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Heavy metal bioaccumulation by mushrooms from artificially fortified soils

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Abstract

Six different species of wild mushrooms growing in the east Black Sea region were analyzed spectrometrically for their trace element (Pb, Cd, Hg, Cu, Mn, and Zn) levels. In the mushrooms supplied from the East Black Sea region, the highest Pb level was 6.88 ± 2.85 mg/kg for the species *Hypholoma fasciculare*, which was collected near the vicinity of the road. The highest Hg level was 0.58 ± 0.16 mg/kg for the species of *Amanite vaginate*, whereas the lowest Hg level was 0.06 ± 0.02 mg/kg in *Russula foetens*. The highest Pb content was 6.68 ± 2.85 mg/kg in the species of *Hypholoma fasciculare*. The lowest Pb level was 0.92 ± 0.27 mg/kg in the species of *Amanita rubescens*. The highest Cd level was 3.16 ± 0.72 mg/kg for *Russula cyanoxantha*. Among the wild mushrooms, the lowest Cd level was 1.08 ± 0.16 mg/kg, for the species *A. rubescens*. The highest Cu and Mn levels were 92.5 ± 14.1 and 56.2 ± 12.4 mg/kg, respectively, for species of *Amanita muscaria*. The highest Zn level (176 ± 31.6 mg/kg) was determined for the species *Amanita vaginate*. The metal bioaccumulation levels of six mushrooms were studied. The Hg level of *A. vaginate* samples increases sharply with increasing Hg concentration in the fortified soil samples. The highest Hg level was 3.16 mg/kg for *Amanita vaginate*, whereas the lowest Hg level was 0.95 mg/kg in *R. foetens*. The Cd level also increased with increasing Cd concentration in the soil samples, but the increase was less distinct than that of the Pb level. However, the Pb levels in mushrooms do not change significantly, despite increasing Pb level in the fortified soil. The highest concentration of Pb found was 9.14 mg/kg in *H. fasciculare* samples.

1. Introduction

Mushrooms are valuable health foods, low in calories, high in vegetable proteins, iron, zinc, chitin, fibre, vitamins and minerals. Mushrooms also have a long history of use in traditional chinese medicine. Their legendary effects on promoting good health and vitality and increasing the body's adaptive response have been supported by recent studies.

Turkey is located in southeastern Europe and Asia. It is bordered in the north by the Black Sea, the south by Iraq, Syria, and the Mediterranean, in the west by the Aegean Sea, in the northeast by Georgia and Armenia, in the northwest by Bulgaria and Greece. Turkey can be separated into seven geographic regions. One of them is the Black Sea region. The Black Sea region can be separated into three smaller geographic regions. The East Black Sea region is one of them. In this region, the climate is mild and rainy. The seasons are normally wet with mild temperatures. The climate during the year, especially, in spring and autumn, is ideal for fungal growth.

Numerous investigations have dealt with the heavy metal contents of mushrooms (Demirbaş, 2000a; Tüzen, Özdemir, & Demirbaş, 1998a, 1998b) and macrofungi (Sesli & Tüzen, 1999) in Turkey. Turkey has a large edible mushroom potential and is becoming an important exporter of wild mushrooms. However, qualified studies have not been carried out on this area in Turkey. In recent years, considerable attention has been focused on the bioaccumulation of heavy metals in fruit bodies of some cultivated mushrooms (Demirbaş, 2000a, 2001; Falandyzs, Bona, & Danisievicz, 1994; Lepsova & Mejstrik, 1998; Liukkonen-Lilja, Kuusi, Laaksovirta, Lodenius, & Piepponen, 1983; Tüzen, et al., 1998a). It is well known that all such cultivated mushrooms have the ability of bioaccumulation of metal ions (Falandyzs, Bona, & Danisievicz, 1994).

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Many investigations have dealt with the metal contents of mushrooms, especially edible ones (Lepsova & Mejstrik, 1998) and numerous data have been published on the contents of heavy metals in mushrooms (Seeger, Meyer, & Schönhut, 1976; Vetter, 1993). Compared with green plants, mushrooms can build up large concentrations of some heavy metals such as Pb, Cd, and Hg (Kuusi, Laaksovirta, & Liukkonen-Lilja, Lodenius, & Piepponen, 1981; Meisch, Schimitt, & Reinle, 1977). It has been reported that Hg(II), Pb(II), and Cd(II) ions, especially, are environmentally harmful to some plants (Tüzen et al., 1998a). This bioaccumulation means that some noxious chemicals, including heavy metals, can accumulate in the fruit bodies of the mushrooms. As these metals are well known for their toxicity at low concentrations, a great deal of effort has been made to evaluate the possible danger to human health from the ingestion of mushrooms (Gast, Jansen, Bierling, & Haanstra, 1988). An especially selected compost can be used for measuring and controlling the metal ion accumulation of a cultivated mushroom.

