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Lead Content in Edible Wild Mushrooms in Northwest Spain as Indicator of Environmental Contamination

M. A. García,[1] J. Alonso,[1] M. I. Fernández,[2] M. J. Melgar[1]

[1] Department of Toxicology, Faculty of Veterinary, University of Santiago de Compostela, 27002-Lugo, Spain
[2] Department of Inorganic Chemistry, Faculty of Sciences, University of Santiago de Compostela, 27002-Lugo, Spain

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Abstract. Lead content was determined in wild growing mushrooms collected from two different areas in the Province of Lugo (NW Spain). It has been analyzed by graphite furnace atomic absorption spectrometry in 95 samples of 13 species (7 mycorrhizals and 6 saprophites). In an assessment of lead concentrations, the following factors have been considered: species and ecology, morphological portion, and traffic pollution. The average lead concentration of the samples was 1 ppm dry weight (dw). Saprophite species presented higher levels than mycorrhizal ones (<1 ppm), *Coprinus comatus* reaching the maximum mean concentration with 2.06 and 2.79 ppm of dw in the hymenophore and the rest of the fruit body. Morphological portion, statistically, did not show significant difference between the two portions; however, *Macrolepiota procera* always presented lead high levels in the hymenophore in all samples. The effect due to traffic pollution has been specially observed in *Coprinus comatus*, presenting the highest concentration with values of 6.51 and 10.43 ppm, respectively, in samples collected in the city center. This species, as other researchers have indicated, could be considered as an indicator by lead contamination. The contribution of mushrooms to the weekly intake of lead was calculated and the posible health risk for the consumer is pointed out. These data are of great importance in view of toxicology and partly environmental protection.

Lead can reach humans through air, water, and food. Lead levels vary from one kind of food to another and from sample to sample by a variety of routes (Zurera-Cosano *et al.* 1987). WHO/FAO (1993) have established a provisional tolerable weekly intake for lead of 0.025 mg/kg of body weight. Edible fungi may contain high amounts of heavy metals such as lead, cadmium, and mercury, as compared with plants (Tyler 1980; Lodenius *et al.* 1981; Quinche 1987).

The principal factors influencing the accumulation of heavy metals in macrofungi are environmental factors (metal concentrations in the soil, pH, organic matter, and contamination by atmospheric deposition) and fungal factors (fungal structure, biochemical composition, decomposition activity, development of mycelium and fruit bodies, morphological portion).

Lead is more uniformly distributed among different species than is cadmium and mercury (Lodenius *et al.* 1981; Zurera-Cosano *et al.* 1987; Mornand 1990). The lead contents of saprophite mushrooms are higher than those of mycorrhizal species. The fruit bodies of mushrooms accumulate remarkably high concentrations of lead, especially in the vicinity of highways or other lead sources (Laaksovirta and Alakuijala 1978; Liukkonen-Lilja *et al.* 1983; Mornand 1990; Jorhem and Sundström 1995). Mushrooms can be used as bioindicators in soil pollution by lead (Quinche 1992; Lodenius *et al.* 1981; Falandysz *et al.* 1993).

The province of Lugo constitutes a part of Galicia, a humid autonomous region in the northwest of Spain. It has a big production of wild mushrooms for commercialization and consumption. Taking into account the economic and gastronomic interests and that high lead levels can harm human health, the aim of the present work was to study the presence of lead on fruit bodies of some edible mushrooms collected in the province of Lugo in relation to some elements: species and ecology (mycorrhizal and saprophite), morphological portion (the hymenophore and rest of the fruit body), and the influence of traffic pollution and the role of fungi as bioindicators.

Materials and Methods

Sampling

Fruit bodies of mushrooms were collected in 1994 and 1995. The areas of the study (Figure 1) included pasture lands and forest (unpolluted areas) distant from potential pollution sources and lawns exposed to pollutants of automobile traffic for many years (polluted areas), the sampling distance to the road being always less than 50 m.

