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High metal contents found in Fulico septica (L.) Wiggers and some other slime molds (Myxomycetes)

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The metal contents of *Fulico septica*, seven other slime molds and two unidentified slime mold plasmodia were studied and compared with the levels in *Vaccinium myrtillus* leaves collected at the same time from the same place. The levels of Cd, Cu and especially Zn were generally far higher in the slime molds. The levels of Zn in *Fulico septica* were so high (4 000–20 000 ppm) that it is difficult to understand how a living organism can tolerate them. The levels of Al and Fe, on the other hand, were higher in *V. myrtillus*. This research underlines the importance of humus as a source of metals for the organisms living in it. It also shows simultaneous peak occurrence of the useful micronutrients Cu and Zn and the toxic Cd, to which the former seems to be antagonistic. The main value of this material is that it provides information for future work.

Key words: Al, Cd, Cu, Fe, *Fuligo septica*, Hg, myxomycetes, slime mold, Zn

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Introduction

Slime molds (Myxomycetes) have commonly been neglected in studies on forest ecology and also in investigations of the role of toxic metals in the multistress disease killing forests in Europe. They are of great potential interest, however, because several kinds of saprophags in the forest ecosystem accumulate metals very vigorously (Andersen 1978, Beyer et al. 1982, Beyer et al. 1985, Pokarzhevskij et al. 1978, Pokarzhevskij et al. 1982). Some changes in the amounts and physiology of soil organisms have also been observed (Bengtson & Rundgren 1984, Beyer & Anderson 1985, Tyler & Westman 1979).

In their natural habitats, the plasmodia of slime molds obtain nutrients by ingesting solid food particles, including bacteria, fungi, and in some cases also algae and other substrates as well (Gray & Alexopoulos 1968). To some degree at least, the metal contents of Myxomycetes can be expected to reflect the metal levels of their food.

Some laboratory experiments have been performed to determine the toxicity of heavy metals to the slime mold *Physarum polycephalum*. These have shown that high contents of heavy metals affect the membrane potential, nuclear biochemistry and survival time of this slime mold (Chin et al. 1973, Chin et al. 1978a, 1987b, Chin & Sina 1978, Terayama et al. 1978).

Preliminary determinations made in our laboratory of the metal contents in some dried museum specimens of *Lycogala epidendrum* (L.) and *Fuligo septica* from the Botanical Museum of Helsinki University showed extremely high Zn levels in *F. septica*, and high metal contents in general (Table 1). It was therefore decided to study the metal levels in material collected from nature.

The metals chosen for study were Al, Fe, Zn, Cu, Cd and Hg, i.e. two common soil metals, two useful plant micronutrients and two poisonous metals. The metal levels in leaves of *Vaccinium myrtillus* were studied in order to have a measure of the general metal load of each locality. *V. myrtillus* has earlier been used successfully by our laboratory as an indicator of this kind (Särkelä & Nuorteva 1987, Kanerva et al. 1988, Koski et al. 1988).

Table 1. Metal levels in specimens of *Fuligo septica* and *Lycogala epidendrum* in the Botanical Museum of the University of Helsinki. The levels are given as mg/kg dwt = ppm of air-dry weight. The values are for individual slime molds.

		Al	Fe	Zn	Cu	Cd
Fuligo septica						
Merimasku	1860	40	27	11000	13	2.1
Lohja	16.10.1909	26	32	5100	12	1.3
Tenhola	23.4.1935	64	57	3600	12	1.6
Kankaanpää	28.8.1935	3.4	22	2200	9.6	2.7
Västanfjärd	6.8.1959	21	92	2600	11	3.2
Lycogala epidendrum						
Porvoo	9.9.1870	35	38	35	5.7	0.28
Espoo	4.10.1909	190	200	49	3.8	0.28
Helsinki	15.4.1938	47	55	69	3.4	1.1
Porvoo	1.5.1964	490	580	82	4.6	0.57

Material

The material was collected from different habitats, mainly a forest near the small town Kokemäki, in southwestern Finland. The study area is about 0.1 x 5 km. The main tree in that area is *Pinus sylvestris*, *V. myrtillus* being a common undershrub. The forest lies approximately 25 km south-east of Harjavalta, an industrial town, known to be a major contributor to metal pollution in the area (Laaksovirta & Silvola 1975, Hynninen 1986, Hynninen & Lodenius 1986, Arstila et al. 1986, Kuokkanen 1986, Sippola & Erviö 1986, Heliövaara et al. 1987). According to the previous investigations, however, the metal pollution does not reach the present study area to any great extent.

