Accumulation of uranium in *Lemna gibba* L. in relation to milieu conditions of tailing waters in abandoned uranium mines in Germany

Martin Mkandawire, E. Gert Dudel and Barbara Taubert

Dresden University of Technology, Institute of General Ecology and Environmental Protection, Pienner Straße 8, D-01737 Tharandt. Germany.
Tel: 49 351 463 31393. Fax: 49 351 463 31399
E-mail: mkanda@forst.tu-dresden.de

Abstract

The influence of the abiotic physicochemical properties of the milieu on uranium accumulation in *Lemna gibba* L. were investigated in tailing waters of two abandoned uranium mining sites in eastern Germany, and in solutions in the laboratory. Enrichment of Uranium into *Lemna gibba* showed relatively high bioaccumulation coefficients in both the field and the laboratory. The uranium accumulation in *Lemna gibba* correlated positively to the milieu concentration of U, P, NO_3^-, and SO_4^{2-}. Conductivity related negatively to the bioaccumulation, which indicated that presence of competing ions influenced uranium enrichment into *Lemna gibba* too. The laboratory experiments revealed that *Lemna gibba* resisted uranium enrichment only in low uranium concentrations but, suddenly shot up and then, decelerated with increasing uranium concentration in the milieu (i.e. sigmoid shaped curve). In contrast the bioaccumulation increased rapidly with PO_4^{3-} supply before levelling off. The results revealed that resistance of *Lemna gibba* to uranium enrichment succumbed to pressure from increasing uranium concentrations in the milieu. They also revealed that PO_4^{3-} influenced the bioaccumulation, not only because of the role of phosphorus in biomass production, but more importantly, due to the interaction between uranyl and phosphate ions. Inorganic NO_3^- and SO_4^{2-} also influenced the bioavailability of uranium to *Lemna gibba*.

Keywords: Uranium, speciation, *Lemna gibba*, tailing waters, bioavailability, bioaccumulation, milieu.

Introduction

Uranium mining and processing during the cold war era has left behind huge environmental and public health problems which remain a particular concern in former East Germany (Diehl, 1995; Diehl, 2003). The legacy from the mining activities includes numerous tailing ponds, low-grade ore heaps and abandoned mines scattered in the states of Saxony and Thuringia (Meinrath et al., 2003). The potential radio- and chemo-toxicity risks come from the potential for contamination of surface water due to the hydrogeochemical behaviour of uranium (Enderle and Friedrich, 1995). In most geological systems uranium is thermodynamically stable in the oxidation state U (VI), which forms uranyl ions (UO_2^{2+}), which in turn react readily with a wide range of ligands in the aquatic environment. Mobilisation and immobilisation of uranium is controlled by at least one of the following processes: redox reactions; changes in pH and Eh; chelation by siderophores and other plant and microbial exudates; inorganic complexation (carbonate, phosphates ligands); bioaccumulation; and demineralisation (Kießig and Hermann, 2000). Formation of carbonate and hydroxycarbonate complexes enhance uranium solubility and mobility, which is one influence on uranium bioavailability (Radgnarsdottir and Charlet, 2000).

The rehabilitation of abandoned uranium mines with conventional engineering technologies is proving costly (Kießig and Hermann, 2000). In addition, long-term stability of rehabilitated sites is uncertain (Mudd, 2000). Consequently ecotechnologies, which are cost-effective and have long-term restoration stability, are advocated as the alternative to conventional engineering technologies. One of the ecotechnologies that has currently gained popularity is phytoremediation (Sadowsky, 1998; Schnoor *et al.*, 1995). The potential for phytoremediation of uranium depends, among a number of other factors, on the plant biomass production and the ability of particular plant species to accumulate large amounts of uranium. Chemical speciation appears to be more important than solubility in determining the effectiveness of uranium uptake (Ebbs et al., 1998; Huang et al., 1998). Both abiotic and biotic factors influence the chemical speciation.

