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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.

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Abstract The knowledge of uranium concentration, in the products entering the human diet is of extreme importance because of their chemical hazard to health. Controlled field experiments with potatoes, beans and lettuce (*Solanum tuberosum* L., *Phaseolus vulgaris* L. and *Lactuca sativa* L.) were carried out in a contaminated soil used by local farmers located near a closed Portuguese uranium mine (Cunha Baixa, Mangualde). The soil with high average uranium levels (64–252 mg/kg) was divided in two plots, and irrigated with non-contaminated and uranium-contaminated water (<20 and >900 µg/L). Uranium maximum average concentration in the edible vegetables parts (mg/kg fresh weight) ranged in the following order: lettuce (234 µg/kg) > green bean (30 µg/kg) > potatoes without peel (4 µg/kg). Although uranium in soil, irrigation water and vegetables was high, the assessment of the health risk based on hazard quotient indicates that consumption of these vegetables does not represent potential adverse (no carcinogenic) effects for a local inhabitant during lifetime.

Keywords Uranium · Vegetable food · Bioconcentration · Health risk · Cunha Baixa (Portugal)

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Introduction

The toxicity of uranium (U) has been under study for over 50 years. Natural U consist primarily of the ^{238}U isotope that is very weak radioactive and is not hazardous as toxicant, but it is a weak chemical poison. The toxicity of this chemical to humans has been of interest since the 1800s when U was used as a homeopathic cure for *Diabetus Mellitus* (Hodge et al. 1973). The early reports demonstrate the susceptibility of humans to the nephrotoxicity of ingested U but provided inadequate basis for estimating the dose for toxic effects (IRIS 2008). Actual Reference Dose (RfD) for chronic oral exposure was established by EPA, since 1989, at 0.003 mg/kg day. In general, the RfD is an estimate (with uncertainty) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious (no carcinogenic) effects during a lifetime (IRIS 2008).

In Portugal the recent closure of uranium mines (2001) has raised concerns regarding the possible chemical and radiological (from ^{238}U daughters alpha-emitting radionuclides) effects on the health of the populations leaving around the mining areas. The Cunha Baixa mine (Mangualde, Central Portugal) where the exploration was undertaken within a rural area is one of these examples. Some soils and shallow groundwater used for farmland irrigation had appreciable U content (Neves 2002; Neves et al. 2005; Neves and Matias 2008), so this element can be expected in agricultural produces. In fact, U has been detected in a variety of foodstuffs (ATSDR 1999). High U accumulation in vegetables food may pose a direct threat to human health if these are being consumed. These health risks will depend on the physicochemical characteristics of soils, plant species, U concentration and human consumption rate (Cobb et al. 2000). Sampling and analysis of representative diets in New York City showed that two categories of plant origin foodstuffs could contribute to U uptake via ingestion: food consisting of vegetables, potatoes or beans and bakery products (as well as cereals, rice and grain) (Lamas 2005).

The implication of contamination to agricultural soils due to irrigation with U-contaminated water and the consequently phyto-accumulation in food crops associated with dietary exposure to U started to be studied recently in Cunha Baixa area (Neves et al. 2008).

The aim of this study was to assess the bioconcentration of uranium in some local Cunha Baixa food vegetables (*Solanum tuberosum* L., *Phaseolus vulgaris* L. and *Lactuca sativa* L.) in order to evaluate potential health risk (no cancer risk) for local adult that are exposure for a long term to uranium through their consumption.

Materials and methods

Study site and experimental work

The study site is located in the village of Cunha Baixa, 20 km from Viseu (Portugal, Fig. 1) where uranium mining activities were extensive for more than 20 years (1970–1993). A detailed description of the geology, mineralogy, underground, open pit and heap leaching mine works was presented elsewhere (Santos Oliveira and Ávila 2001; Neves and Matias 2008).

Controlled field experiments for potato (*S. tuberosum* L.), bean (*P. vulgaris* L.) and lettuce (*L. sativa* L.) cultivation were carried out between the end of April and the end of October 2006, in a sandy-loam Cambisol (Carta de Solos 1978) located in the agricultural area nearby the closed uranium Cunha Baixa mine (Fig. 1). These food vegetables were selected according to local production and use in the inhabitants' diet.

