CONTEÚDOS DE CÁDMIO, CHUMBO E NÍQUEL EM COGUMELOS E RESPECTIVOS SOLOS

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Abstract

The purpose of this investigation was to quantify cadmium, lead, and nickel in wild mushrooms and respective underlying soils from two different regions of Portugal. These metals were quantified by Electrothermal Atomization Atomic Absorption Spectrometry. The values found were: (i) for cadmium, 0.69±1.1 μg/g in caps, 0.39±0.79 μg/g in stalks and 0.011±0.013 μg/g in soils; (ii) for nickel, 1.1±1.6 μg/g in caps, 1.88±1.13 μg/g in stalks, and 74.0±123 μg/g in soils; (iii) for lead, 0.092±0.17 μg/g in caps, 0.15±0.15 μg/g in stalks and 0.45±0.25 μg/g in soils. Lead contents were significantly higher in the soils and mushrooms from the industrialized region of Beira Interior compared to those of the rural region of Trás-os-Montes. Nickel levels were significantly higher in the soils of the rural region, but there were no differences in nickel contents of mushrooms from the two regions. Mushrooms highly accumulated cadmium as shown by the bioconcentration factors (BCF_{cap}=64 and BCF_{stalk}=26) while lead and nickel were excluded (BCFs<<1). The non-edible species Tricholoma acerbum *significantly accumulates more nickel than the edible species* Lactarius deliciosus, Macrolepiota procera *and* Amanita *ponderosa and also accumulates more cadmium than* Lactarius deliciosus*. With this study we contributed for the knowledge of the different capacity of mushrooms in accumulating some metals and found relevant information for nickel, a metal that is still scarcely studied in this much appreciated food.*

Key words: mushrooms; soils; cadmium; nickel; lead; bioconcentration factors.

Resumo

O objectivo da presente investigação consistiu no doseamento de cádmio, chumbo e níquel em cogumelos selvagens e respectivos solos recolhidos em duas diferentes regiões de Portugal. Os metais foram quantificados por Espectrometria de Absorção Atómica com Atomização Electrotérmica. Os valores encontrados foram: (i) para o cádmio, 0,69±1,1 μg/g nos chapéus, 0,39±0,79 μg/g nos pés e 0,011±0,013 μg/g nos solos; (ii) para o níquel, 1,1±1,6 μg/g nos chapéus, 1,88±1,13 μg/g nos pés, e 74,0±123 μg/g nos solos; (iii) para o chumbo, 0,092±0,17 μg/g nos chapéus, 0,15±0,15 μg/g nos pés e 0,45±0,25 μg/g nos solos. Os conteúdos em chumbo foram significativamente mais elevados nos solos e cogumelos colhidos na região industrializada da Beira Interior comparados com os provenientes da região rural de Trás-os-Montes. Os níveis de níquel eram significativamente mais altos nos solos da região rural, mas não houve diferenças significativas nos conteúdos em níquel dos cogumelos das duas regiões. Os cogumelos evidenciaram acumular grandes quantidades de cádmio, como se depreende dos factores de bioconcentração ($BCF_{chapéu}$=64 e $BCF_{pé}$=26), enquanto o chumbo e o níquel são excluídos (BCFs<<1). A espécie não edível Tricholoma acerbum *acumula significativamente mais níquel do que as espécies edíveis* Lactarius deliciosus, Macrolepiota procera *e* Amanita ponderosa *e também acumula mais cádmio que a* Lactarius deliciosus*. Com este estudo contribuímos para o conhecimento da diferente capacidade dos cogumelos para acumular certos metais e encontrámos informação relevante para o níquel, um metal ainda muito pouco estudado neste alimento tão apreciado.*

Palavras-chave: cogumelos; solos; cádmio; níquel; chumbo; factores de bioconcentração.

INTRODUCTION

In recent years the safety of food has become a major concern for consumers. This is due, among other factors, to the increasing awareness about the influence of diet in health. Some groups of pollutants are of special concern when present in foodstuffs and toxic metals are among the most important. This results on one hand, from the recognized toxic properties of some elements and, on the other hand, from the continuing pollution of the environment with dusts and effluents rich in metals as a result of anthropogenic activities. Also, the natural constitution of soils and rocks can be determinant to the transference of metals to vegetables. Metals of interest are those with recognized toxic effects such as cadmium and lead[1]. Others, evidencing some essentiality, can either provoke undesirable effects at high concentrations or elicit allergenic reactions in vulnerable people as is the case of nickel[2].