The aim of the current study was to investigate the influence of milieu physicochemical properties on uranium accumulation in *Lemna gibba* in abandoned uranium mines in Lengenfeld and Neuensalz-Mechelgrün, in eastern Germany. *Lemna gibba* was chosen because it was discovered growing in ponds at both sites. Additionally, *Lemna* sp. is well known as a model plant and polishing species in wastewater treatment (Rose, 2000).

The influence of phosphorus was also a focus of interest because it interacts chemically with uranium, and it was the major growth-limiting element at both study sites. For comparison, other inorganic ligands, SO_4^{2-} and NO_3^-, were also considered. Complementary laboratory experiments were conducted to complement the field results.

Material and Methods

Study sites and field sampling

The field investigations were conducted in tailing ponds of two abandoned uranium mines at Lengenfeld and Neuensalz-Mechelgrün in South-western Saxony, Germany (Figure 1). Three sampling points were established on the tailing ponds in the Lengenfeld between August and December 2001. Six sampling points were established at Neuensalz-Mechelgrün from February to December 2002, on the reservoir on the mine waste and tailings dumps, and below the dump. The arrangement of the sampling points at both sites are illustrated in Figure 1. Reference samples were collected in a stream above the mine tailing dumps at Lengenfeld. The sampling of both plants and water was repeated four times at each sampling point. Conductivity, pH, temperature, and dissolved oxygen concentration were measured on each sampling visit. Water samples were filtered in the field through cellulose acetate filter membranes (0.45-μm pore size), and adjusted to pH 2 with 2 % HNO_3 for uranium determination. A second, unacidified, aliquot was collected for phosphorus analysis.

Laboratory experiments

A strain of *Lemna gibba* L., obtained from the arboretum of Humboldt University at Baumschulweg in Berlin, was used in a two-factor diluted Hutner nutrient solution in a semi-continuous culture mode (Mkandawire and Dudel, 2002; Mkandawire *et al.*, 2004) on a *Lemna* culture equipment placed in ecotron (Plant growth chamber NEMA GmbH, Netzschkau, Germany). The ecotron's operation conditions were set as described elsewhere (Mkandawire et al, 2002; and 2004).

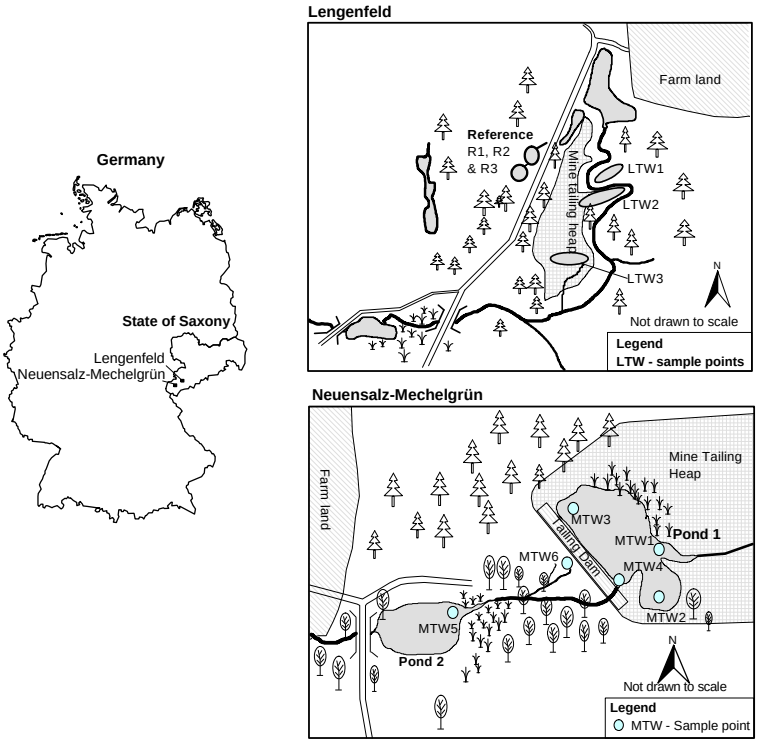

Fig. 1. Location of study sites and sketch plans of sampling points at the study sites

