Bioaccumulation of selected heavy metals by the water fern, *Azolla filiculoides* Lam. in a wetland ecosystem affected by sewage, mine and industrial pollution

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Abstract

The bio-accumulation of the heavy metals, Fe, Cu, Ni, Pb, Zn, Mn and Cr by the water fern, *Azolla filiculoides* Lam. in a wetland ecosystem polluted by effluents from sewage works, mines and industries was investigated. Results showed that the different metals can be accumulated by the water fern at concentration levels not necessarily related to their actual concentrations in the aquatic environment, as measured in this case, in the bottom sediments.

Introduction

It is known that floating aquatic weeds can be used to remove toxic metals from polluted waters (Muramota and Oki, 1983; Abbasi and Nipaney, 1985; Jain *et al.*, 1989). According to the literature, at least two species of *Azolla*, namely *A. pinnata* R.Br. and *A. filiculoides* Lam. have been investigated elsewhere under laboratory and natural environmental conditions for their capacity to absorb certain heavy metals. Sarkar and Jana (1987) investigating the Hill activity of the chloroplasts of *A. pinnata* when exposed to various concentrations of the heavy metals Hg, As, Pb, Cu, Cd and Cr under laboratory conditions, showed the tolerance of this species towards certain concentrations of these heavy metals. Jain *et al.* (1989) who studied the experimental uptake rate of Fe and Cu by *A. pinnata* indicated that the presence of one metal in solution may affect the uptake rate of another metal ion present in the same solution. Mishra *et al.* (1987) showed that the mercury uptake by *A. pinnata* from a culture medium was both concentration and time-dependent and that in addition to Hg accumulation, *A. pinnata* showed a significant decline in growth with increase in concentration of and exposure period to the metal.

According to Ashton and Walmsley (1984), there are three species of *Azolla* present in southern Africa namely *A. pinnata* R.Br. var. *pinnata*, *A. nilotica* Decne. and *A. filiculoides* of which *A. pinnata* var. *pinnata* is the only indigenous species. *A. pinnata* was recorded from small sheltered pans in the Pongola floodplain, extending northwards into the coastal area of Mozambique (Wild 1961), and into Zambia, the Caprivi and northern Botswana. The latter authors also showed *A. nilotica* to be present in the lower Zambezi River, and the Mozambique coastal plain as well as in the Cahora Bassa dam on the Zambezi River where it was found in sheltered backwaters. The third species, *A. filiculoides* was, according to Ashton and Walmsley (1984), introduced from South America (Moore, 1969; Ashton, 1977; Ashton and Walmsley, 1984; Wells *et al.*, 1986) and has until recently been restricted in its distribution in southern Africa (Jacot Guillarmod, 1979). This water fern was originally recorded from the Oorlogspoort Stream (Oosthuizen and Walters, 1961; Jacot Guillarmod, 1979), in the vicinity of Colesberg (northern Cape) (Oosthuizen and Walters, 1961; Wild, 1961) and at Upington (Jacot Guillarmod, 1978) and the Hendrik Verwoerd Dam in the Orange River (Twyman and Ashton, 1972; Ashton, 1982; Ashton and Walmsley, 1984). However, *A. filiculoides* has been observed as recently as 1984 (De Wet, personal observations) to occur in small isolated patches in the Blesbok Spruit wetland ecosystem near Springs and as recently as October 1989 in the Vaal River near Standerton (De Wet and Bezuidenhout, personal observations). Now, five years later, this weed has invaded much of the previously open areas in this wetland, causing a major problem in the ecology of this particular ecosystem, especially so in the Marievale bird sanctuary (Fig. 1) which serves as an important breeding ground for as many as 280 aquatic and semi-aquatic species of water birds, including some endangered species listed in the South African Red Data Book (Brooke, 1984). Birds affected include the greater flamingo, *Phoenicopterus ruber*, the lesser flamingo, *Phoenicopterus minor* and the Goliath heron, *Ardea goliath* which have frequented this sanctuary during recent years (Maclean, 1985).

