Capacity of *Lemna gibba* L. (Duckweed) for Uranium and Arsenic Phytoremediation in Mine Tailing Waters

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ABSTRACT

The potential of *Lemna gibba* L. to clean uranium and arsenic contamination from mine surface waters was investigated in wetlands of two former uranium mines in eastern Germany and in laboratory hydroponic culture. Water and plants were sampled and *L. gibba* growth and yield were monitored in tailing ponds from the field study sites. Contaminant accumulation, growth and yield experiments were conducted in the laboratory using synthetic tailing water. Mean background concentrations of the surface waters were 186.0 ± 81.2 µg l⁻¹ uranium and 47.0 ± 21.3 µg l⁻¹ arsenic in Site one and 293.7 ± 121.3 µg l⁻¹ uranium and 41.37 ± 24.7 µg l⁻¹ arsenic in Site two. The initial concentration of both uranium and arsenic in the culture solutions was 100 µg l⁻¹. The plant samples were either not leached, leached with deionized H₂O or ethylenediaminetetraacetic (EDTA). The results revealed high bioaccumulation coefficients for both uranium and arsenic. Uranium and arsenic content of *L. gibba* dry biomass of the field samples were as follows: nonleached samples > deionized H₂O leached (insignificant ANOVA p = 0.05) > EDTA leached. The difference in both arsenic and uranium enrichment were significantly high between the nonleached and the other two lead samples tested at ANOVA p > 0.001. Estimated mean *L. gibba* density in surface water was 85,344.8 ± 1843.4 fronds m⁻² (≈1319.7 g m⁻²). The maximum specific growth rate was 0.47 ± 0.2 d⁻¹, which exceeded reported specific growth rates for *L. gibba* in the literature. Average yield was estimated at 20.2 ± 6.7 g m⁻² d⁻¹, giving approximately 73.6 ± 21.4 t ha⁻¹ y⁻¹ as the annual yield. The highest accumulations observed were 896.9 ± 203.8 mg kg⁻¹ uranium and 1021.7 ± 250.8 mg kg⁻¹ arsenic dry biomass for a 21-d test period in the laboratory steady-state experiments. The potential extractions from surface waters with *L. gibba* L. were estimated to be 662.7 kg uranium ha⁻¹ yr⁻¹ and 751.9 kg arsenic ha⁻¹ yr⁻¹ under the above conditions.
I. INTRODUCTION

Phytoremediation is receiving increasing attention as a cost-effective alternative to conventional technologies for cleaning heavy metals and radionuclides from aquatic systems. More recently, phytoremediation has been tested or used for removal of uranium and arsenic from contaminated waters; for instance, in former uranium mine sites (Dudel et al., 2001). Potential of the technology depends on the amount of radionuclides and heavy metals immobilized, the biomass turnover (Francesconia et al., 2002; Reeves and Baker, 2000) and the ability of a plant species to tolerate extreme contamination. One of the plant species that has these characteristics is L. gibba L. In laboratory studied, Lemna gibba showed high turnover and yield (Mkandawire and Dudel, 2002). Lemna gibba was also found growing naturally in tailing ponds of former uranium mines in Lengenfeld and Neuensalz-Mechelgrün. Most family members of this species have been used for polishing in waste-water treatment and worldwide distribution of Lemna sp. (Wang et al., 2002; Zayed et al., 1998). In view of these, it was hypothesized that L. gibba L. can play an important role in the immobilization of radionuclides and heavy metal-contaminated surface waters, including environmentally significant concentrations.

To test the hypothesis, field investigations were conducted at former uranium mines of Lengenfeld and Neuensalz-Mechelgrün (Vogtland, Germany). Complementary laboratory experiments were conducted using synthetic surface water based on modification of the Hutner nutrient solution to nutrient and test chemical concentrations of the surface waters from the study sites. These field trial sites are among several sites in former East Germany with radioactive and heavy metal contamination risks due to short- and long-term pollution potential of mine dumps and tailings (Bruske-Hohlfeld, 1999; Enderle and Friedrich, 1995; Juznic et al., 1989; Schuttmann, 1993). Shortfalls of most conventional remediation techniques and high costs already incurred in the sites have increased the need for the use of plants and associated microbes (i.e., a community approach ecotechnology) for remediation (Bech et al., 2002; de Souza et al., 1999; Huang et al., 1998; Miller, 1996). Uranium content of the ore is often only between 0.1% and 0.2%, which resulted in excavating large amounts of ore to get a reasonable amount of uranium. Consequently, apart from the radioactive constituents, tailings also contain other contaminants, e.g., arsenic (Diehl, 2003). High arsenic solubility leads to its leaching from the dump and tailing material, which in turn leads to the enrichment in the environment (Enderle and Friedrich, 1995; Roussel et al., 2000). Consequently, it is important to consider arsenic together with uranium in the remediation process of contaminated waters from uranium mine tailings.