Six uranium test concentrations (10, 50, 100, 250, 500, and 1000 µg l^{-1}) prepared from $UO_2(NO_3)_2 \cdot 6H_2O$ and three phosphate concentrations (0.013, 13.61, 20.0, and 40.0 mg l^{-1}) were prepared from K_2HPO_4 were used. All reagents in the study were of analytical grade. One hundred and fifty fronds of similar size from a seven-day-old pre-culture were inoculated systematically into each vessel containing 700 ml of test solutions. Aliquots were sampled at the start of the experiment and every second day thereafter. The experiments lasted for 21 days. At the end of the experiment *Lemna gibba* biomass was harvested and an aliquot was sampled. All experiments were replicated four times, and parallel control experiments were run.

Sample preparation and determinations

All water samples were handled according to German standards (DIN 38 405-30; 38 404; 38 402 and 38 412 specification). All plant samples

were freeze-dried until constant weight was achieved and were digested using HNO_3–H_2O_2 mixture in a microwave digester (MW-Digestion, CEM MARS 5, Matthews, North Carolina, USA). Uranium in samples was determined by ICP-MS (PQ2+ Thermo, Cheshire, England, UK), and total phosphorus was determined using ICP-OES (Perkin Elmer Plasma 2, Wellesley, USA). Phosphate was determined using spectrophotometric technique (LCK 348, Dr. Lange CADA 50, Düsseldorf, Germany)

Chemical speciation calculation

Chemical speciation in both natural and synthetic tailing waters were calculated with PhreeqC$^+$ 2.8.0.0 Alpha version (Parkhurst and Appelo, 1999) at the beginning and the end of the experiments with a modified thermodynamic database (Mkandawire and Dudel, 2002).

Data analysis

The bioaccumulation coefficient *(b_{acc})* is the frond/solution element concentration quotient. It was estimated by:

$$b_{acc} = \frac{(c_{lem})}{(c_{sol})} \quad \textbf{(1)}$$

where C_{lem} is the uranium (mg l^{-1}) accumulated in the frond and C_{sol} is the uranium concentration (mg l^{-1}) in the solution. The bioaccumulation (q) (mg g^{-1}) is defined as:

$$q = \frac{v(c_i - c_f)}{m} \quad \textbf{(2)}$$

where v is the volume of the solution; c_i and c_f are the initial and equilibrium uranium concentrations, respectively; m is the *Lemna gibba* biomass (g).

The non-parametric Mann-Whitney test was used to analyse the difference in accumulation of uranium in *Lemna gibba*. Correlations were tested using the Spearman Rank test. All statistical analyses were conducted with SPSS 10.1.0 for Windows (SPSS Inc. 2000).

Results and discussion

Uranium speciation

Background chemical quality data for the tailings waters at the two sites are given in Table 1. One pond in Lengenfeld was characterised by low pH, probably associated with dilute acid mine drainage. Slightly reducing conditions were observed from a tailing dam spring. Chemical speciation simulation of the tailings waters with PhreeqC revealed that uranyl carbonate [$UO_2(CO_3)^{2-}$] species were dominant in both Lengenfeld and Neuensalz-Mechelgrün (over 85 %). Figure 2 illustrates the predicted uranium species in tailing waters of both study sites in the Eh-pH diagram for the U-C-O-H system as predicted from the measured pH and the PhreeqC derived Eh.

Table 1: Selected physicochemical condition of waters in the abandoned uranium mines at Lengenfeld and Neuensalz-Mechelgrün, and ponds of reference site.

