concluded that the inhalation of radon decay products have a greater contribution than food and drink pathways for effective human exposure doses (Pereira and Neves 2012).

As previously mentioned, Neves and Matias (2009) attributed the contamination of soils in the Cunha Baixa surrounding fields to the use of groundwater from private wells for irrigation. The groundwater collected 1 km downstream from the mine had low pH values (lower than 4.5–5,0), high levels of electrical conductivity, total dissolved solids, SO_4, F, Ca, Mg, Al, Mn, Ni, U, Zn, and ^{226}Ra. The values for some of these parameters exceeded the quality limits established for water for irrigation purposes and even more for the production of water for human consumption, according to Portuguese—law by decree 236/98 (MA 1998). The situation was particularly concerning in the summer, when the levels of SO_4, Al, Mn, and U were 50–120 times higher than acceptable levels. According to the results of Pereira et al. (2008), the contamination of groundwater, at the Cunha Baixa mine site, was probably caused not only by past heap leaching activities, but also by percolation of rainwater through contaminated soils and sludge, since high concentrations of some of these metals namely U, Al, and Mn were recorded in extracts of mine soils contaminated with sludge, obtained with artificial rainwater.

Although, through a unique or a combination of exposure pathways, significantly high levels of uranium and manganese, and significantly low levels of zinc, were found in the peripheral blood of adults living near the Cunha Baixa uranium mines, in comparison with a control group (Lourenço et al. 2013a). In parallel, a significant decrease in DNA integrity and in DNA content of white blood cells, was detected by the comet assay and flow cytometry, respectively, in individuals aged between 40 and 60 years, and older than 60 years. The individuals with less than 40 years showed a significant decrease in NK and T lymphocytes, giving an indication of potential damages in hematopoietic organs, responsible by the production of immune cells. In the opinion of these authors, the results recorded for the different cellular biomarkers were probably related both with the exposure to uranium and manganese, as well as due to the depletion of zinc in their bodies. Uranium and manganese can both induce DNA damages trough different mechanisms of action namely through the formation and accumulation of reactive oxygen species (ROS), the formation of DNA-adducts and the modification of the activity of DNA polymerase giving rise to DNA damages, through mutations (e.g., Gerber et al. 2002; Stearns et al. 2005). On the other hand, zinc, has a crucial role in the development and maintenance of the immune system, acting as an anti-inflammatory agent and as an antioxidant. This metal protects cells against free radical attack and reduces free radical formation. Since both oxidative stress and chronic inflammations may be responsible by other chronic diseases like cancer and auto-immune diseases (Jomova and Valko 2011), deficiency in zinc and the exposure to uranium and manganese, are probably increasing the susceptibility of Cunha Baixa residents.

In parallel, studies were carried out at the Cunha Baixa, under the scope of research projects aimed in making an ecological risk assessment for the area and in finding new genotoxic endpoints to assess exposures to uranium, radionuclides and ionizing radiation in bioindicator species, respectively.[1] The former project, mainly focused in metal contamination has shown the high sub-lethal toxicity of soils, wastewaters and pond sediments, found in the Cunha Baixa uranium mine, for a battery of freshwater and terrestrial species (Antunes et al. 2007a, b, c; Antunes et al. 2008; Pereira et al. 2009; Marques et al. 2008). Within other aspects it was possible to perceive that the chemical treatment was not effective in the elimination of the toxicity of the mine effluent, since almost all the reproduction parameters tested on two species of cladocerans (*Daphnia magna* and *D. longispina*) were compromised when these organisms were exposed to non-diluted treated effluent (Antunes et al. 2007c). Further, oxidative stress was also recorded in amphibians larvae, exposed in situ, in the effluent treatment pond (Marques et al. 2013a, b). Although, such toxicity could be eliminated by dilution after joining to the receiving freshwater stream, the toxicity for other species as well as potential cumulative effects in natural freshwater communities was little assessed. In fact, Antunes et al. (2007c) have observed that the treatment was not completely effective in removing metals like uranium and manganese, but no information exists for radionuclides in the treated effluent. However, this was probably an explanation for the high radionuclide concentrations found in fish species like barbell (*Barbus bocagei* Steindachner) and nase (*Chondrostoma polylepis* Steindachner) captured in the mid-section of the Mondego River, after the convergence of small streams which have received these treated waters (Ribeira da Pantanha and Rio Castelo) (Carvalho et al. 2007b).