The objective of the current study was to evaluate the potential of Lemna gibba L. for phytoremediation of uranium- or arsenic-contaminated mine surface waters of former uranium mining sites. The evaluation required an understanding of factors that determine the physicochemical processes in the plant milieu; the growth, turnover, and plant requirements; and its ability to withstand toxicity of the contaminant.
II. MATERIALS AND METHODS

A. Field Sampling

Field investigations were conducted in tailing ponds on and below tailing dumps and reference site/pond above tailing dumps at two former uranium mines: Lengenfeld and Neuensalz-Mechelgrün referred thereafter as Site 1 and Site 2, respectively (Figure 1). For reference, repeated sampling was done in a pond about 800 m upstream before the mining dump wetlands in Lengenfeld, having negligible uranium and arsenic contamination. The purposes for field sampling were: (1) to establish *L. gibba* frond density and yield and, consequently, biomass per unit area and, (2) to determine accumulation of uranium or arsenic in *L. gibba* and eventually calculate the extraction capacity.

1. Estimation of *Lemna gibba* Frond Density

For the establishment of mean plant density, a 50 × 50 cm sampling frame was placed randomly in the tailing ponds. In Site 1, sampling was done on three different locations in each of the three ponds between August and November 2001. In Site 2, five spots were sampled at random twice a month from January to December 2002. Plant samples collected within the sampling frame were washed thoroughly in surface waters, placed in plastic bags, and transported to the laboratory. In the laboratory, all debris was removed from the samples. Ten *L. gibba* fronds were isolated from each sample, photographed, and individual frond areas were determined using graphic analysis program (WinCam 2000A, Regent Instruments, Quebec City, Quebec, Canada). The ratio between the total frame area and the mean of the measured individual frond areas was the estimation of the *L. gibba* population density of the frame. Samples were freeze dried as described below. The samples were weighed
after drying to estimate the mass per area. Sampling was repeated four times at each sample location.

2. Sampling for Bioaccumulation Determination

*L. gibba* and water samples were collected from three ponds on the tailing and mine waste dump of Site 1 and five sampling spots in a reservoir of the tailing dam in Site 2. After removing all debris, e.g., leaves, invertebrates, etc., plant samples were divided into three equal portions and treated as follows: 1) nonleached; 2) leached with 2 l deionized H$_2$O; or 3) leached with 2 l of 50 mg l$^{-1}$ EDTA before freeze drying (Christ ALPHA 1-2/LD, Osterode am Harz, Germany). Water samples were handled according to DIN 38 402 (DIN, 2001). Leaching was conducted on the samples from the field to differentiate between temporary fixation through surface sorption and permanent fixation through internalization of uranium or arsenic. Leaching is defined as the removal of removing soluble constituents from a substance or specimen by the action of a percolating liquid. Samples were digested and processed for the determination of uranium and arsenic as below.

B. Field Growth and Yield Trials

Twelve rings of 20 cm in diameter [Figure 2(a)] were floated at random on a reservoir on the tailing pond of Site 2 from February to December 2002. Each ring was inoculated with 200 *Lemna gibba* fronds. The rings were photographed immediately after inoculation and later at weekly intervals. Excess biomass was harvested and weighed every 2 wk. Total *L. gibba* frond area were analyzed using WinCam 2000A. The changes in frond area were used to calculate growth rate. The fresh biomass yield

![FIGURE 2. (a) Lemna field growth monitoring ring floated the tailing waters of Site 2 and (b) L. growth vessel of the Lemna culture equipment used in laboratory experiments. The diameter ring is 20 cm. The growth vessel is composed of an inner pot of 10 cm diameter and perforated at 2 cm from the bottom in which L. gibba grows and, an outer pot of 11 cm diameter.](image-url)
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of *L. gibba* was calculated by subtracting the weight of the inoculums from the total biomass at the harvest. Water and plant samples were also collected during every growth-monitoring time and excess biomass was harvested from the rings. Annual yield (t ha\(^{-1}\) y\(^{-1}\)) in the tailing ponds was extrapolated from the yield per ring.

