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Bioconcentração do urânio em duas variedades de feijão (*Phaseolus vulgaris* L.) e respectivas consequências ambientais

Resumo Alargado

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Abstract

For over two decades, inhabitants of Cunha Baixa (Mangualde, Portugal) have been using water for irrigation purposes collected from privately owned wells, mostly for small scale land farms. There is a uranium mining site close to the village which ceased its activity in 2000. Since then, the slow progress of the mine's environmental rehabilitation, coupled with issues related with the acidified drainage effluent, led to a rising concern on how the presence of the nearby mine might be affecting the health of the local population. This stems from the possible contamination of produce as a result of affected soil and irrigation water from the local wells.

In 2006, two field experiments were carried out in order to assess the repercussions of soil and water irrigation quality in the French bean (*Phaseolus vulgaris* L.) seeded in two soils sited in the vicinity of Cunha Baixa's uranium mine, as well as evaluating possible contaminant uptake (U, Al and Mn) by the plant. The aim of this study is to expand on the relationship between uranium mining and the well-being of surrounding populated areas, determining whether the inhabitants of Cunha Baixa are facing any health risks due to regular bean ingestion — a vegetable that nourishes the native population during part of the year (grown from spring to autumn).

Using two soils with different characteristics, particularly concerning uranium content (40 and 106 mg U/kg) and salinity (340 and 1820 µs/cm), the crops were irrigated with water collected from two distinct private wells — one of them contaminated (U: 1030–1040 µg/L; Al: 7500–8000 µg/L; Mn: 4520 µg/L; F: 1200 µg/L) while the second was not (U: 14–20 µg/L; Al: 17–23 µg/L; Mn: 2,4–5,8 µg/L). Soil from Serra de Sintra (Lisboa) was used as control and irrigation was made using local non-contaminated tap water.

After collecting the plants for analysis, the total uranium content rose significantly in both local soils by more than two-fold of those registered at the beginning, while the available uranium soil fraction had a negligible change. In the second field experiment only, total aluminum and manganese soil content also increased significantly (along with U) in all Cunha Baixa soils, while the available manganese soil fraction had the opposite behavior, registering a significant decrease. The edible bean part has been found to have a high uranium content (up to 71 µg/kg FW), being moderately correlated with the available U soil content. The risk assessment regarding oral exposure through bean consumption revealed that local inhabitants were subject to contaminant levels well below established safety thresholds, suggesting that non-cancer adverse health effects are unlikely to occur. Since Portugal has a fairly large number of uranium mining sites for such a small country, and data regarding uranium levels in food grown around uranium mines in Portugal was nearly non-existent until about a decade ago, these field experiments provide a valuable contribute regarding public health concerns of those who live close to such areas.

Keywords: French bean (*Phaseolus vulgaris* L.), uranium, contamination, oral exposure, risk, Cunha Baixa.

Introduction

Throughout mankind's industrial history, ore mining has always been linked with large landscape modifications. Recently, the scientific community has been raising a higher awareness about environmental risks inherent to mining activity, which can threaten the balance of surrounding ecosystems, possibly affecting the health of local populations. A careful analysis and monitoring of mining sites is, therefore, required in order to control and mitigate these risk factors (Santos Oliveira e Ávila, 2001).

Uranium mining poses a particularly big threat to nearby areas because of its association with chemical and radiological risks inherent to this ore. The remaining debris resulting from mine activity, as well as eventual sludge originating from the mine drainage effluent, are the two most dangerous remainders which, if not properly contained or removed, can be mobilized through wind and rain, contaminating local aquifers, soil or adhering to airborne particles (Pedrosa e Martins, 1999; Machado, 1998; Santos Oliveira e Ávila, 1998). .

In Portugal, the majority of the uranium mining sites are located in the Beiras region, of which the Cunha Baixa mine is part of. From all of the 58 sites in which radioactive ore mining was done, according to preliminary studies made by IGM and ENU, the mining sites of Urgeiriça, Cunha Baixa, Quinta do Bispo and Bica were classified as having the largest environmental repercussions (Magno, 2001; Silveira 2001). Mining impact in Cunha Baixa was mostly reflected in the water quality of some nearby wells, frequently used in crop irrigation, as well as soil contamination which is particularly threatening for agricultural land use (Santos Oliveira *et al.* 1999; Neves *et al.*, 2002; 2003a, 2003b; 2005). For the local population who consumes vegetable food grown in such soils and feeds the cattle with non-edible remaining plant parts, there is a legitimate reason for health concern about the dangers of including such crops in their daily diet due to a higher exposure probability to contaminant compounds.

