A role for *Amanita Muscaria* L. in the circulation of cadmium and vanadium in a non-polluted woodland

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ABSTRACT

The sporophore of the fungus *Amanita muscaria* L. contains greatly elevated levels of cadmium (29.9 μg g^{-1} dwt) and vanadium (344.9 μg g^{-1} dwt) in comparison with the soil in a birch woodland (total (HNO_3-extractable Cd 0.4 μg g^{-1} dwt, V 11.7 μg g^{-1} dwt). The significance of this remarkable concentration of normally rare and dispersed elements in terms of their circulation in the woodland has been investigated. Both elements are released from sporophore tissue in a form which can be taken up by a test plant (lettuce), cultivated in the woodland soil amended with different quantities of sporophore tissue. Cadmium levels in all plant tissues were elevated in comparison to the non-amended controls; only root vanadium levels responded to the amendment of the soil. The results are discussed in terms of their significance for the natural cycling of both elements. It is calculated that an abundant population of sporophores could circulate 1.4% of the total cadmium and 0.65% of the total vanadium pool found in the litter layer and 0–5 cm soil horizon in the sampled woodland over a period of 14 days (mean life span of a sporophore).

INTRODUCTION

Sporophores of the basidiomycete fungus *Amanita muscaria* L. are well known to contain significant accumulations of several rare and dispersed soil-borne elements. Ter Meulen (1931) first indicated the property of this fungus to accumulate significant levels of vanadium from non-polluted soils. This observation has frequently been re-emphasised (Bertrand 1943, Byrne, Ravnik and Kosta 1976, Watkinson 1964, Tyler 1980). Vanadium is present as a unique organo-metallic complex, amavadin (Kneifel and Bayer 1973, Lancashire 1980). The sporophores also contain elevated levels of selenium (Allen and Steinnes 1978, Watkinson 1964), cadmium (Byrne, Ravnik and Kosta 1976) and bromine (Byrne, Ravnik and Kosta 1976). At present, there is no evidence to suggest that these latter elements are present in unique chemical forms.

The sporophores of *A. muscaria* are ephemeral structures, with an average life span of 14 days. They are produced at the end of the north temperate growing season. The accumulation of the aforementioned elements represents their powerful and regular biological focusing to potential toxic levels within an unpolluted ecosystem. It may be that the sporophores of this fungus represent a major stage in the natural biogeochemical cycles of the above elements. The investigation reported below has considered the possible role of the sporophores in the cycling of cadmium and vanadium in some components of an ecosystem containing a large colony of *Amanita muscaria*; remote from man-made emissions of both elements.

MATERIALS AND METHODS

Site

A large colony of *Amanita muscaria* was located in an area of Birch scrub (*Betula pendula*) established on a stabilised sand dune system at Formby Point, Merseyside, UK (NGR SD 277 084). Within this woodland, a representative area 30 × 30 m constituted the sampling site for soil and sporophore collection. The soil is a pararendzina (Hall and Folland 1967), a skeletal soil with no defined 'B' horizon, developed from a blown sand base.

Sampling

Sporophores of *A. muscaria* were collected in early October 1984. The sporophores varied in age and size, but old or damaged specimens were rejected. Specimens were excavated to include the volva at the base of the stipe, but not adjacent soil. Sporophores were not washed, but any adherent debris was removed manually prior to preparation for analysis.

Soil and litter were collected at random from various points within the representative area. At each point, the litter layer, 0–5, 5–10 and 10–20 cm soil depths were sampled, using a stainless steel trowel. All samples were pooled on return to the laboratory, and aliquots of the pooled soils were subsequently prepared for analysis.

Bulk soil samples were collected (0–5 cm layer) for use in plant uptake experiments. These were cleaned of major debris (plant roots and woody material) and stored moist prior to use.

Sample preparation

(a) *Sporophores:*
Fresh weight measurements were made on all sporophores. Each was individually labelled then placed in a labelled plastic petri dish and freeze dried for 14 days. After 7 days, specimens were turned to disturb air pockets which may have prevented drying. The freeze-dried sporophores were individually re-weighed, then all tissues were ground to a fine powder in a Cyclotech sample mill. The powdered sporophore material was pooled, then stored in a desiccator at room temperature until required.

(b) *Plant material:*
All plant material was washed in distilled water, then oven-dried to constant weight at 70°C prior to analysis.

(c) *Soils:*
Soil and litter samples were oven dried at 50°C to constant weight, then ground. Material which passed a 2 mm aperture sieve was used for analysis.