Green frogs (*Pelophylax perezi* previously *Rana perezi*), inhabiting the effluent pond in the Cunha Baixa mine area, displayed significant high levels of Be, Al, Mn, Fe and U in the liver, as well as Pb and U in the kidney, when compared with frogs from a reference area (Marques et al. 2009). The liver was the main target organ for the bioaccumulation of Be, Al, Fe and U. However, the kidneys were more affected, showing dilatation of the lumen of renal tubules and tubular necrosis. Based on previous studies, the authors have explained these effects with the role of kidneys in the excretion of uranium as bicarbonate complex, followed by the reabsorption of the bicarbonate and the release of free uranyl ions. Melanomacrophagic nuclei were also observed in the liver and lungs of frogs living in the Cunha Baixa mine area (Marques et al. 2009). The development of these cells, rich in pigments like melanin, usually occurs as an antioxidant response, since melanin can act as scavenger of free oxygen radicals (ROS). The generation of ROS is one of the cellular mechanisms of toxicity of metals. The exposure to the mine effluent was also suggested as the most likely cause of the high incidence of abnormalities observed in the nucleus of erythrocytes of frogs living in the mine. Other study from the same authors showed that the animals were also under oxidative stress, due to the exposure to metals and radionuclides (Marques et al. 2011) and the lungs were the most compromised organs. The activities of enzymes, involved in the first line of defense against oxidative stress in the cells (catalase—CAT and glutathione reductase—Gred) were significantly inhibited in the lungs of animals captured in the mine area. However, it was also evident that an alternative pathway was activated in the cells, by increasing the activity of another enzyme, the glutathione peroxidase (GPx), which can replace catalase in its function. This was probably one of the mechanisms responsible for preventing the oxidative stress in the other organs of these animals, to levels compatible with their survivorship in the mine effluent, being also an explanation for the absence of lipid peroxidation in the cells of the different organs analyzed (Marques et al. 2011). A study focusing on genes expression profiles by suppressive subtractive hybridization (SSH), on the liver of the same animals found a potential explanation for this fact, recording the up-regulation of genes coding for proteins, some of them from the blood plasma (fibrinogen, hemoglobin and albumin) which within other functions may act as metal scavengers and as antioxidant agents (Marques et al. 2013a). These proteins could have replaced, the anti-oxidative stress enzymes (catalase, Gred and GPx), previously mentioned, giving rise to an alternative response mechanism to chemical stress, and preventing lipid peroxidation, despite the bioaccumulation of metals in the tissues.

---

[1] Projects funded by Foundation for Science and Technology (FCT, Portugal) namely the UraniumRisk (POCTI/AMB/60899/2004) and the project ENGENUR (PTDC/AAC-AMB/114057/2009) (still in progress).

As far as soils are considered, they found to be contaminated with a complex mixture of metals, surpassing different international soil screening values when total concentrations were considered (Pereira et al. 2008). Nevertheless, uranium and aluminum were the elements more prompt to be mobilized to the soils aqueous phase and to become bioavailable. In situ measurements, have shown that the feeding activity of the edaphic fauna, evaluated by the bait lamina assay, was compromised, especially in the soils next the open pit mine, were heap leaching was performed, and at sites were the sludge from the effluent treatment pond was dumped (André et al. 2009). Further, the impairment of the soil microbial community was also recorded through the evaluation of soil enzyme activities and by the analysis of other biomarkers of the metabolic active components of the soil microbial community, namely phospholipid fatty acids (FLPA) and ergosterol, extracted from soil samples collected on the same sites (Antunes et al. 2011; Guedes et al. 2011). The profiles of PFLA recorded for soils from the Cunha Baixa mining area, with different degrees of contamination were completely different, as evaluated using multivariate statistical analysis. The most contaminated soils were characterized by the dominance of PFLA from gram-negative bacteria (Guedes et al. 2011). These bacteria have shown to have high tolerance to stress conditions, due to the presence of cyclo fatty acids in their outer cell membrane, their lipopolysaccharide layer and to their fast-growing ability (Hinojosa et al. 2005, 2010). The dominance of cyclopropyl fatty acids (cyC17:0, cy19:0) was in fact confirmed in the most contaminated soils. The cyclopropane ring of these fatty acids restricts the mobility of the PLFA and reduces the impact of environmental stressors in the fluidity of the membranes (Hinojosa et al. 2005, 2010). However, their dominance can indicate both changes in the composition of the membranes and/or in the soil microbial community (Frostegård et al. 2010). The lipopolysaccharide and the peptidoglycan layers of gram-negative bacteria are also able to immobilize many metal ion species, preventing the disturbances in the integrity of the membranes and thus protecting the cells from these contaminants (Beveridge and Koval 1981). Considering the role of the edaphic fauna and of the soil microbial community in the mineralization of organic debris, one of the most important soil functions, these results unequivocally confirm that this basic function of soils was likely seriously compromised in this area by the contamination with acid, metals and radionuclides.