Trace element concentrations in fungi are considerably higher than those of agricultural crop plants, vegetables and fruit. This would suggest that fungi possess a very effective mechanism that enables them to take up some trace elements from the substrate more readily. This mechanism may be more effective in the parasitic and saprophytic fungi trophic groups than in the mycorrhizal fungi group (Lepsova & Mejstrik, 1988).

Various edible mushrooms are appreciated for their pleasant flavour and their biting texture. In general, their fruiting bodies, on a dry weight basis, contain about 39.9% carbohydrate, 17.5% protein and 2.9% fats, with the rest constituting the minerals (Latiff, Daran, & Mohamed, 1996).

Some living organisms possess the ability to take up and accumulate, in their structure, certain elements (both metallic and non-metallic) at high concentrations. Within the plant kingdom, such species are called indicator plants, because their presence indicates the occurrence of some elements in large quantities in the environment. Other living organisms (such as lichens) came into prominence in environmental protection, because their presence or even extermination could be a good bioindicator of the occurrence of a toxin (Vetter, 1993, 1994).

The decomposition of mushroom samples is an important part of combined analytical methods. Determinations of the heavy metal concentrations have been performed with atomic absorption spectrophotometry (AAS), using flame atomization (Gast, Jansen, Bierling, & Haanstra, 1988). Hg content in mushroom samples has been determined by cold vapour atomic absorption spectrophotometry (CVAAS), using $NaBH_4$ as the reducing agent (Rincon-Leon & Zurera-Cosano, 1986). Al, Pb, and Cd contents have been determined using a carbon rod atomizer in AAS (Mandic, Grgic, & Seruga, 1992). Al, Cu, Mn, Fe, and Zn have been analyzed after

Table 1
Habitat, edibility and the families of mushroom species

Class, family and species of mushroom	Habitat	Edibility
BASIDIOMYCETES Classe		
Amanitaceae Roze		
Amanita muscaria (L.: Fr.) Hook	With bird trees	Poisonous
Amanita rubescens Pers.: Fr.	In woodland	Edible
Amanita vaginate (Bull.: Fr.) Vitt.	In deciduous woods or on heaths	Edible
Russulaceae Lotsy		
Russula cyanoxantha (Sch.) Fr.	Under broad leafed trees	Edible
Russula foetens Pers.: Fr.	Under broad leafed trees or conifers	Inedible
Strophariaceae Sing. & Smith		
Hypholoma fasiculare (Huds.: Fr.) Kumm	On stumps of tree	Inedible

Table 2
Average levels (mg/kg, dry-weight basis) of heavy metal (Pb, Cd, Hg, Cu, Mn, and Zn) in mushroom samples obtained from east Black Sea region

Species	Pb	Cd	Hg	Cu	Mn	Zn
Amanita muscaria	1.95±0.92	2.14±0.32	0.39±0.18	92.5±14.1	56.2±12.4	70.0±9.8
Amanita rubescens	0.92±0.27	1.08±0.16	0.42±0.08	16.8±4.5	7.6±1.8	29.4±6.1
Amanita vaginate	1.62±0.54	2.05±0.41	0.58±0.16	54.0±10.2	36.7±7.0	176±31.6
Hypholoma fasciculare	6.88±2.85	1.36±0.41	0.48±0.26	11.5±3.5	12.6±2.4	19.6±7.2
Russula cyanoxantha	2.05±76	3.16±0.72	0.14±0.05	19.1±5.2	12.6±2.5	26.1±7.1
Russula foetens	2.43±0.78	1.67±0.61	0.06±0.02	35.8±6.9	24.8±5.2	48.0±9.3

dry-ashing and digestion in HCl by flame AAS (Kojo & Lodenius, 1989).

The present study relates to the determination of Hg, Pb, Cd, Fe, Cu, Mn, and Zn contents, using an AAS method for the fruit bodies of six wild mushrooms of Turkish origin. The metal (Hg, Pb, Cd, and Cu) bioaccumulation levels of six mushrooms were also studied.

