Behaviour of two varieties of lettuce (*Lactuca sativa L.*) when exposed to naturally contaminated environment

Marcelino, M. C.[1]

ABSTRACT

In order to evaluate and compare the behaviour of two varieties of lettuce (*Lactuca* sativa L., var. Marady and Romana) when exposed to a contaminated environment and evaluated the risks of their consumption to the human health, there were carried out in autumn 2005 and summer 2006 field experiments in agricultural soil in the surroundings Cunha Baixa (Mangualde) uranium mine.

In the experiments were used two soils (A and B) both divided into two plots, one of them irrigated with water contaminated with uranium, aluminum and manganese and the other with uncontaminated water. The content of these elements were analyzed in irrigation water, soil and in the lettuce tissues (leaf and root) samples.

The greatest production and the highest average content (dry weight) of uranium, aluminum and manganese were observed in the leaf (5.37, 456.33, 374.78 mg/kg, respectively) and root (28.20, 1263.75, 220.50 mg/kg, respectively) of the Romana variety plant. The concentration of these elements was mainly influenced by soil B characteristics and by contaminated irrigation water. As for the coefficient of translocation of the elements, it was found that, of the total absorbed by the lettuce plants, the uranium was, in both experiments, mainly concentrated in the roots and the manganese in the leaf, while the aluminum concentrated on leaf of the Marady variety and on root of the Romana variety. Risk analysis (individual and global) associated with exposure to the three elements, considering only the consumption of this vegetable, showed that the coefficient and the risk index were lower than the unity, contributing little to possible adverse effects on the health of Cunha Baixa´s residents.

Keywords: Lettuce (*Lactuca sativa L.*), Cunha Baixa, soil, water irrigation, contamination, risk.

1. INTRODUCTION

The Cunha Baixa mine (Mangualde - Viseu), which began operations in 1970, was one of the most important uraniferous deposits in Portugal (Ferreira, 2007). After finishing his exploration (1993) were left rock-waste in the open pit, exposed to the action of external of agents geodynamics, such as wind and water seepage causing leaching of polluting materials (Pedrosa and Martins, 1999). In the last years of operation at the Cunha Baixa mine, the acid leaching process, carried out *in situ* to recover uranium from the poor ore as the main

[1] Marisa Carvalho Marcelino. Extended abstract from the thesis to obtain Master degree in Environment Engineering held under scientific supervision of Doctor Maria Orquídia Teixeira Neves, Technical Superior Institute (IST), Technical University of Lisbon (UTL), Portugal, October 2010.

responsible for acid mine drainage, thus causing not only soil contamination, but also surface water and groundwater (Machado, 1998; Neves, 2002, Neves and Matias, 2004; Pedrosa and Martins, 1999; Pereira *et al*., 2008; Santos Oliveira and Ávila, 1998).

The Cunha Baixa mine was already considered one of the mines that pose the most serious environmental impacts (Magno 2001; Silveira 2001). The environmental impacts from operation and the subsequent mine abandonment, are manifested in well irrigation water and in soils that have agricultural use by the population that lies near the mining area (Santos Oliveira *et al*. 1999; Neves *et al*. 1999; 2003a, 2003b, 2005, Neves 2002). The risks to residents and animals associated with exposure to uranium (U) and other metals present in irrigation water and soil, such as aluminum (Al) and manganese (Mn), resulting from the ingestion of vegetable/animal foodstuffs or soil particles, as the case of cattle or children, may cause biological effects as a consequence of their chemical and/or radiological activity (Neves *et al.,* 2008a).

The plants being in direct contact with the soil and through the metal absorbed, reflected local contamination with direct negative effects and may also be an important route of exposure for humans (Athar and Ahmad, 2002; Caussy *et al.,* 2003). The lettuce is considered one of the vegetables most efficient in the uptake of metals (Nicklow *et al*., 1983; Soltanpour & Boon, 1992). Dowdy and Larson (1975) reported that lettuce accumulated easily while metals, such as potatoes and carrots would be excellent non-accumulators.