ANALYSIS

(a) *Sporophore and plant material*
A known dry weight of tissue was digested under pressure at 70°C using 71% Analar grade HNO_3 (Williams 1978). Sample digests were made up to constant volume, and analysed by flame atomic absorption spectrometry (AAS). Cd was measured with an air:C_2H_2 flame on an IL 151 AAS; V was measured with a C_2H_2:NO_2 flame on an IL 151 AAS. Samples were background corrected.

(b) *Soils*
Soil metal levels were obtained following a nitric acid digestion (MAFF 1973). A known weight of dried soil was digested with 71% Aristar grade HNO_3. This procedure identified 'total' soil metal levels. 'Plant-available metal' levels were obtained from a 0.5 M acetic acid extraction (MAFF 1973) of a known weight of dried soil.

Soil V content was measured by flame AAS, using conditions described in (a). Soil Cd determinations were made with a flameless technique on a Pye SP9 AAS, due to the very low levels encountered in the digests and extracts.

Detection limits for both elements were as follows: Cd flame AAS 0.02 μg ml^{-1}, flameless 0.002 μg ml^{-1}; V flame AAS 0.1 μg ml^{-1}.

Where appropriate, soil pH, organic matter content and % moisture were determined, following methods given in MAFF (1973) (organic matter and pH) and Allen (1974) (% moisture).

Statistical analysis of data was carried out using the 'Easy Stats' package on a DEC-20 computer.

RESULTS

1. *Soil analyses at woodland site*
Table 1 details soil pH, organic matter content and total/extractable Cd and V in the woodland soil. The soil can be seen to have a high organic matter content in the litter layer and 0–5 cm horizon, coupled with an acid pH. Total and extractable Cd and V in the soil are very low, indeed no HAC-extractable V was detected in any horizon and total soil V fell below detection limits in all samples collected below the 0–5 cm layer. The 'soil' below the 0–5 cm horizon was largely sand, reflecting the origins of the site.

Table 1 *Characteristics of the woodland soil.*

Parameter	*Litter*	*Soil Depth 0–5 cm*	*5–10 cm*	*10–20 cm*
pH	5.4	4.7	4.6	5.1
Organic matter (%)	35.3 ± 4.1	36.0 ± 3.5	5.3 ± 1.5	2.6 ± 0.3
Cadmium (μg g^{-1})				
Extractable	0.36 ± 0.04	0.23 ± 0.01	0.15 ± 0.03	0.11 ± 0.05
Total	0.38 ± 0.01	0.44 ± 0.01	0.11 ± 0.1	0.12 ± 0.01
Vanadium (μg g^{-1})				
Extractable	ND	ND	ND	ND
Total	6.67 ± 2.2	11.67 ± 6.5	ND	ND

ND – not detected.
Values represent means of 5 replicates ± SE.

2. *Analysis of* A. muscaria *sporophores*
Table 2 gives the mean fresh weight and dry weight of *A. muscaria* sporophores collected from the site, together with data on the Cd and V content of the sporophores. It is evident that fresh sporophore tissues contain >90% H_2O and elevated levels of both elements.

Table 2 *Fresh weight, dry weight and elemental content of* A. muscaria.

Fresh wt (g)	28.83 ± 19.1
Dry wt (g)	2.68 ± 1.35
Cadmium (μg g^{-1} dwt)	29.9 ± 5.6
Vanadium (μg g^{-1} dwt)	344.9 ± 145.2

Sporophore weights n = 29
Sporophore analyses n = 10
Values expressed ± SE

3. *Fate of Cd and V released from sporophore tissues*
The plant availability of both elements, when added to soil in sporophore tissue, was followed in a pot experiment. Soil collected from the 0–5 cm layer at the woodland site was used as the growing medium with the incorporation of known weights of dried and powdered sporophore tissue. Soil was placed in 12 cm plastic pots, and a known weight of sporophore tissue, representing either ½, 1 or 2 sporophores (based on mean dry weight values given in Table 2) was thoroughly incorporated into the upper 2 cm of the soil. The test plant used was lettuce (*Lactuca sativa* L. c.v Webbs Wonderful), not native to the site, but recognised as a known accumulator of soil-borne cadmium (John and Van Laerhoven 1976). There are no reports of vanadium uptake by lettuce. Ten pots were used for each sporophore incorporation, and a further ten pots containing unamended soil acted as controls. The surface of each pot was covered in polythene and 1 seedling (2 leaf stage) was planted through a slit made in this cover. The cover prevented foliar contamination from the upper amended soil layers, and also prevented excessive moisture loss from the very sandy soil. To compensate for the infertility of the soil, all plants received routine applications of a proprietary foliar plant feed during their growing period. Prior analysis showed that neither Cd nor V could be detected in the

dilute preparation as used for foliar feeding. Plants were grown in glasshouse conditions for 11 weeks, then harvested and analysed for Cd and V content.