Previous field experiments made by Neves (2002) using maize crops under a non-controlled scenario, revealed higher than average uranium levels in the plant material (0,6 – 1,2 mg U/kg DW). Although being neither an essential nor benevolent element to plants or animals, many crops can incorporate uranium in their biomass, translocating the element from the soil through absorption at the root system. In general, the uranium plant absorption yields low coefficients: Brooks (1983) proposed a coefficient factor of 0.02; Sheppard *et al.* (1989) suggested a factor of 0.013 whilst AEIA (1994) mentions a coefficient range between 0.001 and 0.01.

Materials and methods

Between early spring and mid-autumn of 2006, two controlled field experiments were conducted with the French bean (*Phaseolus Vulgaris* L.) in two soils (A and B) of agricultural use located in the vicinity of the Cunha Baixa mining site, distanced 50 m from each other and characterized as sandy-loam Cambisols (Carta de Solos, 1978). Based on the investigation work developed by Neves (2002), each soil area (40 m^2) was split into two side-by-side experimental plots (17 m^2) spaced by 1.4 m. In one plot, irrigation was carried out using contaminated water (referenced as A-CW and B-CW) while in the second, non-contaminated water was employed (referenced as A-NCW and B-NCW). All plots had four replicates each in order to be statistically meaningful. Each soil replicate was seeded with 28 bean plants (2 plants side by side x 14), adding to a total of 112 plants per plot. As control, a soil brought from the Serra de Sintra granitic region (located in the Lisbon metropolitan area), stored in 16 plastic containers for seeding, was grouped in four rows of four containers each (referenced as soil C-TW), representative of the other soil replicates' distribution. In this soil, irrigation was done using local tap water. Crops were watered based on their needs; resort to soil fertilizers ("Nitromagnésio 20.5 %") and choice of crop variety (*patareco* and *francês* dwarf bean) was based according with local farming practices.

Composite soil sampling (0-20 cm depth), collected from each soil plot replicate, was done before the plant seeding stage and after the harvest. Soil samples were air-dried, sieved through a 2 mm screen and analyzed for physicochemical parameters of pH (1:2,5 soil/water suspension), salinity (electrical conductivity of the saturated-paste extract – EC_{soil}), cation exchange capacity (CEC), exchangeable cations (Ca, K, Mg, Mn and Na), total organic carbon (TOC) (Walkley & Black, 1934), extractable P and K (Egner *et al.,* 1960) and mineral N (Keeney & Nelson, 1982). Soil sampling elemental analysis of total and available fractions was made using ICP-MS after acid digestion (Code UT-4 Total Digestion, Actlabs Laboratory) and ammonium acetate 0.5 M (Schollenberger & Simon, 1945) respectively.

Water sampling was performed *in situ*, pumped from the private wells (sampling points) and filtered through a mixed cellulose ester 0.45 µm membrane after probing for temperature, pH, Eh and EC measurements, then split into acidified (HNO3, pH < 2) and non-acidified sub-samples used for anionic and cationic analysis. All samples remained refrigerated at 4°C until they reached the laboratory for analysis of the total dissolved solids (TDS), SO_4^{2-}, $Cl^-$and NO_3^- through ionic chromatography and F^- e P through spectrophotometry. Cationic measurement was done through ICP-OES.

When the beans reached their maturity phase (65 to 90 days), the entirety of the plant material was collected and separated into root, leaves/stems and bean pods. After carefully washing the accumulated dirt with tap water, followed by distilled one, plant parts were dried at 40°C, ground and sent for elemental analysis to Actlabs laboratory in Canada. All samples were fresh and dry weighted. Vegetation sample analysis was made through ICP-MS after ashing at 475°C over a 36 hour period followed by a proprietary acid digestion (Code 2D, Actlabs Laboratory). Concentrations of plant elements were calculated through site-specific plant ash/dry and fresh/dry ratios from each soil plot replicate.

Data analysis

The resulting data was statistically tested for significance among the averaged replicate values (n=4) through Kolmogorov-Smirnov (two-sample) non-parametric test for a 5% level. Regarding the control group — because crops in soil C yielded a lower biomass due to little available soil material — plot replicates were grouped in pairs. Statistical correlation was measured using Spearman's rank correlation coefficient using a significance level of 5%.

