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Bulletin of Environmental Contamination and Toxicology

ISSN 0007-4861
Volume 97
Number 6

Bull Environ Contam Toxicol (2016)
97:832-837
DOI 10.1007/s00128-016-1929-x

Bulletin of Environmental Contamination and Toxicology

CELEBRATING 50 YEARS OF PUBLICATION 1966 – 2016

Volume 97 Number 6 December 2016

Contents continued on back cover

128 BECTA6 97(6) 743–888 (2016) ISSN 0007-4861

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Bioaccumulation of Uranium and Thorium by *Lemna minor* and *Lemna gibba* in Pb-Zn-Ag Tailing Water

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Received: 3 July 2016 / Accepted: 21 September 2016 / Published online: 23 September 2016

Abstract This study focused on the ability of *Lemna minor* and *Lemna gibba* to remove U and Th in the tailing water of Keban, Turkey. These plants were placed in tailing water and individually fed to the reactors designed for these plants. Water and plant samples were collected daily from the mining area. The plants were ashed at 300°C for 1 day and analyzed by ICP-MS for U and Th. U was accumulated as a function of time by these plants, and performances between 110% and 483% for *L. gibba*, and between 218% and 1194% for *L. minor*, were shown. The highest Th accumulations in *L. minor* and *L. gibba* were observed at 300% and 600% performances, respectively, on the second day of the experiment. This study indicated that both *L. gibba* and *L. minor* demonstrated a high ability to remove U and Th from tailing water polluted by trace elements.

Keywords Aquatic plants · Bioaccumulation · Uranium · Thorium · Tailing water

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Although uranium (U) and thorium (Th) occur naturally in different geologic environments, drinking water, and food (WHO 2001; ATSDR 2013), they have consequences to human health due to the carcinogenic character and high chemical/radiological toxicities (USEPA 2002; Craft et al. 2004; Bhalara et al. 2014). U ores and their operation are a common anthropogenic source of U. Studies on Th have revealed that Th dust can cause an increase in lung disease, pancreatic cancer, and lung cancer (USEPA 2002). These serious health effects show the need for the decontamination of such areas, which can be achieved through phytoremediation (Lottermoser 2003; Pratas et al. 2014).

Phytoremediation is a method of decontamination that uses plants to substantially or partially remediate the metals or contaminants in sediment, sludge, soil, mining water, waste water, or ground and surface water. This method is also called agro-remediation, green remediation, vegetative remediation and botano-remediation (USEPA 2001). Aquatic macrophytes play a significant role in the protection of aquatic ecosystems, in particular their ability to remove heavy metals makes these plants an attractive candidate for the treatment of sewage, waste water, and industrial effluents (Mkandawire et al. 2004; Sood et al. 2012). The phytoremediation potential of aquatic macrophytes for heavy metals has been studied by Srivastava et al. (2008), Marques et al. (2009), Sasmaz and Obek (2009, 2012), Khan et al. (2009), Goswami et al. (2014), and Tatar and Obek (2014). Some differences in the accumulation potential of heavy metals have been observed.

Aquatic macrophytes have the fastest reproduction and growth rates as compared to terrestrial plants under different climatic conditions (Materazzi et al. 2012). Metal accumulation or uptake by plants has been extensively investigated in the literature, and *L. gibba* and *L. minor*, from the duckweed family, have been used as model plants. *L. minor* and *L. gibba* commonly occur in wetlands. They adapt easily to varying conditions, grow quickly, and have great potential to remove contaminants from water (Dirilgen 2011; Rahman and Hasegawa 2011; Obek and Sasmaz 2011; Materazzi et al. 2012; Bocuk et al. 2013; Rofkar et al. 2014; Tatar and Obek 2014; Pratas et al. 2014; Favas et al. 2014; Goswami et al. 2014; Iqbal and Khera 2015; Babarinde and Onyiaocha 2016; Sasmaz et al. 2016; Babarinde et al. 2016). The contaminant-removing ability of these plants has been studied to investigate the removal of U from the contaminated water of U mining areas (Pratas et al. 2012, 2014; Favas et al. 2014; Wang et al. 2015; Qureshi et al. 2015; Matveyeva et al. 2016; Iqbal 2016; Jha et al. 2016). The aim of this study was firstly to investigate U and Th levels in environmental contaminants in the Keban tailing water, which flows into the Karakaya Dam Lake, secondly to remove these metals from the tailing water by using *L. minor* and *L. gibba*, and finally to detect accumulation abilities of these plants for U and Th.