Table 3 gives the calculated initial Cd and V content of the soil with an without the sporophore amendments at the outset of the experiment. Plant analyses from the treatment are given in Table 4.

Several points emerge from the results. Incorporation of sporophore material causes increased accumulation of Cd and V in the tissues of the test plants. However, the two elements show different patterns of accumulation and redistribution. Cadmium is readily taken up by the test plant, and shows considerable mobility. This is evident at all rates of incorporation. On the other hand, vanadium is much less readily absorbed from the sporophores, and elevated levels are only found in root tissues at the top rate of incorporation. In all cases, there was no detectable translocation of vanadium from the roots.

Lettuce plants grew slightly better in the amended soils, possibly due to the presence of other nutrients in the powdered sporophore. However, even when uptake is expressed on a μg/plant basis, there is still a significant accumulation of cadmium and vanadium as indicated above. Using the calculated total pot Cd and V contents, and pooling the root, stem and leaf contents to give μg element/plant, total % uptake of each element can be calculated in relation to each rate of sporophore amendment (Table 5) over the 11 week growing period.

Table 3 *(a) Calculated initial Cd and V content of amended pots prior to planting (μg/pot).*

Sporophore addition (g)	*Soil*		*Sporophore*		*Total*	
	Cd	*V*	*Cd*	*V*	*Cd*	*V*
O (control)	197.6	5239	–	–	197.6	5239
1.35 (½)	197.6	5239	40.5	465.7	238.1	5705
2.7 (1)	197.6	5239	81	931.5	278.6	6170
5.4 (2)	197.6	5239	162	1863	359.6	7102

Values in brackets represent number of sporophores. The addition represents Dry. wt. soil in 12 cm pot = 449.1 g.

(b) % contribution of sporophore metals to total pot metal pool.

	Rate of addition (g)		
	1.35	2.7	5.4
Cd	17	29.1	45.0
V	8.2	15.1	26.2

Table 4 *(a) Plant uptake of Cd and V in relation to rate of sporophore incorporation (n = 9).*

Sporophore incorporation (g)	*Vanadium (μg g^{-1} dwt)*			*Cadmium (μg g^{-} dwt)*		
	Root	*Stem*	*Leaf*	*Root*	*Stem*	*Leaf*
0	10.6 ± 1.3	ND	ND	2.1 ± 0.35	2.7 ± 0.12	2.7 ± 0.11
1.35	18.4 ± 1.6	ND	ND	4.5 ± 0.16	4.4 ± 0.5	7.2 ± 0.38
2.7	13.2 ± 0.6	ND	ND	4.5 ± 0.26	4.1 ± 0.3	8.9 ± 0.71
5.4	38.2 ± 2.7	ND	ND	5.5 ± 0.29	5.2 ± 0.29	8.8 ± 0.67

ND – Not detected. Values given ± SEs.

(b) Mean tissue dry weight in relation to sporophore incorporation.

Sporophore incorporation (g)	*Mean dry wt (g) (n = 9)*			
	Root	*Stem*	*Leaf*	*Whole plant*
0	0.5 ± 0.18	0.59 ± 1.4	0.97 ± 0.22	2.06 ± 0.5
1.35	1.56 ± 0.2	0.51 ± 0.08	1.49 ± 0.26	3.55 ± 0.5
2.7	1.00 ± 0.17	0.75 ± 0.1	1.75 ± 0.26	3.86 ± 0.2
5.4	1.01 ± 0.17	0.61 ± 0.04	2.04 ± 0.19	3.66 ± 0.3

Values given ± SEs.

Table 5 *Cadmium and vanadium uptake (μg/plant) as a % of total pot metal pool.*

Sporophore addition (g)	*Vanadium*			*Cadmium*		
	μg pot	*μg plant*	*% uptake*	*μg pot*	*μg plant*	*% uptake*
0	5239	7.3 ± 2.6	0.14	197.6	11.4 ± 2.2	5.8
1.35	5705	21.3 ± 5.0	0.37	238.1	17.3 ± 2.8	7.3
2.7	6170	11.2 ± 1.8	0.18	278.6	22.9 ± 4.0	8.2
5.4	7102	39.7 ± 8.7	0.56	359.6	26.1 ± 2.6	7.3

This further substantiates the conclusion that the two elements can be released from decomposing sporophores in a potentially plant-available form.