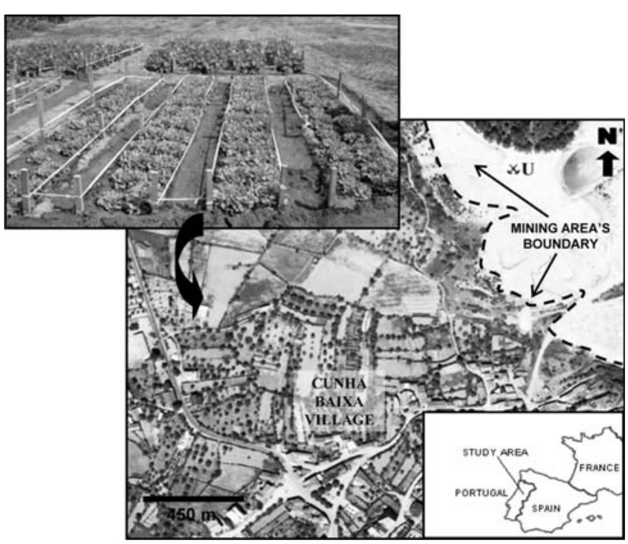

Fig. 1 Geographical location of Cunha Baixa studied site and view of the experimental soil plots

The experimental soil and irrigation water were selected on the basis of developed previous works (Neves 2002; Neves et al. 2003). The soil (40 m^2) was divided in two plots (P1, P2) and both subdivided in four replicates each, as described in Neves et al. (2008). Composite soil samples (0–20 cm) were taken at each replicate before plantation. There were two irrigation treatments: one plot (P1) was irrigated with non-contaminated water (NCW) and the other (P2) with uranium-contaminated water (CW).

During the experiments, plants were watered according to crop irrigation needs and following local agricultural practices as well as the soil fertilizers and the crop varieties selected for this study (Table 1).

Irrigation water samples, pumped from private wells and collected during field experiments, were filtered with a mixed cellulose ester 0.45 µm membrane (GN-6 Metricel®) after temperature, pH, Eh, EC in situ measurements before they were split into acidified (with HNO_3 to $pH < 2$) and non-acidified sub-samples. These water sub-samples were used for cation and anion analysis.

At the end of the growth period all plant productions were collected and edible part (lettuce leaves, green beans and potato tubers) separated from the non-edible parts. Potato tubers were carefully washed in situ to remove soil particles. At the laboratory the tubers were washed with distilled water and dried at room temperature. Tubers were split into two sliced sub-samples: one with and another without peel (1–2 mm layer). Lettuce leaves and green beans were also washed with distilled water and dried (40° C). All samples were fresh and dry weight measured and grinded for analysis. Soil samples were air-dried, sieved at <2 mm and analysed for physicochemical parameters: pH (1:2.5 soil/water suspension); salinity (electrical conductivity of extract saturation (EC); total organic carbon (TOC) following classic methods (Póvoas and Barral 1992).

Uranium chemical analyses were performed at Actlabs Laboratory, Canada. Total uranium concentration in soil and ash plant samples were analysed after acid digestion (Actlabs: Code ultratrace 4 and 2B, respectively) by ICP-MS, as well as the soils uranium available fraction [extracted with 0.5 M ammonium acetate at pH 7 (1:50 mass/volume)]. The water samples were analysed by ICP-OES. The uranium concentration in vegetables was converted to fresh weight (FW) using site-specific wet/dry ratio for plants from each soil plot replicate.

Potential non-cancer risk for exposure to U at Cunha Baixa site scenario was evaluated by comparison of the estimate Exposure Dose from vegetables ingestion pathway

Table 1 Agricultural practices and crop varieties used in the experimental vegetables cultivation

	Potato	Bean	Lettuce
Time period	April to July	July to October	July to September
Crop variety	*Jaerla*	*Patareco*	*Romana*
Density plant[a]	1 × 14 points	2 × 14 points	2 × 14 points
Fertilization	Foskamonium (10-10-10)[b]	Nitromagnesium (20.5)[c]	Nitromagnesium (20.5)[c]
Irrigation[a]			
Handling (10 L)		5 times	3 times
Furrow (60–70 L)	5 times	6 times	11 times

[a] For each replicate

[b] 10% P, 10% K, 10% $N_{ammonium}$

[c] 20.5% N_{total}, 10.25% N_{nitric}, 10.25% $N_{ammonium}$, 12% CaO and 6% MgO

(ED_{ing}) with the Reference Dose (RfD) to yield the hazard quotient (HQ). The exposure dose (mg/kg day) and HQ value was defined as follows (USEPA 1997, 2000).

$$\text{Exposure Dose}\,(ED_{ing}) = (C \times Fi \times Ed \times Ef)/W \times Te$$

$$\text{Hazard Quotient}\,(HQ) = ED_{ing}/RfD$$

where C is the element concentration in vegetable (mg/kg); Fi is the food ingestion rate (kg/person day); Ed is the exposure duration (in this study, equivalent to the average adult lifetime); Ef is the exposure frequency (days/year); W is the average bodyweight (kg); Te is the average exposure time for non-cancer risk (Ed × 365 days) and RfD is the oral reference dose (mg/kg day).