Although the uptake and mobility of Cu, Cd and U by the roots and shoots of *A. filiculoides* have been demonstrated in Israel by Sela *et al.* (1988), no research has yet been done in South Africa on the potential of this species to remove these and other heavy metals from the aquatic environment, especially under those eutrophic conditions currently existing in the Blesbok Spruit. The Blesbok Spruit, which traverses a major gold-mining and industrial area on the Witwatersrand (Fig. 1), has for the past eighty years received effluents and seepage waters from surrounding mines and industries as well as discharges of nutrient-rich treated sewage effluent (Fig. 1). These effluents contain variable concentrations of heavy metals such as Fe, Cu, Ni, Zn, Mn, Pb, Cr, Au and U (Viljoen, 1974; Wessels, 1974; Wittmann and Förstner, 1976 a to c; 1977 a to b; Henzen and Pieterse, 1978; Murray, 1987).

The present study deals with the results obtained on the bio-accumulation of the heavy metals Fe, Cu, Ni, Zn, Pb, Mn and Cr by *A. filiculoides* where it occurs at four localities in the Blesbok Spruit wetland ecosystem. A comparison is also made between the concentrations of these metals in *A. filiculoides* and in the sediments directly underneath the mats of this plant at the same localities.

The main reason why the comparison was made between the bio-accumulation of the metals by *Azolla* and their concentrations in the bottom sediments, and not with the metals in the water itself, was that the accumulated heavy metals in both *Azolla* and the sediments reflected the history of metal contamination over an

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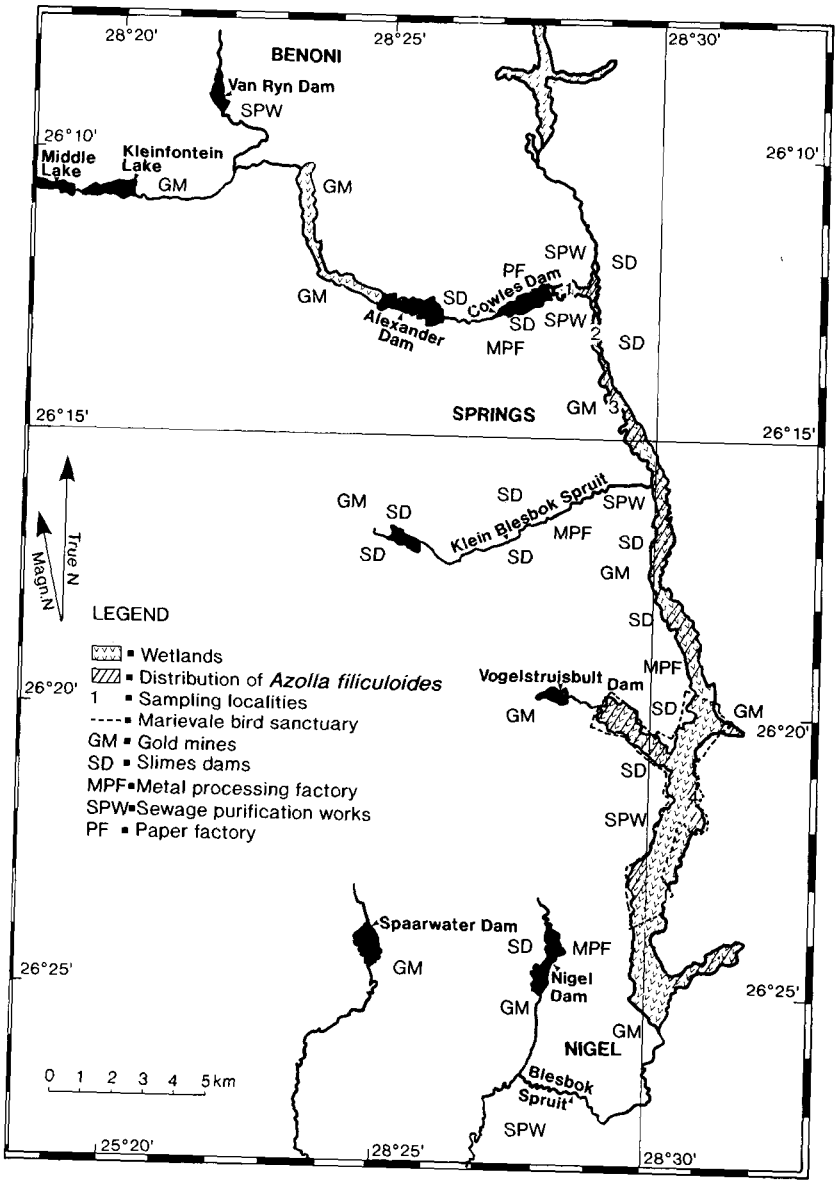