Health risk assessment

The oral risk assessment was based on the EPA (1989) guidelines developed by EPA's Integrated Risk Information System (IRIS), a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants. Unlike the mechanism for carcinogenesis, commonly referred as "non-threshold" since there is theoretically no safe level of exposure for a given chemical that does not constitute a small, but finite, probability of generating a carcinogenic response, chemical toxicity assumes an exposure threshold value that must be overcome for adverse health effects to manifest.

A parameter defined as RfD (Reference Dose) is EPA's preferred toxicity value for the evaluation of non-carcinogenic effects that result from a given pathway of exposure, corresponding to an estimate of a daily exposure level for human population (including sensitive groups) below which is unlikely for them to experience adverse health effects. In absence of other modifiers, it most commonly refers to a chronic timescale length of 365 days or above, specifically developed as a protection for long-term exposure to a compound. The absorbed dose through oral ingestion is calculated using the intake rate and is expressed as mass of a substance entering the body per unit body weight per unit time (mg/kg·day). Risk quantification is made by dividing the estimated chemical intake dose from foodstuff ingestion by the reference dose of the compound (health guideline value).

This ratio is referred as a hazard quotient and the greater its value above unity (HQ > 1), the greater the level of concern. The method is calculated as follows:

$HQ = ID_{ing} / RfD$
$ID_{ing} = C \times I_r \times E_f / bw$
$E_f = F \times E_d / (E_d \times 365)$

Where:
ID_{ing} – Oral intake dose from foodstuff
C – Contaminant concentration
I_r – Intake rate of contaminated food
E_f – Exposure factor
bw – Average body weight
F – Frequency of exposure (days/year)
E_d – Duration of exposure (years)

Results and discussion

Irrigation water

Laboratory analysis of the sampled water from the private wells confirmed one contaminated source (P15) based on a comparison with maximum recommended/allowed values (MRV and MAV, respectively) taken from irrigation water quality guidelines of the Portuguese legislation (DL 236/98). The contaminated well exceeded the MRVs of EC, Al and F, by 1.2 to 1.8-fold margin, except Mn, which was approximately 23-fold higher. While the previous were still within the MAVs, pH was outside the admissible range, located further below the required level. Since uranium guidelines for water were absent in Portuguese and EU law, this study used those established by Australia and New Zealand (ANZECC, 2000) for short-term irrigation water (up to 20 years) in which the U concentration of P15 site exceeded the maximum advised level of 100 µg/L by a 10-fold margin. P24 sampling site and tap water were suitably balanced for culture irrigation.

Soil

The agricultural soils in Cunha Baixa are classified as under-developed cambisols, sandy-loam textured and generally acidic. With a relatively low cation exchange capacity (CEC<13 $cmol_c$/kg), a typical value for low-activity clays (kaolinite > illite; Neves, 2002) and low TOC (<16 g/kg soil), both soils have a low to moderately high salinity levels ranging from 785 to 4224 µS/cm.

In 2005, Santos Oliveira *et al.* reported a local geochemical background level for uranium of 12 mg U/kg. Both field experiment soils (A and B) exceeded this value: soil A had, on average, U content up to 3 times higher while soil B registered as much as 10 times the background concentration for alluvial soils of the region. The only existing soil quality guideline value for agricultural land use regarding U comes from Canada (CCME, 2007), establishing a threshold of 23 mg U/kg, a concentration exceeded in every analysis of both experimental soils A and B.

Soil C differs mainly in regard to TOC and U, registering a higher TOC content (up to a 2-fold margin) and a considerably lower U concentration (total and available) found within the regular range of background U levels (0.3-11.7 mg U/kg) as reported by Bleise *et al.,* 2003.

Both Cunha Baixa and the control soils contained an adequate amount of macro nutrients required for crop development with a fertilizing soil classification between medium and very high (INIA, 2000), based on the soil content of available phosphorus and potassium.

Both field experiments confirm the typical acidity trend of this region’s soil, with values which lie slightly below the ideal pH suited for bean development (pH: 6.0-7.5; INIA, 2000). In the spring experiment, soils ACW and BCW had a statistically significant pH drop after the crop harvest, most likely due to a lower pH value of the contaminated irrigation water.