	Physicochemical Parameters of Tailing Waters			
	pH	**SEC ($\mu S\ cm^{-1}$)**	**Temp. (°C)**	**DO ($mg\ l^{-1}$)**
Reference Site	6,9 – 8,5		7,8 – 14,1	
Lengenfeld	5,1 – 10,3	251 – 3079	7,2 – 15,1	4,0 – 14,8
Mechelgrün	6,9 – 9,0	659 – 1430	5,5 – 20,7	8,8 – 12,3

SEC is specific electrolyte conductivity; and DO is dissolved Oxygen

Speciation models showed that uranium was best dissolved between pH 5,0 and 8,0 in the Hutner based synthetic tailing waters. At pH 5,0 free uranyl (UO_2^{2+}) ions dominated to nearly 80 %. Between 90 to 95 % of uranium was present as hydroxide complexes at pH 6,0. Almost 100 % of uranium formed carbonate complexes at pH 7. With addition of phosphates, uranyl carbonate complexes were replaced by uranyl phosphate species, which had saturation indexes around zero (0). Hence, precipitation of uranyl phosphate in laboratory experiments would likely reduce exposure of *Lemna gibba* to uranium.

Uranium in field plant and water samples

The spatial distribution of uranium accumulation in *Lemna gibba* L. against background concentrations of uranium and a few selected inorganic ligands at the study sites are summarised in Table 2. The results re-

veal that on average the tailing waters in Neuensalz-Mechelgrün had higher uranium than in the Lengenfeld.

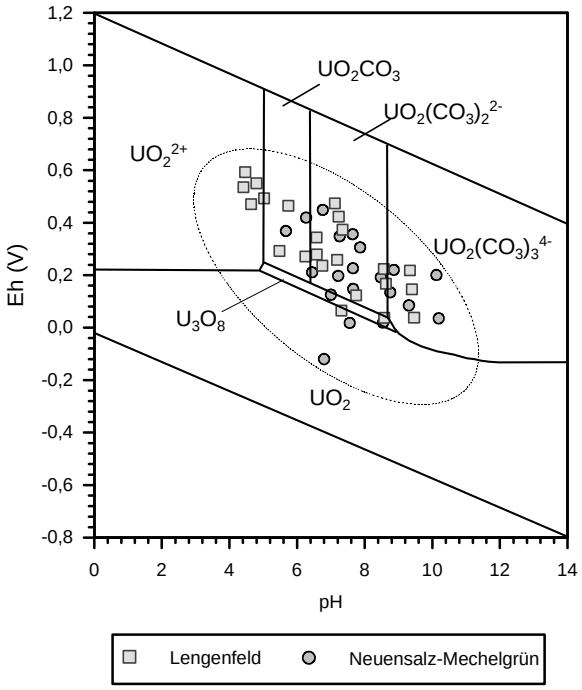

Fig. 2. Distribution of uranium species in tailing waters of Neuensalz-Mechelgrün and Lengenfeld on the Eh-pH diagram for U-C-O-H systems based on PhreeqC chemical speciation modelling of the tailing waters under atmospheric CO_2 pressure, and modification from Brookins (1988).

Contaminant concentrations in the surface mine waters of both mine sites were significantly higher than in waters of the reference site. Contaminant concentrations vary significantly from one section of the tailing ponds to another. All water samples from Lengenfeld differed significantly in uranium content. No temporal variations were noticed, due to the restricted study period in Lengenfeld. In Neuensalz-Mechelgrün, only sample location MTW 6 had significantly higher uranium content than the rest of the sampling points because the water at this location arises from seepages from the dumps. However, no significant differences in uranium accumulation in *Lemna gibba* were observed between the sample locations. However, there were significant temporal variations in the enrichment of uranium into *Lemna gibba* in Neuensalz-Mechelgrün with the highest

observed between July and August. This corresponds to the most productive period in terms of macrophyte growth in the tailing ponds (Mkandawire, 2004). High specific conductivity, dissolved oxygen, and temperature values, but lower PO_4^{3-} and NO_3^- concentrations were observed during this period in Neuensalz-Mechelgrün.