Mushrooms are important in human diet, and some wild species, due to their rare palatability, are highly appreciated world wide as a delicacy. Generally, mushrooms provide carbohydrates, proteins, vitamins, and minerals. A study that evaluated 38 elements in mushrooms collected in non polluted areas demonstrated that they constitute an important source of essential elements, as well as are vehicles of toxic and rare elements[3]. Mushrooms have also been suggested as bioindicators of toxic metal pollutions including, cadmium, lead, mercury and others due to its extensive and close integration in the environment[4]. Thus, the capacity of mushrooms to accumulate toxic metals can have both human health[5,6] and environmental repercussions, revealing potential interesting applications for bioremediation[7,8].

Several elements, including cadmium and lead, have been quantified in mushrooms[9-19]. Nickel quantifications in mushrooms are much scarcer[19–22]. Since the contents of toxic metals in mushrooms are dependent on several variable environmental factors, namely atmospheric pollution, soil constitution and contamination, it is important to monitor their metal contents in the areas where they are traditionally collected for human consumption. This also contributes to clarify the influence of environmental factors in the uptake of metals by different mushroom species.

The purpose of this study was to quantify cadmium, lead and nickel in mushrooms and in the respective underlying soils, from two regions of Portugal where mushrooms are collected both for consumption by local people and also for commercialization. With this study we aimed at contributing for the knowledge of the capacity of mushrooms to accumulate metals and giving relevant information for nickel, that is scarcely studied. Thirty nine wild mushrooms as well as the respective underlying soil samples were collected at two different regions, Trás-os-Montes and Beira Interior. The species were identified by expert mycologists, and were mainly edible specimens. The method for metal analysis included an acid digestion of the samples and subsequent measurement of the metals by Electrothermal Atomization Atomic Absorption Spectrometry (EA-AAS). The levels of the metals in the mushrooms were tentatively correlated with the metal contents in the soils from both regions. The bioaccumulation factors and the distribution of the metals in the different portions of mushrooms (cap and stalk, and also volva for some species) were also determined. A comparison was also made between the metal uptake capacity of the non edible *Tricholoma acerbum* species and the three appreciated edible species, *Lactarius deliciosus*, *Macrolepiota procera*, and *Amanita ponderosa* as well as the ecology of the mushrooms (mychorrhizal and saprophyte).

MATERIALS AND METHODS

Reagents and materials

All solutions were prepared with doubly de-ionized water and the chemicals HCl, HNO_3, $CaCl_2$ were of Suprapure grade (Merck, Germany). Cd, Ni, and Pb standards were prepared daily from the respective 1000 mg/L nitrate solutions (Spectrosol, BDH) in HNO_3 (0.2% v/v). The chemical modifiers were prepared with 1g/L of $Mg(NO_3)_2$ solution and 2g/L of $Mg(NO_3)_2$ + 3g/L of $Pd(NO_3)_2$ solution, Suprapure grade from Merck in 15% (v/v) Suprapure HNO_3.

The Certifed Reference Materials used were Soil NCS ZC73001 (Promochem, Germany) and Lichen CRM 482 – Community Bureau of Reference – BCR (Promochem, Germany).

To avoid contamination of the samples, all PTFE materials (Teflon® vessels, pipettes, micropipette tips and auto-sampler cups) were immersed in freshly prepared 15% v/v *pro analysis* HNO_3 (Merck, Germany) during 24 h, then rinsed thoroughly with doubly de-ionized water, and dried in a dust-free area before use.

Sampling

The mushrooms were collected by hand or with a plastic knife, with residual soil particles manually removed using paper, a brush or a plastic knife, and subdivided into the cap and the stalk parts. For some specimens, also volva was separated. Thirty nine mushrooms pertaining to 10 genus and 16 species were collected in wild growing from different sites in the Trás-os-Montes and Beira Interior regions (NW Portugal). In both areas selected for this study the mushrooms available during the programmed harvesting were collected, focusing mainly on edible species, which were considered, by the mycologist, as representative of the both regions. The identification and edibility of the mushrooms were evaluated by expert mycologists. In Trás-os-Montes 20 mushrooms were collected including, 6 saprophytes and 14 mychorrhizal. In Beira Interior, where potentially pollutant sources are located including, cellulose industry, tobacco plantation, *Eucaliptus* plantation, and railways, 19 mushrooms were collected, 6 saprophytes and 13 mychorrhizal (Table 1).
The underlying soil samples were collected with a plastic spade and the roots, small stones, gravel, leaves, sticks and other external materials were removed.

Table 1 — Species, ecology and sample number (n) of analysed mushrooms of Beira Interior and Trás-os-Montes regions of Portugal.