Soil EC behaved differently in the two field experiments. The first one showed a moderate but significant increase only in soil plots irrigated with contaminated water, while the second experiment revealed an overall decrease across all plots, regardless of the water quality. Given that the second field experiment was carried out during summer season, soil salt content increased in comparison with the measurement of the previous experiment due to higher evaporation rate. This rate decreased throughout crop growth as a consequence of regular irrigation through salt removal from the upper soil layers.

Total U soil content has an overall rising trend across all soil plots in both experiments, confirmed through soil plot analysis before the seeding and after plant harvest, apparently independent of the irrigation water quality. This rise was not reflected on the available U form which, instead, remained unchanged with negligible variations. Throughout the study, soil B samples featured a higher U content than its counterpart — up to 2-fold of total U on average and up to 10 times the available U content in soil A.

Despite having a high U concentration, both soils have a much smaller available fraction, usually below 10% of the total U, with the exception being a measurement made in soil plot B-NCW which reached 18%. Such low available fraction can be attributed to the low activity clay (kaolinite) and low organic matter content, affecting the soil CEC, and consequently the adsorption of the most common U oxidized species of UO_2^{2+} (Laroche *et al.*, 2005).

Total aluminum content in soils ranges from 43 to 93 g/kg. Possessing a wide variability across different soils, Al presence and mobilization are conditioned by soil pH, salinity and chemical species with which it can form complexes.

Plant species and cultivars of the same species differ considerably in their ability to take up and translocate aluminum to above-ground parts (Kabata-Pendias, 1984). It is unclear to what extent aluminum is taken up into root food crops and leafy vegetables. An uptake factor (concentration of aluminum in the plant/concentration of aluminum in soil) of 0.004 for leafy vegetables and 0.00065 for fruits and tubers has been reported (DOE, 1984), but the pH and plant species from which these uptake factors were derived are unclear. Based upon these values, however, it is clear that aluminum is not taken up in plants from soil, but is instead bio diluted. Available Al content is under the limit of the detection method, except in the control soil (soil C), most likely caused by the higher TOC, with as much as twice as the amount found in soils A and B.

Manganese total soil content oscillates between 220 and 800 mg/kg across the two field experiments. Mn, similarly to Al, is found in concentrations with a large variability, usually somewhere between 40 and 900 mg/kg (ATSDR, 2008). Yet most of this is unavailable for plant use since available Mn constitutes a much smaller percentage of the total, ranging from 1 to 4%. It occurs mostly associated with organic matter, or various manganese oxides, influenced by soil pH, moisture and soil aeration (Schulte et al., 1999). Overall, Mn content differs between the two used soils from Cunha Baixa, with soil B plots registering, on average, twice the total Mn detected in soil A. After the crop harvest there was an increase in total Mn while the available diminished across all soil plots.