C. Laboratory Experiments

For laboratory experiments, *L. gibba* L. were obtained from the Arboretum of the Humboldt University at Baumschulweg in Berlin and cultured in a modified Hutner medium according to Mkandawire and Dudel (2002). Thirty fronds of similar size, from 7-d preculture conditions, were systematically inoculated into each test vessel containing 700 ml [Figure 2(b)] of test solutions, which contained either 100 µg l\(^{-1}\) uranium or arsenic. The experiments were conducted in an ecotron (NEMA GmbH Netzschkau, Germany), set in semicontinuous mode, and shaken continuously on special *Lemna* culture equipment. The ecotron was programmed as described in Mkandawire and Dudel (2002) and Mkandawire et al. (2004). *L. gibba* biomass and uranium or arsenic content were quantified at the start and the end of the experiment. To maintain a steady state in the semicontinuous culture, excess biomass was harvested and 20% of the media changed every 48 h. Growth was monitored using total frond count and total frond area through analysis of digital images taken every 2 d, as described above. Sixty ml media and plant samples (all excess biomass) were collected at intervals of 2 d and at the end of the experiment. Two control experiments were set, first without uranium or arsenic but inoculated with *L. gibba* and second with uranium or arsenic but not inoculated. All samples were processed for chemical analysis, as described below.

D. Speciation Calculation

The geochemical computer programme PhreeqC\(^+\) 2.8.0.0 Alpha version (United States Geological Survey, (USGS), 2001) with a modified thermodynamic database (Mkandawire and Dudel, 2002) was used to simulate speciation of component in both nutrient solution and surface waters. Speciation was calculated at the beginning from pH range 4 to 8 to determine optimal pH when most chemical components were soluble in the solution.

E. Element Analysis

Freeze-dried *Lemna* samples were weighed and wet digested (3 ml of 65% HNO\(_3\)/3 ml of 30% H\(_2\)O\(_2\)) in a microwave digester (MDS 2000, CEM, Matthews, North Carolina, USA). Water samples were filtered (0.45 µm pore cellulose acetate filter membrane, Sartorius, GmbH, Göttingen, Germany) immediately after sampling. All samples were diluted with 2% HNO\(_3\) to required concentrations for the determination of uranium and total or total dissolved arsenic in ICP-MS (PQ2+ Thermo VG Elemental, Cheshire, U.K.) and AAS (GF95 with Graphite Furnace, Thermo Elemental, Cheshire, U.K.).

F. Data Analysis

Specific growth rate (µ) was defined as \(\mu = \frac{\ln N_{t2} - \ln N_{t1}}{t_2 - t_1}\) where \(N_{t1}\) is the number of fronds or total area covered by fronds at time \(t_1\) and \(N_{t2}\) is number of fronds at time \(t_2\).
\( t_1 \) and \( t = \text{time (days)}. \) The extraction potential (\( E \)) of contaminant with the biomass is expressed in percentage and given by 
\[
E = 100\frac{\psi Y}{V}\% 
\]
where \( \psi \) is the bioaccumulation coefficients (ratio of mg g\(^{-1}\) plant and mg l\(^{-1}\) solution; in this work, it has also been used as transfer factor). \( Y \) is yield dry biomass (g) and \( V \) is water volume. 

