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Duckweed *Lemna minor* as a Tool for Testing Toxicity of Coal Residues and Polluted Sediments

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Abstract. Duckweed, *Lemna minor,* was used for testing single elements and leachates of coal ashes and sediments by expressing growth as surface coverage. The EC_{50} for the elements Cd, Cu, Zn, As(III), As(V), Se(IV), Se(VI), SeO_2 were 0.86, 2.2, 4.4, 8.4, 297, 21, 67, 37 μM, respectively. Leachates were tested of pulverized coal fuel ash (PFA), including "low NOx" ashes, coal gasification slag (CGS), and, as a reference, the polluted sediments of a canal. The concentrations of elements in leachates of "low NOx" PFA were higher than those in leachates of conventional PFA. The leaching of anions from PFA was more quickly than the cations. CGS showed an absolutely minimal element leaching. Comparison of the effects of conventional PFA with sediments from Rotterdam harbor, River Rhine, and the canal shows PFA to be the far less toxic one. The sediment samples from the canal demonstrated strong growth inhibition, probably due to high zinc concentrations originating from industrial activity.

Duckweed (*Lemna minor*) is used in water quality studies to monitor heavy metals and other aquatic pollutants, because duckweed, like other water plants, may selectively accumulate certain chemicals and may serve as biological monitors (Ray and White 1976; Werff v.d. and Pruyt 1982; Nasu *et al.* 1981, 1984). The possibilities of using water plants as monitoring species were investigated by Roulet (1975), Kenaga and Moolenaar (1979), Rowe *et al.* (1982), Wang (1984, 1986), and Adema and de Zwart (1984). As far as the performance of the test is concerned, both static and flowthrough systems are used (Eichorn von and Augsten 1969; Walbridge 1977; Bishop and Perry 1981).

In the present study the duckweed test is applied to coal residues and polluted sediments. With the construction of new coal burning power stations, the amount of coal waste products, *e.g.*, pulverized fuel ash (conventional and "low NOx" PFA), bottom ash, and coal gasification slag, will increase over the next few years. "Low NOx" PFA is formed at a lower temperature stage in the boiler due to the application of a new type of burners for reduction of NOx emission. As a whole the coal combustion can lead to surpluses of unused fly-ash and CGS. Questions are raised concerning the arrangements for dumping the coal residues regarding the release of heavy metals and other potentially toxic materials.

In The Netherlands, dumping in aquatic environments is out of the question, but large-scale inland deposition on recoverable sites is under study. An important element of the study is to assess the possible environmental impact in connection to various optional management strategies. So far, research at fly-ash basins has been focused on the accumulation of elements in water, sediment, and biota. It is also considered that the potential of duckweed to accumulate heavy metals could also be used to remove and/or relocate elements in the ecosystem (Guthrie and Cherry 1979a, 1979b; Clark 1981; White *et al.* 1986). This is feasable, because in existing fly-ash basins in the USA duckweed is one of the most common plants having been the subject of research (Rodgers 1978). The toxicity of leachates of CGS on *Daphnia,* algae and duckweed was studied by Cushman and Brown (1981) and Klaine (1985). Specific tests for determining the toxicity of PFA leachates were conducted by Epler (1980). Others have carried out research for application of duckweed in toxicity tests of industrial effluents and their conclusion was that bioassays with duckweed are both sensitive and comparable with methods using *Daphnia* or fish (Wang 1986, 1990; Wang and Williams 1990; Taraldsen and Norberg-King 1990).

The main objective of the present study was to obtain insight into the accumulation and toxicity of leached elements. In a previous paper (Jenner and Janssen-Mommen, 1989) we examined phytotoxicity in experiments involving PFA leaching with acetic acid, as prescribed in USEPA (1980), as well as the use of a standard, artificial leachate. The EPA method for leaching resulted in effects attributed to the acetic acid itself rather than to the metals in the leachate. In the study presented here, special attention was paid to "low NOx" ash and to coal gasification slag (CGS) and a number of methodological aspects between the effect of repeated leaching on element concentrations was investigated. Additional monitoring experiments with sediments from a known polluted canal form a case study, providing evidence to support the establishment of a better system for judging the ecotoxicity of coal residues.

Materials and Methods

Lemna minor (duckweed) was obtained from the Department of Plant Physiology of the Wageningen Agricultural University in The Nether-

Table 1. Gorham medium (1950) modified by Rombach (1976)

—$Ca(NO_3)_2 \cdot 4H_2O$	0.5	g
—$MgSO_4 \cdot 7H_2O$	0.25	g
—KH_2PO_4	0.136	g
—H_3BO_3	2.86	mg
—$MnCl_2 \cdot 4H_2O$	1.81	mg
—$ZnSO_4 \cdot 7H_2O$	0.22	mg
—$(NH_4)Mo_7O_{24} \cdot 4H_2O$	0.18	mg
—$CuSO_4 \cdot 5H_2O$	0.07	mg
—$Co(NO_3)_2 \cdot 6H_2O$	0.08	mg
—NH_4VO_3	0.01	mg
—Fe-EDTA solution[a]	5	ml
—Milli-Q water to make up to	1000	ml