Soils from Cunha Baixa uranium mine area have also demonstrated to be toxic and to have their habitat function compromised for invertebrates and plants since effects on different endpoints were recorded after short- and long-term exposures of the earthworm *Eisenia andrei* to these soils, carried out both in laboratory and *in situ* (Antunes et al. 2008; Pereira et al. 2009; Lourenço et al. 2011a, b, Lourenço et al. 2012; Lourenço et al. 2013c). Endpoints at different levels of biological organization (molecular, cellular, tissues, individual, and population level) were seriously impaired in earthworms exposed to soils from the mine area. Hence, in a multibiomarker approach, the organisms displayed DNA damages and changes in the total content of DNA in coelomocyte cells and impairment of the immune system revealed by changes in the frequency of the different types of coelomocytes, (Lourenço et al. 2011a); histopathological changes both in the epidermis, circular and longitudinal muscles, chloragogenous tissue and in the intestinal epithelium (Lourenço et al. 2011b), loss of body biomass, and no production of juveniles (Lourenço et al. 2011a, b, 2012). Although, it was impossible to discriminate if the effects observed were promoted by chemical and/or radiological activity, since both metals and radionuclides were bioaccumulated by earthworms (at high concentrations during *in situ* exposures) (Lourenço et al. 2011a, 2012), the comparison between field and laboratorial exposures have suggested that the exposure to external radiation had no meaning for some of the effects observed in *E. andrei*, because they were similar under both exposure conditions (Lourenço et al. 2010, 2011a). The evaluation of gene expression profile by SSH of *E. andrei* earthworms exposed in situ have also demonstrated that after long-term exposures to contaminated soils, the organisms lost their ability to respond to oxidative stress due to changes in the regulation of genes involved in these mechanisms (Lourenço et al. 2012). This work has improved the understanding of the previous effects observed for the species, at the individual level, as well as to perceive the mechanisms of toxicity of the contaminants found in the wastes of uranium mines. The bioaccumulation of metals and radionuclides by *E. andrei* also gave rise to concerns about potential transferences along food webs, within areas contaminated by radiological material. The sensitivity of invertebrate species to this kind of contamination was probably an explanation for the lower feeding activity of invertebrates recorded in situ, in the mining area, as previously mentioned (André et al. 2009). However, no community level effects in terms of structural biodiversity were recorded for the edaphic fauna of these soils (Antunes et al. 2013). The authors suggested the recent abandonment of the area, as a reason for the homogenization of sites under evaluation. However, it is also possible that the taxonomic identification performed only till the family has masked potential differences in terms of species composition between contaminated and non-contaminated soils. In studies reviewed by Geras′kin et al. (2007), earthworms were in fact the invertebrates showing the most noticeable differences between reference and uranium-radium contaminated sites, from the North of Russia, contributing for a reduction in population size and biodiversity at these sites. Histopathological changes and impaired fecundity were some of the effects observed on earthworms. In opposition, fewer impacts were observed for active-moving arthropods like beetles, spiders, and mites.

The bioaccumulation of metals like Cd especially in the kidney and U, both in the kidney and in the bone was also recorded in European wood mice captured in the Cunha Baixa mine area (Lourenço et al. 2013b). Concomitantly, the animals showed a significant loss of DNA integrity in blood cells, which could have been caused by both exposure to metals, as well as to alpha-emitting radionuclides found in the area (Carvalho et al. 2009). These animals have also displayed an increased expression of a tumor suppressor gene (*P53*) in the liver and in opposition, mutations in another tumor suppressor gene (*Rb*), but this time in the kidney (Lourenço et al. 2013b). Suggesting that the exposure to metals has triggered a defense mechanism in the liver of the animals, but in kidney, it was probably responsible by the inactivation of that mechanism due to damages in the DNA of the cells. The same animals were evaluated for male reproductive parameters [sperm quality (sperm concentration, motility, viability, and morphology), histology of testis and epididymis], and despite a slight increase in the diameter of the seminiferous tubules, in animals captured in the mining area, any other effect was recorded for the tissues analyzed and in sperm quality, suggesting no impairments in the reproductive system of the animals (Marado 2010). These results were not coincident with those reported by Geras′kin et al. (2007) who have observed inhibition of spermatogenesis, caused by a drastic reduction in gonad weight and pathologies in the seminiferous tubules in tundra voles captured in a radium-uranium contaminated site. Nevertheless, these authors suggested the possible existence of several physiological and compensatory mechanisms in this rodent species that could be able to counteract the effects of radionuclides and radiation, maintaining a healthy and fertile population.

In summary, the effects reported on this section and summarized in Table 1 were consistent among different species, and several of them could be used as bioindicator species for these areas.

Environmental remediation

In 2001, the environmental remediation was decided by the Government and entrusted to EXMIN, one of the companies of the EDM holding. As establish by a decree-law published in that year, the remediation works to be carried out should ensure: (1) confinement of radioactive wastes; (2) protection of major rivers and water resources; (3) prevention of radioactive and contaminated materials dispersion from mines and milling facilities; (4) abatement of radiological and chemical risks to human populations and ecosystems; (5) landscape improvement; (6) minimization of the physical risks posed by tunnels, holes, cavities, old mine structures, and buildings; (7) promotion of the industrial architectonic patrimony and new uses for these areas.

After the definition of the objectives of the remediation programs, their implementation started in 2003. Urgeiriça and Cunha Baixa were within the first seven prior areas for intervention, being Urgeiriça in the top of the list (DGEG and EDM 2011). To attain the objectives previously defined, in the Urgeriça mine area, the biggest complex in the country, the process started with the accumulation of all radioactive wastes, from this complex and from another contaminated area, in the "Barragem Vellha" dam which was receiving wastes from the leaching process since 1988. A total amount of 1,600,000 m^3 of extremely heterogeneous wastes were dumped and sealed with clay, screens, geotextiles, gravel, and finally humus. These layers have reduced radiation to background levels,

Table 1 Summary of the main exposure signs and effects recorded on humans and biota (different levels of biological organization) exposed to uranium mine wastes and contaminated water's