The sampling locality in Köyliö was a clear-cut pine forest, in Nummi–Pusula it was a birch forest near the Helsinki–Pori road. The localities in Espoo, Vantaa and Juupajoki were all spruce forests, but in Juupajoki some of the samples were collected from a swamp. The Pyhäranta sampling area was in the yard of a summer cottage located in pine forest near the seashore.

The samples were collected between 5.7.1988 and 22.9.1988.

Methods

The slime molds and the blueberry leaves were collected by hand and placed in plastic containers (100 ccm). Most of the slime mold samples consisted of mature, individual aethalia, a few of plasmodia. The latter were frozen immediately. In the laboratory the samples were dried at room temperature for about a week before the containers were closed. To prevent metal fallout on the samples during this phase they were protected with a sheet of paper. Metal tools were not used during collection or handling of the samples.

Final drying was performed at 40°C for 24 hours. The samples collected as plasmodia, however, — and first treated by freezing — were dried for 3 days in 40°C.

Samples weighing more than 0.6 g were divided into two, one part being analysed for Hg and the other for the other metals. Hg was not determined on small samples. The larger *Fulico septica* samples were also divided into two: the hypothallus and the sporophore. The hypothallus was of course contaminated to some degree by the spores.

For the analyses the samples were digested in 5 ml of 65% HNO_3 (Merck, Suprapur) for 2 h at 50°C, for 4 h at 110°C and for 4 h at 170°C. The samples analysed for Hg were digested in 5 ml HNO_3–H_2SO_4 mixture (1:4) at 85°C for 4 h. All the samples were filtered and diluted to 25 ml with distilled water. The samples were analysed for Cu, Fe and Zn with a Varian spectr AA-40 flame Atomic Absorption Spectrophotometer; for Cd and in the case of the smallest samples Graphite Furnace was used; The Al analyses were done with a Perkin-Elmer 360 flame AAS and the Hg analyses with a Perkin-Elmer MAS-50.

Table 2. Metals in *Fuligo septica* (L.) Wiggers growing on different substrates in the Satakunta (Köyliö and Kokemäki) and Uusimaa (Espoo and Nurmijärvi) provinces. The background levels of metals in the sampling localities are represented by the metal contents in the leaves of *Vaccinium myrtillus* L. The metal levels are given as mg/kg dwt = ppm of air-dry weight. (* = contaminated by *Nectria violacea*, – = not measured, because sample too small, 0 = the level of the metal was almost as low as in the similarly treated 0-standard). The values are for individual slime molds.

Area	Sampling time	Substrate	Metals in leaves of *Vaccinium myrtillus*						Metals in *Fuligo septica* in toto					
			Al	Fe	Zn	Cu	Cd	Hg	Al	Fe	Zn	Cu	Cd	Hg
Kokemäki	5.7.1988	pine stub	150	102	14	5.3	0.020	–	160	300	10000	15	1.32	–
Kokemäki	10.7.1988	pine stub	97	26	25	6.3	0.092	–	200	180	14000	8.6	9.8	0.015
Kokemäki	10.7.1988	pine stub	140	63	43	8.5	0.052	0	190	330	11000	19	0.64	0.034
Köyliö	24.7.1988	pine stub	540	7.8	9.7	5.8	0.18	–	370	410	13000	17	1.0	–
Kokemäki	26.7.1988	needle litter	120	90	13	9.0	0.076	0.026	42	140	12000	6.2	1.5	0.023
Kokemäki	26.7.1988	moss carpet	120	90	13	9.0	0.076	–	67	240	15000	9.6	1.1	–
Kokemäki	2.9.1988	bog-spruce	220	86	17	17	0.45	0.045	26	550	9400	11	1.8	0.037
Kokemäki	2.9.1988	needle litter*	230	100	81	15	0.090	–	38	42	17000	23	3.2	–
Kokemäki	2.9.1988	moss carpet*	200	46	49	4.3	0.038	–	9.3	22	4000	15	0.40	–
Kokemäki	2.9.1988	spruce stub*	260	83	16	9.1	0.16	–	40	190	15000	12	4.0	–
Kokemäki	2.9.1988	needle litter*	360	84	160	9.4	0.066	0.063	55	220	16000	6.3	2.5	0.057
Espoo	20.7.1988	spruce base near the ground	52	23	24	8.9	0.10	–	10	37	18000	5.1	3.4	0.013
Espoo	7.8.1988	spruce base near the ground	230	69	120	8.1	0.047	–	41	450	20000	0	0.53	–
Nurmijärvi	22.9.1988	needle litter*	340	120	79	7.2	0.14	–	170	720	14000	9.0	1.1	–
Nurmijärvi	22.9.1988	spruce stub*	310	110	79	7.8	0.15	–	19	33	4000	13	4.3	–