Materials and Methods

The Keban mining area (Fig. 1) has been chosen because it is one of the biggest and abandoned Pb-Zn-Ag deposits in Turkey. The syenomonzonite and syenitic rocks around Keban also have high Pb, Ag, Zn, Cu, and As concentrations, and poly-metallic mineralizations such as F–Mo, Fe–Cu, Zn–Pb, and Ag-Mn have been observed there. Among the different types of mineralizations in Keban, Pb–Zn ores with high silver concentration are the largest economic deposits, mined for 6000 years according to Seeliger et al. (1985). Pb-Zn-Ag ores have been produced by these mining galleries (Akgul 2015), which were closed because of security reasons, but the galleries have common effluents. The chemical composition of this water can vary, depending on the type of mineralization and the composition of wall rocks.

In this location, the water samples, together with plant samples, were sampled daily. When these samples

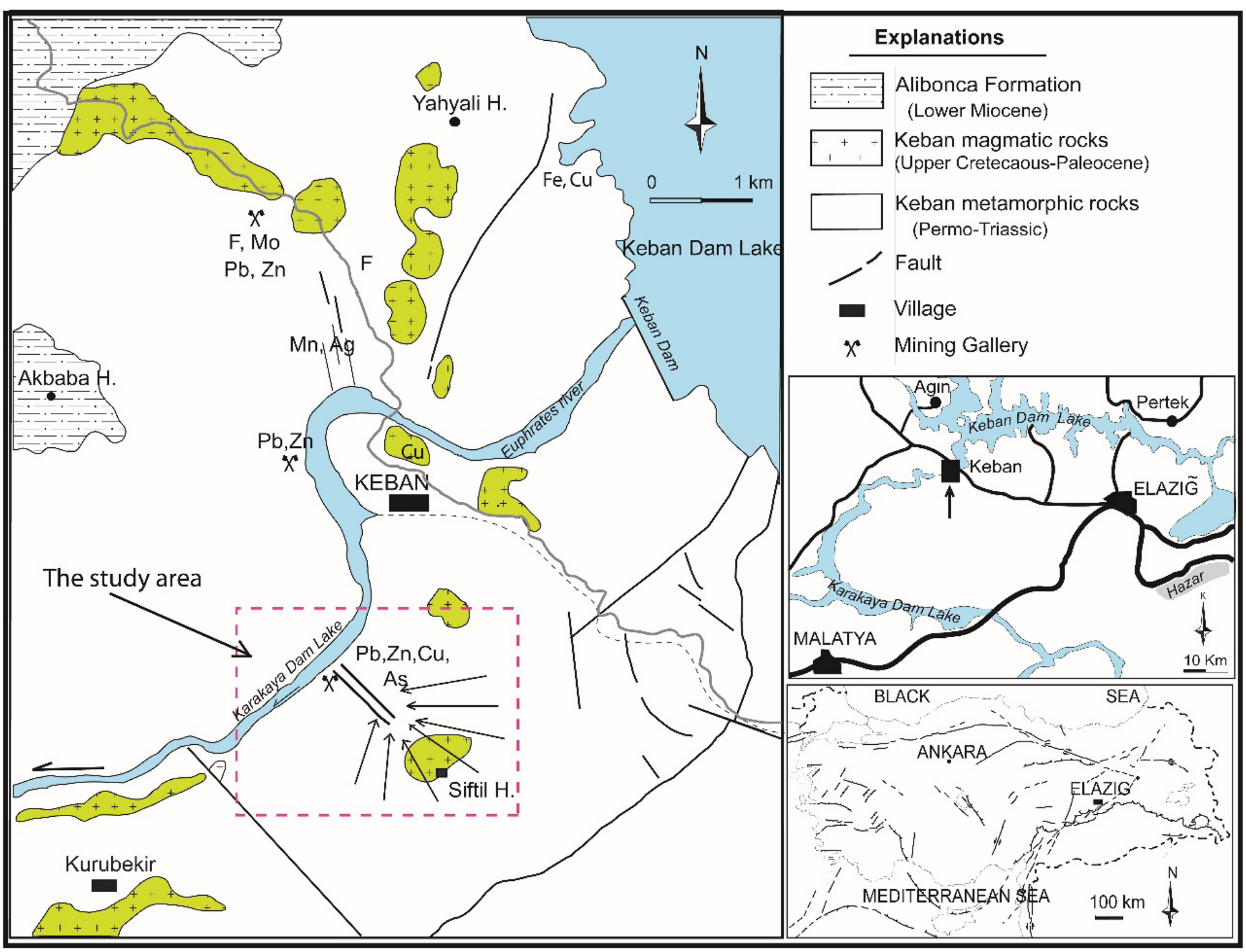

Fig. 1 Geological and location map of the study area (simplified from Akgul 2015)

collected from the study area during an eight-day period, in the same time, the electric conductivity (EC), T °C, and pH of the tailing water were also measured. The T °C, EC, and pH were determined using a digital thermometer, an Orion conductivity electrode, and an Oaktan pH tester 30, respectively. The plants were systematically identified as *L. minor* and *L. gibba*, according to Davis's recommendation (1984).