Regions	Beira Interior (n=19)		Trás-os-Montes (n=20)	
Ecology	Mychoryzial (n=13)	Saprophyte (n=6)	Mychoryzial (n=14)	Saprophyte (n=6)
Species	*Amanita caesarea* (n=1)[a]	*Agaricus sylvicola* (n=3)[a]	*Amanita muscaria* (n=2)[b]	*Lecopaxillus giganteus* (n=1)[a]
	Amanita ponderosa (n=8)[a]	*Volvariella gloicephala* (n=3)[a]	*Lactarius deliciosus* (n=4)[a]	*Macrolepiota procera* (n=4)[a]
	Amanita rubescens (n=1)[a]		*Lactarius piperatus* (n=1)[b]	*Psilocybe fascicularis* (n=1)[b]
	Boletus regius (n=3)[a]		*Lactarius vellereus* (n=1)[b]	
			Suillus granulatus (n=1)[a]	
			Suillus granulatus (n=1)[a]	
			Tricholoma acerbum (n=4)[b]	

[a] Edible

[b] Non edible

Sample preparation

The mushrooms were grouped according to the genus and species. The cap and stalk of all of them, and volva in some species were separated, cut into small portions with a plastic knife, placed in PVC decontaminated tubes and dried to constant weight in stove at 30-35ºC for several days. One representative portion of each soil sample was dried under the same conditions. All the dried samples were reduced to powder in an agata mortar. The dried powered samples (approximately 0.25 g of the dried powdered parts of the mushrooms or the soil samples) were submitted to a wet acid digestion, in a decontaminated closed teflon container, with HF + HNO_3 + HCl and heated at 105ºC overnight to completely dissolve the sample.

Analysis

Instruments

The water purification system was Seralpur PRO 90 CN and Seradest LFM 20.
All analytical weightings were performed with a Mettler Toledo balance AB265-S model. The balance precision is 0.01 mg.

Metal quantifications were carried out in a Perkin-Elmer HGA-850 Furnace installed in a model AAnalyst 300 Spectometer with deuterium arc background correction, equipped with an AS-800 Autosampler. The analyses were performed using Perkin-Elmer HGA Tubes with Integrated Platform.

Analytical conditions

The ashing temperatures of the graphite furnace programme were 700ºC, 1100ºC, and 1300ºC, and the atomization temperatures were 1100ºC, 1800ºC and 2500ºC, for Cd, Ni, and Pb, respectively. The autosampler was programmed to pipette sequentially 10 μL of the modifier solution (0.03 mg $Pd(NO_3)_2$ + 0.02 mg $Mg(NO_3)_2$) and 15 μL of the digested sample/standard solution and dispense them together onto the platform.

Quality assurance and quality control of trace element analysis

The analytical method for the quantification of cadmium, nickel and lead in mushrooms and soils was comprehensively validated under the current international rules[23]. The evaluation of the instrumental precision was determined by measuring 20 times the absorbance signals in the same acid digested sample. For the analytical method, readings of 20 different acid digested aliquots of the same sample were performed.
To calculate the limits of detection (LOD) of the instrumental method, 20 determinations were carried out in 0.2% HNO_3 solution and the value calculated as 3s/m, where "s" is the standard deviation of the blank 0.2% HNO_3 solution measurements and "m" is the slope in the calibration curve. The limit of quantification (LOQ), defined as the strictly lowest concentration of analyte that can be determined with an acceptable level of repeatability, precision, and trueness was calculated as 10 s/m[23]. The accuracy of the analytical method was evaluated by analysing the Certified Reference Materials Lichen CRM 482 and Soil Sample NCS ZC73001. For this purpose, 15 aliquots of Lichen and 10 aliquots of Soil Sample were submitted to the acid pre-treatment under the established conditions and the respective concentrations calculated.

Statistical analysis

The comparison of the obtained results between the two regions and between genera for the several metals was performed. Statistical significant differences were determined by the paired *t* test. A *p* value of $\leq$0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Validation parameters