Figure 1
The Blesbok Spruit wetlands in the vicinity of Springs and Nigel, Transvaal, with an indication of the various potential sources of pollution as well as the four sampling localities

extended period of time more accurately until the day of sampling. The analysis of snap samples of water collected on the day of sampling on the other hand would only reflect the metal concentrations in the water at the specific moment of collection, be it high or low. It was not possible during the present investigation to obtain representative composite samples of the water at the four localities for metal analysis over an extended period of time.

Materials and methods

During the present investigation, four localities were chosen covering the most important possible sources of heavy metal pollution in the Blesbok Spruit wetland ecosystem where *A. filiculoides* also occurs. Water samples for limited chemical analyses were collected at each locality, and the temperature, pH and dissolved oxygen recorded during the collection of *A. filiculoides*. The samples were analysed in the laboratory for ammonia, nitrate, nitrite, phosphate, sulphate and turbidity using a Hach Model No. DR-EL/4 Direct Reading Engineer's Laboratory.

Sampling of *A. filiculoides* took place during June 1989 at the beginning of winter when active growth of the fern was still observed. This forms part of an ongoing program on the study of *A. filiculoides* in the Blesbok Spruit wetland ecosystem. For the determination of biomass, five random samples of *A. filiculoides* were taken at each locality using a 20x20 cm plastic frame. All *A. filiculoides* material within the frame was carefully collected. Excess moisture was removed using a paper towel before the wet mass of each sample was determined. The mean wet mass of *A. filiculoides* from each locality was then determined per m^2 surface area, after which the total estimated biomass of *A. filiculoides* was calculated in metric $t.ha^{-1}$. Another five subsamples of the water fern were also collected at each locality for the determination of heavy metal concentrations. Wet mass determinations of each sample were made. Samples were then individually dried in an oven at

90°C for 48 to 72 h. The dry mass of each sample was then determined.

Core samples of the upper 5 to 10 cm layer of the sediments were collected from the littoral zone of the wetland at each of the four different localities at the same time at which *A. filiculoides* was collected. These samples were also dried at 90°C for at least 48 to 72 h. Both the plant and sediment samples were separately homogenised. Using an electronic Sartorius Model No. R200D balance, approximately 1 g portions of each sample were then accurately weighed to the nearest mg. The samples were then digested according to standard procedures (Anderson, 1974; Agemian and Chu, 1976; Van Loon, 1980), by adding a 10 mℓ 1:1 concentrated perchloric and concentrated nitric acid mixture to the plant and sediment samples. The acid digestion was done in thoroughly cleansed metal-free 250 mℓ pyrex glass beakers covered with watch-glasses on a hotplate at a temperature of approximately 200 to 250°C.

The time of digestion lasted for at least 4 h during which period the total digestion and clearing of the sample occurred. Each digested sample was then separately filtered using a Millipore 6 μm paper filter and a vacuum pump. The filter was finally rinsed with distilled water to remove all traces of the dissolved heavy metals from the filter paper and the volume of each solution then made up to 100 mℓ in a volumetric flask. These solutions were individually transferred to clean glass storage bottles for later analyses of the different heavy metals.

To determine the organic content of the sediments collected at each locality, approximately 1 g of the homogenised dry material from each sample was accurately weighed and ashed in a Labcon muffle furnace Type RM4 at 550°C according to Nalewajko (1966).