The *L. minor* and *L. gibba* plants were brought to Firat University's laboratory from Istanbul University with separate containers, after that, adapted in separate reactors, and placed in each reactor (Fig. 2) as described in the details provided by Sasmaz et al. (2015). These reactors were operated under a sustained regime of flow volume (2.85 L s^{-1}) of tailing water. Both *L. minor* and *L. gibba* samples were collected from the reactors daily throughout the experiment. The plant samples were washed with distilled water and then dried in an oven for 1 day. The dried plants were heated at 300°C to be ashed; then they were digested in HNO_3 (Merck, Darmstadt, Germany), mixed with HNO_3 and HCl at 95°C for 1 h, and analyzed with ICP-MS for U and Th. The operation conditions of A Perkine Elmer Elan 9000 ICP-MS to determine U and Th are given in Sasmaz and Yaman (2008). Minumum detection limits of ICP-MS are 0.01 mg kg^{-1} for U and Th in the plants and 0.02 and 0.05 µg L^{-1} for U and Th in the water, respectively.

The U and Th accumulation potentials for *L. gibba* and *L. minor* were calculated by the following formula. The accumulation potential of Th for the second day in *L. gibba* = (LG2–LG0)/LG0; The accumulation potential of U for the eighth day in *L. minor* = (LM8–LM0)/LM0. The analysis of variance (ANOVA) from SPSS 15.0 software was used. The U and Th values belonging to *L. minor* and *L. gibba* were correlated with Na, Mn, Al, Fe, P, Mg, S, and K, using Spearman's correlation.