The of the Cunha Baixa inhabitants grow lettuce, for its own consumption, in soils near the former mining area and in different seasons (summer and autumn) watering them with water from private wells. This vegetable consumption may be an additional factor of Cunha Baixa public exposure to elements such as U, Al and Mn, that will be able of causing negative effects on their health.

This study aims to evaluate and compare the behaviour of two varieties of lettuce (Marady and Roman), when subjected to naturally contaminated environment (soil and irrigation water), near the disabled Cunha Baixa uranium mine (Mangualde), in different growing seasons (autumn and summer). Another aim is to assess whether the intake of vegetables poses risks (not cancerous) to the health of Cunha Baixa's residents.

2. Materials and Methods

Controlled field experiments were carried out in autumn 2005 and summer 2006, in two local Cunha Baixa soils (A and B, spaced 50 m apart each other) with different amounts of uranium. Each experimental soil, with a total of 40 m^2 was divided into two plots of 17 m^2 (3.4 x 5 m with a space between plots of 1.2 m). Each plot was further subdivided into four replicates with 2.5 m^2 (0.5 x 5 m with 0.4 m between each reply). In each replicate were planted (roughly ten days of growth) 28 lettuce plants (2 plants x 14 points), for a total of 448 plants in each field experiment. It was also used a control soil (soil C), collected in Sintra granite region (Lisbon) and not contaminated with uranium, which was placed in 16 (4x4) plastic containers, representing four replicates. In each container were planted two lettuces, for a total of 32 plants

(Neves *et al*., 2008b). Before and after lettuce growth period composite soil samples were collected from topsoil (0-20 cm) at each replicate.

In each soil A and B a plot was watered with contaminated water (A-C and B-C) and another with uncontaminated water (A-NC and B-NC), extracted from private wells and usually used for irrigation of the selected soils. Soil C was watered with public tap water (C-TW). The local soil and water from wells used in these experiments were chosen based on the research work done earlier by Neves (2002).

To ensure a favorable growth of the culture Nitromagnesium 20.5 fertilizer (20.5% N_{total}, 10.25% N_{nitric}, 10.25% $N_{ammonium}$, 12% CaO and 6 % MgO), was applied to soils 20 days after lettuce transplanting.

In autumn 2005 field experiment, was planted the lettuce variety Marady. The total amount of water used to irrigate the crop during the experiment (36 L/m^2) was influenced by atmospheric conditions observed (a total average of 222.4 mm of rainfall between 21 October and 30 November 2005). In summer 2006 field experiment, was planted the lettuce variety Roman (Neves and Abreu, 2009), watered with a total of approximately 298 L/m^2 (between July 21 and September 20, occurred on average a total of 72.5 mm of precipitation (Meteorological Institute, 2006)).

Plants (leaves and roots) were harvested at the end of the growing season, approximately 60 days after transplantation. The selection of crop varieties, fertilizer used, the frequency and amount of irrigation followed the local agricultural practices.

The experimental data were subjected to analysis of variance (ANOVA) using STATISTICA 7 software for Windows. The average values of the parameters associated with each plot were compared by the Tukey (HSD) test at the 5% level of significance of. To identify statistical correlations between the various parameters, was used the Pearson's coefficient considering significant differences at 5% level. It was not possible to statistically compare the results obtained with the remaining soil C, since the number of replicates was reduced (two replicates).

The plant capacity to translocate an element among organs was assessed by the translocation coefficient defined by the ratio (mg/kg dry weight) between the element concentration in the aerial part (leaves) and its concentration in the plant (leaves and roots).

The chemical risk (not cancerous) to the health of of Cunha Baixa' residents, as a result of ingesting lettuce was evaluated using the hazard quotient (HQ) which relates the estimated exposure dose to the element, by food ingestion, with their oral reference dose (RfD) (USEPA, 1989).

If the estimated dose of exposure by ingestion of food is higher than the oral reference dose (RQ> 1), it is assumed that there may be risks not carcinogenic to the health of the population under study, arising from the consumption of these foods.