The HQ assumes that there is a level of exposure below which it is unlikely for even sensitive populations to experience adverse health effects during lifetime. If the HQ exceeds the unity, there may be concern for potential no carcinogenic effects. In cases where the HQ does not exceed unity, it is assumed that no chronic risk is likely to occur.

Results

Soil and water irrigation

The total and the available fraction U concentration in the soil plots at the beginning of each vegetable field experiment are shown in Fig. 2. Comparing both plots (P1, P2) the range of total uranium concentration was similar for each vegetable but this concentration was higher in soil-lettuce (227–271 mg/kg) than in soil-potato (101–156 mg/kg) or than in soil-bean (35.5–186 mg/kg). However, available fraction U concentration was very similar in both plots for different soil-vegetables (9.6–13.6 mg/kg in soil-potato; 8.75–12.9 mg/kg in soil-bean; 7.05–11.7 mg/kg in soil-lettuce).

Other soil plots characteristics are presented in Table 2. In general, both soil plots were acidic (pH < 5.5), non-saline to very low saline (0 < EC < 4,000 µs/cm) and poor in TOC (<16 g/kg).

During the field experiments the non-contaminated (NCW) and contaminated water (CW) used for soil plots irrigation (P1 and P2, respectively) presented the characteristics summarized in Table 3. Concerning soil characteristics plot P2 showed higher EC and uranium values than

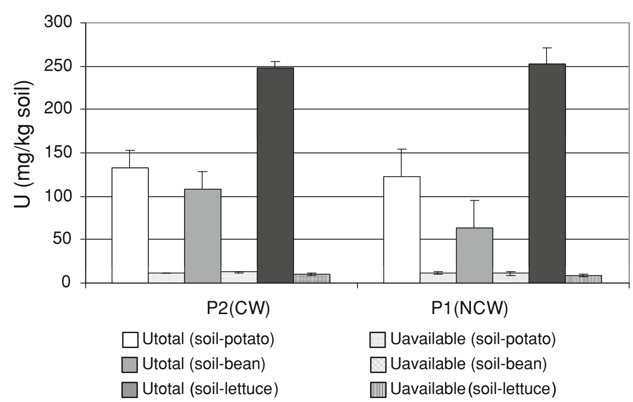

Fig. 2 Total and available average uranium concentration in soil plots (P1 and P2) irrigated with non-contaminated water (*NCW*) and U-contaminated water (*CW*), at the beginning of the vegetable field experiments. *Bars* represent SDs of four replicates

Table 2 Soil characteristics of experimental plots

Soil plot	Vegetable	pH (H_2O)	EC (µs/cm)	TOC (g/kg)
P1	Potato	5.48 ± 0.22	1,539 ± 265	9.9 ± 1.3
	Bean	5.51 ± 0.15	2,121 ± 217	12.0 ± 0.1
	Lettuce	5.72 ± 0.20	1,014 ± 109	11.1 ± 1.3
P2	Potato	5.10 ± 0.14	1,390 ± 120	8.7 ± 1.0
	Bean	4.95 ± 0.08	3,967 ± 91	12.0 ± 0.7
	Lettuce	4.95 ± 0.05	3,590 ± 143	14.48 ± 0.46

The values correspond to average ± SD (n = 4)

Table 3 Water irrigation characteristics during field experiments

Irrigation water (Soil plot)	Vegetable	pH	EC (µs/cm)	U (µg/L)
NCW (P1)	Potato	5.8–5.9	319–360	14–20
	Bean	5.8–5.9	360–412	14–20
	Lettuce	5.8–5.9	360–451	14–20
CW (P2)	Potato	4.2–4.3	1,817–1,823	1,030–1,040
	Bean	4.1–4.2	1,673–1,817	960–1,030
	Lettuce	4.1–4.2	1,817–1,818	940–1,030

plot P1 whereas pH was slightly lower. These differences are the result of the water irrigation quality used before the experiments (Table 3).