Lead levels in 95 samples of edible mushrooms have been analyzed in 13 species (Table 1) of *Basidiomycetes* fungi: 7 mycorrhizals (*Amanita rubescens, Boletus badius, Boletus pinicola, Cantharellus cibarius, Lactarius deliciosus, Russula cyanoxantha,* and *Tricholoma portentosum*) and 6 saprophites (*Agaricus campestris, Agaricus ma-*

Correspondence to: M. J. Melgar

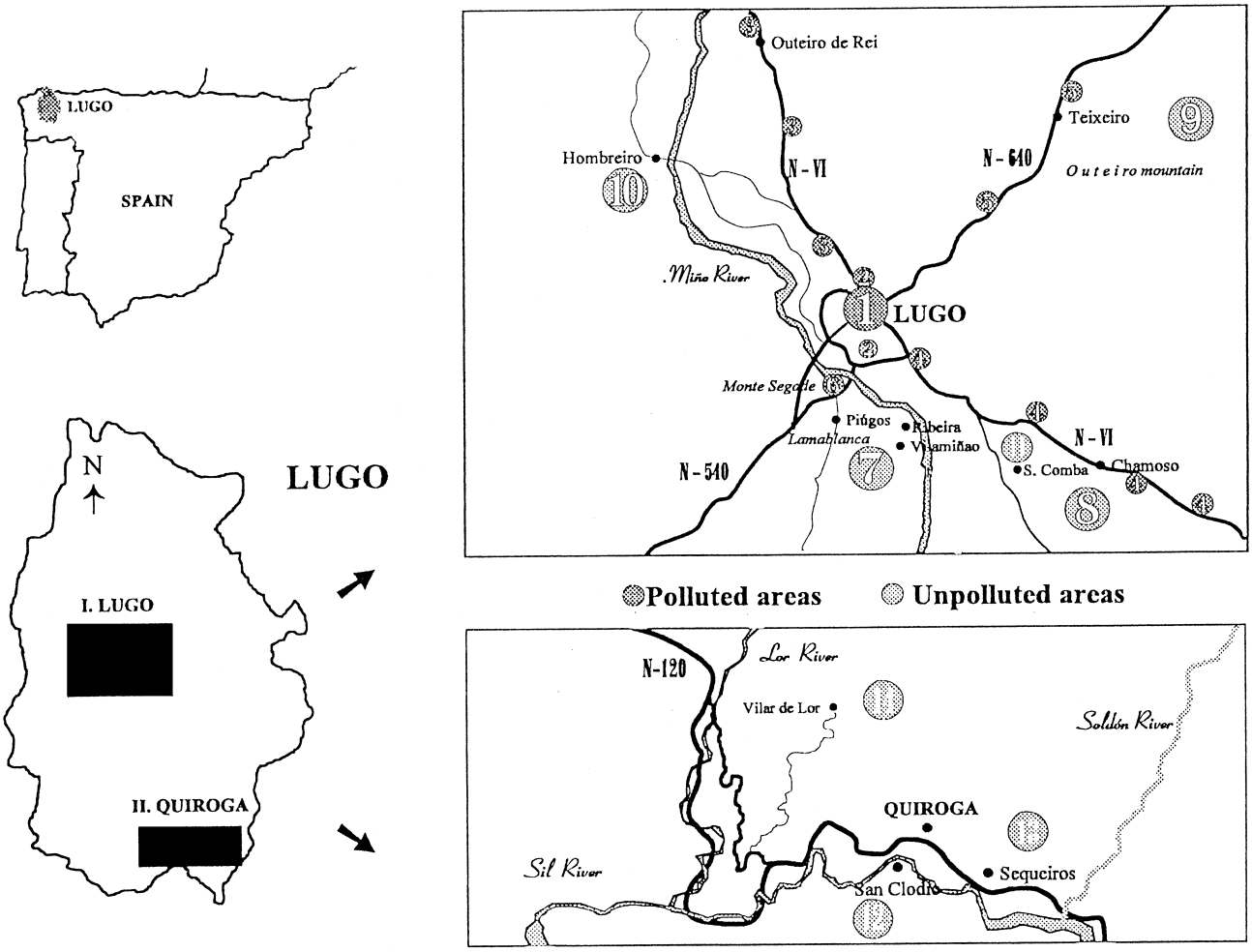

Fig. 1. Sampling areas in Lugo (NW Spain)

Table 1. Species, ecology, and samples number (n)