	Exposure	Effects/physiological responses	
Freshwater aquatic species			
Freshwater green algae (*Pseudokircheneriella subcapitata*)		Growth inhibition promoted by effluent water at concentrations of (EC_{50}: 27.0, 95 % CI 25.5–28.4 %)	Antunes et al. (2007c)
Primary consumer cladocerans (*Daphnia magna and D. longispina*)		Mortality *D. magna* ($EC_{50} = 35.8$; 95 % CI 26.4–50.8 %); *D. longispina* ($EC_{50} = 20.5$; 95 % CI 17.0–24.3 %); *D.* magna—reduction in offspring, number of broods and intrinsic rate of reproduction for effluent concentrations lower than 30.4 %	Antunes et al. (2007a, b)
Benthic invertebrates (*Chironomus riparius*)		100 % mortality in larvae exposed mine effluent as overlying water	Antunes et al. (2007a)
Green frogs (*Pelophylax perezi*)	High levels of Be, Al, Mn, Fe and U in the liver of adults; High levels of Pb and U in the kidney of adults	Histopathological changes in the kidney (dilatation of the lumen of renal tubules and tubular necrosis) Hyperplasia in the alveolar epithelium; discrete thickening of alveolar septa and slight hypoplasia of goblet cells High density of melanomacrophagic nuclei in the liver and lung tissues High incidence of nuclear abnormalities in erythrocytes and lower immature erythrocyte frequency	Marques et al. (2009)
		Oxidative stress in lung tissue (inhibition of catalase and glutathione reductase activity) Increment in the activity of glutathione peroxidase (GP_x and selenium-dependent GP_X)—potential defense mechanism	Marques et al. (2011)
		Adults: up-regulation of genes coding for proteins like fibrinogen, hemoglobin and albumin and L7a protein—potential antioxidant defense mechanism	Marques et al. (2013a, b)
		Tadpoles: growth inhibition; Tail abnormalities; Delayed responses to mechanical stimulus	Marques et al. (2008)
	High levels of Be, Mg, Mn, Al, Co, Ni, Ba and U in the whole body tissues	Tadpoles: about 40 % mortality caused by exposure to the effluent water; Increased GPX activity after exposure to treated effluent	Marques et al. (2013a, b)

Table 1 continued

	Exposure	Effects/physiological responses	
Fish	High levels of radionuclides (^{238}U, ^{226}Ra and ^{210}Po) in *Barbus bocagei* and *Chondrostoma polylepis* captured in the Mondego River, downstream uranium mine areas		Carvalho et al. (2007a, b, c)
Terrestrial species			
Terrestrial plants (*Zea mays* and *Lactuca sativa*)	High bioaccumulation of Co, Cu, Ba, Sr and Ni by lettuce; High bioaccumulation of Ni and Cu by maize	Seed germination and growth inhibition	Pereira et al. (2009)
Earthworms (*Eisenia andrei*)		Avoidance of soils contaminated with mine wastes	Antunes et al. (2008)
		DNA damages and changes in the total DNA content of coelomocyte cells	Lourenço et al. (2011a)
		Immune system impairment (high frequency of eleocyte cells and low frequency of amebocytes/granulocytes)	
		Histopathological changes in epidermis, circular and longitudinal muscles, chloragogenous tissue and intestinal epithelium	Lourenço et al. (2011b)
		Complete inhibition of reproduction	Lourenço et al. (2011a,b) and (2012)
		Loss of body biomass (28 days of exposure)	
		After 14 and/or 56 days of exposure to mine soils:	Lourenço et al. (2013a)
		Up-regulation of genes coding for proteins involved in calcium homeostasis, energy production, DNA repair, tumor genesis, cell survival, antioxidant defense functions and intracellular iron balance	
		Down-regulation of genes coding for proteins involved in ATP synthesis, repair of DNA strand breaks, activation of cellular responses to DNA damage	
		Up–regulation of an EST with homology for a SET oncogene	

Table 1 continued

	Exposure	Effects/physiological responses	
Wood mouse (*Apodemus sylvaticus*)	High levels of cadmium in the kidney and of uranium in the kidney and bone	Loss of DNA integrity in blood cells Up-regulation of the tumor suppressor gene *p 53*	Lourenço et al. (2013b)
Ecosystem diversity and functions			
Soil microbial community composition		Dominance of gram-negative bacteria in contaminated cells Impairment of soil fungus community (low levels of ergosterol on soil samples contaminated with mine wastes) Different PLFA profiles in most contaminated soils	Guedes et al (2011)
Soil microbial activity		Inhibition of soil enzymes activity (dehydrogenase, urease, arylsulphatase, cellulose and acid phosphatase)	Antunes et al. (2011)
Soil macrofauna activity		Inhibition of invertebrates feeding activity measured by bait lamina assay	André et al. (2009)
Organic matter decomposition		Organic matter decomposition impairment at the most contaminated soils (litter bags assay). Biomass loss percentages of about 40 % after 1 year of exposure	Unpublished data
Humans			
	High levels of Mn and U in the blood High levels of ^{210}Po in the scalp hair.	Decrease in DNA integrity and content in white blood cells of individuals older than 40 years living near the uranium mines Decreased number of NK cells and T lymphocytes in individuals with less than 40 years made them more susceptible to infections and cancer High frequency of chromosomal abnormalities High creatinine levels in blood serum Signs of impaired thyroid and reproduction functions	Lourenço et al. (2013a) Falcão et al. (2005a, b, 2007)

Fig. 7 *Urgeiriça mine* poster describing to the general population the materials and the steps followed to seal the pile of radioactive wastes in the Barragem Velha dam (Pereira et al. 2012)

atmospheric dispersion of fine material, and the percolation of rainwater through the contaminated materials (Figs. 7, 8). Before the remediation works, the surface radiometry and the external radiation recorded attained 15,000 cps (7.5 μGy h^{-1}) in some points in the pile. But, the values were reduced for 300cps (0.35 μGy h^{-1}), in all the surface of the pile, after the remedial works have been accomplished in 2008 (DGEG and EDM 2011). In parallel, the natural watercourse of surface waters was diverged to prevent the contact with the radioactive wastes and its subsequent contamination, and new chemical treatment plants were constructed to treat effluent waters generated during the remedial works. Piezometers were installed to monitor the level o subsurface waters and to assess their chemical quality. Further, six stations were created, in different points of the pile, where probes were installed to attain different layers of the sealing material and to control radon concentrations/activity (DGEG and EDM 2011). In this mine area, several efforts were also done to rehabilitate several buildings and facilities, of the biggest national industrial complex dedicated to extraction of uranium and radio salts and whose economic, social, and historic importance could not be forgotten.