Soil Plot		Spring field experiment					Summer field experiment				
		A - NCW	A - CW	B - NCW	B - CW	C - TW	A - NCW	A - CW	B - NCW	B - CW	C - TW
pH (H_2O)	B	6,43±0,26a	6,15±0,23a*	5,86±0,29a	5,42±0,03b*	5,33±0,03	5,5 ± 0,1a	4,8 ± 0,0b	5,5 ± 0,2a	5,0 ± 0,1c	5,0 ±0,1
	A	6,06±0,22a	5,50±0,20b*	5,72±0,20ab	4,95±0,05c*	5,36±0,14	5,6 ± 0,1a	4,8 ± 0,1b	5,6 ± 0,2a	4,9 ± 0,1b	5,1 ± 0,1
CE (µs/cm)	B	2436±1764a	785±364a*	1084±444a	1279±305a*	2488±0	2164 ± 241a*	4224 ± 95b*	2121 ± 218a*	3968 ± 91c*	3568 ± 110
	A	1449±582a	3436±230b*	1014±109a	3590±143b*	2040±275	525±79a*	2818 ± 141b*	308 ± 36a*	2189 ± 216c*	1508 ± 3
COT (g/kg)	B	12,3±0,5a*	15,7±1,3b	10,0±0,5c	11,4±0,4a*	29,5±1,0	13,7 ± 0,7a	14,8 ± 1,2a	12,0 ± 1,1a	12,0 ± 0,7a	30,7 ± 0,3
	A	16,0±1,2ac*	18,2±1,3a	11,2±1,4b	14,5±0,5c*	35,0±1,9	14,7 ± 1,0a	14,4 ± 0,5a	10,2 ± 2,1b	9,6 ± 1,4b	23,5 ± 0,2
$K_{extractable}$ (mg/kg)	B	668±259a	301±45b	257±39b*	259±26b	282±17	322±53a	183±18b*	303 ± 54a	237 ± 14a*	170 ± 4
	A	542±179a	230±46b	203±9b*	195±40b	203±12	247 ± 15a	106 ± 22b*	205 ± 45ac	151 ± 9bc*	91 ± 0
$P_{extractable}$ (mg/kg)	B	133±44a	105±30a	48±13b	22±3c	19±1	111 ± 11a*	93 ± 12abd*	67 ± 18bc*	55 ± 5cd*	57 ± 6
	A	146±45a	128±34a	55±16b	41±14b	19±0	196 ± 5a*	172 ± 5b*	142± 13c*	145 ± 5c*	158 ± 0
$N_{mineral}$ (mg/kg)	B	56±36a	20±7a	28±10a	37±7a	86±2	54 ± 12ab*	65 ±4a*	60 ± 5ab*	74 ± 10b*	106±2
	A	30±12a	26±7a	24±10a	21±9a	67±9	13 ± 1a*	24 ± 4b*	11 ± 1a*	17 ± 4a*	75 ± 0
U_{total} (mg/kg)	B	52±8a*	30±6c*	123±20b*	129±13b*	2±0	31 ± 3a*	53 ± 15ab*	64 ± 18bc*	109 ± 46c*	2 ± 0
	A	100±3a*	92±6a*	252±17b*	248±6b*	10±3	101 ± 5a*	104 ± 4a*	271 ± 4b*	259 ± 3c*	8 ± 1
$U_{available}$ (mg/kg)	B	1,36±0,28a	0,83±0,26a	9,30±0,80b	10,21±0,41b	0,02±0	2,06 ± 0,08a	2,42 ± 0,21a	11,33 ± 1,60b	21,39 ± 0,32b	0,03 ± 0
	A	1,30±0,29a	1,36±0,42a	8,29±0,91b	10,09±0,95b	0,04±0,02	2,15 ± 0,07a	2,84 ± 0,18a	12,69 ± 1,23b	13,31 ± 10,9b	0,07 ± 0,04
Al_{total} (%)	B	5,51±1,02a*	5,31±0,57a*	8,00±1,32a	7,45±1,44a	6,40±0,05	5,10 ± 0,26a*	5,90 ± 0,28bc*	5,61 ± 0,70ac*	5,50 ± 0,29ab*	4,34 ± 0,09
	A	8,09±0,22a*	7,71±0,43a*	9,26±0,14b	9,07±0,25b	6,40±0,47	8,38 ± 0,22a*	8,24 ± 0,38a*	9,17 ± 0,27b*	8,74 ± 0,42ab*	6,84 ± 0,22
$Al_{available}$ (mg/kg)	B	d.l.	d.l.	d.l.	d.l.	3,3±0,3	d.l.	d.l.	d.l.	d.l.	2,75 ± 0,25
	A	d.l.	d.l.	d.l.	d.l.	4,5±0,0	d.l.	d.l.	d.l.	d.l.	4,25 ± 0,75
Mn_{total} (mg/kg)	B	355±35,1a*	324,50±27,3a*	709±91,9b	702,25±75,9b	595,5±3,5	220,8 ± 11,7a*	384,8 ± 58,3b*	469,0 ± 31,2bc*	533,8 ±109,4c*	485,0 ± 29,0
	A	495,8±31,4a*	545,3±45,3a*	787,0±27,2b	759,8±20,6b	410,5±3,5	430,3 ± 9,7a*	458,5 ± 16,8a*	800,0 ± 39,9b*	750,5 ± 7,9b*	398,5 ± 5,5
$Mn_{available}$ (mg/kg)	B	12,6±2,2a*	8,5±1,3a	21,2±2,7b	29,6±5,0b	7,5±0,1	13,1 ± 1,2a*	18,7 ± 0,2c*	23,7 ± 0,9b*	39,4 ± 7,9d*	12,3 ± 1,6
	A	7,2±0,7a*	9,3±1,2a	17,6±2,3b	27,2±3,2c	7,3±0,0	5,8 ± 0,8a*	11,5 ± 0,6bd*	10,2 ± 0,9ad*	23,0 ± 4,5c*	8,7 ± 1,5

Table 1 – Soil parameter values correspond to [average] ± [standard dev.] (n=4 for soils plots A and B, n=2 for soil C); d.l.—detection limit (0.1 mg/kg); values in a row followed by the same letter are not significantly different by Kolmogorov–Smirnov test (pb0.05); test for n=4

Plant yield

Bean plant yield was significantly different between experiments, with a larger overall production of crops grown during the summer.