DISCUSSION

Several points emerge from the data presented above. Initially, the remarkable ability of the fungus to concentrate both elements well in excess of their total soil levels is clearly demonstrated. Furthermore, the data show that cadmium and vanadium released from the sporophore tissue is present in a plant-available form in the woodland soil. The uptake of both elements by the test plant, lettuce, reflected the increase of metal input from increased rates of sporophore incorporation. It is not unreasonable to conclude that *A. muscaria* sporophores can play a major role in the natural biogeochemical cycles of both elements. Table 6 gives a hypothetical illustration of the magnitude of the role of the *A. muscaria* sporophores in the circulation of the woodland soil Cd and V pools. Calculations are based on the metal content of the litter and 0–5 cm soil layer, as it is only in this region of the soil in which the fungal mycelium, the primary site of element acquisition, will

Table 6 *Comparative soil and sporophore metal contents (μg) in 1 m^2 of woodland soil to a depth of 5 cm.*

	Weight	*Cd*	*% Total Cd*	*V*	*% Total V*
Soil	38.25 kg	14100	98.6	351000	99.35
Sporophores	6.75 g	200	1.4	2300	0.65

flourish. A sporophore density of 2.5/m^2 was assumed, as this would reflect the abundance in a healthy, flourishing colony. Assuming that the sporophore has a life span of 14 days, in this time, the 2.5 sporophores will have absorbed and released 1.4% of the total soil Cd pool and 0.65% of the total soil V pool in the square metre of soil. Such a rate of nutrient turnover in a plant organ is surprising, but in the case of such rare and highly dispersed elements as cadmium and vanadium, the present postulation is quite remarkable.

What significance may these accumulations have for the circulation of these elements in the woodland? At first sight, the creation of localised sites of major metal accumulation due to sporophore growth and decay could have serious consequences. However, a number of factors may serve to modify any impact. Firstly, the sporophores are produced at the end of the growing season, at a time when other vegetation is in early stages of senescence, and rates of water and nutrient acquisition from the soil are reduced in comparison to other times in the growing season. Secondly, the elements may not be released in a plant-available form. The pot experiment did not include a series of no-plant control pots, and the temperature at which the sporophores decomposed (25°C) was elevated in comparison to the field temperatures at the time when natural decomposition would occur (10–12°C) (Savidge *et al.*, 1963). Both factors could influence the chemical form of the released elements. As the fungus can accumulate these elements with great efficiency, its mycelium may be able to compete successfully with plant roots for these elements. The mycelial network for each sporophore will be extensive, and may rapidly acquire soluble forms of the elements when they become available in the soil. Alternatively, extracellular secretions from the mycelium may serve to release bound forms of the elements from soil minerals or organic binding sites. Data which could support this has recently been produced by Denny and Wilkins (1987) showing zinc accumulation in cell walls and extra-hyphal polysaccharide slime of *Paxillus involutus*.

A further gap in the present picture is information in the movement of elements in the soil profile, when released from the sporophore tissue. Soil analysis shows accumulation of both elements in the litter and 0–5 cm soil layer, so it is possible that vanadium and cadmium released on sporophore decay accumulate in these layers. Alternatively, due to the low organic matter content of the soil below 5 cm, the loss of soluble forms of the elements from the soil profile may be very rapid. This requires investigation.

In common with other plant material, fungal sporophores are grazed by a variety of primary consumers. In the case of *A. muscaria*, field observations indicated grazing by slugs and by Dipteran larvae. Consuming such a metal rich food source forms a potential entry point for these elements into the food web of the woodland, although the lateness of sporophore appearance may affect the magnitude of the transfer. It is not known if the consumption of elevated cadmium and vanadium from this source has any impact on the physiology of these organisms, or on their life cycles.

A. muscaria forms a mycorrhizal association with birch trees. If the mycelium of the fungus is efficient at removing cadmium and vanadium from the soil, and transporting these elements to the sporophore, then clearly the possibility of their transfer from mycelium → birch tree cannot be ruled out. In the absence of analyses of the elemental content of mycrorrhizal and non-mycorrhizal birch trees, it is not possible to comment further. However, as the association between the fungus and the tree has evolved, the tree may have developed selective barriers to the uptake or accumulation of these elements. A comparison of cadmium and vanadium levels in birch woodlands with and without *A. muscaria* would be of value in this context.

In conclusion, it is apparent that the sporophores of *A. muscaria* act as powerful foci for cadmium and vanadium in natural, unpolluted ecosystems. Some aspects of the fate of these elements, when released from decomposing sporophore tissue, have been considered. It is evident that the fungus constitutes a major step in the natural biogeochemical cycles of both elements, but that there are several aspects of the proposed pathway which require further careful consideration. Further attention should also be focused on the biogeochemical role of other common fungi which are known to accumulate normally dispersed trace elements.

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