Fig. 8 *Urgeiriça mine* the pile of wastes in the Barragem Velha, after rehabilitation works

Fig. 9 *Cunha Baixa uranium mine* a dosimeters and dust meter placed in the terrace of an house located nearby the mine during rehabilitation works

In the Cunha Baixa uranium mine, the former open pit was used for the deposition of dangerous wastes, after stabilization of slopes and after the bottom has been sealed with clay. The construction of ponds and of a drainage system is in progress. All of these works, still ongoing, could be followed in website of the EDM—Empresa de Desenvolvimento Mineiro, S.A. (http://www.edm.pt/html/bemvindo.htm). The remediation project in the Cunha Baixa mine area also involve the remediation of contaminated soils near the old mine, especially those used for agriculture purposes by local inhabitants. The amendment of soils with organic matter (manure) was the strategy followed by EDM to reduce the bioavailability of contaminants. However, the long-term efficiency of such measure is unknown. In addition, the company is constructing a dam to store rainwater in order to provide irrigation water to local inhabitants, allowing to phase out the use of private wells with acidic and radioactive water contaminated by seepage from the old Cunha Baixa mine. During the remedial works, in this area and due to concerns with the impacts of waste mobilization, dosimeters and dust meters (Fig. 9) were installed near the houses to monitor: (1) radon gas fluxes, between soil and the atmosphere; (2) the potential alpha energy of the progeny of radon (^{222}Rn) and of thorium (^{220}Rn); and the concentration of long-life radionuclides of the decay series of uranium and thorium in the dust particles (DGEG and EDM 2011).

In Quinta do Bispo mine, remediation works did not start yet and this will probably be the last old uranium mine to be intervened in the Portuguese territory (EDM, personal communication). In the meantime, the access of public to this mine area is not allowed, and a chemical treatment plant is running to treat the acid mine drainage before releasing it in nearby watercourses.

Conclusions

The exploration of uranium from primary sources is expected to increase in the near future; however, the risks to humans and ecosystems and the costs involved in the mitigation of these risks are frequently forgotten. In fact, the risks caused by the exposures to radiological agents are more easily associated with disasters in nuclear power plants, which are acute and less frequent, while direct and indirect (e.g., through the loss of ecosystem services) risks associated with long-lasting exposures of populations in uranium mining areas are frequently minimized and not included in debates about the transition for energy supplied by nuclear power plants. In Portugal, the great number of abandoned uranium mines and the perception of risks by human populations have driven the authorities to carry out remedial works in the areas of higher concern. Although not finished yet, we may consider that the environmental remediation work implemented shows that the country has been able to successfully deal with the legacies of past uranium mining activity. The primary objectives for physical confinement of radioactive waste are being attained. The abatement of radiation exposure of the populations needs follow-up and confirmation through detailed monitoring programs in the long-term. These programs should include both human health and environmental surveys. The monitoring programs foreseen by the EDM company are based mainly on chemical and radiological parameters, and although fit for the monitoring of chemical and physical waste treatment in place, they do not encompass the assessment of biological effects on the environment. Notwithstanding, ecotoxicological research carried out in these areas during the last few years have demonstrated that there are validated sensitive biological endpoints that could be used to assess the effects on biota caused by exposures to wastes contaminated with metals and radionuclides. This would bring another dimension to the long-term monitoring and ecological risk assessment since only organisms and communities integrate long-lasting exposures to multiple contaminants and express the resultant effects. A consistent body of knowledge about the environmental and human health impacts of these industrial areas was also built in Portugal, under the scope of different research projects, and it is available.

Acknowledgments Foundation for Science and Technology (FCT), Portugal, for the funded research projects ENGENUR (Ref. PTDC/AAC-AMB/114057/2009) and UraniumRisk (Ref. POCI/AMB/60899/2004).

References

André, A., Antunes, S. C., Gonçalves, F., & Pereira, R. (2009). Bait-lamina assay as a tool to assess the effects of metal contamination in the feeding activity of soil invertebrates within a uranium mine area. *Environmental Pollution, 157*, 2368–2377.

Antunes, S. C., Castro, B. B., Moreira, C., Gonçalves, F., & Pereira, R. (2013). Community-level effects in edaphic fauna from an abandoned mining area: Integration with chemical and toxicological lines of evidence. *Ecotoxicology and Environmental Safety, 88*, 65–71.

Antunes, S. C., Castro, B., Pereira, R., & Gonçalves, F. (2008). Contribution for tier I of the ecological risk assessment of Cunha Baixa uranium mine (Central Portugal): II soil ecotoxicological screening. *The Science of the Total Environment, 390*, 387–395.