To check risk (not cancerous) associated with simultaneous exposure to more than one chemical element assuming cumulative risks, the hazard index (HI, USEPA, 1989) was calculated by adding the hazard quotients (HQ) of each element. If the value of risk index is less than unity, it is assumed that there is no concern for potential non-carcinogenic effects.

3. RESULTS AND DISCUSSION

3.1 Water irrigation

The values of the parameters analyzed in contaminated water (C) are above the maximum recommended (VMR) and or the maximum permissible values (VMA) according to Portuguese law (Decree-Law Nº. 236/98) for this propose in opposition to the same parameters in tap water (TW) and uncontaminated water (NC). The absence in national legislation and in other European Union countries of limits for uranium in irrigation water, the trigger value recommended in Australian and New Zealand legislation (ANZECC, 2000) was used.

Contaminated irrigation water presented pH values below the VMR (pH: 6.5 to 8.4) and VMA (pH: 4.5 to 9.0), high EC average (2005: 1700 µS/cm; 2006: 1818 µS/cm), high average contents of U (2005: 1064 mg/L, 2006: 985 mg/L), Al (2005: 8055 mg/L, 2006: 7200 mg/L) and Mn (2005: 4015 g/L, 2006: 4520 mg/L). Thus, this water can eventually cause effects on soil or on lettuce plants.

3.2 Soils

The soil is a support for plants to develop their roots through which obtain the nutrients they need (Botelho da Costa, 2004).

The texture and cation exchange capacity (CEC) of soils A, B and C used in the 2005 and 2006, were determined at the beginning of the first field experiment (autumn 2005). According to the triangular texture classes diagram the soils A and B fall within the sandy-loam texture class while the soil C in loam texture class. According to Varennes (2003) CEC in soils A and C were classified as low (5.0 to 10.0 $cmol_c$ / kg) and as medium in soil B (10.1 to 20.0 $cmol_c$ / kg).

After the 2005 field experiment the total concentration (U <99 mg/kg, Al <6.82%, Mn <591 mg/kg) and available concentration (U <10.77 mg/kg, Al < 17.03 mg/kg, Mn <25.1 mg/kg) in soil (the main elements in the study), did not change significantly when compared with the beginning (U total <117 mg / kg; Al total <6.79%; Mn total <564 mg/kg; U available <14.65 mg/kg; Al available <21.9 mg / kg; Mn available <23.4 mg/kg). It was observed that soil plots B had a higher total U and Mn content, compared with soil A. In relation to the available fraction of these elements it was found that in general, the soil plots B also showed higher concentrations of U and Mn available. The highest average of U available content (14.65 mg/kg) and Al available (21.9 mg/kg) were recorded before planting lettuce, respectively, in B-C and A-C soil plots, and Mn available (25.1 mg/kg) in the soil plot B-C plot after harvest.

In 2006 field experiment in general, the total concentration of U (before <252 mg / kg, after <109 mg/kg), Al (before <9.63%, after <8.52%) and Mn (before <787 mg/kg, after <575 mg / kg) in soils decreased with the lettuce cultivation, in opposition to the available contents that increased, mainly in soil B. Also in the soil B the total and available elements were generally higher than those in soil A. The higher average concentrations of U available (12.75 ± 1.00

mg/kg), Al available (6.18 ± 2.32 mg/kg) and Mn available (33.83 ± 2.52 mg/kg) were recorded in B-C stand at the end of the field experiment.

It was found that the percentage ratio of the available contents of elements in relation to the total content is low in both experiments (2005: U <15.3%, Mn <7.7% and 2006: U <11.7%, Mn <6%), and generally the highest percentages was recorded in soil B.

