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ARTÍCULO

Bioconcentration efficiency of *Lemna minor* L. and *Lemna gibba* L. for trace metals in three southeastern Bulgarian water reservoirs

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Resumen

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Eficiencia de bioconcentration de Lemna minor *L. and* Lemna gibba *L. para metales traza en tres embalses del sureste de Bulgaria*

La lenteja de agua se utiliza en el tratamiento de aguas residuales para eliminar sustancias nocivas, minerales y contaminación orgánica. El propósito del presente estudio fue investigar la eficiencia de bioconcentración de metales pesados en *Lemna minor* y *Lemna gibba* provenientes de tres embalses del sureste de Bulgaria: el canal de la ciudad de Elhovo, el canal del lago Vaja y el canal del lago Mandra. Se analizaron los contenidos de proteína cruda, lípidos y ceniza en las especies estudiadas de *Lemna*. Los metales pesados en agua y plantas acuáticas se determinaron con un espectrómetro de absorción atómica (AAS) "A Analyst 800" - Perkin Elmer. Las concentraciones de metales en *L. minor* y *L. gibba* mostraron una tendencia descendente: Mn>Fe>Zn>Cu>Ni>Cr>Pb>Cd en las tres masas de agua.

Palabras clave: Bioconcentración; Metales pesados; *Lemna minor*; *Lemna gibba*; Proteínas.

Abstract

Duckweed is used in wastewater treatment to remove inorganic substances, mineral and organic contamination. The purpose of present study was to investigate a *Lemna minor* and *Lemna gibba* bioconcentration efficiency of trace metals in three southeast Bulgarian water reservoirs. Three waterbodies located on the territory of South East Bulgaria: water canal town Elhovo, water canal Vaja Lake and water canal Mandra Lake was studied. Crude protein, lipid, ash content in studied species of *Lemna* were analyzed. The trace metal content in water and aquatic plants was determined on an atomic absorption spectrometer (AAS) "A Analyst 800" - Perkin Elmer. The concentrations of metals in both *L. minor* and *L. gibba* and for all sites showed a downward trend: Mn>Fe>Zn>Cu>Ni>Cr>Pb>Cd.

Key words: Bioconcentration; Heavy metal; *Lemna minor*; *Lemna gibba*; Protein.

Introduction

The population of the land is constantly increasing which lead an increased anthropogenic impact on the biosphere. Pollutants such as heavy metals, oil and grease, phenols, sulphide, sulphate, nitrate, phosphate, dissolved solids, suspended solids impact negatively on the environment (Patel & Kanungo 2010, Nayyef *et al.* 2012). These elements in the state of ions, penetrating into the body of the hydrobionates, have a powerful toxic effect, most often as denaturation of important enzyme and transport proteins. Different plants are increasingly used to accumulate and remove pollution from water, soil and air (Gijzen & Kondker 1997). This is eco and efficient technology in which natural properties of the plant are used to remove harmful substances from wastewater (Patel & Kanungo 2010, Ugya 2015). Aquatic plants respond even to temporary deviations of the chemical and physical reference values, which is sufficient to suggest that these organisms could be used as an important biological marker for the purity of a given hydro-ecosystem. One of the most common hydrobionites that serve as a filter for toxins of a different nature is the species of the genus *Lemna* L. (Chaudhary & Sharma 2014, Axtell *et al.* 2003). This species has been successfully used to assess the heavy metal content of freshwater ecosystems. *Lemna minor* L. and *Lemna gibba* L. are aquatic plants floating on the surface of still or slow-moving fresh water bodies and often form dense floating mats in eutrophic ditches and ponds (Driever *et al.* 2005). These plants adapt easily to different environmental terms. They have high protein content and are a source of food for waterfowl, fish, and small invertebrates and provide habitat for a number of small organisms (Van Hoeck *et al.* 2015). It is therefore very important to analyze how much of the pollution in the *Lemna* pool accumulates in its tissues. Duckweed is also used in wastewater treatment to remove mineral and organic contamination (Velichkova *et al.* 2017). It has a high metal accumulation potential, which is particularly important for local dirt. It is considered a reliable bioindicator for both passive and active biomonitoring of water bodies (Jayasri & Suthindhiran 2017). The purpose of present study was to investigate the *L. minor* and *L. gibba* bioconcentration efficiency for heavy metals of three southeastern Bulgarian water reservoirs.