[a] Fe-EDTA solution consists of: 934 mg $FeCl_3 \cdot H_2O$ and 800 mg Na_2-EDTA (Titriplex III) in 1 L Milli-Q water. The solution is aerated for several hours to accomplish complete oxidation

lands. Duckweed was disinfected by immersing the fronds in 70% ethanol, followed by 1% NaOCl and rinsing with sterilised water (Bowker, 1980). The sterilized (120°C; 20 min) growth medium used in the experiments was a medium modified by Rombach (1976) according to Gorham (1950), see Table 1. Instead of 10 μM EDTA as applied originally in the "Gorham" medium was reduced to a concentration of 2.5 μM, since preliminary experiments demonstrated that 10 μM EDTA caused growth decline. The pH value of the medium is 5.0 ± 0.1. For the culture of *L. minor* 200 ml of the medium is transferred to high petri dishes (ϕ 9.5 cm, height 5 cm). The stock culture was kept in glass petri dishes, which was sterilized at 150°C for 2 h. The stock culture was maintained sterile and transferred to fresh medium every 2 wk. All glassware and the disposable high petri dishes, used for the experiments, were cleaned in approx. 0.1 N HNO_3 for 24 h and were rinsed with demineralized water (Milli-Q). The experiments were performed in triplicate, each experiment was inoculated with 10 fronds, and lasted for 2 wk. All incubations were performed in a laminar flow chamber.

For the aseptic culture of *L. minor* a specially designed cabinet was used. In the cabinet the illumination was provided by cool white fluorescent lamps (16 h light: 8 h dark) with a light intensity of 80 $\mu mol/m^2/s$ at water level. Inside the cabinet the temperature is measured at two locations, recorded continuously, and maintained at 23 ± 1°C. The cabinet itself is placed in a room climatized at 18°C.

The conventional PFA originated from KEMA batch A8850 (Amer power station); the "low NOx" ash was sampled at the Borssele power station. The coal gasification slag (CGS) was "Drayton slag" produced according to the Shell process at a plant in Houston, USA. The "low NOx" PFA was divided into a course fraction and a fine fraction by a wind shifter (Heyd Sichter D1,2, type DNC, Germany). The composition of the fine fraction is 90% finer than 24 μm, and the course fraction is 90% finer than 146 μm.

The sediment samples from the Apeldoornsch Kanaal were taken using a "Van Veen" sediment sampler. Samples were taken from three locations: a control site (outlet of a brook into the canal) called the "Veldhuizer spreng"; a pigment factory near the city of Apeldoorn; and a electroplating plant in the town of Dieren. One sample was taken from the control site and three were taken from each of the other locations. The samples were dried at 105°C and heated to establish the organic weight loss (at 600°C). The organic content of the control was 0.9%; Apeldoorn 20.3%; Dieren 9.1%.

The coal residues and sediments were leached, according to the extraction procedure (EP) described by USEPA (1980), but nitric acid instead of acetic acid was used due to the toxic action on *L. minor* of the latter. The conventional PFA was leached three times in succession. The "low NOx" and CGS was leached at three different L/S ratios (10, 20, and 40) at pHs 5, 7, and 9 in two separate successive leaching experiments.

Of the coal residue/sediment samples 100 g was added to 1,600 ml (16 × weight) of demineralized water. The sample was stirred for 24 h at ambient temperature while the pH was kept constant at 5, 7, and 9 with 0.5 N HNO_3/NaOH. After stirring and checking the pH, demineralized water was added to make up the final volume of the sample to 2,000 ml (L/S 20) in correspondence to the formula:

$$V = 20(W) - 16(W) - A$$

V = volume demineralized water added (ml); W = weight of the solids (g); A = volume added 0.5 N HNO_3/NaOH (ml)

For the purpose of studying the influence of pH and solid-liquid ratio on the susceptibility to leaching of elements in the waste products, the concentrations were converted to leached percentages, *i.e.*, the fraction of the total amount of the given element present in the solid material that had been leached out. The leached percentage was calculated as follows:

$$\frac{(L/S) \times \text{conc. in leachate } (\mu g\ L^{-1})}{\text{conc. sediment } (mg\ kg^{-1} \text{ dry matter})} = \%\ \text{leached}$$