Species	Ecology	n
Agaricus campestris Linneo	Saprophite	6
Agaricus macrosporus (Möll.:Schaef.) Pil.	Saprophite	1
Amanita rubescens (Pers.: Fr.) S.F. Gray	Mycorrhizal	10
Boletus pinicola (Vitt.) Venturi	Mycorrhizal	12
Boletus badius Fr. [*Xerocomus badius*] (Fr.) K. ex G.	Mycorrhizal	7
Cantharellus cibarius Fries	Mycorrhizal	8
Clitocybe nebularis (Batsch.: Fr.) Kummer	Saprophite	6
Coprinus comatus (Müller: Fr.) S.F. Gray	Saprophite	8
Lactarius deliciosus (L.) S.F. Gray	Mycorrhizal	6
Lepista nuda (Bull.: Fr.) Cooke	Saprophite	6
Macrolepiota procera (Scop.: Fr.) Singer	Saprophite	9
Russula cyanoxantha Schaeff.: Fr.	Mycorrhizal	10
Tricholoma portentosum (Fr.) Quélet	Mycorrhizal	6
Total samples		95

crosporus, *Clitocybe nebularis*, *Coprinus comatus*, *Lepista nuda*, and *Macrolepiota procera*). These species were selected in relation to edible quality, commercialization, and frequency in the areas of the study.

Samples were cleaned (not washed), cut, and separated in two portions: the hymenophore (lamellas in the species of genus *Agaricus*, *Amanita*, *Cantharellus*, *Clitocybe*, *Coprinus*, *Lactarius*, *Lepista*, *Macrolepiota*, *Russula*, and *Tricholoma*, and tubes and porus in the species of genus *Boletus* and *Xerocomus*) and the rest of the fruit body (the cap, except hymenophore, and the stalk), homogenized and dried at 105°C for 6 h. Approximately, 3-g aliquots of homogenized dry mushrooms were placed in a porcelain crucible and ashed in an oven at 425–440°C for 15–40 h (depending on the species, for a complete mineralization). The filtered product was transferred into a 25-ml volumetric flask making up the level with 1 N HCl. All samples were run in triplicate.

Analysis

Lead analysis was performed with a Hitachi model Z-8100 Graphite furnace atomic absorption spectrophotometer with Zeeman background correction and a wavelength of 283.3 nm.

The accuracy of the method was analyzed in relation to the study of analytical recovery. The mean recovery was 89.3%. Precision and reproducibility of the method were assessed by analyzing 12 replicates of one representative sample and calculating the coefficient of variation, which was 5.24%. According to these results, the method can be considered reproductive and precise. Limit of detection was determined according to the Decision of the Commission of CEE (DOCE 1990), as equal to three times the standard deviation of the mean of blank determinations. The concentration limit obtained was 0.35 µg/L. The examination and elimination of matrix effects (interferences) were realized by applying the method of standard additions.

Lead concentrations in the samples were calculated by the following formula (US EPA 1989):

$$\mu g \text{ lead/kg dry weight} = A \times V/W$$

where A = µg/L of lead in processed sample from calibration curve; V = final volume of the processed sample, ml; W = dry weight of sample, g. The final results were expressed in mg/kg (ppm)

Results and Discussion

Mean lead concentrations in the mushroom species are given in Table 2, where samples number (n), minimum, maximum, and mean concentrations and the standard deviations are indicated.

In an assessment of the concentrations of the metal the following factors, among others, have been considered: species of mushroom and ecology, morphological portion (hymenophore and the rest of the fruit body), and traffic pollution.

Species and Ecology

Saprophite species showed the maximum lead levels (Figure 2) with high mean concentrations in *C. comatus* with 2.06 and 2.79 ppm of dry weight in hymenophore and the rest of the fruit-body, respectively. However, according to other authors (Fagot *et al.* 1988; Kalač *et al.* 1989, 1991, 1996; Mornand 1990; Grzybek 1991–92; Mandić *et al.* 1992), *L. nuda* collected in unpolluted area was the most accumulative species, with mean levels of 2.03 ppm for hymenophore and 1.64 ppm for the rest of the fruit body. The rest of the saprophite species showed mean values between 1.25 and 2.05 ppm for hymenophore and 0.73–1.32 ppm for the rest of the fruit body.

Saprophite species presented higher mean levels of lead than mycorrhizal ones (<1 ppm) in traffic-polluted areas as well as in unpolluted areas; the difference was higher even in the road areas. In fact, the maximum level of the mycorrhizal species was for *C. cibarius* (1.16 ppm for hymenophore and 1.26 ppm for the rest of the fruit body). We agree with Kuusi *et al.* (1981) in relation to the ecology, but they did not find significant differences in the rural area.