2. Materials and methods

In this study, 36 samples of wild-growing mushrooms, corresponding to different species, were used. The mushroom specimens were collected from locations in the east Black Sea region of Turkey in 2000. Species include *Amanita muscaria, Amanita vaginata, Hypholoma fasciculare, Russula cyanoxantha* and *Russula foetens*. These samples were washed with demineralized water. Each sample was dried at 50°C overnight and crushed in a mortar with achate beaker and pestle.

For the identification of specimens, the colour, odour and other apparent properties of the mushrooms and vegetation were noted. The mushrooms were identified using the reference books of European Flora (Breitenbach & Kränzlin, 1984, 1986, 1991).

Digestion of mushroom samples was performed using an oxi-acidic mixture of HNO_3:H_2SO_4:H_2O_2 (4:1:1, 12 ml for 2-4 g sample) and heating at 75°C for 3 h. After

Table 3
Average concentrations (mg/kg, dry-weight basis) of heavy metals (Hg, Pb, Cd and Cu) of fortified soils and mushrooms (*Amanita muscaria, Amanita rubescens, Amanita vaginate, Russula foetens, Russula cyanoxantha, Hyphofolama fasciculare*) samples obtained from east Black Sea region

Sample	Metal concentration of soil				Metal concentration of mushroom			
	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu
Amanita muscaria	0.52	0.82	0.26	0.55	0.23	1.54	1.87	72.8
	1.21	1.66	0.85	1.24	0.48	1.73	2.69	129
	1.84	2.85	1.97	3.09	0.66	2.13	4.33	197
	3.76	3.51	2.67	5.63	1.94	2.28	11.6	231
	6.44	6.35	3.51	14.8	1.78	1.84	8.56	251
	10.2	12.3	8.05	33.4	0.55	1.37	2.34	159
Amanita rubescens	0.54	0.85	0.23	0.56	0.32	0.78	0.86	14.0
	1.25	1.72	0.78	1.21	0.51	1.07	1.42	23.8
	2.18	2.94	2.02	3.12	0.79	1.36	1.80	37.3
	3.72	3.42	2.74	5.70	2.15	1.46	5.45	48.2
	6.21	6.50	3.82	15.1	2.11	1.34	4.13	56.8
	10.6	12.2	8.12	32.9	0.64	0.98	1.35	29.1
Amanita vaginate	0.53	0.84	0.25	0.60	0.38	1.34	1.73	14.6
	1.38	1.76	0.81	1.23	0.67	2.32	2.56	25.4
	2.20	2.74	1.86	3.10	0.92	2.81	3.14	45.0
	3.50	3.54	2.78	5.76	3.16	3.09	8.75	58.7
	6.47	6.34	3.69	14.9	2.86	2.70	6.92	71.4
	10.5	12.3	7.94	33.1	0.81	1.95	2.63	41.9
Russula foetens	0.50	0.45	0.26	0.53	0.08	1.96	1.41	28.5
	1.25	0.68	0.83	1.25	0.18	3.15	2.36	50.9
	2.18	1.12	1.84	3.05	0.26	3.54	2.91	84.3
	3.52	2.41	2.75	5.77	0.95	3.75	6.87	106
	6.45	5.88	3.70	15.1	0.85	3.56	5.42	138
	10.2	9.91	7.98	32.8	0.25	1.76	2.13	76.8
Russula cyanoxantha	0.49	0.46	0.22	0.62	0.12	1.67	2.58	18.0
	1.26	0.95	0.85	1.23	0.26	2.81	3.65	27.9
	2.13	1.36	1.90	3.18	0.38	3.23	4.15	43.2
	3.40	3.11	2.83	5.79	1.12	3.66	11.6	58.6
	6.58	6.08	3.72	15.2	0.99	3.48	9.57	71.3
	10.4	11.4	8.09	33.0	0.35	1.60	3.85	47.5
Hypholoma fasciculare	0.54	0.83	0.24	0.65	0.34	5.18	1.15	10.6
	1.26	1.86	0.86	1.24	0.68	7.55	2.19	19.1
	2.26	2.77	1.87	3.14	0.86	8.41	2.77	30.2
	3.45	3.32	2.85	5.81	2.08	9.14	8.34	43.8
	6.64	6.47	3.76	15.1	1.78	8.70	7.10	52.7
	10.5	12.8	7.92	32.8	0.62	4.32	2.68	35.9

cooling, 20 ml demineralized water was added, the digest was again heated up to 150°C for 4 h and brought to a volume of 25 ml with demineralized water (Demirbaş, 2000b; Tüzen, Sesli, & Demirbaş, 1999).

