ACCUMULATION OF VARIOUS METALS BY *FULIGO SEPTICA* (L.) WIGGERS AND BY SOME OTHER SLIME MOULDS (MYXOMYCETES)

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During the last 25 years it has been noted that there are many macromycetes which selectively concentrate metals and even some non-metals from their substrates (Mjestrik & Lepsová 1992, Stijve 1993). For example, many representatives of the genus *Agaricus* are notorious for their high mercury and cadmium content (Meisch *et al.* 1977, Stijve & Besson 1976), whereas the non-metal arsenic is encountered at high concentrations in such unrelated fungi as *Entoloma lividum* and *Sarcosphaera coronaria* (Stijve *et al.* 1990). Indeed, there exists now an extensive literature on this subject including reports on the accumulation of radionuclides, *e.g.* the caesium isotopes which were widely diffused in the Chernobyl disaster. Many investigators have used mushroom species such as *Laccaria amethystina*, *Xerocomus badius*, *Paxillus involutus*, *Rozites caperatus* and others as bioindicators for the contamination of sites with radioactive caesium (Stijve & Poretti 1990).

However, virtually nothing has been published on the ability of slime moulds (myxomycetes) to concentrate metals and other elements from their substrates, except for a single paper by Setälä & Nuorteva (1989). In a study of environmental pollution in south-west Finland, these investigators compared metal contents of several slime molds to the levels encountered in blueberry leaves collected at the same sites. For this purpose, the following metals were selected: aluminium and iron (major soil constituents), zinc (Zn) and copper (essential plant nutrients), and mercury and cadmium (notoriously toxic metals). The results of this investigation are summarised in Table I. It is clear that aluminium, copper and mercury are not accumulated by the two slime moulds. On the other hand, *Fuligo septica* has an enormous affinity for zinc, because it contains on the average 240 times more than the blueberry leaves. The iron and cadmium content are also higher than the amounts measured in the substrate, but the concentration rates are much smaller. *Tubifera ferruginosa* apparently only concentrates zinc, cadmium and (perhaps) copper, but the levels are far less spectacular. This proved also to be the case for *Symphytocarpus flaccidus*, *Amaurochaete atra*, *Ceratiomyxa fruticulosa* and a *Stemonitis* species, which were also analysed by the Finnish authors. The high amount of zinc in *Fuligo septica* is rather intriguing, since it is much more than ever encountered in macromycetes which contain on the average 100 mg/kg on dry matter. Some examples of zinc-accumulating species are *Macrolepiota procera*, 386 mg/kg (Byrne *et al.* 1976) and *Hygrophorus nitratus* in which not less than 1,025 mg/kg was found (Tyler 1980). Although the slime mould concentrates cadmium, the levels found are well below those of macromycetes like *Agaricus* spp. containing more than 100 mg/kg of this toxic metal (Meisch *et al.* 1977). Analyses of herbarium collections indicated that the zinc and cadmium concentrations in *F. septica* collected in 1860, 1909, 1935 and 1959 were as high as those found in 1989, and are therefore not a consequence of environmental pollution. Setäla & Nuorteva (1989) pointed out that the zinc levels in this slime mould are so high that it is difficult to understand how a living organism can tolerate them. They suggested that the metal probably affords protection from some more dangerous factor by acting as an enzyme activator in detoxification systems. An obvious first step in verifying this hypothesis would be the elucidation of the chemical form(s) in which zinc is present in the slime mould. It is somewhat puzzling that the paper of the Finnish authors has not received the attention it surely merits, because in the 10 years since its publication nobody has apparently bothered to investigate metals in slime moulds.

Table I. Metal concentrations in slime moulds growing on various substrates in south-west Finland (in mg/kg on dry matter) (Setälä & Nuorteva 1989)

	***Vaccinium* (leaves) N = 15**		***Fuligo septica* N = 15**		***Tubifera ferruginosa* N = 3**	
	Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**
Aluminium	52–540	230	9–370	96	9–99	51
Iron	8–120	73	22–720	264	13–96	55
Zinc	10–160	50	4,000–20,000	12,160	150–570	310
Copper	4.3–17	8,7	n.d.–23	11.3	19–26	21
Cadmium	0.02–0.18	0.12	0.40–9.8	2.44	1.2–4.6	2.8
Mercury	n.d.–0.06	—	0.013–0.057	~ 0,03	—	—

n.d. = not detectable; N = number of samples.