Fig. 2 *L. gibba* L. and *L. minor* L. were separately placed in each reactor

Results and Discussion

The physicochemical parameters, anions, and cations of tailing water have been given by Sasmaz et al. (2015), except for U and Th concentrations. The mean U and Th concentrations in the tailing water were detected to be 42 and 0.22 µg L^{-1}, respectively, in this study ($p < 0.5$). The mean pH, temperature, and EC values were 7.36, 19.7°C, and 2.29 mS cm^{-1}, respectively; these values remained fairly consistent/regular throughout the experiment. The mean U concentration in the study area was higher than the limit value (15 µg L^{-1}) of drinking water established by the WHO (2005). While Palmer and Edmond (1993) reported that the average U value in river water is ~0.3 µg L^{-1}, Favas et al. (2014) indicated that it has high concentrations (139 µg L^{-1}) such as two mining areas in central Portugal. At these points, U concentrations could be directly linked to mining activities since these streams were directly fed by mine drainage (Favas et al. 2014). U (VI) predominately occurs in an acidic environment (pH < 4.0) as UO_2^{2+}; at higher pH ranges (4.0 < pH < 7.0), composite hydrolyzed ionic species occur, such as $(UO_2)_3(OH)_5^{+}$, $(UO_2)_2(OH)_2^{2+}$, and UO_2OH^{+}. The average pH (7.36) of the tailing water in the study area is higher than 7.0 and, therefore, Wang et al. (2010) indicated that U (VI) easily precipitated in the water when the pH of tailing water was above 7.0.

Based on common cations (Mg^{2+}, Ca^{2+}, K^{+}, and Na^{+}) and anion (Cl^{-}, HCO_3, NO_3^{-}, and SO_4^{2}) contents, Mg, Na, and Ca are the dominant metals and represent greater than 90 % of the composition of total cations. Sulfate and bicarbonate are the most dominant anions in the studied tailing water and represent 88–95 % of the total anion composition in the tailing water. In this study, the water in the selected area has been classified as calcium-magnesium-sulfate-bicarbonate water, based on total ion content.

The U levels before the experimental study of *L. gibba* (LG-0) and *L. minor* (LM-0) are 0.42 and 0.33 mg kg^{-1}, respectively ($p < 0.5$). These U levels for both species are defined as the control group values of this study. From the first day, *L. gibba* and *L. minor* accumulated, respectively, 0.88 and 1.05 mg U kg^{-1} on a daily basis. In the study, the values of U that *L. gibba* removed from low U concentration tailing water increased to 110 % the first day, to 131 % the second day, and to 200 %, 252 %, 293 %, 326 %, 381 %, and 483 % the following days ($p < 0.5$). The amounts of U absorbed by *L. minor* were 218 % the first day, 282 % the second day, and 406 %, 530 %, 497 %, 797 %, 945 %, and 1194 % the following days ($p < 0.5$). As presented in Fig. 3, the accumulation of U by *L. gibba* and *L. minor* increased linearly throughout the experiment. Although the tailing water had very low U concentrations (mean U concentration: 42 µg L^{-1}), *L. gibba* and *L. minor* accumulated, respectively, 58 and 102 times more U than was originally

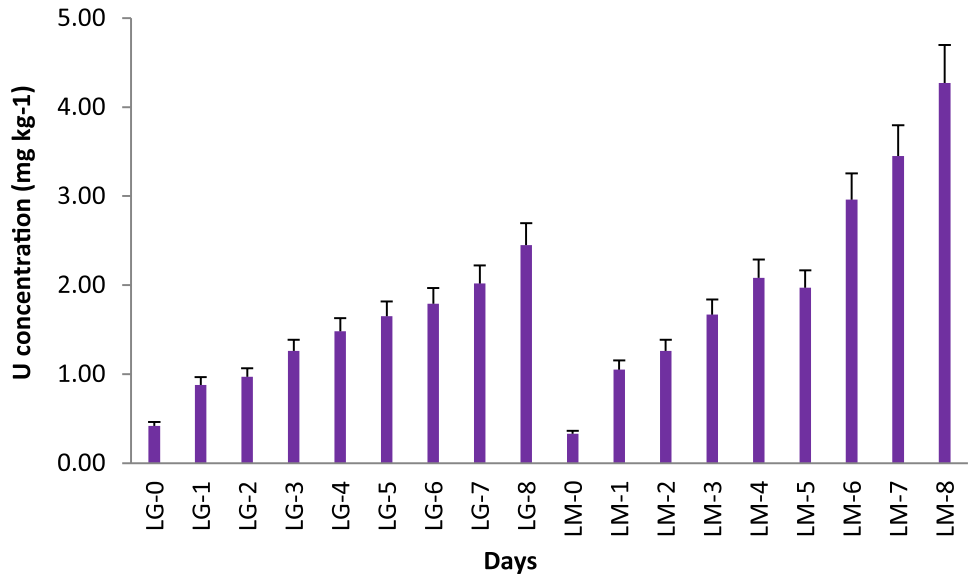

Fig. 3 Uranium accumulations by *Lemna gibba* (LG) and *Lemna minor* (LM) under a sustained regime of flow volume (2.85 L s^{-1}) of tailing water