3.3 Plants

Field experience in 2005 in soils A and B, the use of contaminated irrigation water had a negative influence (reduction of 53% and 42% respectively) on the production of lettuce variety Marady (A-C: 0.85; A-NC: 1.80, B-C: 0.82, B-NC: 1.41 kg, fresh weight) while soil characteristics of the had little effect. In 2006, the soils A and B production was on average also lower (reduction of 75 and 87% respectively) in plots irrigated with contaminated water (A-C: 5.16, A-NC: 20.60, B-C: 2.53, B-NC: 19.20 kg, fresh weight), also suggested a greater influence by the irrigation water quality on the production of summer leaf variety. In this field experiment, the soil plots A and B watered with contaminated water showed a reduction in leaf production; 51% in soil B compared with A,. Compared to 2005, the average production of lettuce leaf was higher in 2006 experiment (the biggest increase of production: 1044% (A-NC)), suggesting that the summer variety (Romana) naturally trend to have a higher production, when compared with the Marady variety (autumn).

In soil C, there was an increase of 2.40 kg (3000%) between the 2005 production (0.08 kg, fresh weight) and 2006 (2.48 kg, fresh weight), showing also the higher productivity of the of summer variety, whatever soil characteristics and irrigation water quality.

It was found that root production in field experiment 2005 in soil A (AC: 0.09, A-NC: 0.15 kg, fresh weight) and B (BC: 0.11, B-NC: 0.13 kg, fresh weight) was not affected neither by the soil nor by irrigation water. In the experiment with the Roman variety, as in the aerial part, it is concluded that there was influence of irrigation water (reduction: 68% in A-C and 81% in B-C) and the of soil higher content in U (reduction: 59% in B-C and 30% (B-NC)) in the root production. Comparing the two experiments, it was found that with the exception of the soil plot B-C (2006) the production of AC: 0.22, A-NC: 0.69, B-C: 0.09, B-NC: 0.48 kg fresh weight was always significantly different and higher than in 2005.

In 2005 the average content of uranium in the plant leaf grown in soils A and B, showed more influence of the soil than of the irrigation water. In 2006 cultivation, both water irrigation and the contaminated soil with higher U concentrations resulted in higher contents of this element in lettuce Romana variety leaf (0.19 to 5.37 mg U/kg, dry weight). Compared with 2005, the U content in leaf lettuce was significantly higher than in 2006 only in the of B-C soil plot, as soil U content available in this plot before planting was significantly lower than in 2006. This behavior may be related to the greater amount of irrigation water supplied to the plant during the summer experiment. In soil C in the plants showed the lowest U content in leaves (2005: 0.04; 2006: 0, 10 mg/kg dry weight), when compared with the other plots.

Soil B influenced the concentration of uranium in the root of the Marady variety (BC: 4.37, B-NC: 4.64 mg/kg dry weight), that was higher than concentration of root lettuce grown in soil A (2.9 and 4.3 the value recorded in A-C and A-NC, respectively) since uranium levels in this soil were higher. In summer experiment both irrigation water and soil characteristics, such as the U content available influenced the content of U in the root of the variety Romana (AC: 7.26, A-NC: 7.35, BC: 28.20, B-NC: 18.45 mg/kg dry weight). By comparing the two studied lettuce varieties, the Romana variety tends to concentrate and retain more U in roots than Marady variety, possibly due to greater exposure to the element through irrigation and factors related to the plants themselves. Compared with other soil plots the roots from plants developed in soil C recorded the lowest U content (2005: 0.11 and 2006: 0.69 mg / kg dry weight).

The calculated translocation coefficient of uranium in the plant (2005: <0.33, 2006: <0.16) showed that these lettuce varieties in the mainly concentrate the U in the inedible parts (roots).

The average content of Al in lettuce Marady variety leaf (A-C: 350.28, A-NC: 210.80, B-C: 331.36, B-NC: 225.01 mg/kg, weight dry) showed that the use of contaminated irrigation water contributed to a higher Al concentration in the aerial parts. In the summer trial, apart from the quality of irrigation water, soil characteristics also seems to have influenced the Al leaf content (A-C: 191.72, A-NC: 83.43, B-C: 456.33, B-NC: 123.34 mg/kg, dry weight). In soil A the autumn variety concentrated more Al than the summer variety. In soil B this trend was only observed in the plot B-NC. Thus was probably an influence of the irrigation water Al content registered in 2005, that was higher than in 2006. In soil C, the Al average content in the leaves of lettuces grown in 2006 (169.77 mg/kg, dry weight), in opposite to the contents recorded in 2005 (43.00 mg/kg, dry weight), was higher for the plants grown in plots irrigated with uncontaminated water, showing the interference of higher Al content in tap water relatively uncontaminated water.