Materials and methods

Plant material and water

Three water-bodies located on the territory of South East Bulgaria: water canal town Elhovo (Tundzha River) (42°09'57"N, 26°33'23"E), water canal Vaja Lake (42°30'37"N, 27°24'17"E), water canal Mandra Lake (42°24'43"N, 27°20'24"E) were studied. These water-bodies pass through big settlements, industrial and agricultural regions and is a precondition for their pollution with toxicants of different nature. Waters of these water-bodies are used for irrigation and fish farming.

Water samples from the studied water bodies were collected on June 2016 in accordance with the requirements of EN ISO 5667-1/2007. Water samples were collected from a depth of 0.5-1 m using 1.5 L PET bottles. The water samples were stored in accordance with EN ISO 5667 - 3/2006. The measurement of pH, temperature and conductivity was made with a portable combined meter and with a pH probe (Hach Lange, Germany). Other analyzed hydrochemical parameters ammonium (mg l^{-1}), nitrites (mg l^{-1}), nitrates (mg l^{-1}), and phosphate (mg l^{-1}) were measured spectrophotometrically with a spectrophotometer DR 2800 (Hach Lange, Germany) with appropriate cuvette tests (Hach Lange, 2007).

The samples of the studied aquatic plants from water bodies were collected, stored and analyzed. From each pond plant samples were put in clean plastic water tanks (10 L) and labeled carefully by permanent marker. In the laboratory, the plant material (10 g) was washed carefully to remove dirt, sludge, and other adhesive debris from it. All the collected plant samples were placed in newspapers for the absorption of excess water and dried.

Water and aquatic plants samples were analyzed in the laboratories of the Environment Research Center at the Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

Experimental method

Crude protein content (%) was calculated by converting the nitrogen content, quantify by Kjeldahl's method, using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content (%) was determined by the method of Soxhlet, using an automatic system (Soxtec 2050, FOSS, Sweden). Ash content (%) was investigated by incineration in a muffle furnace (MLW, Germany) at 550 °C for 8 h. Crucibles were brought about the room temperature and weighed.

The heavy metal in water and aquatic plants was determined on an atomic absorption spectrometer (AAS) "A Analyst 800" - Perkin Elmer. Analyses for heavy metal in surface water samples were conducted in graphite tube or flame (depending on the concentration of these elements), at a definite wavelength and preliminary preservation of water in samples with 5 cm^3 concentrated HNO3 per sample (ISO 8288, BS EN ISO 5667-3/2006). The contents of heavy metal in water samples were measured in mg kg^{-1}. The capacity of plants to absorb and accumulate metals from the water was evaluated using their bioconcentration factor (BCF). BCF was calculated as the ratio of the concentrations of metals in aquatic plant and water (Hawker & Connell 1991): BCF = [Metal] plant / [Metal] water .

The samples of aquatic plants were prepared for analysis by combustion in a microwave oven Perkin Elmer Multiwave 3000. The extracts were extended up to 25 ml with distilled water. The metal concentrations in the acid solutions were amended of AAS in accordance with ISO 11047. The concentrations of the investigated element of aquatic plants were expressed as mg kg^{-1} dry weight.

The instrument was periodically calibrated with standard chemical solutions prepared from commercially available chemicals (Merck, Germany). An air-acetylene flame and hollow cathode lamp for all samples were used. Calibration curves were prepared using dilutions of stock solutions. The samples (water and aquatic plants) were measured three times and the mean values were calculated.

Data analyses were conducted by using one-way Analysis of Variance ANOVA (MS Office, 2010).

Results and discussion

The results of the chemical composition of the studied duckweed are given in table 1.

The highest amount of protein was measured in *L. minor* (21.80 %), which was 14.2 % more than the least measured in *L. gibba* (18.71). With regard to crude lipid *L. minor* has higher quantity lipids (0.93 %), which is 30 % more than *L. gibba*. The content of NFE is a little higher in *L. gibba*. With regard to the raw fiber is observed at a large quantity in *L. gibba* (10.17 %), and less in *L. minor* (7.50 %). All these results show the good nutritional value of species of the genus *Lemna* and are prerequisite for their use in food rations of fish, birds, swine and other animals (Drost *et al.*, 2007).