For the toxicity tests with *L. minor*, the leachate was diluted with Gorham's medium containing 2.5 μM EDTA, pH 5, with leachate concentrations in the range 0–100%. The number of fronds was enumerated twice a week, viz., on days 4, 7, 11, and 14. A distinction was made between fully grown (1), near-fully grown (3/4), half-grown (1/2), and newly formed (1/4) fronds. The multiplication rate (MR) of *L. minor* was calculated for various concentrations of metal additions. The multiplication rate is a measure for the rate of increase in the number of fronds (Rombach 1976). $MR = 1000(\log n_1 - \log n_0)/t$; $t = t_1 - t_0$ (days); n_1 = number of fronds on day t_1; n_0 = number of fronds on day t_0. Growth was also measured and expressed as a percentage of total surface covering of the petri dish (= 100%), using image processing (PC Vision Plus framegrabber) and software package TIM (Difa Measuring Systems BV, Breda, The Netherlands). The surface coverage parameter can be established rapidly and easily, and this parameter has a much greater resolution than can be achieved by the conventional way of counting the number of fronds. In experiments with the leachates, the counting of fronds is therefore omitted. The effects on growth are expressed as the percent difference of the leaf area coverage between the exposed culture and the control. At the end of each experiment, fresh weight by fast but careful blotting, and dry weight (60°C for 24 h) were determined. Concentrations of elements in the duckweed were determined by standard methods. Dried duckweed was digested in a pressure bomb with a Teflon liner (Berghof, Germany) with 1:1 diluted nitric acid. Depending on concentrations in the samples, elements in PFA, CGS, sediments, growth medium, leachate, and duckweed were measured either by means of atomic absorption spectrophotometry (AAS) or by inductively coupled plasma (ICP). The element concentration is exposed in μg/L for the solutions and in μg/g dryweight for sediments and in μg/g fresh weight for duckweed samples. With these data the concentration factor (CF) in the duckweed was calculated (CF = concentration duckweed/concentration "initial" medium).

Results

Element Composition in the Coal Ashes

The concentrations of most of the analyzed micro-elements in the fine fraction of the "low NOx" PFA were comparable with those in conventional PFA (Table 2). However, the As, Cr, and Ni concentrations in the fine fraction were lower, while that of Pb was higher. In the coarse fraction of the "low NOx" PFA, all analyzed micro-element concentrations were about twice as

Table 2. Element concentrations in coal ashes (dry wt).
— = not measured

		“low NOx” PFA		
Element	Conventional PFA	Fine	Coarse	Coal gasific. slag
Macro-elements (%)				
Si	25.6	—	—	15.8
Al	15.2	—	—	10.5
Fe	4.8	—	—	7.3
Ca	1.29	—	—	23.2
Mg	0.56	—	—	0.48
Na	0.32	—	—	0.06
K	1.94	—	—	0.10
Ti	0.91	—	—	0.79
P	0.18	—	—	0.23
S	—	—	—	1.30
C	5.1	—	—	0.58
Micro-elements (μg/g)				
As	42	14	<10	21.6
B	170	187	105	143
Cr	150	71	37	216
Cu	156	139	69	65
Mo	25	28.0	9.8	<3
Ni	142	80	35	190
Pb	88	255	62	<3
Se	17	24.8	13.5	5.4
Zn	120	128	26	25

low as those in the fine fraction. In the CGS the concentrations of most of the macro-elements were lower than in the conventional fly-ash, but the calcium concentration in particular was appreciably higher. The micro-element concentrations in CGS were in general also lower than in conventional PFA, except for chromium and nickel.

Leaching Behavior of Elements

Conventional PFA: When the PFA was subjected to repeated leaching (three times), the element concentrations in the first leachate (I) proved to be the highest, dropping rapidly in the second and third leachates (II and III) (see Table 3).

The leaching percentages of the anionic elements are clearly higher than those of the cationic elements. B mainly leached out in the first step. The total cumulative leaching percentages given in Table 3 show that at the final liquid-soil ratio of 60 (3 times 20), 38% of Se was leached out. The percentages for As and Mo amounts to 21% and 22%, respectively.

To summarize, the final leached element concentrations after the three steps vary between 10% and 40% for the anionic elements and are less than 1% for the cationic elements.

"Low NOx" Ashes and Coal Gasification Slag: In previous leaching experiments, with conventional PFA, pH was kept at pH 5, although, as is well known, the behavior of elements in PFA changes at higher pH values. Especially As and Se are leaching in higher concentrations at higher pH values. The pH of the PFA produced in The Netherlands is normally between 9 and 11. The element concentrations in leachates of "low NOx" PFA and CGS at pHs 5, 7, and 9, of two successive leaching procedures were performed, at solid-liquid ratios of 10, 20, and 40. The pH was kept at the above-mentioned values during the leaching procedure (Table 4).

With the "low NOx" PFA, the leached percentages of B calculated were over 100%, which, of course, should not be possible. However, the composition of PFA is not homogeneous, resulting in deviations in the calculations for element distribution in the solid material.

The anionic elements in the "low NOx" PFA leached out very well. After the first leaching, the leached percentages of B were between 50% and 100%, of Se and Mo 30–60% and 20–50%, respectively. The leached percentages of arsenic were lower (2–35%). The second leaching resulted in a further 10–20% leaching of B and Se, 5–15% of Mo, and 5–10% of As. The leaching of the cationic elements (Cu, Ni, Pb, and Zn) is generally in the order of a few percent. Only when the coarse fraction was leached at pH 5, with liquid-solid ratios of 20 and 40, really high leached percentages for Cu were measured. Such values not being credible, Cu contamination has been suspected. Cr can leach out either as a cation or as an anion, and its leached percentage is comparable with the cationic elements.