A Varian atomic absorption spectrophotometer, Series No. 875, was used to measure the concentrations of the metals in each sample. During the atomic absorption analysis, use was made of Fe, Cu, Ni, Zn, Pb, Mn and Cr analytical standards. Values obtained, expressed as mg.ℓ^{-1}, were in turn recalculated to μg.g^{-1}, using the initial dry mass of each plant or sediment sample before digestion.

A bio-accumulation factor (BF) calculated for the various metals (expressed as %) was determined by using the formula:

$$(BF(\%) = \frac{\text{Concentration of the metal in } A.\ filiculoides\ (\mu g.g^{-1})}{\text{Concentration of the metal in the sediments } (\mu g.g^{-1})} \times 100$$

This formula corresponds to the Goldschmidt enrichment principle (Goldschmidt, 1937) as used by Hutchinson (1943) and Cannon (1960). The reason why the metal concentrations in the sediment were used, but not those in the water, has already been explained earlier.

Statistical evaluation of the results was made using Lotus 123 and Harvard Graphics software packages.

Results

Results on the selected physical and chemical parameters analysed for at the four localities (Fig. 1), are summarised in Table 1. Winter water temperatures, measured between 12:00 and 15:00, were found to be fairly constant, fluctuating between 12°C and 13°C. Although the water of the wetland was clearly affected by effluent and seepage waters from mines, as reflected by the mineral loads in the water at the various localities, pH values had recovered and were constantly above seven at all four localities, exceeding 8 at Locality 1. Values for dissolved oxygen varied between 4,7 mg.ℓ^{-1} (Locality 1) and 6,6 mg.ℓ^{-1} (Locality 3). The concentrations of ammonia, nitrate, nitrite and orthophosphate showed the effects of the treated sewage effluents discharged into the Blesbok Spruit wetlands upstream from Localities 1 and 2 and in the vicinity of Locality 4 (Fig. 1).

The effect of mine effluents on the water chemistry is reflected to some extent by the concentration of sulphates in the water which equalled or exceeded 300 mg.ℓ^{-1} at three of the four sampling localities. The relatively high turbidity value of 15 FTU at Locality 1 can be ascribed to organic fibre in the water originating from the effluent of a paper and pulp factory (Fig. 1).

The concentrations of the heavy metals Fe, Cu, Ni, Zn, Pb, Mn and Cr from the sediments at the four localities in the Blesbok Spruit wetlands (expressed as μg.g^{-1}) are listed in Table 2. According to these findings the sequence in concentrations of the metals in descending order was Fe > Zn > Mn > Ni > Cr > Cu > Pb. Extremely high values for iron occurred in the sediments at all four localities, varying between 7 528 μg.g^{-1} (Locality 4) and 59 598 μg.g^{-1} (Locality 1). These high concentrations are directly related to the pyrite (FeS_2) occurring in the unearthed gold-bearing ore and which, when exposed to atmospheric oxygen and moisture, results in the oxidation of the sulphidic components (S_2^{2-}) in pyrite to sulphate (SO_4^{2-}) as a result of which acidic H^+ and ionic Fe^{2+} (aq) are released into the aquatic environment (Harrison, 1958; Berner, 1970; Thompson, 1980; Förstner and Wittmann, 1981; Murray, 1987). The concentrations of Cu in the sediments were reasonably similar for all four localities, fluctuating between 89 μg.g^{-1} (Locality 3) and 97 μg.g^{-1} (Locality 4; Table 2). Although Ni exceeded 100 μg.g^{-1} in the sediments at all four localities, downstream there was a progressive increase of this metal in the sediments at the successive sampling localities, with a significantly higher concentration at Locality 4 (232 μg.g^{-1}). This can possibly be ascribed to effluents originating from a metal processing factory in the vicinity of this locality (Fig. 1). In contrast, values for Pb in the sediments, which fluctuated

TABLE 1
PHYSICAL AND CHEMICAL CONDITIONS IN THE BLESBOK SPRUIT WETLANDS AT FOUR DIFFERENT LOCALITIES DURING EARLY WINTER (JUNE 1989)