	Moisture	Dry matter	Crude protein	Crude lipid	Crude fiber	Ash	*NFE
***L. minor* (Vaja)**	5.64	94.36	21.80	0.93	7.50	22.53	41.60
***L. gibba* (Elhovo)**	5.58	94.42	20.36	0.80	10.05	21.94	41.27
***L. gibba* (Mandra)**	5.89	94.11	18.71	0.56	10.17	21.87	42.80

*NFE - nitrogen free extract

Tabla 1. Composición química de *L. minor* y *L. gibba* en los cuerpos de agua estudiados. En porcentaje.

Table 1. Chemical composition of *L. minor* and *L. gibba* in the studied water bodies. As percentage.

The chemical composition of the water of the Vaja, Elhovo, Mandra are presented in a table 2.

According to various authors (Chawla *et al.* 1991, Körner *et al.* 2001, Kaur *et al.* 2013), there is a correlation between the values of pH, temperature and the accumulation of heavy metals of

	pH	Temperature °C	Conductivity µS cm^{-1}	NH_4^+ mg l^{-1}	NO_3^- mg l^{-1}	NO_2^- mg l^{-1}	PO_4^- mg l^{-1}
Vaja	6.46 ± 0.1	22 ± 0.02	109 ± 8	0.041 ± 0.11	0.038 ± 0.01	0.003 ± 0.002	0.36 ± 0.03
Elhovo	6.27 ± 0.2	22.5 ± 0.03	98 ± 6	0.038 ± 0.01	0.04 ± 0.01	0.002 ± 0.001	0.38 ± 0.02
Mandra	6.32 ± 0.1	23 ± 0.02	116 ± 5	0.04 ± 0.02	0.042 ± 0.02	0.003 ± 0.001	0.35 ± 0.03

Tabla 2. Composición química de los cuerpos de agua estudiados. Media ± SD.

Table 2. Chemical composition of the studied water bodies. Average ± SD)

	Vaja			Elhovo			Lake Mandra		
	Water	***L. minor***	**BCF**	**Water**	***L. gibba***	**BCF**	**Water**	***L. gibba***	**BCF**
Fe	0.6630 ± 0.09	1309.95 ± 3.43	1975.80	1.5630 ± 0.08	1324.71 ± 4.9	847.50	1.4550 ± 0.08	1317.26 ± 4.8	905.30
Zn	0.4980 ± 0.023	90.91 ± 0.54	182.60	0.5210 ± 0.009	98.07 ± 40.2	188.20	0.6280 ± 0.009	92.63 ± 41.2	147.50
Mn	0.2750 ± 0,09	2985.80 ± 3.53	10857.50	0.2610 ± 0.07	2776.47 ± 5.3	10637.80	0.3480 ± 0.07	2792.34 ± 4.6	8024
Ni	0.0930 ± 0.02	0.4678 ± 0.08	5.03	0.0748 ± 0.025	0.4957 ± 0.07	6.60	0.0657 ± 0.025	0.4838 ± 0.07	7.40
Cu	0.1260 ± 0.05	8.93 ± 2.5	70.90	0.1090 ± 0.04	8.07 ± 1.6	74.04	0.1390 ± 0.04	9.82 ± 2.6	70.60
Pb	0.0325 ± 0.014	0.1871 ± 0.07	5.60	0.0267 ± 0.01	0.1984 ± 0.04	7.40	0.0201 ± 0.01	0.1935 ± 0.06	9.60
Cd	0.0083 ± 0.002	0.0465 ± 0.01	5.61	0.0065 ± 0.002	0.0498 ± 0.01	7.70	0.0051 ± 0.002	0.0480 ± 0.03	9.40
Cr	0.017 ± 0.01	0.1997 ± 0.06	11.70	0.017 ± 0.015	0.2104 ± 0.08	12.40	0.009 ± 0.015	0.2056 ± 0.08	22.80

Tabla 3. Concentraciones medias (mg kg^{-1}) ± (SD) (n=3) de metales en agua de Vaja, Elhovo y lago Mandra, en *L. minor* y *L. gibba*, y factor de bioconcentración (BCF)(planta/agua) para cada localidad.