Referring to the validation parameters of the analytical method adopted for Cd, Ni, and Pb quantification in mushrooms and soils, the coefficient of variation for the instrumental precision was 2.2, 9.6 and 2.2% for Cd, Ni and Pb respectively. For the analytical method, the coefficient of variation was 7.7, 9.7 and 8.7%, for Cd, Ni and Pb, respectively. These values show that the method is precise for the three elements. Linearity was achieved in the working ranges (μg/L) 0.062-2.5, 0.63-50.0, and 0.45-20.0 for Cd, Ni, and Pb, respectively. The LODs of the instrumental method were 0.062, 0.63 and 0.45 μg/L for Cd, Ni, and Pb, respectively. The LOQs were 0.21, 2.08 and 1.48 μg/L, for Cd, Ni, and Pb, respectively. Considering 0.25 g of the dried powdered parts of the mushrooms or the soil samples in an 8 mL final volume, the LODs, expressed in ng/g dry weight, were 1.98, 20.0 and 14.3 ng/g and the LOQs were 6.61, 66.7 and 47.5 ng/g for Cd, Ni, and Pb, respectively. The maximum limit values established by the European regulation for Cd and Pb in mushrooms are 1.0 and 0.3 μg/g wet weight, respectively[24]. In this study we quantified the metals in dried mushrooms. Thus, these European limits expressed as dry weight are approximately 10.0 and 3.0 μg/g, assuming that the dry weight of mushrooms is about 10% of the fresh weight. The LOQ values found for the present method are well below these maximum allowed levels, thus enabling the monitoring of these toxic metals in mushrooms.
The accuracy data are presented in Table 2. It can be observed that the measured values are in good agreement with the certified values. In relation to the mean certified value for Lichen CRM 482, the deviation of the mean measured value was 4.1 for Cd, 0.81 for Ni, and 3.7%, for Pb. For Soil Sample NCS ZC73001 it was 2.9, 1.2, and 3.6%, for Cd, Ni, and Pb, respectively.

Table 2 — Contents (mean values, μg/g dry weight ± SD) of Cd, Ni and Pb in lichen and soil sample reference materials.

Certified Material	Certified value (μg/g)			Trás-os-Montes (n=20)		
	Cd	Ni	Pb	Cd	Ni	Pb
Lichen CRM 482 (n=15)	0.56±0.02	2.47±0.07	40.9±1.4	0.54±0.03 (cv=4.1%)	2.45±0.05 (cv=0.81%)	39.4±1.3 (cv=3.7%)
Soil Sample NCS ZC73001 (n= 10)	0.105±0.013	26±1	22±2	0.102±0.007 (cv=2.9%)	26.3±1.4 (cv=1.2%)	22.7±0.4 (cv=3.6%)

cv - deviation of the mean measured value found in relation to the mean certified value

Metal contents in the total mushrooms and in the underlying soils

The levels of Cd, Ni, and Pb in the caps and stalks of the 39 mushrooms, as well as in the corresponding underlying soil samples are summarized in Table 3. Considering the mean values (μg/g dry weight) of the metals in all samples, Pb was present at the lowest levels (0.092±0.17 μg/g and 0.15±0.15 μg/g, for caps and stalks, respectively), followed by Cd (0.69±1.1 μg/g and 0.39±0.79 μg/g, for caps and stalks, respectively), and Ni (1.1±1.6 μg/g and 1.88±1.13 μg/g, for caps and stalks, respectively). A similar profile for these three metals in wild mushrooms was found by Ouzouni *et al*., 2009[21] and by Soylak *et al*., 2005[22], with even higher Ni contents (ranging from 1.72 to 24.1 μg/g) in the later study but no explanation was presented by the authors for these high Ni levels[22]. As it can be observed, the coefficient of variation of the mean metal contents is high, and this can be attributed to the relatively high number of different mushroom species included in our study. Concerning the metal contents of all the underlying soil samples, Cd was present at the lowest concentration (0.011±0.013 μg/g), followed by Pb (0.45±0.25 μg/g), and Ni (74.0±123 μg/g). Significant differences were observed in the metal composition of soils from the two regions, both for Pb and for Ni. Referring to Pb, the soil contents were 0.53±0.15 and 0.39±0.29 μg/g, in Beira Interior and Trás-os-Montes, respectively, being the values significantly higher for the industrialized Beira Interior region ($p<0.05$). Nickel concentration was about 10 times higher in Trás-os-Montes (125±150 versus 13.5±12.3 μg/g in Beira Interior, $p<0.01$). This can be attributed to the constitution of the serpentinized soils of Trás-os-Montes, which are especially rich in Ni, present as NiO[25]. This Ni species has a low solubility and is poorly uptaken by almost all the serpentine flora[25].

Table 3 — Metal mean value contents (μg/g dry weight) in all the soil samples and mushrooms collected in Beira Interior and Trás-os- Montes areas. Values are also presented for the mychorrhizal and saprophyte ecological groups.