A comparison of the leaching of elements from the fine fraction of the "low NOx" PFA with the leaching of elements from the coarse fraction shows that at a pH of 5, the anionic elements (As, B, Mo, and Se) leach out better from the fine fraction. There is little difference in the leaching of Mo from the two fractions at pHs 7 and 9. Less of the Se was leached from the fine fraction at pH 7, and less of the B was leached from the fine fraction at pH 7 and pH 9. Differences in the leaching of the cationic elements from the two fractions were limited, the only difference being that Cu and Zn appeared to leach out better from the coarse fraction at pH 5. One must bear in mind, however, that these observations are not credible, especially where Cu is concerned.

The leaching of the elements from the CGS was low compared with the leaching from the "low NOx" PFA. Leaching of the anionic elements As, Mo, and Se was not demonstrable, and leaching of boron was in most cases only $\leqslant 1\%$. Similarly, almost no leaching of cationic elements was detectable, except for Ni and Zn, the leaching of which was comparable with their leaching from "low NOx" PFA.

Toxicity Tests with Lemna minor

Single Elements: An assessment was made of the toxic effects on growth of the cations Cd, Cu, and Zn and the anions As and Se to duckweed. The parameter surface coverage showed to be far more sensitive (EC_{50}) by a factor 15–20 for Cd and Zn than the parameter MR (Table 5). For the NOEC values no difference can be demonstrated between the two parameters for the cations. With the anions the values differ up to a factor 10, whereby the parameter surface coverage also turns out to be far more sensitive. From this point onwards it was decided only to use surface coverage as a parameter.

The EC_{50} values show a clear difference in toxicity between the cations and the anions (Table 5). On the basis of their toxicity the elements analyzed can be subdivided into three groups of toxicity:

—the cations Cd, Cu, and Zn with $EC_{50} < 0.5$ mg/L
—the anions As (III) and Se (IV) with EC_{50} 0.5–5 mg/L
—the anions As (V) and Se (VI) with $EC_{50} > 5$ mg/L

Table 3. Element concentrations in the leachates of conventional PFA after three successive leaching procedures using HNO_3. The last group of columns shows the leached percentage

	Conc. in leachate (μg/L)			Percentage leached (%)			
Element	I	II	III	I	II	III	Total
As	266	118	52	12.7	5.6	2.5	21
B	834	54	<15	9.8	0.6	<0.2	10.5
Cr	35	3.8	1.3	0.46	0.05	0.02	0.5
Cu	5.4	2.8	2.4	0.07	0.04	0.03	0.1
Fe	3	2.1	<2	<0.01	<0.01	<0.01	<0.01
Mo	181	77	<50	14.5	6.2	<4	22
Ni	70	9.5	5.8	0.99	0.13	0.08	1.2
Sb	31.2	9.9	6	4.8	1.5	0.9	7.2
Se	222	73	31	26.1	8.6	3.7	38

Table 4. Percentages of elements leached from "low NOx" PFA and CGS at different pH values and L/S ratios in two separated successive leaching experiments. (— = below detection level. All the values obtained for lead were beneath detection limits, so this element has been omitted from the table)

		Fine fraction						Coarse fraction						CGS					
		L/S 10		L/S 20		L/S 40		L/S 10		L/S 20		L/S 40		L/S 10		L/S 20		L/S 40	
Element[a]	pH	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
B	5	115	18	106	15	124	12	99	16	108	12	112	10	1.1	1.5	0.6	0.3	1.1	0.8
(1) —	7	73	18	74	12	89	16	87	17	94	11	102	13	0.5	0.4	—	—	0.7	—
(2) <20	9	45	11	45	14	52	11	63	17	66	14	78	13	0.4	0.3	—	—	—	—
Cu	5	0.3	0.7	14	43	5.3	23	2.4	3.6	113	128	90	162	—	—	—	—	—	1.9
(1) —	7	—	—	0.1	0.2	—	—	0.1	0.1	1.2	0.6	0.3	0.1	—	—	—	—	—	—
(2) <2	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.1	—
As	5	33	6.1	32	4.7	36.3	4.5	—	—	—	—	—	—	—	—	—	—	—	—
(1) <10	7	9.3	—	8.5	5.7	19	6.4	—	—	—	—	—	—	—	—	—	—	—	—
(2) <3	9	1.7	2.9	3.6	5.7	8.7	11	—	—	—	—	—	—	—	—	—	—	—	—
Cr	5	0.1	—	0.1	—	0.2	—	0.1	—	—	—	0.1	0.1	—	—	—	—	—	—
(1) —	7	2.2	0.4	1.5	0.1	2.7	0.4	1.0	0.1	0.2	—	1.2	0.2	—	—	—	—	—	—
(2) 0.7	9	2.0	0.5	2.1	0.6	2.6	0.7	1.0	0.3	1.0	0.3	1.4	0.4	—	—	—	—	—	—
Mo	5	30	10	51	13	47	11	16	2.6	22	3.4	16	4.9	—	—	—	—	—	—
(1) <3	7	43	15	59	11	49	11	40	11	53	8.0	45	8.0	—	—	—	—	—	—
(2) <2.5	9	35	9.3	37	13	37	8.6	38	9.0	38	7.8	42	9.0	—	—	—	—	—	—
Ni	5	2.7	0.7	4.1	1.2	2.5	0.7	2.2	0.5	2.4	1.1	3.1	1.6	2.7	5.0	1.8	1.2	2.9	3.8
(1) —	7	0.6	0.1	0.9	0.3	1.0	0.1	0.9	0.2	1.1	0.2	0.9	—	0.2	0.3	0.3	0.2	0.8	0.6
(2) <3	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Se	5	57	13	45	16	57	14	27	3.8	—	—	38	9.2	—	—	—	—	—	—
(1) —	7	30	12	33	18	53	15	46	17	58	16	75	20	—	—	—	—	—	—
(2) <4	9	35	13	48	13	58	14	31	13	45	17	53	13	—	—	—	—	7.7	—
Zn	5	1.3	0.9	2.3	2.1	6.4	3.9	4.9	2.3	15	26	15	11	2.4	6.6	1.9	2.7	—	6.2
(1) —	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(2) <15	9	0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] (1) Detection level solid (μg g^{-1} dry wt)
(2) Detection level liquid (μg L^{-1})