Extraction for a longer period and changes of contaminant concentration with time, considering removal by \( L. \) gibba, is given by 
\[
C_{tn} = C_{t_0}e^{-Et} \text{ where } C_{tn} \text{ is concentration at time } t = n \text{ and } C_{t_0} \text{ is initial concentration (at } t = 0). 
\]
For frond density dependent contaminant extraction potential, the change in concentration \( \Delta[C] \) in the solution was calculated as 
\[
\Delta[C] = \frac{1}{\rho} \int_0^t F_d T C_{t_0} \psi dt 
\]
where \( \rho = \text{solution density (g cm}^{-3}\)), which in this study has been assumed to be 1 g cm\(^{-3}\)), and, \( F_d = \text{frond density fraction} \) and \( T = \text{water use (l day}^{-1}\)). All the calculations were in accordance with ISO/CD 20079, EPA OPPTS 850.4400, and OECD 221 (ISO, 2001; OECD, 2002; Sims et al., 2000).

Data were analyzed with one-way analysis of variance (ANOVA) or Student’s \( t \)-test for pair-wise analysis. Where the data did not fit a normal distribution, a non-parametric Mann–Whitney test using the computer package SPSS\textsuperscript{®} version 11 (SPSS Inc.©2002, Chicago, IL, USA). Significance was regarded at \( p \leq 0.05 \).

### III. RESULTS AND DISCUSSIONS

#### A. Lemna Density in Surface Ponds

The \( L. \) gibba stands were observed as being very dense in stagnant surface waters achieving density range 84,360–87,927 fronds m\(^{-2}\) (Table 1). Field observation revealed that the density of \( Lemna \) calculated here is just arbitrary because there existed 1.0–6.0-cm-thick \( L. \) gibba mat composed of many layers, i.e., some fronds were under others but contributed significantly to the biomass only and not to the area. Thus, it was more sensible to use \( Lemna \) dry biomass in population density calculation than frond numbers. Using mass as the parameter, there were no significant differences between Sites 1 and 2, while compared with reference sample points, it was significantly different. The reference sample points had a mean background concentration of 7.9 \( \mu g \) l\(^{-1}\) uranium and 3.02 \( \mu g \) l\(^{-1}\) arsenic, which seems to have had a negligible effect on \( Lemna \) gibba growth. Day length and global radiation measured in Sites 1 and 2 and the reference points were insignificantly different.

<table>
<thead>
<tr>
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<th>Mean Fronds m(^{-2})</th>
<th>Mean biomass (g m(^{-2}))</th>
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<tbody>
<tr>
<td>Site 1</td>
<td>84360.7 ± 1671.2</td>
<td>1081.5 ± 224.9</td>
</tr>
<tr>
<td>Site 2</td>
<td>83746.2 ± 1211.4</td>
<td>936.5 ± 214.0</td>
</tr>
<tr>
<td>Reference site</td>
<td>87927.5 ± 2012.9</td>
<td>1941.2 ± 632.3*</td>
</tr>
</tbody>
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\( ^*p \leq 0.05, \ p \leq 0.01, \text{ and Implies } p \leq 0.001. \)
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The dense *L. gibba* mat on the surface of the ponds has important implications in immobilization of uranium and arsenic. Generally, plant density has physical effect on flow rate of the water. However, *L. gibba* as a floating macrophyte had very low impact on the water flow, but its density created a large surface for contact with the contaminants. Extraction of contaminants is a function of plant density and surface area in contact with water (Robinson, Fernandez *et al*., 2003; Robinson, Lombi *et al*., 2003). Dissolved oxygen saturation was lower in waters under the *L. gibba* mat (85.0 ± 5.3%) than in open tailing ponds (112.1 ± 7.4%) at similar temperature, thereby creating slight reducing conditions. Influence of dissolved O\(_2\) in the water–sediment chemistry and biology has been reported, e.g., U(VI) immobilization in anaerobic nitrate-dependent Fe(II) oxidation by *Dechlorosoma* species (Atun and Kilislioglu, 2002); reduced solubility of organic arsenic species under the oxidizing condition (Carbonell-Barrachina *et al*., 2000); and uranium immobilization in the presence of sulphur-reducing bacteria (Phillips *et al*., 1995; Schippers *et al*., 1995; Spear *et al*., 1999). *L. gibba* is a host to and is associated with microbial flora (unpublished data) and definitely uranium or arsenic immobilization benefited from the *Lemna* microbes association in surface water. This requires further investigations.