Table 2: Uranium bioaccumulation and background concentrations of selected chemical variables in the tailing ponds of abandoned uranium mines. Values are mean of several repeated sampling ± standard deviation.

Site	Sampling location	U in dry biomass (mg kg^{-1})	Mean Background Concentrations			
			U (µg l^{-1})	SO_4^{2-} (mg l^{-1})	P (µg l^{-1})	NO_3^- (mg l^{-1})
Reference	RW1	29,9***	2,0***	18,4	2,9	-
		±9,7	±1,5	±12,5	±1,6	
	RW2	30,4***	2,5***	46,9	2,4	-
		±9,9	±0,9	±28,1	±1,1	
	RW3	20,3***	0,5***	117,0	1,3	-
		±7,3	±0,5	±96,6	±1,4	
Lengenfeld	LTW1	262,3	49,3	76,7	0,5	-
		±588,6	±11, 9	±31,6	±0,3	
	LTW2	369,9	50,2	142,0	0,1	-
		±115,6	±44,2	±106,3	±0,1	
	LTW3	792,1	186,1	69,9	0,6	-
		±85,8	±98,6	±26,0	±0,7	
Mechelgrün	MTW1	599,4	147,9	196,2**	2,2	20,1
		±148,2	±54,6	±20,1	±1,8	±8,3
	MTW2	669,9	153,5	166,1**	1,6	29,7
		±240,5	±75,7	±26,2	±1,2	±17,2
	MTW3	613,3	214,1	155,5**	2,1	39,0
		±163,5	±143,6	±12,2	±2,0	±21,8
	MTW4	462,6	187,9	125,9**	1,5	46,9*
		±212,7	±86,8	±15,1	±1,4	±8,7
	MTW5	501,1	109,5	162,8*	1,3	29,2
		±127,0	±66,5	±19,7	±1,1	±13,7
	MTW6	-	291,7**	197,5	1,8	27,7
			±20,6	±63,9	±1,5	±13,8

*** $p = 0.001$; ** $p = 0.01$; and * $p = 0.05$

Influence of the background uranium concentration

Figure 3 (a) shows that there is an exponential increase in uranium concentration of dry biomass with increasing uranium concentration in the tailings waters (correlation coefficient $r = 0,88$ at $p < 0,001$ within the 95 % confidence interval). This is attributed to the general principle that plants are able to exclude the non-essential metals in low concentrations, but this barrier breaks down with increasing milieu concentrations. This phenome-

non has been described to be associated with plants, which indulge on active exclusion in metal uptake (Robinson et al. 2003). However, Figure 3 (b) shows that as the milieu uranium concentration increase to 1000 µg l^{-1}, the bioaccumulation coefficients might decelerate. Unfortunately, in the current results it was difficult to conclude because of the high standard deviations at higher initial uranium concentrations in the experiments. The relationship fitted into a sigmoid regression with $r = 0{,}955$ at $p < 0{,}01$ within the 95 % confidence interval. Because the nutrient solution had high initial PO_4^{3-} (13,6 mg l^{-1}), the decreases in bioaccumulation coefficients were due to the formation of uranyl phosphate species, which resulted into non-bioavailability of uranium.

Influence of the milieu Phosphorus Concentration

The phosphorus concentrations in the tailings waters ranged from $0{,}13 \pm 0{,}07$ to $5{,}76 \pm 2{,}01$ µg l^{-1}, which are within the growth limiting range at both study sites. There was a linear relationship between phosphorus concentration and uranium bioaccumulation ($r = 0{,}927$ at $p < 0{,}001$ within the 95 % confidence interval) (Figure 4 (a)). This is of little surprise since uranium accumulation is biomass related. However, in laboratory culture experiments uranium bioaccumulation was very rapid at very low PO_4^{3-} supply up to a threshold level, beyond which no influence of increasing PO_4^{3-} supply is evident (Figure 4 (b)). The influence of PO_4^{3-} on *Lemna gibba* growth observed by Mkandawire et al. (2004) coupled with uranyl phosphate complexation, may be responsible for the bioaccumulation trend. Thus, the influence of PO_4^{3-} in uranium accumulation in *Lemna gibba* is most noticeable in the PO_4^{3-} limiting conditions such as those found in tailing waters.