The NOEC values of the cations are also considerably lower than those of the anions, with the exception of arsenite and selenite.

The data on the accumulation of metals by duckweed and the corresponding concentration factors are also presented in Table 5. It turns out that, especially for Cu and Se, low concentrations in the duckweed are toxic (approx. 20–50 μg/g), followed by Cd, As (III), and finally Zn and As (V), with concentrations ranging from 200 to 300 μg/g. As with the EC_{50} and NOEC values, there is a clear difference in concentration factor (CF) between the cations and the anions, with the exception of arsenite. The CF for the cations and the anion arsenite is many times greater than that of the remaining anions (Table 6).

Conventional PFA and "Low NOx" PFA: In the case of the conventional PFA, only the leachate from the first leaching

Table 5. EC_{50} and NOEC values (expressed as mg/L) of the elements studied in both surface covering and multiplication rate and the accumulated concentration of elements (µg/g fresh weight) at the EC_{50} (surface covering) with the corresponding concentration factor (CF)

Element	Surface coverage EC_{50}	Surface coverage NOEC	Accumulation (µg/g)	CF	Multiplication rate EC_{50}	Multiplication rate NOEC
Cd	0.1	<0.06	88	816	1.6	<0.06
Cu	0.14	0.06	25	194	0.32	0.06
Zn	0.29	0.16	296	825	5.6	0.16
As (III)	0.63	<0.75	140	±210	1.6	<0.75
As (V)	22.2	<4	214	9	86	37
Se (IV)	1.7	0.08	38	21	3.5	0.8
Se (VI)	±5.3	0.8	±45	±8	±11.5	>2.4
SeO_2	2.9	0.8	46	15	8.6	0.8

Table 6. Leachate percentage associated with 50% growth reduction (EC_{50}) in duckweed, and the associated element concentrations in the "low NOx" leachates. These concentrations are compared with the EC_{50} and NOEC figures obtained in tests carried out using the individual elements. (10–1 = L/S 10, first leaching)

Leachate L/S	EC_{50} % leachate	Element concentrations in leachate at EC_{50} (µg L^{-1})									Visual effects
		As	B	Cu	Cr	Mo	Ni	Pb	Se	Zn	
Fine											
10–1	36.0	167	8060	24.9	1.2	370	78	<1	506	92	a
10–2	72.4	62	2580	73.1	<0.5	233	41	<2	239	94	a/b
20–1	15.5	35	1960	168	0.3	201	25	<1	86	65	b
20–2	5.9	2	550	192	<0.1	111	3	<1	12	55	b
40–1	51.5	6.5	3200	103	1.9	52	25	<2	179	130	a/b
40–2	17.2	2.7	510	154	<0.2	101	2.3	<1	15	63	b
Coarse											
10–1	53.2	3.9	5770	95	1.3	134	41	<1	197	92	a
10–2	38.2	<2	950	106	<0.3	75	7	<1	20	54	a/b
20–1	5.9	0.2	800	246	<0.1	106	2.5	<1	0.3	58	b
20–2	5.5	<1	510	260	<0.1	101	1.1	<1	3.3	66	b
40–1	8.0	<1	700	141	<0.1	101	2.2	<1	10	54	b
40–2	5.8	<1	490	179	<0.1	101	0.8	<1	1.8	51	b
EC_{50}	630	8400	140	—	8700	450*	—	1650	290		
NOEC	—	540	65	—	—	45*	—	>80	165		

* = Wang 1986

[a] Fronds smaller and lighter in colour, necrosis (effect presumably caused by B)

[b] Losing of fronds, edges of and eventually whole fronds turn yellowish brown (effect presumably caused by Cu)

(step I) was demonstrated to have a toxic effect on growth of *L. minor*. The 75% leachate was associated with the EC_{50} (Figure 2).