Samples	Cap			Stalk			Soil		
	Cadmium (μg/g dw)	Nickel (μg/g dw)	Lead (μg/g dw)	Cadmium (μg/g dw)	Nickel (μg/g dw)	Lead (μg/g dw)	Cadmium (μg/g dw)	Nickel (μg/g dw)	Lead (μg/g dw)
All samples (n=39)	0.69±1.1 (0.013-5.0) Med. 0.31	1.1±1.6 (<0.067-7.3) Med. 0.65	0.092±0.17 (<0.048-1.0) Med. 0.054	0.39±0.79 (0.007-4.0) Med. 0.14	1.88±1.13 (0.73-5.13) Med.1.38	0.15±0.15 (<0.048-0.82) Med. 0.11	0.011±0.013 (<0.007-0.038) Med. 0.009	74.0±123 (1.84-348) Med. 31.7	0.45±0.25 (0.20-1.0) Med. 0.30
Beira Interior (n=19)	0.23±0.51 (0.015-1.7) Med. 0.28	1.3±1.8 (0.13-7.3) Med. 0.63	0.15±0.24 (<0.048-1.0) Med. 0.079	0.21±0.28 (0.008-1.1) Med.0.11	1.96±1.12 (0.96-5.13) Med.1.38	0.23±0.20[d]) (<0.048-0.82) Med. 0.20	0.008±0.006 (<0.007-0.014) Med. 0.013	13.5±12.3[e] (1.84-40.3) Med. 10.0	0.53±0.15[g] (0.26-0.71) Med.0.57
Beira Interior Mychorrhizal (n=13)	0.31±0.55 (0.070-1.7) Med. 0.28	1.5±2.0 (0.13-7.3) Med. 0.66	0.16± 0.27 (<0.048-1.0) Med. 0.064	0.25±0.30 (0.014-1.1) Med. 0.15	1.94±1.25 (0.96-5.13) Med.1.29	0.23±0.22(h) (<0.048-0.82) Med. 0.20	0.007±0.006 (<0.007-0.013) Med. <0.007	15.2±13.6 (1.84-40.3) Med. 10.0	0.51±0.17 (0.26-0.71) Med.0.57
Beira Interior Saprophyte (n=6)	0.093±0.30 (0.015-0.63) Med. 0.19	0.47±0.25[a] (0.13-0.72) Med. 0.52	0.13±0.048 (<0.048-0.20) Med. 0.13	0.048±0.077 (0.008-0.16) Med. 0.010	2.05±0.61[a] (1.24-2.73) Med.2.11	0.21±0.12 (<0.048-0.32) Med. 0.20	0.013±0.007 (0.013-0.014) Med. 0.013	7.66±2.75 (5.28-10.0) Med. 2.75	0.58±0.012 (0.57-0.59) Med.0.57
Trás-os-Montes (n=20)	0.90±1.4 (0.013-5.0) Med. 0.39	0.98±1.4[b] (<0.067-6.4) Med. 0.72	0.046±0.066 (<0.048-0.25) Med. <0.048	0.55±1.0 (0.007-4.0) Med.0.15	1.82±1.17[b] (0.73-4.92) Med.1.42	0.084±0.063[d] (<0.048-0.18) Med. 0.082	0.014±0.016 (<0.007-0.038) Med. 0.009	125±150[e] (2.84-348) Med. 41.4	0.39±0.29[g] (0.20-1.0) Med. 0.27
Trás-os-Montes Mychorrhizal (n=14)	1.1± 1.6 (0.013-5.0) Med. 0.38	1.2±1.6 (<0.067-6.4) Med.0.83	0.025±0.038[f] (<0.048-0.13) Med. <0.048	0.70±1.21 (0.007-4.0) Med. 1.7	2.04±1.30 (0.73-4.92) Med.1.55	0.086±0.064[f h] (<0.048-0.18) Med. 0.082	0.015±0.018 (<0.007-0.038) Med. <0.007	146±156 (2.84-348) Med. 41.4	0.34±0.24 (0.20-1.0) Med.0.27
Trás-os-Montes Saprophyte (n=6)	0.46± 0.31 (0.013-0.31) Med. 0.44	0.45±0.60[c] (<0.067-1.6) Med.0.22	0.096±0.092 (<0.048-0.25) Med. 0.089	0.20±0.18 (0.058-0.48) Med. 0.11	1.29±0.53[c] (0.89-4.92) Med.1.15	0.082±0.068 (<0.048-0.15) Med. 0.093	0.011±0.014 (<0.007-0.038) Med. 0.010	77.0±13.3 (2.84-348) Med. 35.9	0.49±0.39 (0.20-1.0) Med. 0.27

[a] p= 0.0285 (*); [b] p= 0.0468 (*); [c] p= 0.0272 (*); [d] p= 0.0047 (**); [e] p= 0.0041(**); [f] p= 0.0046 (**); [g] p= 0.0497(*); [h] p= 0.0253 (*); Med=Median; Values in parenthesis are minimum and maximum.