Table 1 Spearman's correlation coefficients between some metals with U and Th in *Lemna gibba*

	Mn	Fe	Ca	Mg	Na	Al	K	P	S
U	0.87	−0.56	−0.61	0.47	0.35	0.86	−0.81	0.88	0.93
Th	0.96	−0.53	−0.28	0.33	0.13	0.78	−0.64	0.65	0.68

Table 2 Spearman's correlation coefficients between some metals with U and Th in *Lemna minor*

	Mn	Fe	Ca	Mg	Na	Al	K	P	S
U	0.66	−0.11	0.15	−0.26	0.14	0.64	−0.58	0.26	0.95
Th	0.89	−0.13	0.14	−0.17	−0.48	0.36	−0.13	0.19	0.69

contained in the tailing water. *L. minor* demonstrated a higher ability to remove U compared to control group concentrations (Fig. 3). U had a strong positive correlation ($p<0.5$) with the S, P, Mn and Al in *L. gibba* (Table 1) and S, Mn and Al in *L. minor* (Table 2). U showed strong negative correlations with K, Ca and Fe in *L. gibba* (Table 1) and K in *L. minor* (Table 2).

For both *L. gibba* (LG-0) and *L. minor* (LM-0), Th values before the experimental study were 0.01 mg kg^{-1}, which was also defined as a control group value in this study (Fig. 4). From the first day, *L. gibba* and *L. minor* accumulated, respectively, 0.02 and 0.02 mg Th kg^{-1} on a daily basis ($p<0.5$). Th concentrations in *L. gibba* increased to 100% the first day, to 600% the second day, and to 400%, 200%, 500%, 600%, 500%, and 800% the following days

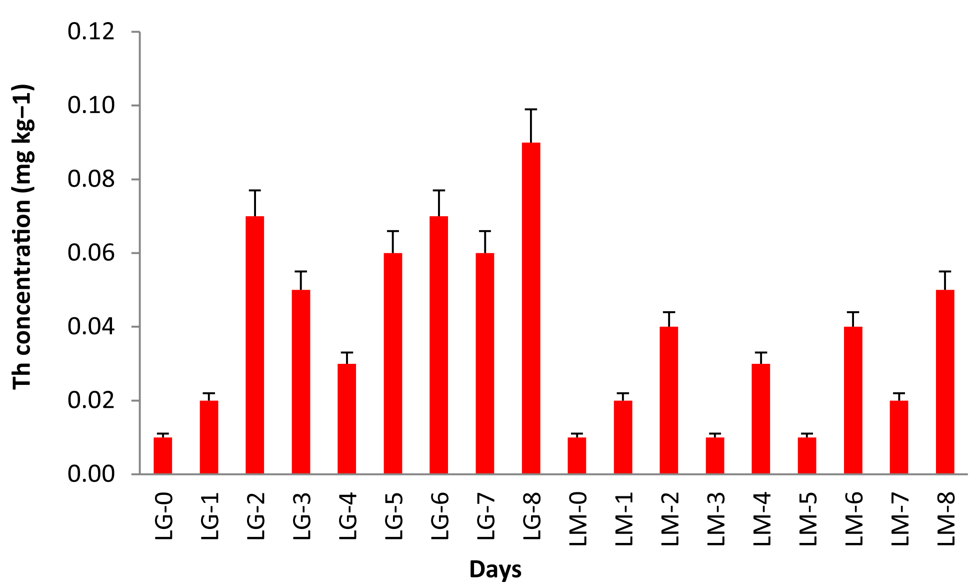

Fig. 4 Thorium accumulations by *Lemna gibba* (LG) and *Lemna minor* (LM) under a sustained regime of flow volume (2.85 L s^{-1}) of tailing water