Antunes, S. C., de Figueiredo, M. D. R., Castro, B. B. S. M., Pereira, R., & Gonçalves, F. (2007a). Evaluation of water column and sediment toxicity from an abandoned uranium mine using a battery of bioassays. *The Science of the Total Environment, 374*, 252–259.

Antunes, S. C., Pereira, R., & Gonçalves, F. (2007b). Evaluation of the potential toxicity (acute and chronic) of sediments from an abandoned uranium mine ponds. *Journal of Soils and Sediments, 7*(6), 368–376.

Antunes, S. C., Pereira, R., & Gonçalves, F. (2007c). Acute and chronic toxicity of effluent water from an abandoned uranium mine. *Archives of Environmental Contamination and Toxicology, 53*(2), 207–213.

Antunes, S. C., Pereira, R., Marques, S. M., Castro, B. B., & Gonçalves, F. (2011). Impaired microbial activity caused by metal pollution: A field study in a deactivated uranium mining area. *The Science of the Total Environment, 410*, 87–95.

AR—Assembleia de República. (2001). *Resolução da Assembleia da República no 34/2001, de 2 de Maio de 2001*. http://siddamb.apambiente.pt/publico/documentoPublico.asp?documento=23703&versao=1. Accessed Nov 2012.

ASTDR—Agency for Toxic Substances & Disease Registry. (2011). *Toxicological profile for uranium. Draft. U.S. Department of Health and Human Services*. Public Health Service and Agency for Toxic Substances and Disease Registry. 452 pp + annexes.http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=440&tid=77. Accessed Nov 2012.

Beveridge, T. J., & Koval, F. (1981). Binding of metals to cell envelopes of Escherichia coli K-12. *Applied and Environmental Microbiology, 42*(2), 325–335.

Carvalho, F. P. (2011). Marie Curie and the discovery of radium. In B. Merkel & M. Schipek (Eds.), *The new uranium mining boom. Challenge and lessons learned* (pp. 3–13). Berlin: Springer-Verlag.

Carvalho, F. P., Falcão, J. M., Machado Leite, M., Alarcão, M., Cordeiro, E., et al. (2007c). *Uranium mines and their residues (MINURAR): Assessment of Exposure radioactivity and toxic metals and their health effects in a Portuguese population*. International Agency of Atomic Energy. IAEA-CN-145/137P. http://curem.iaea.org/envrad2007/content/rsrc/Envrad2007/137P/137P-Fernando-S1formatted.pdf. Accessed Nov 2012.

Carvalho, F. P., Madruga, M. J., Reis, M. C., Alves, J. G., Oliveira, J. M., Gouveia, J., et al. (2007a). Radioactivity in the environment around past radium and uranium mining sites of Portugal. *Journal of Environmental Radioactivity, 96*, 39–46.

Carvalho, F. P., Oliveira, J. M., & Faria, I. (2009a). Alpha emitting radionuclides in drainage from Quinta do Bispo and Cunha Baixa uranium mines (Portugal) and associated radiotoxicological risk. *Bulletin of Environmental Contamination and Toxicology, 83*, 668–673.

Carvalho, F. P., Oliveira, J. M., Lopes, I., & Batista, A. (2007b). Radionuclides from past uranium mining in rivers of Portugal. *Journal of Environmental Radioactivity, 98*, 298–314.

Carvalho, F. P., Oliveira, J. M., & Malta, M. (2009b). Analyses of radionuclides in soil, water, and agriculture products near the Urgeiriça uranium mine in Portugal. *Journal of Radioanalytical and Nuclear Chemistry, 281*, 479–484.

Carvalho, F. P., Oliveira, J. M., & Malta, M. (2011). Radionuclides in plants growing on sludge and water from uranium mine water treatment. *Ecological Engineering, 37*, 1058–1063.

Carvalho, F. P., Oliveira, J. M., Neves, M. O., Abreu, M. M., & Vicente, E. M. (2009c). Soil to plant (*Solanum tuberosum* L.) radionuclide transfer in the vicinity of an old uranium mine. *Geochemistry: Exploration Environment Analysis, 9*, 275–278.

Cordeiro Santos, J., Freira, A. P., & Antunes Fiúza, A. M. (1983). Tratamento de minérios pobres na mina da Cunha Baixa. *Boletim de Minas, 20*(3), 130–145.

DGEG—Direção Geral de Energia e Geologia, EDM - Empresa de Desenvolvimento Mineiro S.A. (2011). *The legacy of abandoned mines. The context and the actions in Portugal*. Edition supported by Direção Geral de Energia e Minas and Empresa de Desesnvolvimento Mineiro, SA, Lisboa, Portugal. http://www.edm.pt/html/files2/assets/seo/page1.html. Accessed Nov 2012.

Falcão, J. M., Carvalho, F. P., Leite, M. M., Alarcão, M., Cordeiro Ribeiro, E. J., et al. (2005).*MinUrar—Minas de Urânio e seus resíduos. Efeitos na Saúde da População*. Relatório Científico I. Instituto Nacional de Saúde Dr° Ricardo Jorge, Instituto Tecnológico e Nuclear e Instituto Nacional de Engenharia, Tecnologia e Inovação, Lisboa. http://www.itn.pt/docum/relat/minurar/2005-MinUrar-relatorio1.pdf. Accessed Nov 2012.