Metal contents in the mushrooms collected in the two different regions

In Table 3 are also presented the data obtained separately for the mushrooms collected in the two regions, Beira Interior and Trás-os-Montes, and ecologically, into mychorrhizal and saprophyte. Globally, Beira Interior is a more industrialized region (with cellulose industry, tobacco plantation and railways) than Trás-os-Montes and consequently, potentially more polluted. The analysis of the obtained results for the three metals in the mushrooms collected in the two regions showed significantly higher Pb contents in the stalks of the mushrooms in the industrialized Beira Interior region (0.23±0.20 μg/g versus 0.084±0.063 μg/g in the rural Trás-os-Montes region, $p<0.05$). This is in accordance with the significantly higher concentrations of Pb in the soils in Beira Interior. However, neither in terms of mean nor individual values (ranging from <0.048 to 1.0 in the caps and from <0.048 to 0.82 μg/g in the stalks), did the Pb concentrations surpass the maximum limit values established by the European rules for cultured mushrooms (0.30 μg/g wet weight or 3.0 μg/g dry weight)[24]. Our data agree with other studies in which Pb mushroom contents were evaluated in polluted areas. However, the lead contents in mushrooms seem to be dependent on the different pollution sources. For example, much higher Pb levels were found in mushrooms collected 1-2 meters from a high-traffic road (ranging from 18.3 μg/g in Boletus badius to 29 μg/g dry weight in Macrolepiota procera)[9]. In other edible species collected near a smelting polluted area very high levels of Pb were also found (60 and 165 μg/g dry weight in caps and stalks, respectively), showing the importance of the type of pollution in fungi Pb contamination[14]. No differences for the Cd and Ni contents were noted between the mushrooms collected in the two regions, in spite of the significantly higher Ni contents found in the soils of Trás-os-Montes region. Ni in these soils is mainly present as NiO, a scarcely soluble compound, limiting the uptake of this metal by botanical species[25]. In a study that evaluated the transference of Ni from these soils to several serpentine flora, the authors found

very different abilities of serpentinophytes to uptake Ni. Nickel uptake was generally very low, and only one species was identified as a hyperaccumulator of Ni[25]. In the present study, none of the collected mushroom species showed high affinity for the Ni present in the soils of this region.

Bioconcentration factors for the three metals

Table 4 summarizes the mean BCF values for Cd, Ni, and Pb, calculated separately for caps and stalks, and obtained by the ratio between the levels of the metals present in the respective parts of the mushrooms and the contents in the soils. When analysing the obtained BCFs, and considering the mean values of all the 39 studied mushrooms, we can observe that these values are much higher for Cd than for Ni and Pb. The same trend was noticed when BCFs were analysed per region and ecology of the mushrooms. This confirms the findings of other studies showing the high transference of Cd from soils to mushrooms, as compared with Ni and Pb[7, 17, 19, 20]. Recently published studies confirm the low capacity of mushrooms to uptake Pb from the underlying soils, concluding that they are bioexclusors (BCF < 1)[12]. Also Chudzynski and Falandysz , 2008, evaluated the BCFs for Pb and Ni in Larch Bolete and verified that they were lower than 1, classifying this mushroom species as an exclusor of both elements[20]. In the present study, the BCF values found for caps were 64.1±710, 0.024±0.19, and 0.082±0.65 for Cd, Ni, and Pb, respectively. For stalks, the BCFs were 26.4±662, 0.073±0.26, and 0.21±0.54 for Cd, Ni, and Pb, respectively. The data show that Cd mainly accumulates in the caps, in accordance with results obtained in previous studies[20]. The high contents of nickel present in the soils of Trás-os-Montes region do not significantly contaminate the analysed mushroom species. It seems that their ingestion by humans does not present a risk of exaggerated ingestion of this allergenic metal. The very low BCF levels found for Pb also indicate that consumption of these mushroom species is safe.

Table 4 — Bioconcentration factors (BCF values) (geometric mean ± SD), and cap/stalk quotient (arithmetic mean ±SD).