The present paper reports the results of an investigation on the occurrence of metals in five common slime mould species, collected in Australia, Canada, New Mexico and Switzerland. The analytical method used was wet digestion, followed by inductively coupled plasma mass spectrometry (ICP-MS) determination of 60 elements (Zbinden & Andrey 1997). Some elements for which this method is somewhat problematic, *e.g.* potassium, phosphorus, iron and calcium (Ca) were determined by ICP-AES (AOAC official method 1996).

The results obtained for the 17 samples are listed in Table II. For about 50 elements including the rare earth metals, and also for some non-metals as arsenic, antimony and selenium, the concentrations found were uninterestingly low, and are therefore not reported here. The results for *Fuligo septica* proved to be most remarkable. In accordance with Setälä & Nuorteva (1989) we found enormous zinc concentrations, which fluctuated within a factor of 10. However, what is new is that *Fuligo* not only accumulates calcium, but also barium (Ba) and strontium (Sr), *i.e.* metals belonging to the same chemical group. The presence of much lime in some slime moulds had already been observed in the 19th century (Schweinitz 1832), but the role of calcium in the formation of the peridium was only studied more recently by Schoknecht & Keller (1977, 1989). We observed that the calcium content apparently fluctuates with the life cycle of the slime mould: the yellow plasmodium contained much less than older collections consisting mainly of spores. The preference for lime is not shared by the four other slime moulds (at least not to that extent), although the calcium concentrations, except that of *Tubifera*, are significantly higher than those measured in macromycetes (Seeger & Hütter 1981). Calcium is undoubtedly a major essential element for *F. septica*, but the presence of high concentrations of the mildly toxic metals strontium and barium is somewhat surprising. Indeed, as said above, these metals are chemically rather close, and could, therefore, be simultaneously absorbed with the calcium from the soil. Since the element radium also belongs to this group, we subjected a pooled *Fuligo* sample to gamma spectrometry, and found indeed evidence of a low, but significant concentration of Ra 226 (670 Bq/kg).

Even more amazing is the presence of a staggering amount of manganese (Mn) compared with a relatively low iron content. These metals are chemically close, but in most organisms, *e.g.* mushrooms, iron predominates over manganese except for many Phallales (Schmitt *et al.* 1977) and representatives of the genus *Panaeolus* wherein the ratio iron:manganese is often <1 (Stijve & Blake 1994).

Lycogala epidendrum seems to bioaccumulate copper, whereas the four other slime moulds appear to exclude this metal. It is worth noting that in the five slime moulds the essential element magnesium (Mn) is present at the same concentrations as those generally observed in macromycetes. The magnesium content of mushrooms is subject to little variation. Even within one genus, the fluctuation rarely exceeds a factor 2 (Seeger & Beckert 1979). The metal potassium, the principal cation in green plants and mushrooms is very low in slime moulds. The concentrations listed in Table II are even lower than those of the Polyporaceae which contain generally about one per cent of potassium on dry matter (Seeger 1978).

In mushrooms the non-metal phosphorus plays a key role in the intracellular transport of many metals (as soluble complex phosphates), and its level is indeed positively correlated with the heavy metal concentrations present. Mycorrhizal mushrooms contain on the average 0.61% P, whereas saprophytes need at least twice as much. This would explain why members of the genus *Agaricus* contain often far more mercury, cadmium, silver, lead and other metals than symbionts such as russulas and boletes (Quinche 1997). These observations do not seem to apply to slime moulds, since the phosphorus content of *Tubifera ferruginosa* (which is poor in metals) is hardly different from that of *F. septica* which, especially in the sporulating stage, is loaded with both light (Ca, Ba, Sr) and heavy (Zn, Mn) metals.