Falcão, J. M., Carvalho, F. P., Leite, M. M., Alarcão, M., Cordeiro Ribeiro, E. J., et al. (2005). *MinUrar—Minas de Urânio e seus resíduos. Efeitos na Saúde da População*. Relatório Científico I. Instituto Nacional de Saúde Dr° Ricardo Jorge, Instituto Tecnológico e Nuclear e Instituto Nacional de Engenharia, Tecnologia e Inovação, Lisboa. http://www.itn.pt/docum/relat/minurar/2007-MinUrar-relatorio2.pdf. Accessed Nov 2012.

Frostegård, A., Tunlid, A., & Bååth, E. (2010). Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry, 43*(8), 1621–1625.

Geras′kin, S. A., Evseeva, T. I., Belykh, E. S., Majstrenko, T. A., Michalik, B., & Taskaev, A. I. (2007). Effects of non-human species inhabiting areas with enhanced level of natural radioactivity in the north of Russia: A review. *Journal of Environmental Radioactivity, 94*, 151–182.

Gerber, G. B., Léonard, A., & Hantson, P. (2002). Carcinogenicity, mutagenicity and teratogenicity of manganese compounds. *Critical Reviews on Oncology/Hematology, 42*, 25–34.

Guedes, M. J., Pereira, R., Duarte, K., Rocha-Santos, T. A. P., Antunes, S. C., Gonçalves, F., et al. (2011). Sterols and fatty acid biomarkers as indicators of changes in soil microbial communities in a uranium mine area. *Journal of Environmental Science and Health, Part A, 46*, 659–668.

Hinojosa, M. B., Carreira, J. A., García-Ruíz, R., & Dick, R. P. (2005). Microbial response to heavy metal-polluted soils: Community analysis from phospholipid-linked fatty acids and ester-linked fatty acids extracts. *Journal of Environmental Quality, 34*, 1789–1800.

Hinojosa, M. B., García-Ruíz, R., & Carreira, J. A. (2010). Utilizing microbial community structure and function to evaluate the health of heavy metal polluted soils. In I. Sherameti & A. Varma (Eds.), *Soil heavy metals. Soil biology* (pp. 185–224). Berlin, Heidelberg: Springer Verlag.

IAEA—International Atomic Energy Agency. (2001). *Analysis of uranium supply to 2050*. International Atomic Energy Agency, Viena. http://www-pub.iaea.org/MTCD/publictions/PDF/Pub1104_scr.pdf. Accessed Nov 2012.

Jomova, K., & Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology, 283*, 65–87.

Kahouli, S. (2011). Re-examining uranium supply and demand: New insights. *Energy Policy, 39*, 358–376.

Kleiner, K. (2008). *Nuclear energy: Assessing the emissions*. Nature Reports Climate Change, *2*, 130–131. http://www.nature.com/climate/2008/0810/pdf/climate.2008.99.pdf. Accessed Nov 2012.

Lierre, C., & Pio Leite, A. (1934). *L' industrie du Radium au Portugal*. Lisboa: Anais do Instituto Superior Técnico, Universidade Técnica de Lisboa.

Lourenço, J., Castro, B. B., Machado, R., Nunes, B., Mendo, S., Gonçalves, F., Pereira, R. (2010). Genetic, biochemical and individual responses of the teleost fish *Carassius auratus* to uranium. *Archives of Environmental Contamination and Toxicology, 58*(4), 1023–1031.

Lourenço, J., Pereira, R., Gonçalves, F., & Mendo, S. (2013a). Metals bioaccumulation, genotoxicity and gene expression in the European wood mouse (*Apodemus sylvaticus*) inhabiting an abandoned uranium mining area. *The Science of the Total Environment, 443*, 673–680.

Lourenço, J., Pereira, R., Gonçalves, F., & Mendo, S. (2013b). SSH gene expression profile of *Eisenia andrei* exposed in situ to a naturally contaminated soil from an abandoned uranium mine. *Ecotoxicology and Environmental Safety, 88*, 16–25.

Lourenço, J., Pereira, R., Pinto, F., Caetano, T., Silva, A., Carvalheiro, T., et al. (2013c). Biomonitoring a human population inhabiting nearby a deactivated uranium mine. *Toxicology, 305*(8), 89–98.

Lourenço, J., Pereira, R., Silva, A., Carvalho, F., Oliveira, J., Malta, M., et al. (2012). Evaluation of the sensitivity of genotoxicity and cytotoxicity endpoints in earthworms exposed in situ to uranium mining wastes. *Ecotoxicology and Environmental Safety, 75*, 46–54.

Lourenço, J., Pereira, R., Silva, A., Morgado, J., Carvalho, F., Oliveira, J., et al. (2011a). Genotoxic endpoints in the earthworms sub-lethal assay to evaluate natural soils contaminated by metals and radionuclides. *Journal of Hazardous Materials, 186*, 788–795.

Lourenço, J., Silva, A., Carvalho, F., Oliveira, J., Malta, M., Mendo, S., et al. (2011b). Histopathological changes in the earthworm Eisenia andrei associated with the exposure to metals and radionuclides. *Chemosphere, 85*, 1630–1634.

MA—Ministério do Ambiente. (1998). *Decreto-Lei no 236/98 de 1 de Agosto*. Diário da República I Série A, no 176: 3676-3722. http://dre.pt/pdf1s/1998/08/176A00/36763722.pdf. Accessed Nov 2012.