Influence of selected inorganic ligands and physical properties on uranium uptake

The background concentrations of SO_4^{2-} related positively, but non-linearly, to the accumulated uranium in *Lemna gibba* ($r = 0{,}901$ tested at $p < 0{,}001$ within the 95 % confidence interval) (Figure 5 (a)). The enhancement of bioavailability of metals by SO_4^{2-} has been demonstrated by several researchers (Gu et al., 2000; Zhimang et al., 2000). Although conductivity correlated positively to SO_4^{2-} concentration in the tailing waters (mean overall correlation coefficient for log-transformed values $r^2 = 0.601$), high conductivity reduced the uranium bioaccumulation. Con-

ductivity has been observed to reduce nutrient availability to some plants (Economakis, 1992; Mpelasoka and Nichols, 2003). Therefore, the conductivity effect on the bioaccumulation was seems likely to be due to uranium ion competition with others in the cocktail of ions in the milieu.

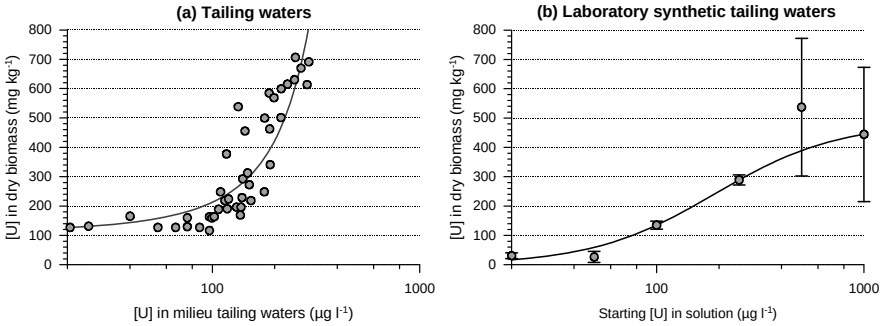

Fig. 3. The uranium bioaccumulation against (a) uranium background concentration in tailing waters in Lengenfeld and Neuensalz-Mechelgrün, and (b) different initial uranium concentrations in culture solution in laboratory experiment. The initial PO_4^{3-} concentration in the solution was 13,6 mg l^{-1}.

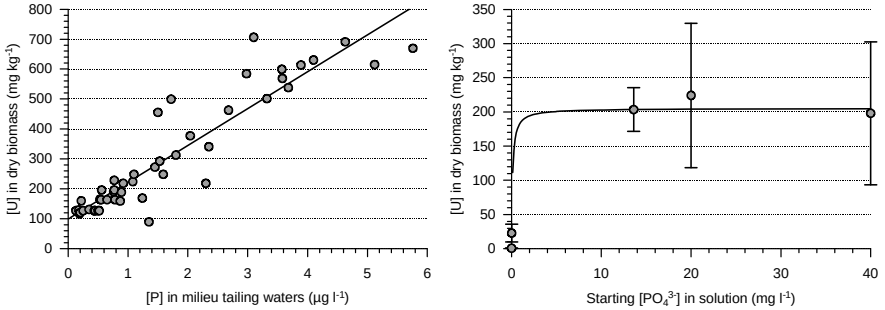

Fig. 4. The uranium bioaccumulation against (a) phosphorus background concentration in tailing waters in Lengenfeld and Neuensalz-Mechelgrün, and (b) different start PO_4^{3-} concentration in culture solution in laboratory experiment. The initial uranium concentration in the culture was 100 µg l^{-1}.