At present, little is known about the chemical forms in which the metals occur in the slime moulds. Upon treating the *Fuligo* sporemass with diluted hydrochloric acid, carbon dioxide was produced under effervescence, indicating that an important part of the calcium should be present as the carbonate, similarly to the peridia of two important slime mould families (Schoknecht & Keller 1989). The calcium oxalate crystals which can be readily observed in tissues of many green plants and, to a lesser extent, also in macromycetes, proved microscopically and chemically absent in *F. septica*. However, the possibility that part of the metals could be present as silicates, sulfates or even salts of organic acids is still under investigation. Two more metals found in the slime moulds invite some comments. The concentrations of toxic cadmium are rather low and do not vary much. In *L. epidendrum* we observed not less than 20 mg/kg tin, a metal that usually occurs only in traces (<1 mg/kg) in plants, animals and fungi. It is noteworthy that no accumulation of elements from the same group such as arsenic and antimony could be found. It would be interesting to look for tin in some related slime moulds.

The occurrence of high levels of heavy metals in edible mushrooms has been a cause of some concern, especially in Germany, where guidelines to limit consumption have been published (Anonymous 1978). It should therefore be pointed out that plasmodia of *Fuligo septica* and developing aethalia of *Enteridium lycoperdon* are fried and eaten by some parts of the population around Veracruz (Mexico). The large fruitings which are periodically collected in this area are called 'caca de luna'(!) by the locals (Villarreal 1983, Montoya-Esquivel 1992) Since these exotic food items are probably only a negligible part of the population's diet, there should be no danger of chronic zinc, manganese or barium poisoning.

This tentative study shows that myxomycetes also distinguish themselves from macrofungi and microfungi in their affinity to various metals, and their placement in a separate Kingdom is therefore also justified from this point of view.

Table II. Concentrations of phosphorus and 11 metals in five species of slime moulds

	Fuligo septica N=8	*Tubifera ferruginosa* N = 2	*Enteridium splendens* N = 2	*Enteridium lycoperdon* N = 3	*Lycogala epidendrum* N = 2
Potassium %	0.22–0.39 (0.33)	0.21–0.29	0.38–0.48	0.27	0.13–0.17
Phosphorus %	0.56–1.03 (0.82)	0.70–0.81	0.65–0.67	0.73	0.48–0.65
Calcium %	**4.80–11.2 (8.76)**	0.028–0.061	0.14–0.16	0.11	0.27–049
Magnesium %	0.073–0.10 (0.093)	0.061–0.068	0.08–0.081	0.078	0.064–0.08
Barium	**294–15,190 (2,550)**	2–3	5–6	32	12–20
Strontium	**237–2,190 (1,290)**	5–6	13–16	15	33–46
Manganese	**116–4,570 (1,600)**	11–13	5–7	27	9–31
Iron	–478 (232)	67–115	65–78	188	118–132
Copper	3–14 (6.9)	7–11	4–4	7	**52–84**
Zinc	**395–3,600 (1,490)**	74–83	69–69	119	72–83
Cadmium	0.88–1.90 (1.15)	0.68–0.98	1.05–1.35	0.62	0.90–1.12
Chromium	1.5–72 (26)	12–23	3–4	18	2.9–8.4
Nickel	1.6–34 (13.3)	6–11	0.34–0.46	10	0.55–1.3
Tin	0.05–1.1 (0.41)	0.07–0.13	<0.05	<0.05	**19–30**
Bismuth	<0.05	<0.05	<0.05	20	<0.05

All values in mg/kg dry matter, except for those expressed as a percentage.
Average concentrations between brackets.
Values in bold type indicate that the concentration of the metal in the slime mould is well above the average soil level.

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Summary
Fifteen collections of five slime mould (myxomycetes) species (*Fuligo septica* (L.) Wiggers, *Enteridium splendens* Morgan, *E. lycoperdon* Bull., *Tubifera ferruginosa* (Batsch) J.F. Gmel. and *Lycogala epidendrum* (L.) Fries) were analysed for about 60 chemical elements. It was confirmed that *Fuligo septica* strongly accumulates zinc (up to 3600 mg/kg on dry matter), and to a far lesser extent also iron and cadmium. For the first time, it is reported that *F. septica* not only strongly concentrates calcium (4.8–11.2%), but also barium (294–15,190 mg/kg), strontium (237–2,190 mg/kg) and manganese (116–4,570 mg/kg). On the other hand,

bismuth was observed in a single collection of *Enteridium lycoperdon*, but this finding still requires confirmation. In their affinity for metals, slime moulds differ strongly from micromycetes and macromycetes. Their placement in a separate Kingdom is therefore once again justified.

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