The toxic effects of the "low NOx" PFA on *L. minor* were tested using the leachates obtained at pH 5 with liquid-solid ratios of 10, 20, and 40, respectively (Figure 1). The element concentrations in the leachates obtained at pH 5 were generally higher than those obtained at pH 7 and 9, which implies that the toxicity tests may be regarded as "worst case" testing. The toxicity of the leachates obtained from the CGS was not tested because of the low element concentrations in the leachates. Only Ni may be expected to have toxic effects.

An erratic relationship appeared to exist between the toxicity of the leachates of the fine fraction and the liquid-solid ratio (Table 6). The growth reduction of the duckweed was greatest with the leachate obtained using a liquid-solid ratio of 20. The tests using leachates from the coarse fraction did seem to reveal a tendency for the toxicity to be greater at higher liquid-solid ratios, but again the growth reduction was greatest with the leachate obtained at a liquid-solid ratio of 20 (Figure 1).

The effects of the leachates from both fine and coarse fraction increased as the copper concentrations in the leachates rose. The copper concentrations in the leachates are to be considered as potentially toxic. Only the leachates obtained from the (first) leaching of the fine fraction with L/S 10 displayed a toxicity higher than one would expect from the copper concentrations alone. Since the leachates contain a mixture of a large number of elements, other elements are likely to contribute to the toxicity as well.

Sediment Testing: Low element concentrations were found in the leachates of the Veldhuizer brook sediments (Table 7). At both other sampling points the Fe, Cr, and As concentrations were a factor of 2–12 higher, Cd and Ni concentrations were 20–30 times higher, Pb (Apeldoorn) was 65 times higher and Zn was even 170–370 times higher.

The leachate of the brook sediment has no clear toxic effect on duckweed growth. However, at higher concentrations the fronds become smaller and the roots shorter. *L. minor* grew very poorly on the leachates of the samples from Apeldoorn and

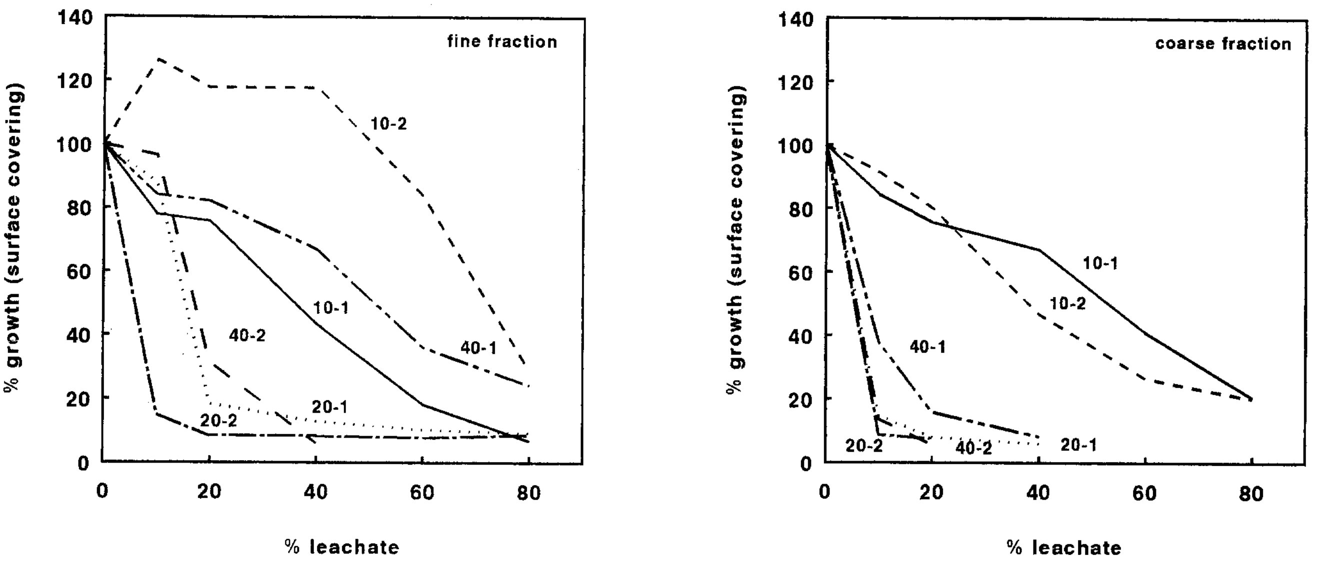

Fig. 1. Growth of *L. minor* exposed to leachates of "low NOx" PFA obtained at pH 5 and various liquid-solid ratios. 10-1 = liquid-solid ratio of 10, first leachate