Table 3. Average concentrations (mg kg^{-1}) ± (SD) (n=3) of metals in water from Vaja, Elhovo and lake Mandra, in *L. minor* and *L. gibba,* and bioconcentration factor (BCF) (plant/water) for every locality.

the species from genus *Lemna*. The highest bioconcentration potential of heavy metal was observed by *Lemna* sp. at pH 6 and temperature higher than 22 °C (Singh *et al.* 2012). In our studied reservoirs the measured pH (6.27-6.46) and temperatures (22-23 °C) are close to the optimum for the accumulation of the species. At the level of ammonium and nitrate ions in the water reservoirs 0.04-0.68 mg l^{-1} the accumulation capacity of the *Lemna* sp. gradually increases (Hammouda *et al.*, 1995). Similar low concentrations of these ions were also measured in our studied ponds.

On the protein content also influences and the amounts of heavy metals in water bodies in which duckweeds are located. Minimal amounts of the metals (0.05 mg l^{-1}) have an inhibitory effect on the protein content (Miranda *et al.* 2000, Hou *et al.* 2007). Probably that is the reason for the lower protein content in our tested species compared to the higher values reported for pure cultures.

The concentrations of metals in the *L. minor* followed a downward trend (Table 3):

Mn>Fe>Zn>Cu>Ni>Cr>Pb> Cd

The bioconcentration (BCF) capacity of *L. minor* is shown through its bioconcentration factors, indicating a decreasing order as follows:

Mn>Fe>Zn>Cu>Cr>Cd>Pb> Ni

It is noticeable that the bioconcentration capacity of *L. minor* for Mn is thousand times higher than it is for the other metals.

There is no difference in the order of the Mn, Fe, Zn and Cu content in a plant compared to the sequence of their bioconcentration ability. The difference in the order of another metal content in a plant compared to the sequence of their accumulation ability can be seen in table 3. This tendency suggests the different accumulation capacity of macrophytes for certain metals. Plants accumulate certain metals irrespective of their concentrations in the water, which is obviously a characteristic provided by its capacity for the accumulation of each individual element (Kastratović *et al.* 2015).

The concentrations of metals in the *L. gibba* followed a downward trend (table 3):

Mn>Fe>Zn>Cu>Ni>Cr>Pb>Cd

Comparison of levels of elements in each of the examined groups of plants proved that the highest concentrations of Mn, Fe, and Zn are found in *L. minor* and *L. gibba*. The bioconcentration (BCF) capacity of *L. gibba* from water Elhovo is shown through its bioconcentration factors, indicating a decreasing order as follows:

Mn>Fe>Zn>Cu>Cr>Cd>Pb>Ni

These results show a correlation between a higher metal content in the water and hence a greater bioconcentration factor. Accumulation of metal in macrophytes depends on elements concentration in the water.

The bioconcentration capacity of *L. gibba* from water Mandra Lake is shown through its bioconcentration factors, indicating a decreasing order as follows: Mn>Fe>Zn>Cu>Cr>Pb>Cd>Ni

The differences of concentrations order of microelements in *L. gibba* from the two water bodies is only for Cd and Pb. This can be explained by the eventuality exhibit synergism by Cd and Ni. Positive correlations were found between Fe, Zn, Mn and Cu contents in water and in plants.

The results obtained in table 3 show that *L. gibba* is more effective in the accumulation of metals compare to *L. minor*. Again, the bioconcentration capacity of *L. gibba* for Mn is thousand times higher than it is for the other metals.

Similar results for the order of accumulation concentration of the metals in *L. minor* have been obtained Kastratović *et al.* (2015) in the study of Skadar Lake. Doganlar *et al.* (2012) investigated the effects of manganese and nickel on *L. gibba* under laboratory conditions. They received that Mn accumulation higher than Ni, which agree with our results. Each of these heavy metals in any way negatively influencing on the duckweeds. Cadmium inhibited duckweed growth even at low concentrations. Copper was an essential element and has an important role in cellular metabolism at low concentrations (Khellaf *et al.* 2008). Zinc does not show any visible signs of toxicity but showed a reduction of biomass. Ni was toxic for the macrophytes and decreased considerably the growth rate. Chromium is dangerous heavy metal which causes membrane damage and growth inhibition (Sinha *et al.*, 2005). Pb produced toxic effects on chlorophylls content in *L. gibba* (Miranda *et al.* 2000). In these studied species, *L. minor* and L. *gibba*, the strongest bioaccumulation was observed in manganese and iron. These metals are necessary for the metabolism and can more easily to be absorbed from surrounding environment and transported to the green parts of the plants (Lasat 2010).

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