Characteristics as pH, EC and U in contaminated and non-contaminated irrigation water were similar during the period of the field experiments. Contaminated water presented pH and EC values that exceeded Maximum Allowable Value (5.0–9 and 1,000 µs/cm, respectively) established by Portuguese legislation (DL 236/98) for this purpose. An irrigation water guideline value is not established in Portugal for U, so the trigger value of 100 µg/L established in Australia and New Zealand legislation (ANZECC 2000) for short-term irrigation (20 years) was used for comparison; U concentration in Cunha Baixa contaminated water reached levels 9- to 10-fold higher. Water salinity (750 < EC < 3,000 ms/cm) presents for soil a medium to high salinity hazard so detrimental effects on sensitive crops as field and string beans, lettuce, bell pepper, onion and carrots could be expected (IWQC 1997). In dry season, the temperature and the high sulphate and calcium concentration detected in the contaminated irrigation water (970–1,012 and 227–259 mg/L, respectively) provided the precipitation of a fine layer of gypsum at the surface of the watered soils.

Uranium in vegetables

The uranium concentration in potatoes grown in the soil plots (P1, P2) exposed to different water quality is presented in Fig. 3. The tubers (with peel) from P2 irrigated with contaminated water concentrated more uranium (110.5 ± 26 µg/kg FW) than tubers from P1 (57 ± 19 µg/kg FW). However, in potato tubers without peel this trend was not followed; in both soil plots element concentration was at the same level (P1: 3.5 ± 0.6 µg/kg FW; P2: 4.0 ± 0.6 µg/kg FW).

Bean growing in both soil plots showed lower U concentration in green bean tissues (pods) than potato tubers with peel; but presenting similar amounts of U when bean was growing either in soil plots irrigated with contaminated or non-contaminated water (P1: 30 ± 6 µg/kg FW; P2: 26 ± 2 µg/kg FW) (Fig. 4).

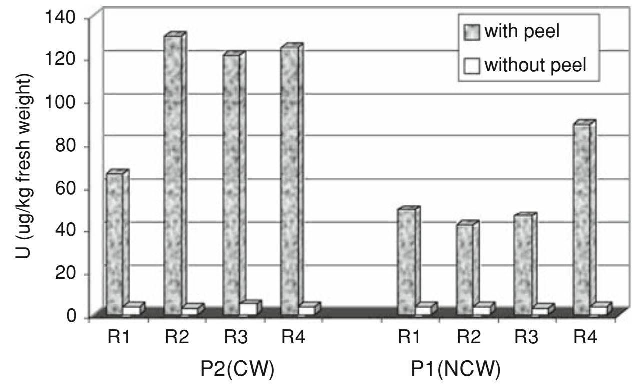

Fig. 3 Uranium concentration on potato tubers with and without peel, after vegetable growth in soil plots (P1 and P2 with four replicates each) exposed to different irrigation waters (*NCW* non-contaminated water; *CW* contaminated water)

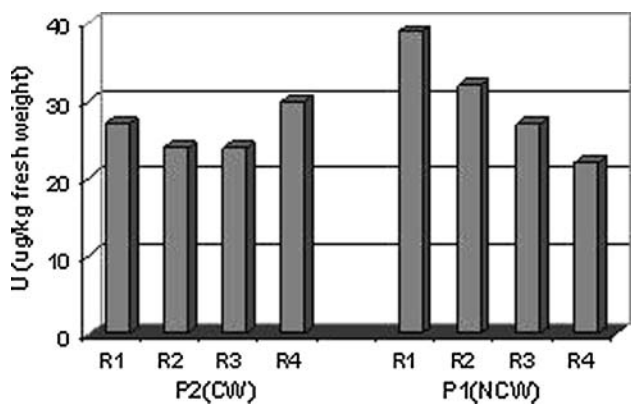

Fig. 4 Uranium concentration on green bean tissues (pods), after vegetable growth in soil plots (P1 and P2 with four replicates each) exposed to different irrigation waters (*NCW* non-contaminated water; *CW* contaminated water)