Physical and chemical parameters	Localities 1	2	3	4	$\bar{X}$ ± SD	
Temperature (°C)	13	13	12	12,5	12,6	0,4
pH	8,1	7,9	7,7	7,2	7,6**	0,3
Dissolved Oxygen *	4,7	5,4	6,6	6,3	5,8	0,8
Ammonia N *	3,7	3,0	2,8	6,2	3,9	1,4
Nitrate N *	2,6	2,2	1,8	1,8	2,1	0,4
Nitrate N *	0,15	0,17	0,08	0,06	0,12	0,05
Orthophosphate *	0,6	0,5	0,4	0,1	0,4	0,19
Sulphate *	240	300	300	335	293,8	34,2
Turbidity (FTU)	15	7	0,1	5	6,8	5,4
Iron *	0,02	0,02	0,02	0,03	0,02	0
Copper *	0	0,1	0,1	0,15	0,09	0,05
Manganese *	0,5	0,4	0,4	7,2	2,1	2,9
Chromium *	0,09	0,07	0,05	0,02	0,06	0,03

* Values in mg/ℓ
** Mean of -log [H^+]

TABLE 2

VALUES ($\mu g.g^{-1}$) OBTAINED FOR THE DIFFERENT HEAVY METALS IN *AZOLLA FILICULOIDES* AND THE BOTTOM SEDIMENTS WITH BIO-ACCUMULATION FACTOR (BF) VALUES AT THE FOUR DIFFERENT LOCALITIES IN THE BLESBOK SPRUIT WETLANDS

		Localities				
		1	2	3	4	
		$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
Iron ($\mu g.g^{-1}$)	*Azolla*	2 455 ± 68	7 906 ± 861	1 893 ± 115	4 710 ± 41	4 241 ±2 364
	Sediment	59 598	45 650	23 898	7 528	34 169 ±19 960
	BF (%)	4	17	8	63	23 ± 23
Copper ($\mu g.g^{-1}$)	*Azolla*	93 ± 4	67 ± 9	37 ± 1	60 ± 4	64 ± 20
	Sediment	91	94	89	97	93 ± 3
	BF (%)	102	72	42	62	70 ± 22
Nickel ($\mu g.g^{-1}$)	*Azolla*	80 ± 4	168 ± 15	189 ± 11	89 ± 6	131 ± 48
	Sediment	118	137	170	232	164 ± 43
	BF (%)	67	123	111	38	85 ± 26
Lead ($\mu g.g^{-1}$)	*Azolla*	120 ± 14	119 ± 14	121 ± 13	109 ± 0,3	117± 5
	Sediment	51	39	6	0	24 ± 21
	BF (%)	237	308	1 883	0	607 ± 745
Zinc ($\mu g.g^{-1}$)	*Azolla*	49 ± 2	109 ± 5	91 ± 11	120 ± 1	92 ± 27
	Sediment	66	376	415	613	368 ± 196
	BF (%)	74	29	22	20	36 ± 22
Manganese ($\mu g.g^{-1}$)	*Azolla*	3 746 ± 100	3 679 ± 257	5 535 ± 13	5 459 ± 32	4 607 ± 893
	Sediment	390	307	242	143	271 ± 90
	BF (%)	959	1199	2 287	3 814	2 065 ±1 127
Chromium ($\mu g.g^{-1}$)	*Azolla*	14 ± 1	21 ± 3	7 ± 0,3	4 ± 0,2	12 ± 7
	Sediment	129	128	98	94	112 ± 16
	BF (%)	11	17	7	4	10 ± 5
Sediment organic	content (%)	0,8	6,9	1,2	3,6	3,1 ± 2,4