**B. In-Situ Growth and Yield**

The monthly biomass production, based on fortnight biomass determination, was estimated only in Site 2. There were significant differences in yield of *L. gibba* among the months in 2002, with a tendency for an increase from February to August and a decrease from September to December (Figure 3). Highest growth rate of

![Graph showing mean monthly yield estimates for *L. gibba* in mine tailing reservoir at Neuensalz-Mechelgrün](image)

**FIGURE 3.** Mean monthly yield estimates for *L. gibba* in waters of mine tailing reservoir of an abandoned uranium mine at Site 2 (Neuensalz-Mechelgrün). The values are mean of four-repeated sampling and measurements and error bars are standard deviation.
0.29 ± 0.17 d\(^{-1}\), which resulted in a yield of about 30.1 ± 12.3 g m\(^{-2}\) d\(^{-1}\), was observed in August, while lowest growth rates were observed during the winter period. The \textit{L. gibba} attained maximum biomass production between the months of July and August. During this period, the \textit{L. gibba} mat was observed to be very thick and was estimated to have a thickness between 4–6 cm. Mean maximum temperature recorded between July–August was 21.4 ± 6.7°C. Climate data, obtained from a nearby weather station (German Meteorology Service) in Plauen, showed that the sites from June to August has the longest mean monthly sun hours (156–188 h) and mean temperature range from 11.5 to 18.0°C. Inhibition by nutrient limitation and space has been formally assessed (Mkandawire \textit{et al.}, 2004). The high standard deviations in the yield calculation in August (Figure 3) were likely due to the high rainfall recorded during the study period. The fronds might have been washed away from the sampling rings. \textit{L. gibba} grew throughout the winter, but with very low growth rate (estimated to mean 0.01 ± 0.004 d\(^{-1}\)), probably because the lowest water temperature obtained during the winter period was 11.0°C. Nevertheless, production found in this study here still remains above most literature values and equal to values obtained in tropical environments, e.g., southeast Asia (Khang, 2000). Hence, \textit{L. gibba} may qualify for phytoremediation in the mine surface water based on its net primary production, even under of the influence of the mentioned contaminants.

C. Growth Efficiency in the Laboratory

In the laboratory experiments, \textit{L. gibba} attained a mean maximum specific growth rate of 0.37 ± 0.2 d\(^{-1}\) with a yield of 35.7 ± 7.4 g m\(^{-2}\) d\(^{-1}\) in control experiments. It was observed that \textit{L. gibba} exhibited symptoms of toxicity (e.g., reduced growth, chlorosis) when exposed to high concentrations of arsenic in hydroponic culture. Arsenic was observed to be more toxic to \textit{L. gibba} than uranium. Arsenic significantly inhibited the growth rate, while both uranium and arsenic significantly affected the final yield. Biomass production and growth rates in the laboratory experiment were significantly higher than in the field. This is attributed to optimal culture conditions for \textit{L. gibba} growth that prevailed in the laboratory. These ideal conditions included: 1) reduced resource competition, e.g., with algae; 2) no or very little parasite, pathogen, and herbivore attack; and 3) influence of balanced growth in semicontinuous culture mode that, among other things, reduced intraspecific competition for a resource and accumulation of metabolic waste. If, indeed, phytoremediation of uranium and arsenic in surface water is dependent on biomass production, then \textit{L. gibba} qualifies for phytoremediation species because the growth performance in both laboratory experiments and field trials confirmed high turnover.

D. Uranium and Arsenic Bioaccumulation and Stability of Fixation in \textit{Lemna gibba}

The mean uranium and arsenic bioaccumulation were 514.5 ± 83.7 and 519.6 ± 95.4 mg kg\(^{-1}\) dry biomass, respectively, in Site 1 and 612.4 ± 143.6 mg kg\(^{-1}\) and 301.5 ± 64.8 mg kg\(^{-1}\), respectively, in Site 2, while the reference point had 31.5 ± 12.1 and 44.6 ± 17.3 mg kg\(^{-1}\), respectively. The mean background concentrations in surface waters for uranium and arsenic were 186.0 ± 81.2 μg l\(^{-1}\) 47.0 ± 21.3 μg l\(^{-1}\)
FIGURE 4. Influence of uranium and arsenic (100 µg l⁻¹) on the yield and specific growth rates of *L. gibba* under in hydroponic cultures. The nutrient culture was based on standard Hutner solution. The values are mean of four replicates and the error bars are standard deviations.