In the 2005 field experiment of, the irrigation water and soil characteristics did not influence the concentration of Al in lettuce roots (A-C: 224.25, A-NC: 209.00, B-C: 172.25, B-NC: 157.50 mg/kg dry weight). The summer experiment there was a trend to concentrate more aluminum in root lettuce (1263.75 mg/kg dry weight) that grown in soil watered with the uncontaminated water. In general, the average Al levels of in lettuce roots were higher in 2006 trial (A-C: 957.00, A-NC: 1263.75, B-C: 712.75, B-NC: 987.75 mg/kg weight dry) than the 2005 trial. In soil C, the Al root contents (2005: 118.00 2006: 428.50 mg/kg dry weight) was lower than concentration observed in the other plots, however it was lower than in 2005.

Coefficient translocation of Al in plants grown in 2005 (<0.65) showed that in soils A and B, the lettuce Marady variety concentrates more Al on leaves than in the roots, in opposite that was found in lettuce Romana variety (<0.39). In soil C, the two studied lettuce varieties had the same capacity to translocate the Al to the aerial part (0.27).

Regarding Mn the highest concentrations was recorded in lettuce leaves grown in soils A and B of the 2005 experiment and when watered with contaminated water (A-C: 164.94, B-C: 185.90 mg/kg, dry weight). The same behavior was in the 2006 essay (A-C: 252.33, B-C: 374.78 mg/kg, dry weight). The Mn content in lettuce leaf grown in soil plots A and B was higher in 2006 than in 2005. This can be the result of Romana variety intrinsic characteristics and/or the fact that in 2006 experiment Mn content was higher in contaminated and uncontaminated water. In 2005 the lettuce leaf Mn content (75.30 mg/kg, dry weight) in soil C was lower than in any other plot, while in 2006 it was the higest (477.95 mg/kg, dry weight).

In soils A and B at 2005 and 2006 field experiments of in, the average Mn content in the roots apparently no suffered influence either the soil or irrigation water. In general, the lettuce grown in 2006 concentrate more Mn on the roots (A-C: 171.75, A-NC: 71.68, B-C: 155.25, B-NC: 220.50 mg/kg, dry weight) than lettuce grown in 2005 (A-C: 59.48 A-NC: 34.20, B-C: 79.63, B-NC: 38.43 mg/kg, dry weight). This results may indicate that root Mn concentrations were related with the amount of irrigation water, that was higher in 2006 and/or possible characteristics of the varieties used in the study. In soil C was also found a trend to the lettuce concentrate more Mn in root in summer (91.70 mg/kg, dry weight) than in autumn (29.05 mg/kg, dry weight) experiments.

Coefficient translocation of Mn showed that, in general, the two studied lettuce varieties concentrated more Mn in the leaves. The Marady variety showed the same capacity to translocate to the leaf Mn (0.73), whatever the farming conditions, while in Roman variety this capacity was more variable (40-84%).

3.4 Health risk associated with lettuce consumption

The results of the risk analysis (for no carcinogenic effects) for human health from exposure to each element of concern (U, Al and Mn), considering only the consumption of lettuce and taking into account the dietary habits of Cunha Baixa's residents, indicate that the intake of this vegetable not represent a potential health risk. The same applies to the combined risk associated with cumulative exposure to all three elements. On the three age groups considered, children are the population group where the risk quotient (<0.0143) and risk index (0.0264) were higher, although both were less than unity.

It was also observed that lettuce that concentrate more U (B-C plot, 2006) when consumed by children, adolescents and adults representing respectively 24.2%, 17 7% and 16.7% of the tolerable daily limit intake set by WHO for this element (0.6 mg/kg body weight daily).