In conclusion, the levels of lead obtained showed significant differences between saprophite and mycorrhizal species ($p < 0.001$), with higher values in saprophite species because they probably have higher decomposing activity and considerable catalase activity values have been found earlier (Lamaison *et al.* 1975; Goulas 1987; Kojo and Lodenius 1989). The similar phenomenon was observed for the levels of other heavy metals (Lodenius *et al.* 1981; Zurera-Cosano *et al.* 1987; Kojo and Lodenius 1988).

Morphological Portion

In this work, we have selected hymenophore and the rest of the fruit body portions because hymenophore is the hymenium-bearing structure (Hawksworth *et al.* 1995) or fertile portion in contrast with the rest of the fruit body.

Fifty-five of the total samples presented a higher level of lead in the hymenophore than in the rest of the fruit body; however, statistically there was no significant difference between the two portions (Figure 2) in contrast with the other heavy metals such as cadmium and mercury (Seeger *et al.* 1978; Kojo and Lodenius 1989; Zródlowski 1995). Nonetheless, *M. procera* species always presented high lead concentrations in the hymenophore in all samples.

Table 2. Determination of lead. Results are expressed as minimum, maximum, and mean concentrations (ppm d_i/w) with standard deviation

Species	n	MP	Dry Weight (%)	Mini-mum	Maxi-mum	Mean	SD
Agaricus campestris	6	H	8.03	0.59	2.85	1.89	0.95
		RF	7.82	0.35	2.25	1.40	0.69
Agaricus macrosporus	1	H	9.22			1.25	
		RF	9.35			0.73	
Amanita rubescens	10	H	8.58	0.33	1.15	0.59	0.25
		RF	7.86	0.30	1.05	0.73	0.27
Boletus pinicola	12	H	11.18	0.19	1.09	0.51	0.32
		RF	10.25	0.14	1.09	0.45	0.27
Boletus badius	7	H	9.57	0.20	0.50	0.37	0.12
		RF	8.68	0.17	0.78	0.52	0.21
Cantharellus cibarius	8	H	12.48	0.38	1.16	0.81	0.32
		RF	10.32	0.34	1.26	0.79	0.37
Clitocybe nebularis	6	H	8.90	0.99	3.00	1.96	0.85
		RF	8.17	0.64	2.76	1.31	0.77
Coprinus comatus	8	H	6.05	0.53	6.51	2.06	2.05
		RF	6.49	0.46	10.43	2.79	3.39
Lactarius deliciosus	6	H	12.07	0.46	0.96	0.71	0.19
		RF	10.84	0.34	0.70	0.46	0.20
Lepista nuda	6	H	7.82	0.59	6.89	2.03	2.41
		RF	7.40	0.66	3.50	1.64	1.04
Macrolepiota procera	9	H	12.08	0.47	5.67	2.05	1.87
		RF	12.36	0.36	2.05	0.92	0.61
Russula cyanoxantha	9	H	10.19	0.33	0.83	0.54	0.18
		RF	9.87	0.25	0.99	0.59	0.26
Tricholoma portentosum	6	H	8.18	0.15	0.67	0.38	0.18
		RF	7.78	0.22	0.76	0.50	0.22
Global levels	95	H	9.69	0.15	6.89	1.10	1.24
		RF	9.20	0.14	10.43	0.97	1.22

n, number of samples; MP, morphological portion; H, hymenophore; RF, rest of the fruit body

Table 3. Multifactor analysis of variance to determine the significant differences of the three factors in lead accumulation

Factors	Variables	Average	F Ratio	P
Ecology	Saprophite species	1.691	47.06	[a]*
	Mycorrhizal species	0.604		
Morphological portion	Hymenophore	1.223	0.99	NS
	Rest of fruit body	1.073		
Traffic pollution	Polluted areas	1.448	15.31	[a]*
	Unpolluted areas	0.847		

NS, no significant difference
[a] Significant difference ($p \leq 0.001$)

Traffıc Pollution

This factor is very important, mainly in the samples collected in the city center, which showed the maximum level. This effect has been observed in *C. comatus* collected in areas with