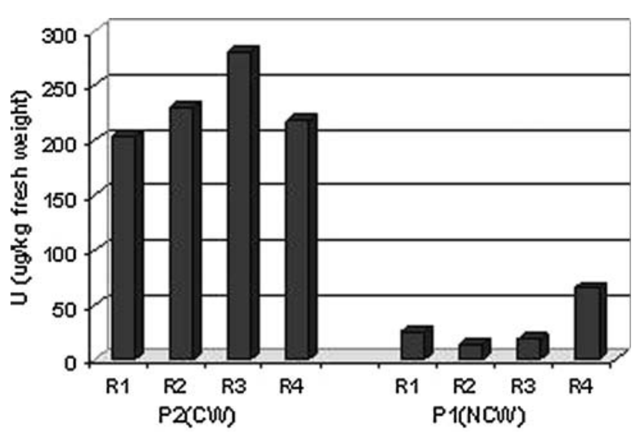

Fig. 5 Uranium concentration in lettuce leaves, after vegetable growth in soil plots (P1 and P2 with four replicates each) exposed to different irrigation waters (*NCW* non-contaminated water; *CW* contaminated water)

Table 4 Uranium vegetable concentration (C), vegetable ingestion rate (Fi), exposure frequency (Ef) and Exposure Dose trough ingestion (ED_{ing})

Vegetable	C (mg/kg)	Fi (kg/day)	Ef (days/year)	ED_{ing} (mg/kg day)
Whole potato tubers	0.136	0.10	52	2.77E10-5
Potato tubers without peel	0.004	0.10	312	4.89E10-6
Green beans	0.036	0.05	24	1.69E10-6
Lettuce leaves	0.263	0.03	72	2.22E10-5

The concentration of uranium in lettuce leaves (Fig. 5) followed the same trend of potato tubers with peel; lower in soil plot P1 (31 ± 21 μg/kg FW) and higher in soil plot P2 (234 ± 29 μg/kg FW) irrigated with uranium contaminated water.

Health risk from vegetables consumption

The health risk assessment for Cunha Baixa' inhabitants were evaluated considering only uranium exposure through a single pathway: ingestion of the studied vegetables (potatoes, green beans and lettuce). Uranium chemical exposure dose (ED_{ing}) was estimated considering an average adult of 70 kg body weight, an adult lifetime of 50 years (Ed) and data expressed in Table 4 as vegetable uranium concentration (maximum content); local vegetable ingestion rate (Fi) and local exposure frequency (Ef). It was also assumed that cooking has no effect on the potato and green bean U concentration.

The ED_{ing} divided by uranium RfD (0.003 mg/kg day) yield for individual vegetable consuming the following Hazard Quotient values: 0.0092 for whole potato tubers, 0.0016 for potato tubers without peel, 0.0005 for green beans and 0.007 for lettuce leaves.

Discussion

The soil used in these field experiments had higher total U concentration than those reported in literature and considered as the normal concentration range in soils (0.3–11.7 mg/kg; Bleise et al. 2002). A soil collected at 1.5 km SW from Cunha Baixa mine area, used for agricultural practices and irrigated with non-contaminated water, which can be considered as the regional reference level, presented U concentration of 9.8 mg/kg (Neves 2002) that also lies within the range referred above. Despite the differences in the total U concentration (Fig. 2), the element fraction attributed to soil water plus exchangeable soil fraction (available fraction) was similar in all soil-vegetable plots. Consequently, the uranium fraction available in soil that could be uptake by the studied vegetables was similar in all the experimental plots.

The concentration of U in the studied edible vegetables tissues was very different; the highest level was found in lettuce leaves (234 ± 29 μg/kg FW) followed by whole potato tubers (110.5 ± 26 μg/kg FW) green bean tissues (26 ± 2 μg/kg FW) and potato tubers without peel (4.0 ± 0.6 μg/kg FW). These results agree with the works of some authors (Sarić et al. 1995; Hakonson-Hayes et al. 2002; Zirovski 2006) that found significant differences in U concentration among several food plant species.

In this experiment, U lettuce leaves concentration was 40-fold higher than mean range concentration reported for vegetable leaves (0.5–5 μg/kg FW; Schnug et al. 2005). The experimental work on lettuce (*L. sativa* L.) carried out by Sarić et al. (1995) on a barren soil deposit (17 mg U/kg average concentration) of the Kalna-Gabrovnica U mine (Serbia), detected on the aboveground parts an average level of 2.15 mg U/kg dry weight (DW). Also, Hakonson-Hayes et al. (2002) found a similar U concentration (2.30 ± 0.39 mg U/kg DW) in the edible crop tissues of lettuce (*Lactuca scarriola* L.) grown in a soil pot with an average concentration of 2.3 mg U/kg and irrigated with well water containing 1,200 μg U/L. In the present study U lettuce concentration was comparatively higher (5.37 ± 0.85 mg U/kg DW) in soil plot P2(CW) also irrigated with a similar U concentration water. Although lettuce in soil plot P1(NCW) presented lower U concentration (0.76 ± 0.37 mg U/kg DW) than in soil plot P2(CW) the obtained values exceed those reported by the former authors (0.44 ± 0.14 mg U/kg DW) for plants also irrigated with 150 μg U/L.