4. CONCLUSION

Quality analysis of irrigation water and soil used in field experiments on agricultural Cunha Baixa (Mangualde) soils concluded that irrigation with contaminated water and the soil with higher U, Al and Mn concentrations had negative effects on the studied lettuce varieties (Marady and Romana) as production reduction (53% and 87% in the 2005 and 2006 experiments, respectively) and concentration increasing of U, Al and Mn in lettuce tissues (leaf and root).

In general, the lettuce Roman variety presented a trend to have a greater leaf and root average production of than lettuce Marady variety. The higher U, Al and Mn mean contents (dry weight) were also observed in the leaf (5.37, 456.33, 374.78 mg/kg, respectively) and root (28.20, 1263.75, 220.50 mg/kg, respectively) plants of the Roman variety.

Coefficient translocation of the elements within the plant showed that in the two field experiments the U was concentrated preferentially in the root and Mn in the leaf, , while Al was concentrated mostly in leaf of Marady variety and in root of Roman variety.

Risk analysis (individual and combined) associated with the exposure to three elements, considering only the consumption of this vegetable, indicated that the risk quotient (<0.0143) and risk index (0.0264) were below the unit, suggesting that lettuce intake not represent a potential health risk for Cunha Baixa's residents.

REFERENCES

ANZECC, Australian and New Zealand Environmental and Conservation Council. (2000). *Water quality for irrigation and general water use. Water Guidelines,* Vol. 1, Chap. 4, pp. 4.2-11. Acceded 7 April 2010, http://www.mincos.gov.au/publications/australian_and_new_zealand_guidelines_for_fresh_and_marine_water_quality

Athar, R., & Ahmad, M. (2002). Heavy metal Toxicity: Effect on plant growth and metal uptake by wheat, and on free living Azotobacter. *Water, Air an Soil Pollution , 138*, pp. 165-180.

Boon, D. Y., & Soltanpour, P. N. (1992). Lead, cadmium, and zinc contamination of aspen garden soils and vegetation. *Journal of Environmental Quality , vol. 21*, 82-86.

Caussy, D., Gochfeld, M., Gurzau, E., Neagu, C., & Ruedel, H. (2003). Lessons from case studies of metals: investigating exposure, bioavailability, and risk. *Ecotoxicology and Environmental Safety , 16*, pp. 45-51.

Costa, J. B. (2004). *Caracterização e constituição do solo* (7.ª ed.). Lisboa: Fundação Calouste Gulbenkian.

Dowdy, R. H., & Larson, W. E. (1975). The availability of sludge-borne metals to various vegetable crops. *Journal of Environmental Quality , vol. 4*, 278-282.

Ferreira, M. J. (2007). *Toxicidade de solos uraníferos em cogumelos e plantas comestíveis.* Dissertação de mestrado: Universidade de Aveiro, Departamento de Biologia.

Instituto de Meteorologia. (2006). *Boletim Meteorológico para a Agricultura, n.º1975-1980.* Retrieved 20 April 2010, from Instituto de Meteorologia, IP Portugal: http://www.meteo.pt/pt/index.html

Machado, M. J. (1998). *Estudo de impacto ambiental em minas abandonadas - comportamento dos metais dissolvidos nas águas da Cunha Baixa e Quinta do Bispo.* Secção de Hidroquímica. Instituto Geológico e Mineiro, Lisboa, Portugal.

Magno, C. E. (2001). O sistema de gestão territorial e os recursos geológicos em Portugal. *Bol. Minas , 38 (3)*, pp. 151-160.

Neves, M. O., Matias, M. J., Abreu, M. M., Magalhães, M. C., & Basto, M. J. (2005). Abandoned mine site characterization for remediation: the case of the Cunha Baixa uranium mine (Viseu, Portugal). International Workshop on Environmental Contamination from Uranium Production Facilities and their Remediation. *IAEA Proceeding Series* , 159-169.

Neves, O. (2002). *Minas desactivadas e impactos geoquímicos ambientais: O caso da mina de urânio da Cunha Baixa (Viseu).* Tese de Doutoramento, Depart. Eng.ª Minas e Georrecursos, Instituto Superior Técnico, Universidade Técnica de Lisboa, Portugal.