Samples	Cadmium			Nickel			Lead		
	BCF Cap	BCF Stalk	Cap/ Stalk	BCF Cap	BCF Stalk	Cap/ Stalk	BCF Cap	BCF Stalk	Cap/ Stalk
All samples (n=39)	64.1±710	26.4±662	3.5±6.0	0.024±0.19	0.073±0.26	0.55±0.56	0.082±0.65	0.21±0.54	0.98±2.3
Beira Interior (n=19)	51.9±206	18.8±135	4.7±8.7	0.078±0.25[c]	0.193±0.32[d]	0.64±0.73	0.13±0.94	0.30±0.75	0.61±0.63
Beira Interior Mychorrhizal (n=13)	96.9±216	41.8±144	2.6±1.2	0.088±0.15[a]	0.17±0.36[b]	0.77±0.79	0.11±1.1	0.30±0.86	0.48±0.41
Beira Interior Saprophyte (n=6)	6.8±20.7	1.4±5.3	12±18	0.055±0.030	0.27±0.15	0.22±0.10	0.22±0.08	0.30±0.22	1.0±1.0
Trás-os-Montes (n=20)	76.8±945	35.3±894	2.5±1.2	0.009±0.040[c]	0.032±0.11[d]	0.47±0.36	0.057±0.22	0.15±0.26	0.30±0.49
Trás-os-Montes Mychorrhizal (n=14)	81.1±1117	40.7±1063	2.3±1.2	0.009±0.047[a]	0.025±0.090[b]	0.57±0.37	0.041±0.14	0.17±0.28	0.10±0.19
Trás-os-Montes Saprophyte (n=6)	67.8±217	25.1±40.0	2.9±1.3	0.008±0.017	0.054±0.15	0.26±0.25	0.13±0.35	0.12±0.19	0.78±0.67

[a] p=0.0183 (*); [b] p=0.0128 (*); [c] p=0.0091 (**); [d] p=0.0033 (**)

Distribution of Cd, Pb, and Ni in the different mushroom parts

Referring to the distribution of the metals in the mushroom parts, the three metals behave differently. While Cd is present at higher concentrations in the caps, Ni and Pb are more concentrated in the stalks, as shown by the cap/stalk BCF ratios of 3.5, 0.55, and 0.98 for Cd, Ni, and Pb, respectively (Table 4). These results show that Cd, besides being highly transferable from soils to mushrooms, is also highly translocated in the mushrooms, concentrating in the caps. The presence of this metal in human diet is of concern and requires

tight control given its high transference from soils to edible vegetables, its kinetics in humans with a long half-life of about 30 years, and its recognized highly toxic effects. It is of note that in the present study all the mushroom samples showed Cd contents lower than the maximum level established by the European regulation for fungi (1.0 μg/g wet weight)[23]. For Ni, the accumulation in the stalks of the mushrooms, as compared with the caps, is very consistent across the different species, as evidenced by the significantly higher concentrations found both for the total samples collected in Trás-os-Montes and for the saprophytes of both regions (See Table 3). Referring to lead, although without significance, there is a clear tendency for lead to accumulate in the stalks as is shown in Table 3 and by the BCF cap/stalk ratio almost always lower than 1, as depicted in Table 4.

To study the metal distribution in the mushrooms, we considered their two main constitutive parts, caps and stalks. However, certain species possess also volva, a part that involves mainly the stalk and is fundamental for the ecological identification. Eight samples of the mychorrhizal species Amanita ponderosa exhibiting volva were collected in the Beira Interior region and the three parts, cap, stalk and volva were separately analysed for the metal contents. The results are presented in Table 5. Interestingly, for Ni we found a significantly higher concentration in the volva as compared with the Ni contents of caps and stalks (3.5±2.3, 1.0±1.2, and 1.4±0.53, respectively in volva, cap, and stalk). It is not common the separated analysis of this part of the mushrooms as evidenced from the absence of published data. Our results show that it can be recommended to discard the volva before cooking the mushrooms, because it can accumulate some metals as observed for Ni in the present study.

Table 5 — Metal contents (μg/g dry weight) in the three parts (volva, cap, and stalk) of eight mushroom samples (Amanita ponderosa species and Mychorrhizal ecology) collected in the zone of Beira Interior region.