in Site 1, respectively, and 293.7 ± 121.3 µg l⁻¹ and 41.4 ± 24.7 µg l⁻¹ in Site 2, respectively. Generally, bioaccumulation coefficient for uranium and arsenic in the field were high (Figure 5). *L. gibba* accumulates uranium or arsenic through biosorption onto the frond surface and metabolic driven uptake into the fronds. The latter is usually regarded as a permanent contaminant sink, while the former might act as either temporary or permanent, depending on the biosorption mechanism involved (Mkandawire *et al.*, 2003). Figure 5 indicates that nonleached field samples revealed highest transfer from milieu to *L. gibba* (significant tested with ANOVA *p* ≤ 0.001). Leaching with deionized H₂O was insignificantly higher than EDTA-leached samples. The high accumulation measured in the nonleached samples represents total uranium
and arsenic immobilized by *L. gibba* L. in the surface water through both temporary and permanent processes. The loosely bound contaminants could be simply removed with deionized H$_2$O, while strongly bound surface sorption could be mobilized with decreasing pH (Mkandawire *et al.*, 2003). The difference between deionized H$_2$O and EDTA-leached samples might have been caused by uranium or arsenic bound to low molecular organic compounds exuded by *L. gibba* and associated microbiota.

Differences in the physicochemical environment and temporal variation between the sites may account for higher arsenic accumulation in Site 1 than in Site 2.
Generally, other studies have observed higher background concentration for arsenic in Site 1 than current observed results (Dienemann et al., 2003). For instance, Site 1 was found to have extraordinary amount of Fe ($\sim$300 mg l$^{-1}$), higher P and S concentrations than Site 2. The arsenic bioavailability varies with the pH in the milieu, nature of minerals constituting the sediments, and presence of competing ions. The plant availability of arsenic is influenced by the presence of competing ions such as phosphate, sulphate, and dissolved organic carbon. Phosphate has been earlier shown to decrease the uptake of arsenic in *L. gibba* L. from aquatic environment (Mkandawire et al., 2004). The chemical speciation simulated with PhreeqC predicted high solubility of arsenic above pH 6.5 in the presence of aluminium. This was presumably the pH, at which most of the arsenic was bioavailable and dissolved from sediments. However, simulation of arsenic speciation in the presence of Fe ions under oxidizing conditions predicated reduced soluble species and, consequently low arsenic bioavailability.

### E. Uranium and Arsenic Immobilization Ability

The results show that, under laboratory experimental conditions, *L. gibba* immobilized 84.5% uranium and 88.2% arsenic from 1 L of nutrient solution during a 21-d growth period. Bioaccumulation values are presented in Table 2. Differences of uranium and arsenic concentrations in residual aliquot and on filter residues measured after filtration through a 0.45-$\mu$m pore filter membrane are presented in Figure 6. Similar results were observed and reported (Mkandawire and Dudel, 2002). Since the contribution of microorganisms was estimated at less than 2%, the filter residue uranium and arsenic might be the precipitated large organic uranyl or arsenic complexes.

Inorganic arsenic forms stable complexes with dissolved natural organic matter-bound metals (DeMarco et al., 2003; Gallagher et al., 2001; Kalbitz and Wennrich, 1998). In the current study, the total dissolved organic matter in the *L. gibba* culture after 21 d ranged from 0.30–10.0 mg l$^{-1}$. Arsenic is known to efficiently induce the biosynthesis of metal-binding phytochelatins ([gamma-glutamate-cysteine](n)-glycine) (Lombi et al., 2002; Zhang et al., 2002). It has been reported that *L. gibba* exudes low molecular weight organic acids (e.g., oxalic acid) when exposed to uranium or grown under phosphate deficient environments (Mkandawire and Dudel, 2002). Consequently, they suggested that low molecular weight organic ligands such as oxalic acid exuded by *L. gibba* were responsible for uranium uptake in batch nutrient solutions. This increases the potential of *L. gibba* for uranium or arsenic phytoremediation because it seems that the species immobilizes uranium and arsenic

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<tr>
<th>Bioaccumulation value (mg kg$^{-1}$)</th>
<th>Phytoextraction potential (%)</th>
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<tr>
<td>Arsenic 1021.65 ± 250</td>
<td>48.3 ± 15.1</td>
</tr>
<tr>
<td>Uranium 896.88 ± 203</td>
<td>41.4 ± 11.9</td>
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through additional mechanisms to those known accumulation (uptake) and biosorption. Thus, organic bound uranium or arsenic complexes in tailing ponds, perhaps sink into the sediments contributing to high sediment accumulation found in the study sites by Fritzsche (2003).