Results and discussion

Slime molds are not infrequent in the field in Finland, but being scattered they are difficult to observe and they are often too small for metal analyses. It is not possible for an amateur field worker to obtain a large material during a single summer and the present material is merely intended to provide preliminary information for future work. The results are given in detail in Tables 2–4.

In order to illustrate the occurrence of metals in the different slime mold species, the metal levels in the individual slime molds were divided by the metal levels in the indicator plant *V. myrtillus*. The mean value and standard deviation of the ratios for each species were calculated. If the mean value was under 1, the ratio was recalculated using the metal content of the slime mold as a divisor. This is a convenient way of describing the behavior of different metals in different slime mold species. Figs. 1–3 show that the levels of the common soil metals Al and Fe are higher in *V. myrtillus*, whereas all the other metals have higher contents in the slime molds. The levels of Al and Fe in *F.septica* are the only clear exception to this rule. Zn and Cd are accumulated by slime molds remarkably vigorously.

The trends described above can be attributed to the fact that *V. myrtillus* takes its nutrients from both humus and the mineral soil, whereas slime molds are considered to be confined to humus and decaying material.

The high levels of Zn in *F. septica* cannot be explained at present. Zn has not been observed to protect *Physarum polycephalum* against Cd, though this is the case in *Eschericia coli*, mammals and insects (Chin & Sina 1978, Elinder & Piscator 1987, Mailman 1981, Pihlajamäki et al 1989, Rantataro et al. 1989, Reddy et al. 1987, Vogel et al. 1988, Yamoto et al. 1987). Zn possibly affords protection from some more dangerous factor by acting as a coenzyme or enzyme activator in detoxification systems (Migula & Kedziorsky 1986). The mechanisms of the accumulation and toleration of zink are also obscure.

The high levels of Cd are also difficult to understand. In studies of the toxicity of Cd to *P. polychephalum* effects on the biochemistry of the nucleus, membrane potential and survival time have been noted (Chin et al. 1973, Chin et al. 1978a, b, Chin & Sina 1978, Terayama et al. 1978). When do the toxic metals affect the slime molds in nature?

Though the number of samples was small the levels of Fe and Zn in *F. septica* proved to be statistically significantly higher in nature than in the museum samples (Fe, $p = 0.05$ and Zn, $p = 0.01$).

Analyses of museum samples (Table 1) show that high metal levels existed in slime molds before the environment was subjected to the recent pollution load.

Table 3. Scattered observations of the occurrence of metals in seven species of slime molds in different Finnish localities. The metal levels are given as mg/kg dwt = ppm of air-dry weight. (– = not measured because sample too small, 0 = the level of the metal was almost as low as in the similarly treated 0-standard). The values are for individual slime molds, except for *Amaurochaete atra* in Kokemäki, where three slime molds of this species were found in an area approximately 5 m^2 in size.