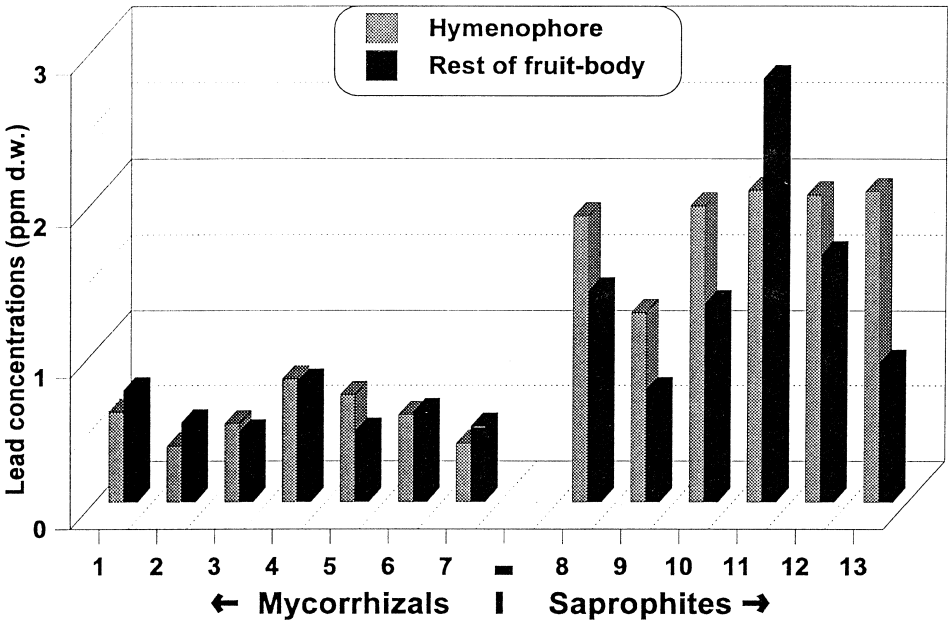

Fig. 2. Mean levels of lead in the analyzed mushroom species in relation to ecology and morphological portion

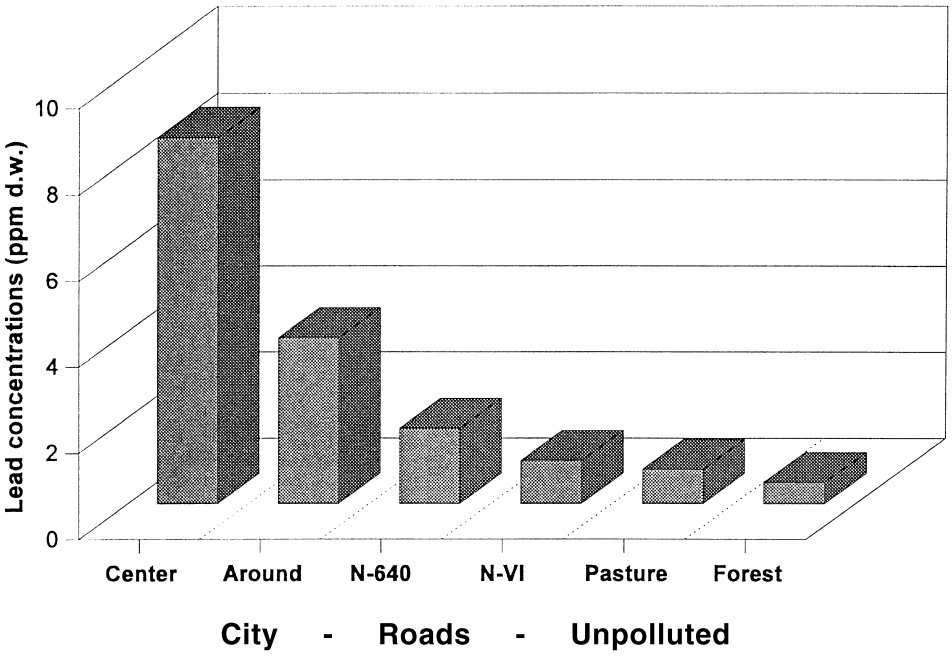

Fig. 3. Lead levels in *Coprinus comatus* collected in different areas

different traffic pollution (Figure 3). The highest concentrations for this species were 6.51 and 10.43 ppm for hymenophore and the rest of the fruit body, respectively, in the city center. Other samples collected in the city presented levels of 2.90 ppm for hymenophore and 4.66 ppm for the rest of the fruit body. This species, as other researchers (Quinche 1992) have indicated, can be considered a bioindicator for lead contamination. Jorhem and Sundström (1995) frequently found this species in urban areas, but the levels were very low in relation to the high levels expected by the deposition of lead emitted from car exhaust. With the exception of the samples collected in the city areas, the species that presented the highest level was *L. nuda.*