Metal	Volva (n = 8)	Cap (n = 8)	Stalk (n = 8)	Soil (n = 8)	BCF volva	BCF cap	BCF stalk	Cap/ Stalk	Cap/ Volva	Stalk/ Volva
Cd	0.58±0.71 (0.043-2.2) Med. 0.33	0.52±0.53 (0.089-1.7) Med. 0.35	0.30±0.34 (0.032-1.1) Med. 0.18	0.0029±0.0026 (0.0019-0.0092) Med. 0.0019	136±136	153±96	73±75	2.3±2.1	1.2±0.44	0.58±0.27
Ni	3.5±2.3[ab] (1.0-6.4) Med. 3.0	1.0±1.2[a] (0.13-3.6) Med. 0.64	1.4±0.53[b] (0.96-2.6) Med. 1.27	19±17 (1.8-40) Med. 19	0.290±1.05	0.066±0.29	0.14±0.45	0.74±0.88	0.35±0.32	0.56±0.30
Pb	0.19±0.18 (0.073-0.60) Med. 0.12	0.20±0.36 (<0.048-1.0) Med. 0.070	0.23±0.28 (<0.048-0.82) Med. 0.090	0.47±0.21 (0.25-0.71) Med.0.43	0.430±0.42	2.00±3.47	2.2±3.7	0.59±0.48	1.7±3.5	1.8±2.7

[a] p=0.0182 (*) ; [b] p=0.0275 (*); Med=Median; Values in parenthesis are minimum and maximum

Different accumulation of the metals by the different mushroom species and genera

Previous studies have identified some mushroom species as high accumulators of toxic elements. One example is the accumulation of Pb by *Macrolepiota procera*[9]. We tried to identify a potential metal accumulator species in our study. With this purpose, we analysed the data obtained for the three metals in the studied species for which 4 or more samples of the same species were available including the edible species, *Amanita ponderosa*[8], *Lactarius deliciosus*[4], Macrolepiota procera[4], and the non edible species *Tricholoma acerbum*[4]. Some interesting differences were found, namely the significantly higher capacity of the non edible species *Tricholoma acerbum* to accumulate Cd in the caps, about 5 times higher than the edible species, *Lactarius deliciosus* (respectively 0.73±0.40 and 0.16±0.044 μg/g in terms of mean values). Additionally, it is of note that these two mushroom species are both mychorrhizal and were collected in the rural region of Trás-os-Montes. Both nutritionally and in a safety perspective this is a very interesting information. When metal contents were compared between the two edible species *Lactarius deliciosus* and *Macrolepiota procera*, it was found that both Cd and Pb levels were significantly higher in the cap of *Macrolepiota procera*. *Macrolepiota procera* significantly accumulated more Pb in the cap (0.14±0.08), 20 times higher than the values found in the cap of the non edible *Tricholoma acerbum* (0.0071±0.0001). This finding confirms that this saprophyte species accumulates heavy metals as reported by others[9]. When we analysed the Ni contents of all the 39 mushrooms, we found that this element specially accumulates in the stalk, as shown in Table 3. Comparing the Ni contents per species, we found significantly higher contents in the stalks of the non edible *Tricholoma*

acerbum (3.55±1.44) than in the three edible species ($p<0.02$ for *Lactarius deliciosus*, 1.00±0.21 and *Macrolepiota procera*, 1.02±0.22 and $p<0.01$ for *Amanita ponderosa*, 1.4±0.53 μg/g). As for Pb, the non edible species significantly accumulates more Ni than these three edible species.

Considering the genera of the mushrooms, we found that in the industrialized region of Beira Interior, the stalks of the mychorrhizal samples presented significantly higher contents of Pb (0.23±0.22 μg/g) than the stalks of the mychorrhizal samples from Trás-os-Montes (0.086±0.064 μg/g). These data show that the capacity of mychorrhizal species for accumulating Pb depends on its concentration in the underlying soil. In fact, lead concentration in Beira Interior soils was also significantly higher than in Trás-os-Montes. No significant differences were found between mychorrhizal and saprophyte in what refers to the accumulation of the three studied metals.

CONCLUSIONS

In the present study it was found that the soils of the industrialized region of Beira Interior were significantly richer in Pb than those of the rural region of Trás-os-Montes. This correlated with the significantly higher concentrations of Pb in the stalks of the mushrooms collected in the industrialized region.

Although the soils of the rural region had significantly higher concentrations of Ni, this did not influence the contents of this metal in the analysed mushroom species.

It was shown that Cd presents a high mobility from the soils to the mushrooms, accumulating mainly in the cap, the elected part for human consumption, while Ni and Pb were mainly concentrated in the stalks.

In none of the analysed mushroom samples did the Pb and Cd contents surpass the maximum limit values established for mushrooms by the European regulation. The edible mushroom species analysed in the present study behave as bioexclusors for Pb and Ni, and therefore undesirable effects are not expected from these metals in the studied mushroom species.

The *Tricholoma acerbum* species highly accumulates Cd and Ni. Being a non edible species, it is of no concern for human safety and its potential use in soils bioremediation could constitute an interesting issue.

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