Water irrigation quality seems to have a great influence on U lettuce leaves content. In fact, during September to November (autumn season) 2005, an equal field experiment was carried out in the same Cunha Baixa soil and U concentration in lettuce was lower (100 ± 20 μg/kg FW) in soil plot irrigated with contaminated water (Neves et al. 2008) than the observed in lettuce cultivated in 2006 summer season (this study). As soil conditions, namely U soil available fraction concentrations and irrigation water quality were similar, the differences in U plant leaves concentration can be explained by the lower frequency and lower amount of water irrigation used in autumn season (8 handling times, due to meteorological conditions), in opposition to 2006 summer season when plants were watered 3 and 11 times by handling and furrow, respectively (Table 1).

In Cunha Baixa, as in others places around the world, potato is one of the most important staple crops in inhabitants' diet. In a recent study Zirovsky (2006) detected 7 μg U/kg FW in potatoes bough in Montreal stores, which is a concentration 8- to 11-fold lower than those found in potatoes from Cunha Baixa experiments. Also Sarić et al. (1997) detected for potato U concentrations (average of 0.07 mg U/kg DW) lower than those obtained in the present study; U concentration (DW) in potato tubers ranged between 0.59 ± 0.15 mg U/kg in P2(CW) and 0.30 ± 0.08 mg U/kg in P1(NCW). Otherwise, comparing U concentration in potato tubers with or without peel, 90–97% of total U was concentrated in the peel (Fig. 3). In potato tuber the peel seems to be a storage tissue for U and Cunha Baixa inhabitants should minimize uranium oral intake by consuming this food vegetable peeled. These results are also in agreement with those obtained by Sarić et al. (1997) for various vegetables as bulbous (onions), tuberous plants (potatoes) and thickened roots (carrots, radish, red beet and sugar beet), which presented highest U concentration in the peel of the surface root.

Among the studied food vegetables, green beans tissues had the lowest U concentration (Fig. 4), although higher than the values found by Zirovski (2006) also for beans (<7 μg U/kg FW).

The effect of water irrigation U content on vegetable edible parts was only verified on whole potatoes tubers and lettuce leaves; a significant U concentration increase was observed in the plants watered with uranium-contaminated water (P2(CW); Figs. 3, 5). Differences in vegetables U content suggest that absorption is related to the different plants ability for uptake regulation and probably with uranium distribution within the plant tissues.

Under normal conditions, the ingestion of U by an adult Cunha Baixa' inhabitant can occur through food and drinking water. Generally, the water collected in the area from private wells is not used for human consumption and the local tap water presents U concentration (<2 μg/L) below the safe provisional guideline of 15 μg/L considered by WHO (2004) for drinking-water quality. So, in Cunha Baixa village health adverse effects are not expected from drinking water route and consequently in this study, the potential U chemical health risk assessment was only based on the first available U food data (potato, green bean and lettuce) for the mine area.

Estimated ED_{ing}, based on site-specific vegetable ingestion were lower than RfD (Table 4). Consequently HQ values were less than 1 for both individual or combined ingested vegetables (HQ ≅ 0.018); lettuces leaves gave the main contribution.

In conclusions, the results of this study indicate that potato tubers, green beans and lettuce that grew in soils presenting total and available U up to 252 mg/kg and 14 mg/kg, respectively and irrigated with contaminated water (up to 1,050 μgU/L) absorb and translocate U to the edible plant parts. Their U enrichment ranged in the following order: lettuce leaves > potatoes with peel > green beans > potatoes without peel. Hazard quotient values (HQ < 1) suggest that U exposure trough this vegetables ingestion by an adult Cunha Baixa' inhabitant will not present potential adverse human health risks (nephrotoxicity) during a lifetime. However, for a complete risk characterization in order to peaceful Cunha Baixa' inhabitants more data on other basic food components (fruit, other vegetables, meat products) is necessary, as well as an assessment of the health cancer risk.

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