In general, the mean values of lead in traffic-polluted areas were 1.55 and 1.33 ppm for hymenophore and the rest of the fruit body, respectively, and in the unpolluted areas were 0.63 ppm for hymenophore and 0.58 ppm for the rest of the fruit body. These results agree with the opinion of other authors (Lodenius *et al.* 1981; Zurera-Cosano *et al.* 1987; Kalač *et al.* 1989) who considered that the lead content in fungi was influenced by the traffic pollution.

Table 4. Ranges of lead concentration in mushroom species

		Levels of Lead							
		<1 ppm		1–3 ppm		3–5 ppm		>5 ppm	
Species	n	H	RF	H	RF	H	RF	H	RF
Ag. campestris	6	1	2	5	4	—	—	—	—
Ag. macrosporus	1	—	1	1	—	—	—	—	—
Am. rubescens	10	9	9	1	1	—	—	—	—
B. pinicola	12	11	11	1	1	—	—	—	—
B. badius	7	7	7	—	—	—	—	—	—
Ca. cibarius	8	4	5	4	3	—	—	—	—
Cl. nebularis	6	1	4	4	2	1	—	—	—
Co. comatus	8	4	4	2	2	1	1	1	1
La. deliciosus	6	6	6	—	—	—	—	—	—
Le. nuda	6	2	2	3	3	—	1	1	—
M. procera	9	4	6	3	3	1	—	1	—
R. cyanoxantha	10	10	10	—	—	—	—	—	—
T. portentosum	6	6	6	—	—	—	—	—	—
Global	95	65	73	24	19	3	2	3	1
		68.4%	76.8%	25.2%	20.0%	3.2%	2.1%	3.2%	1.1%

n, samples number; H, hymenophore; RF, rest of the fruit body

Finally, the statistical study (ANOVA) is shown in Table 3, where it can be observed the influence of the three factors considered in lead accumulation.

Toxicological Repercussions

The occurrence and distribution of different toxic components in certain mushrooms are not only a mycological theoretical problem, but also have practical toxicological aspects. According to WHO/FAO (1993), the acceptable human weekly intake of lead is 0.025 mg/kg of body weight (1.5–1.75 mg of lead for an adult of 60–70 kg). Actually, there is no Spanish regulation about the content of lead in wild or cultivated mushrooms. The Czech and Slovak safe minimum limit for lead in mushrooms is 5 mg/kg dry weight. Polish legislation established the permitted lead value at 0.3 mg/kg of fresh mushroom weight.

In this work, lead levels have been grouped in a range of concentrations for evaluating the percentage of samples that exceed the safe minimum level according to the Czech and Slovak legislation (Table 4). Only one sample, corresponding to the species *C. comatus* exceeded the level of 5 ppm in the rest of the fruit body (1.1%) and three samples corresponding to the species *C. comatus*, *L. nuda*, and *M. procera* in the hymenophore (3.2%).

In relation to the pollution source (main roads), Jorhem and Sundström (1995) investigated the presence of lead in *A. arvensis* growing between 25 m and 250 m from main road (~50,000 vehicles/day) and they showed that lead concentrations were twofold higher in the shorter distance. They concluded that lead was derived mainly from the contaminated roadside soil rather than from atmospheric deposition.

In general, considering that mushrooms are not the only source of lead, it is wise to restrict the consumption of mushrooms from most of the polluted areas, especially saprophite species; within a radius of approximately 50 m from the source of contamination (main roads), mushrooms should not be consumed.