F. Extrapolations of Uranium and Arsenic Phytoextraction

Dry biomass production in kg ha\(^{-1}\) was calculated from the dry weight per vessel, culture time (21 d) and extrapolated to a year. Hence, it was possible to estimate the residence time of surface water in a surface pond covered by \(L.\ gibba\) required to reduce uranium or arsenic to standard uranium and arsenic ambient surface water limits with knowledge of the desired contamination reduction factor (e.g., 2–50). Based on the calculated yield and extraction potential rates of 41% and 48% for uranium and arsenic per day, respectively (Table 2), and with assumption that no remobilization takes place, \(L.\ gibba\) L. can remove a maximum of 662.7 kg uranium and 751.9 kg arsenic ha\(^{-1}\) yr\(^{-1}\) in surface waters after desorption of surface complexed uranium or arsenic with EDTA. This prognosis indicates that \(L.\ gibba\) can reduce 100 \(\mu\)g l\(^{-1}\) uranium or arsenic contamination in 1000 L of waters to the German recommended limits of 30.0 \(\mu\)g l\(^{-1}\) uranium in ambient surface waters and World
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Health Organization (WHO) recommended limits of 10.0 µg l\(^{-1}\) arsenic in drinking water limits (Robinson *et al.*, 2003) within a week under the laboratory yield and extraction potential.

Similarly, under the established background concentration for uranium and arsenic in the surface waters, *L. gibba* can extract 37.6 and 6.6 kg l\(^{-1}\) y\(^{-1}\) uranium and arsenic from the surface water, respectively. Lemnaceae are able to remove and accumulate high levels of heavy metals from polluted water (Babu *et al.*, 2001; Hasar and Obek, 2001; Nasu and Kugimoto, 1981). The removal of uranium and arsenic were, however, related to the physicochemical properties of the aquatic systems like pH, redox potential, conductivity, dissolved oxygen, and ions in the solution particularly Fe, Al, S, and P. As much as these results look promising for phytoremediation, a few research groups have reported that *L. gibba* is relatively sensitive to changes in the environment, particularly to organic compounds (Fairchild *et al.*, 1997; Wang, 1986, 1990). Hence, its phytoremediation capacity can be limited in situations where the *L. gibba* yield is affected by toxicity, e.g., arsenic.

IV. CONCLUSION

The amount of uranium and arsenic removed, the yield, and the rapid turnover obtained in this study suggests that *L. gibba* is potentially a phytoremediation species for uranium, arsenic, and other heavy metals of concern in surface waters of abandoned uranium mines. The plant acted as both short- and long-term sink of uranium and arsenic from the contaminated surface waters. If uranium and arsenic in the *L. gibba* biomass are trapped permanently, then biomineralization of uranium and arsenic could be facilitated upon death and the sedimentation of the plant biomass. However, there still exist some uncertainties with the potential, capacity, and limits of uranium and arsenic immobilization, particularly during decomposition or humification of dead *L. gibba* fronds. Therefore, the stability of the long-term contaminant sink in the presence of microbial activities and during the decay process should be carefully considered. Further, the effectiveness of *L. gibba* in large and deep water reservoirs and during high runoff in storm weather might be limited due to its size. Due to its floating and size, *L. gibba* easily can be transported with flowing water, which results into the transfer of contaminants once remobilization takes place through either desorption or decay. Hence, further studies to ascertain the extent of uranium and arsenic remobilisation through decay and other microbial process, influence of geochemical changes following physical and metabolic gaseous exchanges, and exudates in the total aquatic system under *Lemna gibba* mats are necessary. Nevertheless, *L. gibba* presents good quality for a model plant to study phytoremediation processes and a mechanism of uranium and arsenic contaminated surface waters. It can also be used as a member of the phytoremediation plant community in a constructed wetland.

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