Amongst the soils, bean pod yield in soil B registered, on average, three times of soil A, possibly caused by soil differences related with the amount of exchangeable Mg (2-fold higher in soil B) and external factors of air temperature and humidity, given the preference of bean plants for dryer and less temperature oscillations (Bouwkamp & Summers, 1982). As for plant production in the same soil, irrigation water type didn't seem to have a discernible effect in bean pod production.

The summer field experiment, however, showed a different behavior related to influence of irrigation water, with crop yield in between same soil plots being significantly different, with ACW having 45% less that ACNW and BCW with 27% lower yield than BNCW. Most likely, since this time growth was less conditioned by limiting factors of climate, water quality type had greater impact in crop yield. A strong negative correlation was obtained between pod yield and EC (r_{soil_A}=-0.96; r_{soil_B}=0.85), reflecting the plant's low tolerance of moderate to high salinity levels (Bernardo, 1996). Additionally, although not as expressive, a slight trend can be observed in soil B yielding a bigger plant production compared to soil A in both field experiments.

Stem/leaf yield showed also a negative influence of contaminated water irrigation in the summer experiment, with significantly lower plant material in both soil plots ACW (less 50%) and BCW (26% lower). Again, both were strongly correlated with soil salinity (EC) and soil pH.

Element concentration found in bean pod and stem/leaf

Uranium

Comparison between plots irrigated with different water quality revealed slightly higher concentrations although with non-significant differences. Identically, stems and leaves show a similar trend except for soil A summer trial, where significantly different concentrations can be found between the two plots (2-fold more U for contaminated water plot). Globally, it appears to be a slight influence of the water quality on the U plant concentration.

Soil influence appears to be a larger influencing factor in U plant concentration uptake. Such impact can be seen on U content of plants developed in soil B, where these systematically reveal higher concentration than those grown in soil A. In the first field experiment (spring), soil B pods had 2 times the U content of those in soil A and 1,8 times more in the second trial (summer). Stem/leaf also showed a consistent higher U concentration in soil B, comparing with those detected for soil A.

Aluminum

Both experiments revealed no significant differences of Al concentration in plant parts in all soil plots, suggesting that the different water qualities do not play a preponderant part in Al plant uptake despite high Al content in the CW (Table 2).

Manganese

Manganese plant content was generally higher in plots irrigated with CW as well as in control soil C for both field experiments. In the summer trial plots ACW and C-RP stand out, having concentration 2,9 and 1,8-fold higher respectively in comparison with the previous field experiment. Plants grown in soil C registered the overall maximum content (144,6 ± 7,1 mg/kg DW), possibly due to higher CEC, which favors chelation, increasing the amount of exchangeable and soluble Mn ready for root uptake. There was a strong correlation between Mn plant concentration and soil salinity (EC), with an average of r=0.93, for Cunha Baixa soils.

Oral risk assessment

Determination of exposure dose to Al, Mn and U was based on dietary habits of Cunha Baixa inhabitants. Ingestion rate for bean pods was assumed to be 50 g/day, with 24 day/year consumption, for a 1 year exposure length, based on a previous study related to uranium in vegetable foodstuff in Cunha Baixa (Neves *et al.*, 2011). Risk classification was divided in two age groups: adults (>20 year old) and children (5-11 year old)**,** with a mean body weight of 70 and 32 kg respectively. The concentration of each element used in exposure dose calculation corresponds to the averaged maximum content detected of all soil plots (plot ACW Mn: 15.5 mg/kg; plot BCW U: 71 μg/kg; plot BCW Al: 17.0 mg/kg). Intake of these elements was based on the premise that its concentrations would not be affected by cooking.

Based on results illustrated in table 2, bean pods consumption clearly show lower exposure to these elements than their established RfD (U: 0.003 mg/kg day; Mn: 0.14 mg/kg day and Al: 1.0 mg/kg day), with a hazard quotient lower than 1, with a contribution of 0.24% 0.17% and 1.1% for U, Al and Mn respectively, for the most sensitive age group (children). Therefore, Cunha Baixa inhabitants are not expected to be negatively affected due to regular bean ingestion in both age groups. Even taking in account the lower TDI (tolerable dose intake) guideline for U intake of 0.6 μg/kg day recommended by WHO (1998), considering the same intakes rates and frequency of consumption, dietary intake of bean pods makes up for 0.6 and 1.2% of the dose for adults and children age groups respectively.