considerably between the various sampling stations, showed an overall decline downstream in the sediments. A maximum of 51 $\mu g.g^{-1}$ was obtained for Pb at Locality 1, followed by 39 $\mu g.g^{-1}$ at Locality 2 and 6 $\mu g.g^{-1}$ at Locality 3. Pb concentrations in the sediment at Locality 4 were so low that they could not be measured. The concentration for Zn, in contrast with Pb, increased at the sampling localities downstream from Locality 1, reaching a maximum of 613 $\mu g.g^{-1}$ at Locality 4. The concentration of this metal at Locality 1 was also markedly lower (66 $\mu g.g^{-1}$) than those found at sampling Localities 2 to 4. As was the case for Ni, the increase of Zn at Locality 4, can perhaps also be ascribed to the effect of effluents from the above-mentioned metal-processing factory, but some contribution certainly originated from the surrounding mining areas, as Zn was used in the past in the gold extraction processes (Murray, 1987). The higher values for Zn at Localities 2 and 3 may have originated from the effluent of a sewage purification works which entered the wetlands upstream from these localities (Fig. 1). Values for Mn in die sediments showed a progressive decline from a maximum of 390 $\mu g.g^{-1}$ (Locality 1) to 143 $\mu g.g^{-1}$ (Locality 4; Table 2). Like Fe, Mn mainly originated from the ore of surrounding gold-mines (Murray, 1987). Cr concentrations were the highest in the sediments at Localities 1 and 2, and in all cases exceeded 128 $\mu g.g^{-1}$. Values declined to below 100 $\mu g.g^{-1}$ at sampling Stations 3 and 4.

Values obtained for the bio-accumulation of the different heavy metals by *A. filiculoides* at the four localities in the Blesbok Spruit wetlands are listed in Table 2. The bio-accumulation factor (BF) of Fe by *A. filiculoides* at the four localities showed variable levels, fluctuating between 4% at Locality 1 and 63% at Locality 4 (Table 2), with a mean value of 23% for all four stations. Even so, this metal, as with Mn, clearly occurred in the highest concentrations of all metals analysed for in *A. filiculoides* with a maximum of 7 906 $\mu g.g^{-1}$ at Locality 2. *A. filiculoides* showed an even better ability to accumulate Mn in its tissues with a BF fluctuating between 959% (Locality 1) and 3 814% (Locality 4). Of interest was the downstream increase in concentration of Mn in *A. filiculoides* with peaks at Localities 3 and 4, whereas the concentrations of this metal in the sediments gradually declined at the successive downstream sampling stations. The actual concentration of Cu in *A. filiculoides*, which varied between 37 $\mu g.g^{-1}$ (Locality 4) and 93 $\mu g.g^{-1}$ (Locality 1), showed a close relationship with the BF, varying between 102% (Locality 1) and 42% (Locality 3), respectively.

TABLE 3

ESTIMATED DENSITIES OF *AZOLLA FILICULOIDES* IN t.ha^{-1} EXPRESSED AS DRY MASS WITH AN INDICATION OF THE QUANTITIES OF THE DIFFERENT HEAVY METALS ACCUMULATED BY THIS PLANT AT THE FOUR LOCALITIES IN THE BLESBOK SPRUIT WETLANDS

			Metals in kg per ton dry mass (a) : per hectare (b)													
Localities	g.m^{-2} dry mass	t.ha^{-1} dry mass	Fe		Cu		Ni		Pb		Zn		Mn		Cr	
			a	b	a	b	a	b	a	b	a	b	a	b	a	b
1	655,85	6,56	2,45	16,10	0,09	0,61	0,08	0,52	0,12	0,79	0,05	0,32	3,75	24,57	0,01	0,09
2	77,00	0,77	7,91	6,09	0,07	0,05	0,17	0,13	0,12	0,09	0,11	0,08	3,68	2,83	0,02	0,02
3	224,90	2,25	1,89	4,26	0,04	0,08	0,19	0,42	0,12	0,27	0,09	0,21	5,54	12,45	0,007	0,02
4	968,10	9,68	4,71	45,60	0,06	0,58	0,09	0,86	0,11	1,06	0,12	1,16	5,46	52,85	0,004	0,04