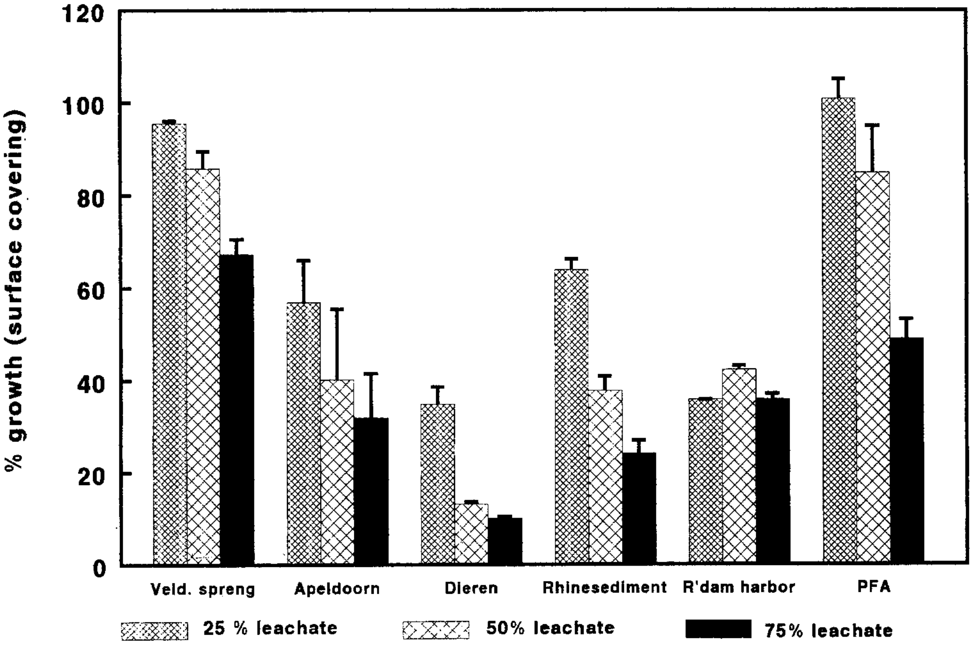

Fig. 2. Comparison between the growth percentages of *L. minor* at 25%, 50%, and 75% added leachate of different bottom sediments and conventional PFA

Dieren. The fronds were fewer in number, smaller and lighter in color, and displayed a tendency to overlap. At high leachate concentrations, the old fronds were yellow, and the roots white and liable to drop off. In the experiments using leachates of samples obtained from the brook location, the concentrations of added leachate associated with EC_{50} were over 75%. Leachates of Apeldoorn and Dieren showed an EC_{50} at concentrations of 35% and 19%, respectively (Figure 2).

The Zn concentrations in the leachates of the samples from both Apeldoorn and Dieren clearly exceeded the EC_{50} concentration as determined in the experiments with the individual elements. The concentrations in the duckweed associated with the EC_{50} of the Apeldoorn and Dieren leachates were 350 and 450 μg/g, respectively. These concentrations correspond fairly well with the concentrations in duckweed at EC_{50} concentrations (= 300 μg/g) that were determined in the experiments using the individual elements. The Zn concentrations in the Apeldoorn and Dieren leachates at EC_{50} were, however, clearly higher (1,500 μg/L and 1,900 μg/L respectively), indicating that the availability of Zn in the leachates was less than in the growth medium at single element tests (290 μg/L).

Discussion and Conclusions

Duckweed, *Lemna minor*, is well suited for testing the toxicity of leachates, and the experiments described here support three conclusions:

Table 7. Element concentrations in the sediments (dry weight) and leachates with the associated leached percentages (mean with SD).
A: Veldhuizer brook (control);
B: Apeldoorn (pigment factory);
C: Dieren (electroplating plant)

Element	Location	Sediment (μg/g)	Leachate (μg/L)	Leached (%)
As	A	6.8 ± 2.3	<5	—
	B	27.2 ± 3.4	10.5 ± 2.7	0.8 ± 0.3
	C	15.9 ± 0.8	37.1 ± 11.5	4.7 ± 1.2
Cd	A	0.1 ± 0.1	0.3 ± 0.1	6.2
	B	8.1 ± 1.6	11.5 ± 2.2	2.9 ± 0.6
	C	24.3 ± 0.4	7.5 ± 1.2	0.6 ± 0.1
Cr	A	7 ± 1	<8	—
	B	748 ± 196	55 ± 24	0.16 ± 0.08
	C	126 ± 6	36 ± 8	0.58 ± 0.11
Fe*	A	10 ± 1	0.1 ± 0.1	0.02
	B	34 ± 6	1.4 ± 0.5	0.09 ± 0.03
	C	12 ± 1	0.9 ± 0.1	0.15 ± 0.01
Hg	A	<0.5	<0.5	—
	B	0.7 ± 0.1	<0.5	—
	C	1.1 ± 0.4	<0.5	—
Ni	A	9 ± 1	<10	—
	B	98 ± 24	242 ± 49	5.0 ± 0.8
	C	71 ± 21	194 ± 39	5.6 ± 0.5
Pb	A	8 ± 4	<3	—
	B	2479 ± 692	197 ± 44	0.17 ± 0.05
	C	189 ± 30	7 ± 2	0.08 ± 0.01
Se	A	<4	<5	—
	B	5 ± 2	<5	—
	C	<4	<5	—
V	A	⩽5	<10	—
	B	45 ± 7	<10	—
	C	56 ± 4	14 ± 3	0.50 ± 0.06
Zn	A	36 ± 2	25 ± 4	1.4
	B	1050 ± 251	4247 ± 767	8.2 ± 1.1
	C	1682 ± 139	9200 ± 339	11.0 ± 1.3

*Iron concentration in mg g^{-1} and in mg L^{-1}

1. Toxicity of conventional and "low NOx" PFA is only related to the anionic elements and the toxicity of the tested dredged sediments is caused by cations.
2. The leaching of the elements from CGS is very limited.
3. The toxicity of the tested coal residues is far lower than that of sediments from the Apeldoornsch Kanaal.