Global exposure parameters		**Children**	**Adults**	**Unity**
Average body weight	m	32	70	kg
Exposure frequency	f_{exp}	24		days/year
Duration of exposure	t_{exp}	365		Days
Intake rate	t_{ing}	0,05		kg/day
Toxicological parameters				
RfD	U	0,003		mg/kg·day
	Al	1		
	Mn	0,14		
Maximum average concentration (fresh weight)				
C	U	71		μg/kg
	Al	17,0		mg/kg
	Mn	15,5		
Exposure dose				
DE_{ing}	U	$7{,}30\text{x}10^{6}$	$3{,}30\ \text{x}10^{6}$	mg/kg·day
	Al	$1{,}70\ \text{x}10^{3}$	$8{,}00\ \text{x}10^{4}$	
	Mn	$1{,}60\ \text{x}10^{3}$	$7{,}30\ \text{x}10^{4}$	
Hazard quotient				
HQ	U	$2{,}40\ \text{x}10^{3}$	$1{,}10\ \text{x}10^{3}$	adimensional
	Al	$1{,}70\ \text{x}10^{3}$	$8{,}00\ \text{x}10^{4}$	
	Mn	$1{,}10\ \text{x}10^{2}$	$5{,}20\ \text{x}10^{3}$	
Tolerable Intake Dose				
TDI (OMS)	U	0,11(18%)	0,05 (8%)	μg/kg·dia

Table 2 – Reference parameters and data results of the oral risk assessment derived from bean consumption for Cunha Baixa inhabitants.

Additionally, an assessment of the overall potential for non-carcinogenic effects posed by more than one vegetable foodstuff could provide a further realistic scenario. By including potato and lettuce intake in health risk assessment using the data taken from previous studies (Figueiredo, 2009; Marcelino, 2010), under similar experimental conditions, in the same soil plots and irrigation water quality, the calculated risk revealed that the combination of these three vegetables still does not point to any worrying exposure levels, meaning that adverse health effects are very unlikely to occur since the sum of the hazard quotients corresponds to a value greatly below 1.

Combined Hazard Quotient $HQ_c = \Sigma HQ_i$			
	Children	Adults	Unity
U	0,0433	0,0268	adimensional
Al	0,0122	0,0073	
Mn	0,034	0,019	

Table 3 – Cumulative risk factors of the combined oral risk assessment derived from bean, potato and lettuce consumption for Cunha Baixa inhabitants.

Conclusion

Overall, the soil content of the three potentially toxic compounds U, Al and Mn did not appear to be influenced directly by the irrigation water type. The main prominent change was related to total U content in soil, which rose significantly from the instant before seeding by 3-fold and 2.2-fold more, on average, for spring and summer field experiments respectively. On the other hand, soil salinity and pH seemed to be affected by the quality of water, with an increase of EC in the CW irrigated plots (EC_A: 785→3436 µS/cm; EC_B: 1279→3590 µS/cm) and acidity (pH_A: 6.15→5.50; pH_B: 6.15→5.50).

Plant material yield also did not appear to be influenced by the water type since no significant differences were found between plots belonging to the same soil. However, soil B plots showed a larger overall productivity in comparison with soil A. Pods had a bigger development in the second field experiment (summer), which led to higher yield, while stems/leaves and roots yielded higher during spring season.

Plant U concentration showed a greater variability on edible parts (pods) reaching higher values in plots watered with CW (up to 433.2 ± 167.6 mg U/kg in plot B-CW), despite overall mean concentration not being significantly different. Uranium soil content was found to be strongly correlated with U in plant material.

Risk assessment for oral exposure regarding non carcinogenic health effects revealed no reasons for alarm since the obtained risk quotients were very far from the unity, with a contribution 0.24%, 0.17% and 1.10% for U, Al and Mn respectively, for the established threshold exposure level in most sensitive age group. Though this study did not investigate the radiological hazard risk due to vegetable consumption, previous studies (Carvalho *et al.,* 2009) suggest it might be an issue worthy of further analysis in the future, making a special remark concerning radium content in soil and water, and it's tendency for accumulation in agricultural foodstuff and consequent mobilization to human through the trophic chain.

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