For analysis of mercury, the technique described was as follows: 0.5 g was taken from the dried homogenized sample and its digestion was carried out using 7 ml of a HNO_3:H_2SO_4:H_2O_2 acid mixture at a ratio of 4:1:1; digestion was done at 60°C in a thermostatic bath, being completed in about 1.5 h. For oxidation of the sample, a solution of potassium permanganate was reduced with a solution of hydroxylamine sulfate (Demirbaş, 2001).

Pb and Cd levels in the mushroom samples were determined using a GBC 3000 graphite furnace for AAS. Determination of heavy metal (Fe, Cu, Mn, Zn) contents was carried out with a GBC 905 model AAS, using flame atomization. For the determination of Pb and Cd contents, deuterium and Smith-Hieftje background correction have been used. The standard-addition procedure was used in all determinations.

The wavelength and slit values, as nm, used for the determination of Pb, Cd, Fe, Cu, Mn, and Zn were: 283.3 and 0.5, 228.8 and 0.5, 248.3 and 0.2, 324.7 and 0.5, 279.5 and 0.2, and 213.9 and 0.5, respectively.

3. Results and discussion

The habitat, edibility and the families of mushroom species are given in Table 1. The heavy metal levels of the 18 selected species of uncultivated and cultivated mushrooms are given in Table 2. The average concentrations (mg/kg, dry-weight basis) of heavy metal (Hg, Pb, Cd and Cu) of fortified soil and mushroom (*A. muscaria, A. rubescens, A. vaginate, R. foetens, R. cyanoxantha, H. fasciculare*) samples obtained from East Black Sea region are shown in Table 3. The metal levels of fortified soil samples and mushrooms are separately given in Figs. 1–4.

The heavy metal levels in the mushrooms are hardly affected by pH and organic matter content of the soil (Gast, Jansen, Bierling, & Haanstra, 1988).

The trace element contents of the species depend on the ability of the species to extract elements from the substrate, and on the selective uptake and deposition of elements in tissues. An interesting aspect of our study is that different samples of the same species differ considerably in their trace element contents. According to our studies (Demirbaş, 2000a, 2001; Tüzen et al., 1998a, 1998b), no difference between saprophytic and mycorrhizal-forming species was observed.