Area	Sampling time	Slime mold substrate	Metals in leaves of *Vaccinium myrtillus*						Metals in slime mold					
			Al	Fe	Zn	Cu	Cd	Hg	Al	Fe	Zn	Cu	Cd	Hg
Lycogala epidenrum L. (Fries)														
Espoo	7.8.1988	spruce stub	14	7.8	8.3	4.0	0.020	–	15	25	76	7.2	2.0	–
Espoo	14.8.1988	spruce stub	130	70	19	5.9	0.094	–	14	34	120	4.9	3.6	–
Espoo	14.8.1988	spruce stub	250	120	28	9.5	0.13	–	0	22	130	0	12	–
Juupajoki	8.9.1988	birch trunk	84	46	11	4.1	0.48	–	4.8	7.0	100	3.4	0.38	–
Juupajoki	12.9.1988	pine stub	240	62	16	8.7	0.50	–	45	23	62	3.3	0.64	–
Juupajoki	16.9.1988	pine stub	200	63	16	5.6	0.025	–	17	25	160	3.5	0.48	–
Juupajoki	17.9.1988	trunk in swamp	300	52	21	8.1	0.037	–	6.1	19	68	17	1.1	–
Symphytocarpus flaccidus (Morgan)														
Pyhäranta	20.7.1988	spruce stub	160	100	16	5.3	0.034	0.023						
		Aethalium A							11	32	270	14	11	0.035
		Aethalium B							32	65	200	8.6	7.7	0.032
		Plasmodium A							6.5	11	150	6.4	7.2	0.020
		Plasmodium A, after 3 h							11	20	170	8.3	9.7	0.10
Espoo	14.8.1988	pine stub	130	41	13	5.0	0.046	–	28	43	180	7.8	5.6	–
Juupajoki	8.9.1988	birch trunk	84	46	11	4.1	0.048	–	34	32	120	5.7	3.6	–
Tubifera ferruginosa (Batsch)														
Kokemäki	2.9.1988	spruce stub	240	82	15	10	0.12	–	44	57	210	26	1.2	–
Kokemäki	2.9.1988	moss carpet	200	46	49	4.3	0.38	–	9.3	13	150	19	1.6	–
Kokemäki	2.9.1988	moss carpet	120	77	12	8.4	0.38	–	99	96	570	19	4.6	–
Amaurochaete atra (Albert.& Schweinitz)														
Nummi–Pusula	15.7.1988	birch stub	110	4.2	20	6.0	0.080	–	75	33	240	8.4	3.6	–
Kokemäki	26.7.1988	pine stub	71	35	250	7.3	3.5	0.085	38	32	240	9.4	3.8	0.12
Ceratiomyxa fruticulosa (O.F. Müller)														
Espoo	7.8.1988	spruce stub	120	47	28	4.5	0.036	–	16	36	150	14	0.94	–
Stemonitis sp.														
Vantaa	21.8.1988	spruce stub	62	130	20	9.8	0.62	–	290	110	23	8.9	0.051	–
Plasmodia														
Espoo	7.8.1988	spruce stub	230	69	120	8.1	0.047	–	12	31	96	12	1.0	–
Vantaa	21.8.1988	spruce trunk	290	110	23	8.9	0.51	–	64	63	210	23	0.93	–

When the metal contents in the hypothallus and sporophore of *F. septica* were compared, hypothallus was found to contain more Zn (statistically significant, p = 0.01) than the spore bearing part. This raises the question whether Zn is necessary for the formation of the hypothallus or is secreted as a waste to the hypothallus in order to protect the sporophore and spores against damage by the metal.

The contents of Fe were also higher in the hypothallus the difference being statistically almost significant. The reason for this may be one of those suggested above.

When the Cd levels of the slime molds were compared with the Cd levels in lawn decomposing and mycorrhizal fungi (Kuusi et al. 1981) the slime molds, mostly collected from rural districts, were found to have higher Cd levels than the fungi collected from the rural districts, and approximately as high Cd contents as the fungi collected from urban areas.

Table 4. Comparison between metal levels (ppm of air-dry weight) in hypothallus and sporophore in *Fuligo septica* individuals.

	Al	Fe	Zn	Cu	Cd	Hg
10.7.1988 Kokemäki						
Hypothallus	26	480	28000	7.2	1.0	–
Sporophore	74	140	12000	8.8	11	0.015
10.7.1988 Kokemäki						
Hypothallus	70	570	18000	19	0.69	–
Sporophore	210	280	9700	19	0.63	0.034
26.7.1988 Kokemäki						
Hypothallus	190	480	24000	3.8	0.54	–
Sporophore	36	120	11000	6.4	1.5	0.023
26.7.1988 Kokemäki						
Hypothallus	130	250	27000	11	0.58	–
Sporophore	50	240	12000	9.3	1.2	–
20.7.1988 Espoo						
Hypothallus	39	120	31000	4.7	11	–
Sporophore	1.9	14	14000	5.3	1.1	0.013