Neves, O., & Abreu, M. M. (2009). Are uranium-contaminated soil and irrigation water a risk for human vegetables consumers? A study case with *Solanum tuberosum L., Phaseolus vulgaris L. and Lactuca sativa L. Ecotoxicology , Vol. 18*, pp. 1130-1136.

Neves, O., & Matias, M. J. (2004). Focos de poluição na área mineira da Cunha Baixa (viseu, Portugal). *Caderno Lab. Xeolóxico de Laxe* (Vol. 29, pp. 187-202). Coruña.

Neves, O., Abreu, M. M., & Matias, M. J. (2003a). Comportamento do urânio, alumínio e manganês no milho cultivado em solos na área da mina de urânio da Cunha Baixa. *Memórias e Notícias* , 2 (Nova Série), 265-278.

Neves, O., Abreu, M. M., & Vicente, E. M. (2008a). Transferência do urânio no sistema água-solo-planta (*Lactuca sativa L.*) na área mineira da Cunha Baixa. *Revista Electrónica de Ciências da Terra, Geosciences On-line Journal, GEOTIC - Sociedade Geológica de Portugal , Vol. 5 - nº 3*.

Neves, O., Abreu, M. M., & Vicente, E. M. (2008b). Uptake of Uranium by Lettuce (*Lactuca sativa L.*) in Natural Uranium Contaminated Soils in Order to Assess Chemical Risk for Consumers. *Water Air Soil Pollut , Vol. 195*, pp. 73-84.

Neves, O., Abreu, M. M., Basto, M. J., & Matias, M. J. (1999). Contribuição para o estudo da contaminação resultante da exploração e abandono da mina da Cunha Baixa. II. Solos. *Actas XI Semana de Geoquímica / II Congresso Ibérico de Geoquímica dos Países de Línguas Portuguesa* , Lisboa, Portugal, 483-486.

Neves, O., Matias, M. J., & Cores Graça, R. (2003b). Efeitos da actividade mineira na qualidade da água de rega: um caso de estudo na envolvente da mina de urânio da Cunha Baixa.

Comunicações Seminários Sobre Águas Subterrâneas. APRH-LNEC , Lisboa, 27 e 28 de Fevereiro.

Nicklow, C. W., Comas-Haezebrouck, P. H., & Feper, W. A. (1983). Influence of varying soil lead levels on lead uptake of leafy and root vegetables. *Journal of the American Society for Horticulture Science , vol. 108*, 193-195.

Pedrosa, M. Y., & Martins, H. M. (1999). *Hidrologia da Mina da Cunha Baixa - Estudo Preliminar.* Estudo de Impacto Ambiental em Minas Abandonadas. Instituto Geológico e Mineiro, Lisboa, 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.

Santos Oliveira, J. M., & Ávila, P. F. (1998). *Estudo geoquímico na área da mina da Cunha Baixa (Mangualde, no Centro de Portugal).* Instituto Geológico e Mineiro, Lisboa, Portugal.

Santos Oliveira, J. M., Canto Machado, M. J., Neves, O., & Matias, M. J. (1999). Estudos de impacte químico associado a uma mina de urânio no centro de Portugal. *Anais do V Congresso de Geoquímica dos Países de Língua Portuguesa & VII Congresso Brasileiro de Geoquímica* , Porto Seguro, Baía, Brasil, 170-1730.

Silveira, B. C. (2001). Impacte radiológico da exploração de urânio em Portugal. *Geonovas, Rev. Assoc. Portg. Geólogos , n.º 15*, 71-86.

USEPA, U. S. Environmental Protection Agency. (1989). *Risk Assessment Guidance for Superfund Volume I Human Health Evaluation Manual (Part A) Interim Final.* EPA/540/1-89/002, Office of Emergency and Remedial Response, U. S. EPA, Washington, DC. Acceded 25 September 2010, de http://www.epa.gov/swerrims/riskassessment/ragsa/index.htm