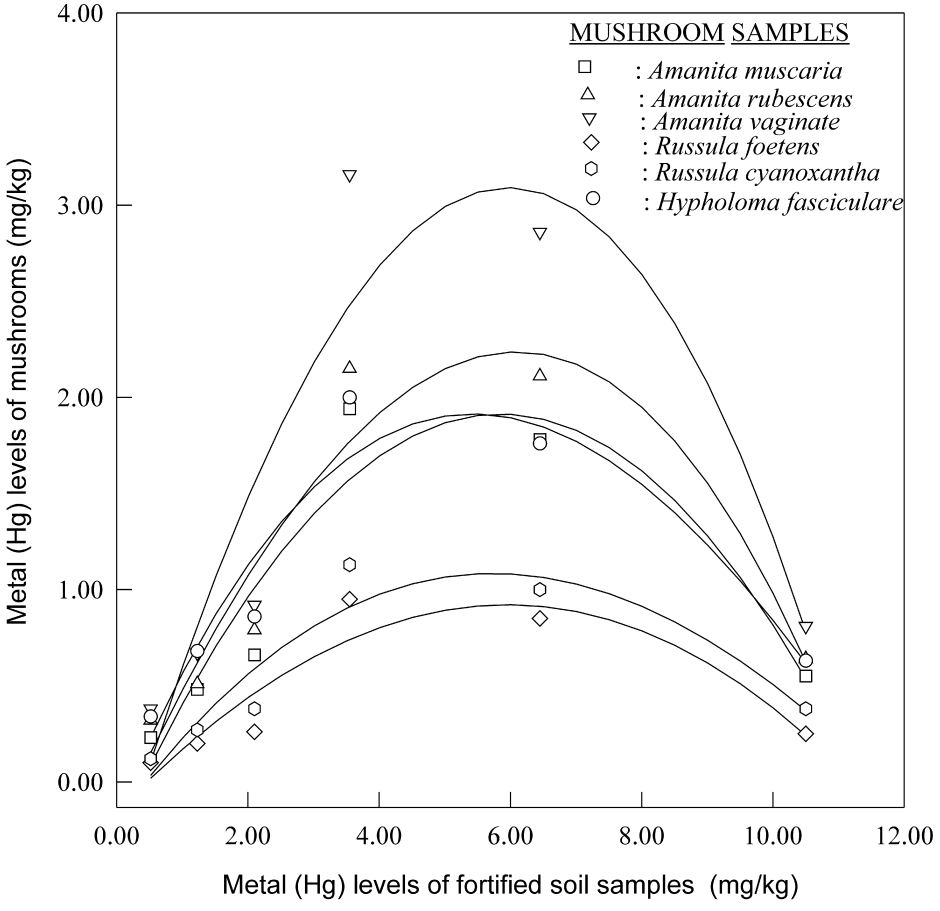

Fig. 1. Hg levels of fortified soil samples and mushrooms.

Trace element concentrations in the species analyzed (Table 2) are in good agreement with earlier values reported within Europe (Seeger, Meyer, & Schönhut, 1976).

From Table 2, in the mushrooms supplied from the east Black Sea region, The highest Hg level was found as 0.58 ± 0.16 mg/kg for the species *A. vaginate*, whereas the lowest Hg level was 0.06 ± 0.02 mg/kg in *R. foetens*. The highest Pb content was found as 6.68 ± 2.85 mg/kg in the species of *H. fasciculare*. The lowest Pb level was 0.92 ± 0.27 mg/kg in the species of *A. rubescens*. The highest Cd level was determined as 3.16 ± 0.72 mg/kg for *R. cyanoxantha*. Among the wild mushrooms, the lowest Cd level was 1.08 ± 0.16 mg/kg for the species of *A. rubescens*. The highest Cu and Mn levels were 92.5 ± 14.1 and 56.2 ± 12.4 mg/kg, respectively, for species of *A. muscaria*. The highest Zn level (176.1 ± 31.6 mg/kg) was determined for the species *A. vaginata*.

Cd is known as a principal toxic element, since it inhibits many life processes (Vetter, 1987, 1989, 1993). It is documented that the cadmium content in other species, originating from the same habitats as the macrofungi with high cadmium concentrations, is lower by an order of magnitude. There are two important questions: does the high cadmium concentration in certain species of *Agaricus* reflect abnormally high levels of cadmium in the environment or is the element preferentially accumulated by these species, perhaps because of a specific biochemical function of the metal? With what kind of compounds is cadmium bound in these organisms? Following Schmitt and Meisch (1985), the presence of a specific cadmium transport system may be postulated for certain toadstools, especially for *Agaricus* species. Schmitt and Meisch (1985) demonstrated the fact of cadmium accumulation. From the fruit bodies of *Agaricus macrosporus,* a cadmium-binding phospho-glycoprotein, cadmium mycophosplatin, was isolated (Schmitt & Meisch, 1985). This protein has a molecular weight of 12 000 daltons, containing phosphorus, but not sulfur, and contains glucose and galactose. Another group of mycologists analyzed taxonomic groups/species with a higher cadmium content and in general, the phenomenon of its accumulation (Kojo & Lodenius, 1988; Santoprete & Innocenti, 1984; Stijve & Besson, 1976; Vetter, 1987). The ability to accumulate cadmium is closely correlated with the presence of the binding compound, which is a genetically-coded feature (Vetter, 1993).