The bio-accumulation of Ni by *A. filiculoides* exceeded the concentrations of this metal in the sediments in two cases (Localities 2 and 3). At Localities 1 and 4, however, the Ni bio-accumulation values of *A. filiculoides* were lower. The ability of *A. filiculoides* to accumulate Pb from the aquatic environment appeared to be very good, and exceeded in all cases the concentrations of this metal in the sediments (Table 2). A fairly consistent level of bio-accumulation was recorded at Localities 1 to 3 and even at Locality 4, where the concentration of Pb could not be measured in the sediments, comparatively high values were recorded in *A. filiculoides*, with the concentration of Pb in the tissues of *A. filiculoides* still exceeding 109 $\mu g.g^{-1}$. Although there was a tendency for *A. filiculoides* to bio-accumulate Zn in large quantities at the localities downstream from Locality 1, the accumulation of Zn, relative to the concentration of this metal in the sediments, gradually declined to 20% (Locality 4), compared to 71% at Locality 1. Cr occurred in the lowest concentrations of all seven metals in the tissues of *A. filiculoides*, varying between 4 $\mu g.g^{-1}$ (Locality 4) and 14 $\mu g.g^{-1}$ at Locality 1, despite appreciable concentrations recorded for this metal in the sediments (Table 2).

Discussion

Of the seven metals analysed for, Mn and Fe are the two metals with the highest bioconcentrations in *A. filiculoides* (Table 2) in terms of $\mu g.g^{-1}$ dry mass. Comparing the BF values, Mn (2 065 %) followed by Pb (607 %), Ni (85 %) and Cu (70 %) were the best absorbed by *A. filiculoides*, with the BF for Cr (10 %) being the lowest of all the metals.

Of the metals investigated in the present study Fe, Cu, Mn and Zn, which occurred in appreciable quantities in both the sediments and in *A. filiculoides*, are considered to be essential elements (Hutchinson, 1943; Cannon, 1960) for plant growth and metabolism. According to Goldschmidt (1937) and Brooks (1972), the extent of accumulation of specific heavy metals by terrestrial plants allows them to be classified into biogenic, intermediate and non-biogenic elements. The non-biogenic elements are also considered as ballast elements (Frey-Wyssling, 1935). These metals are accumulated without any known physiological requirements for them by plants. Biogenic metals, on the other hand, play important physiological roles in plants such as in protein synthesis (Zn) (Devlin, 1975), as enzyme activators (Cu and Mn) (Devlin, 1975; Ting, 1982), as metalloenzymes (Zn) (Brooks, 1972; Ting, 1982) and as prosthetic groups in respiratory enzymes (Fe) (Brooks, 1972). Elements with an enrichment coefficient (Goldschmidt, 1937) or BF greater than 10% are, with the exception of ballast elements, considered as biogenic, whilst those with coefficients less than 1% are considered as non-biogenic.

Judged by present results obtained on the BF by *A. filiculoides* of Cu, Ni, Pb and Zn, these metals clearly fall within the framework of biogenic metals as defined by Hutchinson (1943) and Cannon (1960) for terrestrial plants, with Fe being the best placed as an intermediate element, even although a relatively high BF value was recorded for Fe in *A. filiculoides* at Locality 4 (Table 2). The BF for Cr, on the other hand, fits in with the classification by the latter authors for this element as non-biogenic. From these results it is clear that, in terms of the bio-accumulation of the various metals, the aquatic weed *A. filiculoides* behaved in a way similar to that described for terrestrial plants (Hutchinson, 1943; Cannon, 1960).

Looking at the estimated concentrations of the heavy metals in the standing crop of *A. filiculoides* in the Blesbok Spruit wetland ecosystem (Table 3) and taking into consideration the massive growths and vegetative reproduction that this plant is capable of throughout the year, even during the winter, the total potential mass of metal that can be bio-accumulated from the aquatic environment by this plant may be considerably higher than the calculated firgures given in Table 3. It appears that *A. filiculoides*, having a comparatively short life cycle (Ashton, 1982; Ashton and Walmsley, 1976; Ashton, 1977), rapidly grows to maturity and eventually dies off, sinking to the bottom where the metals can be trapped in the sediments from where they may eventually be released again into the aquatic environment during the process of decay. Alternatively, *A. filiculoides* and the bio-accumulated metal loads can be washed downstream during floods and deposited in the sediments of streams and rivers great distances from where the metal loads originated. However, if an active cropping programme of *A. filiculoides* can take place throughout the year, substantial quantities of these metals concerned can effectively be removed with the plant material from the aquatic environment. Some of the metals can then be reclaimed (Tel-Or, undated report; Clark, 1984).

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