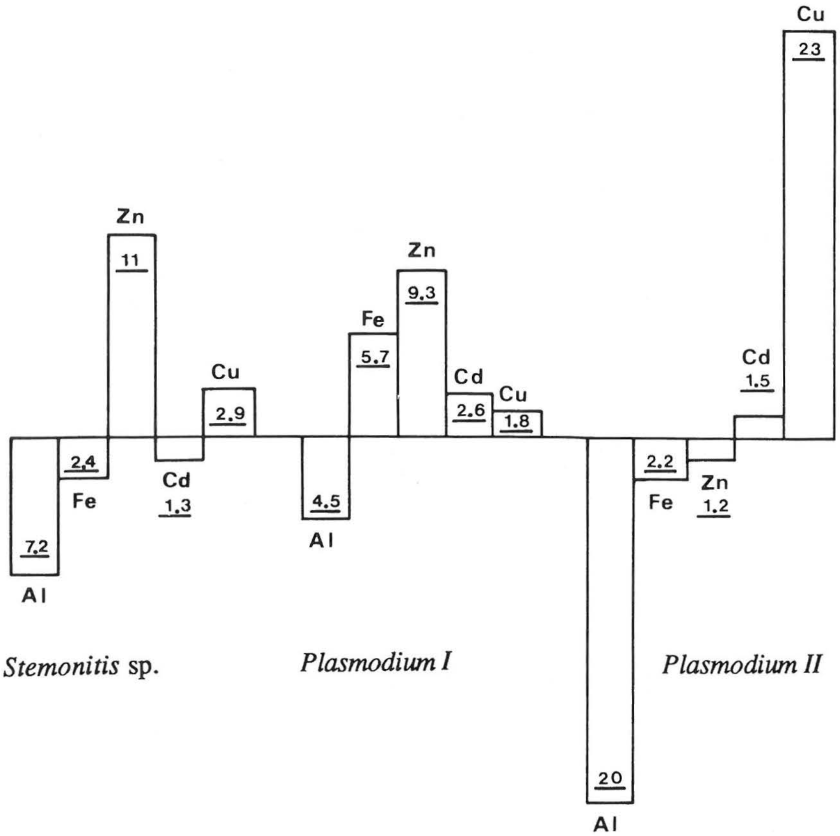
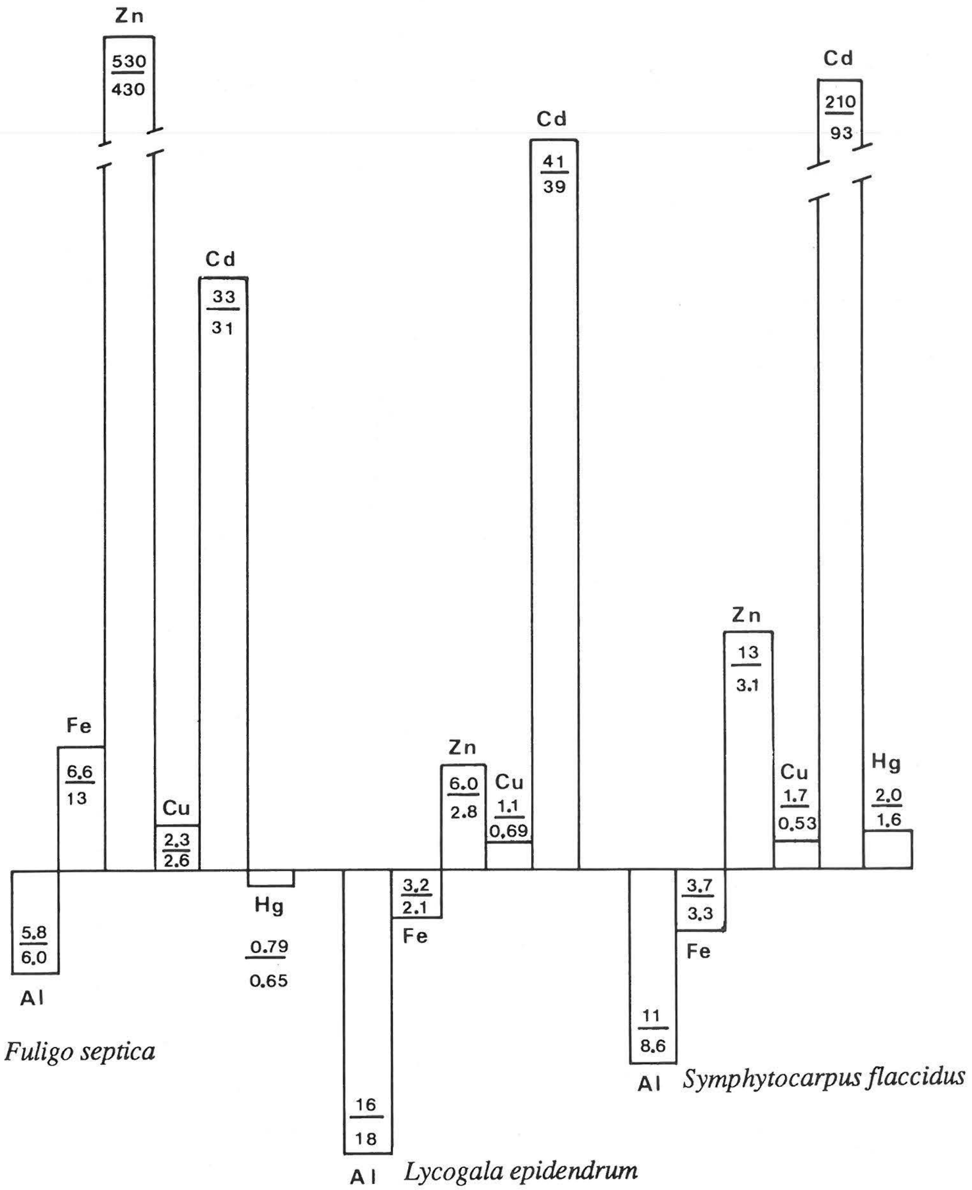