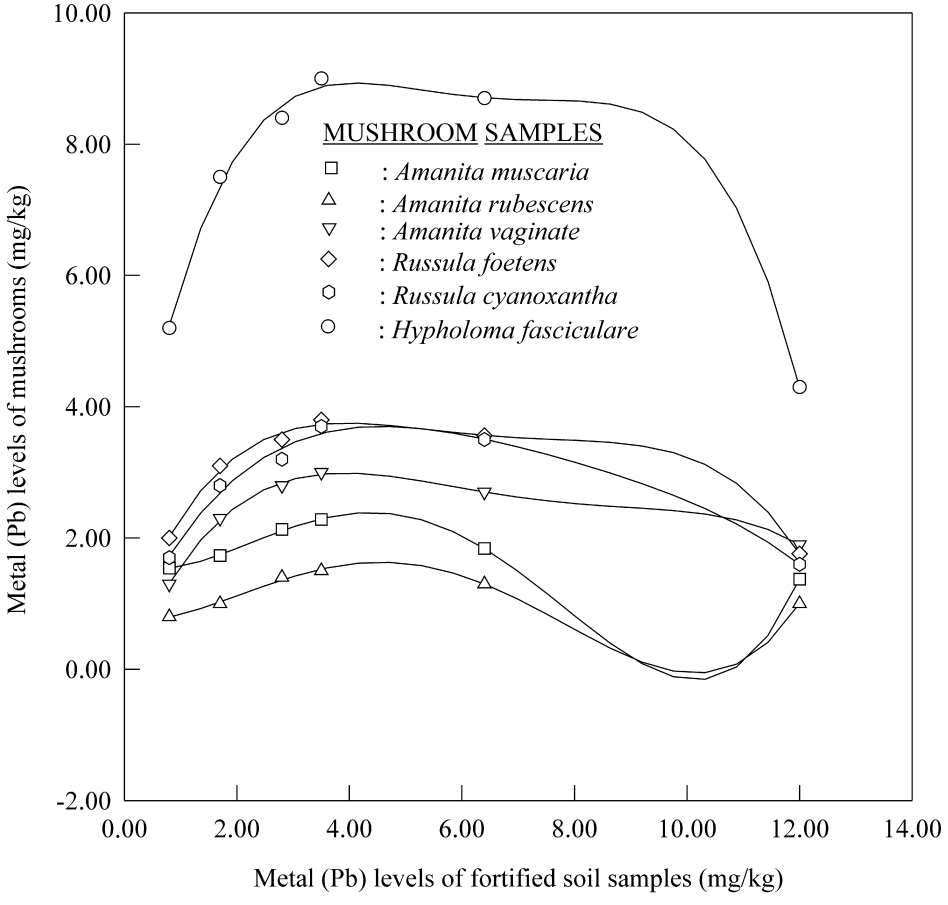

Fig. 2. Pb levels of fortified soil samples and mushrooms.

The mean mercury level in macrofungi surpasses, by two orders of magnitude, those in the green plants (green plants: 0.015 ppm; macrofungi: 1–1.5 ppm) and varies according to the type of fungi, since litter-decomposing species (*Agaricus, Marasmius*) have higher mercury concentrations (0.1–72 ppm) than the wood-destroying species and general (1.5–2.0 ppm) (Laaksovirta & Lodenius, 1979). Taking into account the standard of FAO-WHO and the actual mercury concentration in mushroom in Finland, consumption of 40–370 g fresh mushrooms per week, for adults, is the maximum recommended.

Lead contamination varies and manifests itself in other ways than in the green plants. Lead-accumulating species of fungi are not known; the lead concentration of the analyzed mushroom species and the samples was 0.1 to 40 ppm (on dry weight basis; Laaksovirta & Alakuijala, 1978; Seeger, Meyer, & Schönhut, 1976). Imported lead contaminants were registered in some urban fungi samples from England (Thomas, 1992). Beside busy streets, the concentrations were so high that the fungi could not be recommended for food use (Laaksorvirta & Alakuijala, 1978).

The copper content in macrofungi is significantly higher than that of the green plants (the average of Hungarian fungi samples amounts to 44–48 ppm; Vetter, 1987). This toxic metal is accumulated by the genera *Macrolepiota* and *Agaricus*.

Metal-accumulating ability of mushroom can have two origins: (1) bioaccumulation, supposing the presence of certain metal binding compounds (for cadmium, vanadium, etc.); (2) higher element content, in consequence of higher metal content, in the environment.

Bioaccumulating ability can be coupled with supernormal concentrations occurring in the environment. The metal-accumulating ability is a characteristic and differentiating biological feature.

In Fig. 1, the Hg level of *A. vaginate* samples increases sharply with increasing Hg concentration in the fortified soil samples. The highest Hg level was 3.16 mg/kg for of *A. vaginate*, whereas the lowest Hg level was 0.95 mg/kg in *R. foetens* (Table 3). The Cd level (Fig. 3) also increased with increasing Cd concentration in the soil samples, but the increase was less distinct than that of the Pb level (Fig. 2). However, the Pb levels in