There was a positive linear relationship between uranium bioaccumulation and background NO_3^- ($r = 0{,}875$ tested at $p < 0{,}001$) (Figure 5 (b)). Speciation simulation of uranium solutions prepared from solid $UO_2(NO_3)_2 \cdot 6H_2O$ predicted that uranium existed mostly as free uranyl ions. Hence, the enhancement of biological driven uptake in *Lemna gibba* due to availability of growth limiting nitrogen appears to be more significant than chemical speciation in determining the relationship between

NO_3^- and uranium bioaccumulation. This study had found real influence between pH and uranium bioaccumulation in the tailing waters. However, pH is related to nitrogen acquisition and NH_4^+ toxicity by *Lemna gibba* (Caicedo et al., 2000; Körner et al., 2001).

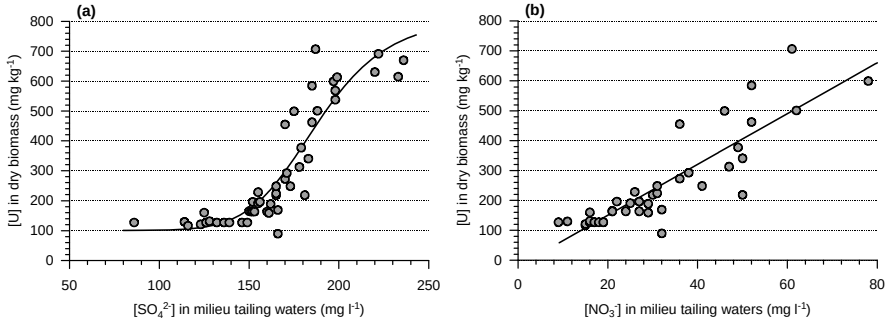

Fig. 5. Enrichment of uranium in *Lemna gibba* dry biomass against (a) sulphate, and (b) nitrate background concentrations in tailing waters of Lengenfeld and Neuensalz-Mechelgrün abandoned uranium mines

The distribution of uranium in water and sediments depends on the pH because the pH influences uranium speciation, and 5–20 % of uranium exist the form of fulvic and humic acid species (Franke *et al.*, 2000; Kupsch *et al.*, 1998). Dissolved oxygen influenced chemical speciation due to its direct link to Eh in the aquatic environment.

Conclusion

This study has demonstrated that there are elevated uranium concentrations in tailings ponds of the two abandoned uranium mines in eastern Germany and that *Lemna gibba* in these ponds also has elevated concentrations, apparently due to bioaccumulation, which may be influenced by other chemical variables of the water. Thus, uranium might be a risk through contamination of the surface water pathway. It also indicated that accumulation of uranium by *Lemna gibba* is site specific, and depends on the particular geochemistry of the waters in question. Limited bioaccumulation of uranium by *Lemna gibba* appears to be due to active exclusion. Hence, biosorption as demonstrated in an earlier study (Mkandawire et al., 2003) seems to be the main accumulation mechanism in low milieu uranium concentrations, until the control gates collapse with increasing uranium concentrations. *Lemna gibba* appears to be able to immobilise a significant

amount of uranium in tailing waters, such that it may be a potential phytoremediation species. These results also suggest that manipulation of PO_4^{3-} availability may be the key to enhancing uranium attenuation by *Lemna gibba* and other macrophytes. Further investigations are required to establish the influence of other chemical variables, such as competing cations, and the influence of dissolved oxygen and dissolved inorganic carbon in the milieu.

Acknowledgements

The study was financed under German Federal Ministry of Education and Research (BMBF) project grant No. 02WB0222. Arndt Weiske did ICP-MS and DOC/TN analyses with laboratory assistance from Karin Klinzmann and Annet Jost. Phosphate was analysed with ICP-OES at *Landesforstpräsidium* of the State of Saxony in Graupa.

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