($p<0.5$). As *L. minor* removed Th, these concentrations increased to 100 % the first day, to 300 % the second day, and then varied to 200 % the fourth day, 300 % sixth day, 100 % seventh day, and 400 % eighth day ($p<0.5$). But it was observed to release back Th into the water on the third and fifth days. Soldo et al. (2005) reported that the reason of the de-accumulation of metals to the external environment by organisms are the interception of the potential toxic effects of metals to the structures as DNA and protein. Although the tailing water had very low Th content (mean Th concentration: 22 μg L^{-1}), *L. gibba* and *L. minor* removed 409 and 227 times more Th than was originally contained in the tailing water.

As seen in Fig. 4, the removal of Th by *L. gibba* significantly increased in the second, sixth, and eighth days of the experiment; afterwards, this was seen by decreases and increases in the removal of Th. *L. minor* was also observed to have considerable increases in Th levels during the second, fourth, sixth and eighth days, and decreases were shown on the third, fifth, and seventh days. *L. gibba* was detected to have a higher Th-removal ability than *L. minor*, compared to control group Th concentration for both *L. minor* and *L. gibba* (Fig. 4). Th had a strong positive correlation ($p<0.5$) with the Mn, Al, P and S in *L. gibba* (Table 1) and Mn and S in *L. minor* (Table 2). Th showed weak negative correlations with K and Fe in *L. gibba* (Table 1) and Na in *L. minor* (Table 2).

Based on their study of U mining in Saxony, Germany, Mkandawire and Dudel (2005) pointed out that *L. gibba* was one of the best examples of accumulator plants, showing that its U concentration ranged between 514 and 612 mg kg^{-1} U dry biomass. Sasmaz and Obek (2009) studied the accumulation performance of *L. gibba* for U from secondary effluents, and they indicated that *L. gibba* significantly accumulated the highest concentration (122 %) of U in the first 2 days. However, in the following days, some decreases and increases were seen in the accumulation level, depending on the saturation level of the plants. Members of *Lemnaceae* (duckweeds), including *Lemna, Spirodella, Wolffia*, and *Wolffiella*, are the most favored plants for phytoremediation, and they have been intensively described in the literature (Miretzky et al. 2004; Rahman et al. 2007; Pratas et al. 2012). Pratas et al. (2012) found a set of vegetable species with the ability to accumulate U in higher values despite very low U content in the fresh water of central Portugal. The highest U concentration was found in filamentous algae in the residual water of the tailing pond at Jaduguda, India. U level in filamentous algae was detected to change between 0.1 and 97.8 mg kg^{-1} (Khalid et al. 2004). These species showed great potential for phytoremediation because they were rampant and easy to grow in their native conditions. Among free floating and submerged plants filamentous algae, *Jussiaea* and *Pistia* had high bioproductivity, biomass and uranium accumulation (Jha et al. 2016).

The aquatic plants *L. gibba* and *L. minor* were used for the accumulation of U and Th in tailing water belonging to a Pb–Zn mining area as an alternative method for cleaning and rehabilitating water contaminated with U and Th. The results demonstrate that U was absorbed by *L. gibba* and *L. minor* with a linear increase during the eight-day period. Th was absorbed effectively by both *L. gibba* and *L. minor* on the first 2 days of the experimental study. On the following days, increases and decreases in the accumulation performances of Th were seen, likely due to the saturation levels of the plants being reached. As a result, this study was revealed to be a feasible and cost effective method for the removal of U and Th by the phytoremediation from radioactively contaminated water. Therefore, it is recommended that these plants should be harvested at the right time for the protection of human health and the environment because they contained more concentrations of U and Th in stated days. Future studies should be focused on cleaning and rehabilitating waters contaminated with U and Th in mining areas and municipality wastewater treatment plant.

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