Fig. 1. See the next page for the explanation text.



Figs. 1–3. Metal contents in some Myxomycetes compared with levels in *Vaccinium myrtillus* leaves. The horizontal line represents the metal content in the reference plant, *Vaccinium myrtillus*. Above this line, the columns and numbers show the ratio of the metal content of the Myxomycete to the metal content of *V. myrtillus*. Below this line, the columns and numbers show the ratio of the metal content of *V. myrtillus* to the metal content of Myxomycete. The number underlined is the mean value of the ratio and the number under it is the mean deviation.

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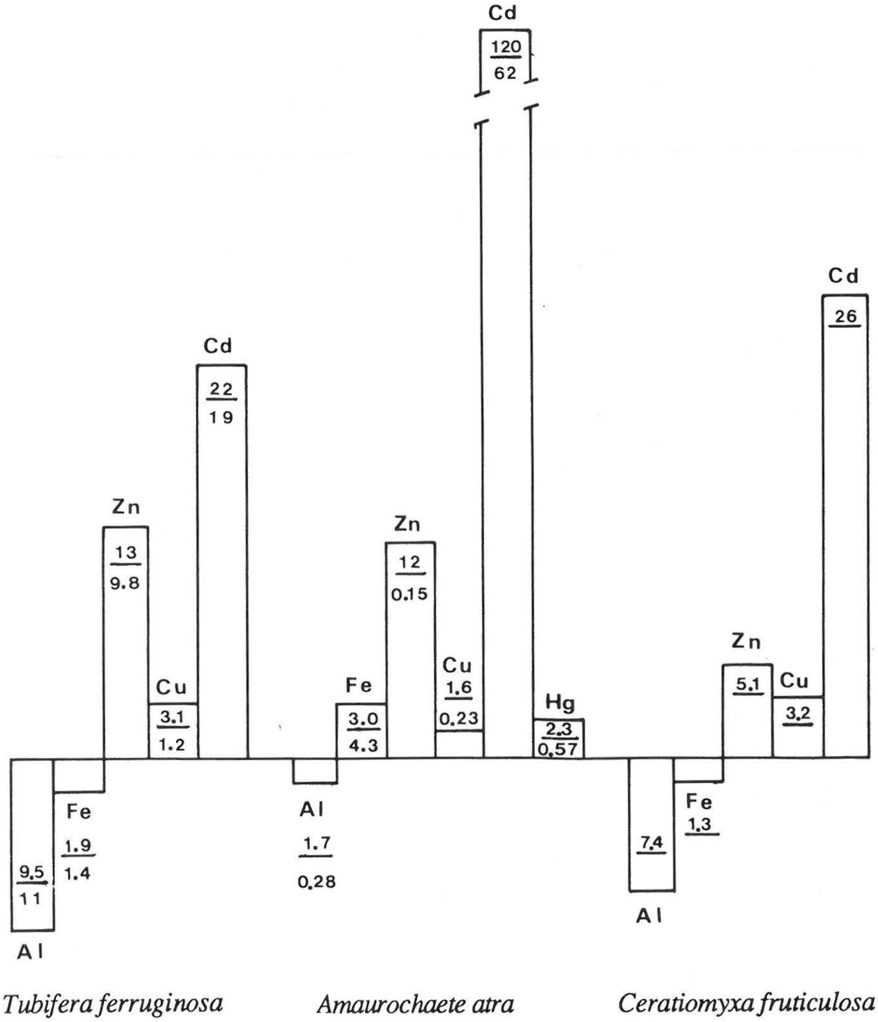

Figs. 1–3. See left for the explanation text.

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