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References

DOCE (Diario Oficial de las Comunidades Europeas) (1990) Decisión de la Comisión de 26 de septiembre de 1990, por la que se establecen los métodos de referencia para la investigación de residuos de metales pesados y de arsénico (90/515/CEE)

Fagot J, Guillitte O, Delcarte, E (1988) Contamination en métaux lourds des champignons supérieurs sur sites industriels et naturels en Belgique. Arch Int Phys Biochimie 96:36

Falandysz J, Niestój M, Danisiewicz D, Bona H, Pempkowiak J (1993) Cadmium and lead in wild mushroom *Agaricus campestris* L. from different locations in Northern Poland. Bromat Chem Toksykol 26:285–280

Goulas JP (1987) Production de peroxyde d'hydrogène par le mycélium du champignon de couche et sa contribution à la dégradation des constituants humiques des composts. Agronomie 7:853–858

Grzybek J (1991–92) Estimation of lead, cadmium, and nickel content by means of atomic absorption spectroscopy in dry fruitbodies of some macromycetes growing in Poland. II. Acta mycologica 27:213–220

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995) Dictionary of the fungi, 8th ed. Cab International, New York

Jorhem L, Sundström B (1995) Levels of some trace elements in edible fungi. Z Lebensm Unters Forsch 201:311–316

Kalač P, Wittingerová M, Stašková I, Šimaák aak M, Bastl J (1989) Contents of mercury, lead and cadmium in mushrooms. Cs Hyg 34:568–576

Kalač P, Burda J, Stašková I (1991) Concentrations of lead, cadmium, mercury and copper in mushrooms in the vicinity of a lead smelter. sci tot Environ 105:109–119

Kalač P, Niznanska M, Bevilaqua D, Stašková I (1996) Concentrations of mercury, copper, cadmium and lead in fruiting bodies of edible mushrooms in the vicinity of a mercury smelter and a copper smelter. Sci Tot Environ 177:251–258

Kojo MR, Lodenius M (1989) Cadmium and mercury in macrofungi. Mechanisms of transport and accumulation. Angew Botanik 63:279–292

Kuusi T, Laaksovirta K, Liukkonen-Lilja H, Lodenius M, Piepponen S (1981) Lead, cadmium, and mercury contents of fungi in the Helsinki area and in unpolluted control areas. Z Lebensm Unters Forsch 173:261–267

Laaksovirta K, Alakuijala P (1978) Lead, cadmium and zinc contents of fungi in the parks of Helsinki. Ann Bot Fennici 15:253–257

Lamaison JL, Pourrat H, Pourrat H (1975) Activité catalasique des macromycetes. Obtention d'une catalase purifiée. Ann Pharm Fr 33:441–446

Liukkonen-Lilja H, Kuusi T, Laaksovirta K, Lodenius M, Piepponen S (1983) The effect of lead processing works on the lead, cadmium and mercury contents of fungi. Z Lebensm Unters Forsch 176:120–123

Lodenius M, Kuusi T, Laaksovirta K, Liukkonen-Lilja H, Piepponen S (1981) Lead, cadmium and mercury contents of fungi in Mikkeli, SE Finland. Ann Bot Fennici 18:183–186

Mandić ML, Grgić J, Grgić Z, Šeruga M (1992) The natural levels of aluminium, cadmium and lead in wildlife mushrooms in Eastern Croatia. Deutsche Lebensmittel-Rundschau 88:76–77

Mornand J (1990) Présence de métaux lourds dans les champignons. Bull oc Myc Fr 106:31–46

Quinche JP (1987) Le cadmium, un élément présent en traces dans les sols, les plantes et les champignons. Revue Suisse Agric 19:71–77

Quinche JP (1992) Les teneurs en huit éléments traces des carpophores de *Coprinus comatus.* Mycol Helv 5:133–142

Seeger R, Nutzel R, Dill U (1978) Cadmium in Pilzen. Z Lebensm Unters Forsch 166:23–34

Tyler G (1980) Metals in sporophores of Basidiomycetes. Trans Br Mycol Soc 74:41–49

U.S. Environmental Protection Agency (1989) The methods for evaluating solid waste physical/chemical methods, 3rd ed. Proposed update package, U.S. Environmental Protection Agency, Forms 51001, PB 89-14807, Washington DC

WHO/FAO (1993) Evaluation of certain food additives and contaminants. WHO Technical Report Series 837, Geneva

Źródłowski Z (1995) The influence of washing and peeling of mushrooms *Agaricus bisporus* on the level of heavy metal contaminations. Polish J Food Nutr Sci 4/45:26–33

Zurera-Cosano G, Rincón-León F, Pozo-Lora R (1987) Lead and cadmium content of some edible mushrooms. J Food Qual 10:311–317