Roughly, the chemical composition of the PFAs is reasonably alike; CGS showed higher chromium and nickel contents, and significantly lower lead, selenium, and molybdenum concentrations.

The concentrations of elements leached from conventional PFA (USEPA extraction procedure) can be presented as follows:

< 10 μg/L: Cu, Fe
10–50 μg/L: Cr, Sb
50–100 μg/L: Ni
100–500 μg/L: As, Mo, Se
> 500 μg/L: B

Cations as Cu, Fe, and Zn are well bonded in conventional PFA, and appear only in low concentrations in the leachate. The anions As, B, Mo, and Se on the contrary leach well and quickly from the PFA and can appear in relatively high concentrations in the leachate, especially in the short term. Further distinction is possible within the anionic group of elements, which may be categorized on the basis of the rate of leaching and the extent to which they are leached out, as follows: Se > Mo ⩾ As > B. An explanation for these elements' different leaching characteristics may lie in the extent to which each is bonded to the surface of the PFA particles (van der Sloot 1989). For it would appear that Se is 80–100% surface bonded, As 50–80%, and Mo 50–70%. The rate of leaching for "low NOx" PFA was in the order of B >> Mo ⩾ Se > As. Percentual leaching concentrations are higher of "low NOx" PFA compared with conventional PFA. Element leaching of CGS is neglectable with the exception of Ni.

The influence of the pH on the susceptibility to leaching was demonstrable for most elements. Leaching of the anionic elements B and As is lowering as pH becomes higher. Leaching of the elements Mo and Se was highest at pH 7, though in the case of Se this was only so with the coarse fraction. Leaching of Cr increased as the pH increased. A clear reduction in the leaching of the cationic elements Cu, Ni, and Zn was observed as the pH rose. There was generally no detectable leaching of these elements at pHs 7 and 9.

The influence of the L/S ratio on the elements' susceptibility to leaching was less clear than the pH effect. With the "low NOx" PFA, there was increased leaching of B, As and Se at higher liquid-solid ratios, but in respect of As and Se this was only detectable at pHs 7 and 9. Leaching of Mo was highest with a liquid-solid ratio of 20. Among the cationic elements, leaching of Zn increased at higher liquid-solid ratios. The solubility product of the different elements plays an important role in this context (de Groot *et al.* 1989).

In their free ionic form, the cationic elements are easily taken up by *L. minor* (high CF) and can therefore be toxic in low concentrations; this is also true of As (III). The biological availability of the rest of the anionic elements examined is lower for *L. minor* (low CF), with the result that relatively high concentrations are needed in the leachate before a toxic effect occurs.

The results of toxicity tests on the fine and coarse fractions of the "low NOx" ash using *L. minor* do not reveal the expected relationship between the liquid-solid ratio and growth inhibition. Growth was most strongly inhibited at a liquid-solid ratio of 20. The results are likely to have been dominated by the effects of Cu. Acute effects such as loss of mother/daughter fronds were visually detectable, as were non-acute effects such as fronds being stunted, lighter in color, or yellowish brown and necrotic.

Toxicity experiments with CGS leachates on *Spirodela oligorhiza* and the algae *Selenastrum capricornutum* showed an EC_{50} of 76% and a NOEC 20% (Klaine 1985). Klaine obtained the CGS from a pilot plant and followed the USEPA (1980) leaching procedure, using acetic acid. A substantial part of the effects found may be attributed to the use of acetic acid. In our experiments the element concentrations in the CGS leachates were low and consequently no toxicity experiments were carried out.

The growth effects on *L. minor* with leachates from the Apeldoornsch Kanaal are probably caused by the high Zn concentrations. Zn has an NOEC of circa 160 μg/L and an EC_{50} of 290 μg/L. Concentrations of Zn in both sediments and leachates were relatively high. Leaching of most other elements was relatively low (less than 1%). Strong growth inhibition was also observed in earlier studies and pilot experiments using sediments from the River Rhine and harbor sludges from Rotterdam treated in the same way as the canal sediments. The effects were probably also caused by Zn. In the harbour and River Rhine sediments Zn concentrations were 1,050 and 650 μg/g (d.w.) respectively. The leached percentage was about 10%. It can be concluded that PFA is less phytotoxic and if there are any effects they are caused by the anions compared to the effects of the tested sediments.

In a review of the accumulation of toxic trace elements by freshwater vascular plants, Outridge and Noller (1991) concluded that the free-floating species may be useful for biomonitoring elements in water. Problematic is the absence of a standardized testing protocol covering leaching tests and effect tests adjusted for the biological availability of elements. The present paper may be a step ahead in the proper direction.

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