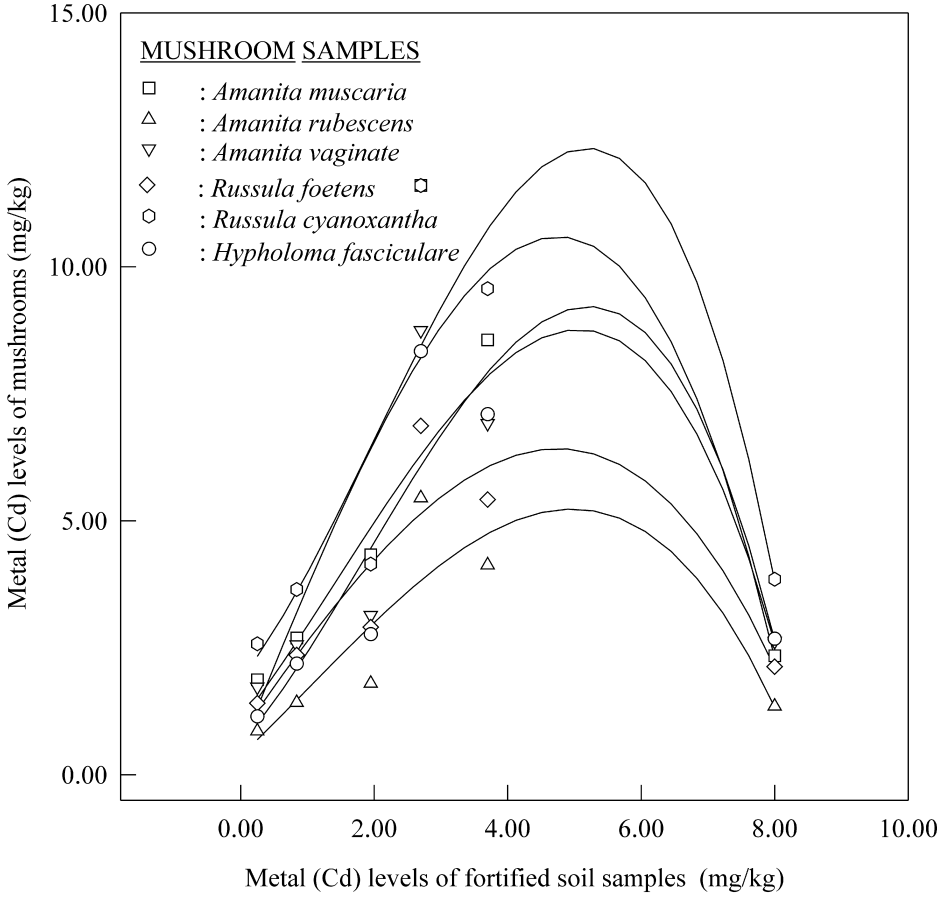

Fig. 3. Cd levels of fortified soil samples and mushrooms.

mushrooms do not change significantly, despite increasing Pb level in the fortified soil. As can be seen in Table 3, the highest concentration of Pb found was 9.14 mg/kg in *Hypholoma fasciculare* samples.

In the wild mushroom samples, the highest copper level was 251 mg/kg for *A. muscaria*, whereas the lowest copper level was 56.8 mg/kg in *A. rubescens* (Table 3).

The results show that the level of mercury differs according to species analyzed and anatomical groups. In a previous study (Zurera-Cosano, Rincon-Leon, Arcos, & Pozo, 1986), there were found to be significant differences between species, although not between anatomical groups. The significance of the species factor in the capacity for concentration of heavy metals has been pointed out by other authors (Bowen, 1996; Crowley, 1978; Hopwood, 1975). According to Stijve and Besson (1976), the mechanism by which some heavy metals are accumulated is somewhat obscure although it seems to be associated with a chelation reaction with the sulfhydryl groups of protein and especially with methionine. However, these same authors found very low levels of lead, cadmium and mercury in samples of *Posalliota bispora* cultivated with a high content of methionine in relation to other species.

Uptakes of cadmium, copper, lead, and zinc in mushrooms and their relationship with soil characteristics (Gast, Jansen, Bierling, & Haanstra, 1988) have been reported. The fact that toxic metals are present in high concentrations in the fruiting bodies of both edible and inedible fungi from an area greatly favoured by mushroom pickers is of particular importance in relation to the FAO/WHO Standards (1976) for lead and cadmium as toxic metals. The maximum permissible dose for an adult is 3 mg lead and 0.5 mg cadmium per week. Hg and Cd levels of the mushrooms generally increased sharply with increasing Hg and Cd concentrations in the soil samples (Figs. 1 and 3). In general, the mushrooms bioaccumulated low amounts of Hg, Pb and Cd; any higher concentrations of those metals showed a phytotoxic effect, causing a lower yield. It appears that the mushrooms take up the heavy metals readily. This might cause a threat to health of consumers and demand soil redemption programs.