Marado, A. C. C. (2010). *Effects of uranium contamination on male reproductive parameters of the wood mouse Apodemus sylvaticus*. MSc Thesis. Coimbra, Portugal: University of Coimbra.

Marques, S. M., Antunes, S. C., Nunes, B., Gonçalves, F., & Pereira, R. (2011). Antioxidant response and metal accumulation in tissues of Iberian green frogs (*Pelophylax perezi*) inhabiting a deactivated uranium mine. *Ecotoxicology, 20*, 1315–1327.

Marques, S. M., Antunes, S. C., Pissarra, H., Pereira, M. L., Gonçalves, F., & Pereira, R. (2009). Histopathological changes and erythrocytic nuclear abnormalities in Iberian Green Frogs (*Rana perezi* Seoane) from a uranium mine pond. *Aquatic Toxicology, 91*, 187–195.

Marques, S. M., Chaves, S., Gonçalves, F., & Pereira, R. (2013a). Differential gene expression in Iberian green frogs (*Pelophylax perezi*) inhabiting a deactivated uranium mine. *Ecotoxicology and Environmental Safety, 87*, 115–119.

Marques, S. M., Chaves, S., Gonçalves, F., & Pereira, R. (2013b). Evaluation of growth, biochemical, bioaccumulation parameters in *Pelophylax perezi* tadpoles. *Following an in situ acute exposure to three different effluent ponds from na uranium mine Science of Total Environment, 445–446*, 321–328.

Marques, S. M., Gonçalves, F., & Pereira, R. (2008). Effects of a uranium mine effluent in the early-life stages of *Rana perezi* Seoane. *The Science of Total Environment, 402*(1), 29–35.

ME—Ministério da Economia. (2001). *Decreto-Lei no 198/A, de 6 de Julho de 2001*. Diário da República, I Série-A, no 155, Suplemento, 4084(1)–4084(7).

ME—Ministério da Economia, MAOT—Ministério do Ambiente e Ordenamento do Território. (2002). Despacho Conjunto no 242/2002, de 5 de Abril. *Diário da República II Série, 80*, 6270–6271.

Nero, J. M. G., Dias, J. M. M., Pereira, A. J. S. C., Godinho, M. M., Neves, L. J. P. F., & Barbosa, S. V. T. (2003). *Metodologia integrada para caracterização do cenário ambiental em minas de urânio desativadas*., Vila Real, Portugal: III Seminário sobre Recursos Geológicos, Ambiente e Ordenamento do Território, Departamento de Geologia da Universidade de Trás-os-Montes.

Nero, J. M.G., Dias, J. M., Torrinha, A. J., Neves, L. J., Torrinha, J. A. (2005). Environmental evaluation and remediation methodologies of abandoned radioactive mines in Portugal. In: *Proceedings of an international workshop on environmental Contamination from uranium production facilities and remediation measures held in Lisbon 11-13*. International Atomic Agency pp. 145–158.

Neves, M. O., Abreu, M. M., & Figueiredo, V. (2012a). Uranium in vegetable foodstuffs: Should residents near the Cunha Baixa uranium mine site (Central Northern Portugal) be concerned. *Environmental Geochemistry and Health, 34*, 181–189.

Neves, M. O., Figueiredo, V. R., & Abreu, M. M. (2012b). Transfer of U, Al and Mn in the water-soil-plant (*Solanum tuberosum* L.) system near a former uranium mining area (Cunha Baixa, Portugal) and implications to human health. *The Science of the Total Environment, 416*, 156–163.

Neves, O., & Matias, J. M. (2009). Assessment of groundwater quality and contamination problems ascribed to na abandoned uranium mine (Cunha Baixa region, Central Portugal). *Environmental Geology, 53*, 1799–1810.

Oliveira, J. (2002). *A energia nuclear em Portugal*. Uma esquina de História. Ed. "O Mirante", Santarém, Portugal.

Pereira, R., Antunes, S. C., Marques, S. M., & Gonçalves, F. (2008). Contribution for tier I of the ecological risk assessment of Cunha Baixa uranium mine (Central Portugal): I soil chemical characterization. *The Science of the Total Environment, 390*, 377–386.

Pereira, R., Marques, C. R., Silva, F. M. J., Neves, M. F. J. V., Caetano, A. L., Antunes, S. C., et al. (2009). Phytotoxicity and genotoxicity of soils from an abandoned uranium mine area. *Applied Soil Ecology, 42*(3), 209–220.

Pereira, A. J. S. C., & Neves, L. J. P. F. (2012). Estimation of the radiological background and dose assessment in áreas with naturally occurring uranium geochemical anomalies—a case study in the Iberian Massif (Central Portugal). *Journal of Environmental Radioactivity, 112*, 96–107.

Pereira, R., Barnosa, S., & Carvalho, F. (2012). Field trip guidebook. Uranium mining in Portugal: the environmental legacies of the greatest explorations. *9th ISEG international Symposium on Environmental Geochemistry*. 15–21 of July, Aveiro, Portugal

Stearns, D. M., Yazzie, M., Bradley, A. S., Coryell, V. H., Shelley, J. T., Ashby, A., et al. (2005). Uranyl acetate induces hprt mutations and uranium DNA adducts in Chinese hamster ovary EMS cells. *Mutagenesis, 20*(6), 417–423.