4. Conclusion

The occurrence and distribution of different toxic elements in certain mushrooms is not only a theoretical

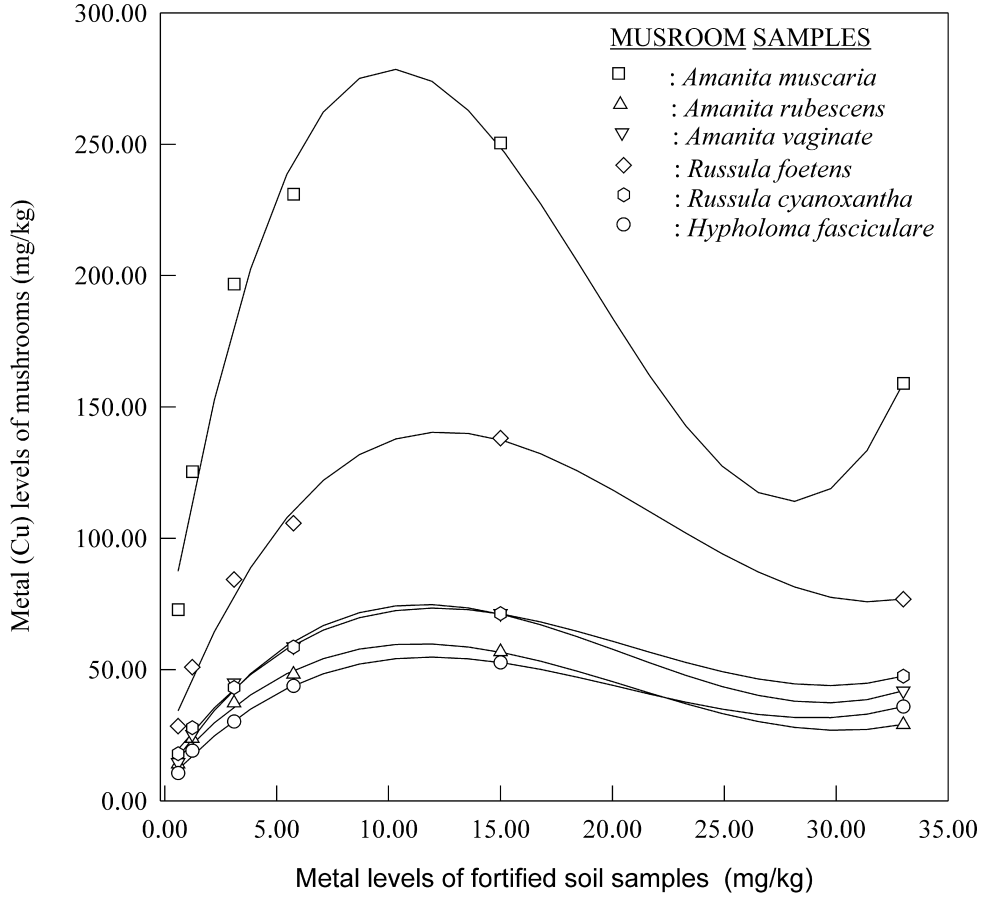

Fig. 4. Cu levels of fortified soil samples and mushrooms.

mycological problem, but also a practical toxicologicalone. Therefore, the investigation of the biological mechanisms of uptake and binding (accumulation) could play an important role in the future. The problem of uptake and accumulation of these elements has environmental and toxicological aspects.

The metal (Hg, Pb, Cd and Cu) bioaccumulation levels of six mushrooms (*A. muscaria, A. rubescens, A. vaginate, R. foetens, R. cyanoxantha, H. fasciculare*) samples obtained from the east Black Sea region was investigated. The Hg level of *A. vaginate* samples increases sharply with increasing Hg concentration in the fortified soil samples. The highest Hg level was 3.16 mg/kg for *A. vaginate*, whereas the lowest Hg level was 0.95 mg/kg in *R. foetens*. The Cd level also increased with increasing Cd concentration in the soil samples, but the increase was less distinct than that of the Pb level. However, the Pb levels in mushrooms do not change significantly, despite increasing Pb levels in the fortified soil. The highest concentration of Pb found was 9.14 mg/kg in *H. fasciculare* samples.

The results obtained were verified by UV vis spectrophotometric methods of AOAC (Horwitz, 1970). The results obtained from AA and UV vis spectrophotometric methods were compared, and agreement was found on average to be $\pm 5\%$.

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