Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. VI, 2017
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

BIOACUMULATION AND PROTEIN CONTENT OF *LEMNA MINUTA* KUNTH AND *LEMNA VALDIVIANA* PHIL. IN BULGARIAN WATER RESERVOIRS

Katya VELICHKOVA, Ivaylo SIRAKOV, Elica VALKOVA, Stefka STOYANOVA, Gergana KOSTADINOVA

Trakia University, Students Campus, 6000 Stara Zagora, Bulgaria

Corresponding author email: genova@abv.bg

Abstract

Lemna sp. have an important role indifferent aspect of aquatic ecosystems serving as a food source, by providing shelter to fish and aquatic invertebrates, changing water quality by regulating oxygen balance, nutrient cycles, and accumulating heavy metals.The aim of present study was to investigate a new found species of Lemna in Bulgarian water reservoirs regarding their protein content and bioaccumulation of heavy metals. Two water bodies located on the territory of South East Bulgaria –Tvardica Dam Lake with growing L. minuta and Nikolaevo fishpond with growing L. valdiviana were studied. Crude protein, lipid, ash contentin two species of Lemna were analysed. The heavy metal in water andaquatic plants was determined on an atomic absorption spectrometer (AAS) "A Analyst 800" - Perkin Elmer. The highest quantity of protein was measured in L. minuta (26.42%), which was 8.71% more compared to L. valdiviana (24.12). The concentrations of metals in the L. minuta and L. valdiviana followed a downward trend: Mn>Fe>Zn>Cu>Ni>Cr>Pb>Cd. The series of bioaccumulation is in descending order: Mn>Fe>Cu>Zn>Cr> Cd>Pb>Ni for L. minuta, and Mn>Fe>Zn>Cu>Cd>Pb>Cr>Ni for L. valdiviana.

***Key words**: bioaccumulation, heavy metal, Lemna minuta, Lemna valdiviana, protein.*

INTRODUCTION

The increasing population and rapid industrialization, fertilizer and pesticide use led to an increased anthropogenic impact on the environment. These contaminants diffuse into aquatic environments through industrial discharges, petroleum spills, urban and atmospheric fall-out (Olajire et al., 2005). The heavy metals are of major importance among various water pollutants because of their persistent and bio-accumulative nature (Chang et al., 2009; Yadav et al., 2009). Most of the heavy metals are toxic in nature and can be a threat to human health and the environment at higher concentrations (Vinodhini and Narayanan, 2009). Copper (Cu), nickel (Ni), cadmium (Cd) and zinc (Zn) are considered as toxic since they cause deleterious effect in plants, animals and humans (Chaudhary and Sharma, 2014). Water as vital for people is need to develop a low cost and eco-friendly technology to remove pollutants particularly heavy metals, thereby improving water quality. Conventional remediation systems based on physical and chemical methods are efficient but have economical and technical limitation. Bioaccumulation of various heavy metals in aquatic and wetland ecosystems has important significance globally (Greger, 1999). Some aquatic macrophytes take up heavy metals from aquatic environment and are being used in wastewater renovation systems (Prasad et al., 2001). Such an excellent candidate for removal, disposal, and recovery of heavymetals from the polluted aquatic ecosystems is *Lemna* sp. These free-floating, fast growing, and nitrogen fixing pteridophyte also have a higher content of protein. *Lemna* sp. are found in temperate climates and serve as an important food source for various water birds and fish (Drost et al., 2007). *Lemna* sp. have an important role indifferent aspect of aquatic ecosystems serving as a food source, by providing shelter to fish and aquatic invertebrates, changing water quality by regulating oxygen balance, nutrient cycles, and accumulating heavy metals. The protein content of *Lemna* sp. is one of the highest in the plant kingdom - 6.8 – 45.0 (Lehmanet al., 1981; Landolt and Kandeler,

1987) and it is dependent on growth conditions. The biotic and abiotic stressor on plant mobilizes diverse signaling molecules and regulates many processes that amplify and specify the physiological response through metabolic changes (Zhao et al., 2005). The nutrients taken up by duckweed are assimilated into plant protein (Abouel-Kheiret al., 2007). Wastewater ammonia was converted into a protein rich biomass, which could be used for animal feed or as soil fertilizer. *Lemna* species are of ecological significance as they are primary producers being a source of food for waterfowl, fish and small invertebrates and provide habitat for a number of small organisms (Van Hoeck et al., 2015). They are adapted to a wide variety of climatic regions where they, under favorable conditions, can grow extremely rapidly predominantly via asexual reproduction (Lemon et al., 2001). Therefore, except for bioaccumulation they are used for food and feed. The aim of present study was to investigate a new found species of *Lemna* in Bulgarian water reservoirs regarding their protein content and bioaccumulation of heavy metals.

MATERIALS AND METHODS

Water and aquatic plants samples

Two water bodies located on the territory of South East Bulgaria –Tvardica Dam Lake (42°24'29"N27°28'19"E) with growing *L. minuta* and Nikolaevo fishpond (42°37'1"N 25°49'1"E) with growing *L. valdiviana* were studied. These water bodies passage 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 samplesof the studied waterbodies and plant sampleswere collected on June 2016. 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 samples of the studied aquatic plants from water bodies were driedand archived.

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

Methods for analysis

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 andaquatic 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 wave length and preliminary preservation of water in samples with 5cm^3 concentrated HNO_3 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 samples of aquatic plants were prepared for analysis by wet 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.

The capacity of plants to absorb and accumulate metals from the water was evaluated using their bio-concentration factor (BCF). BCF was calculated as the ratio of the concentrations of metals in aquatic plant and water: BCF = [Metal] plant/[Metal] water (Hawker and Connell, 1991). Data analyses were conducted by using one-way Analysis of Variance ANOVA (MS Office, 2010).

RESULTS AND DISCUSSIONS

The results of the chemical composition of the two dstudie duckweed are given in Table 1.

Table 1. Chemical composition of *L. minuta (L.m)* and *L. valdiviana (L.v)* in the studied water bodies

Sample	Moisture %	dry matter %	crude protein %	crude lipid %	crude fiber %	Ash %	*NFE %
L. v	5.46	94.54	24.12	0.95	10.42	24.75	34.3
L. m	5.72	94.28	26.42	0.95	8.82	23.71	34.4

*NFE – nitrogen free extract

The highest quantity of protein was measured in *L. minuta* (26.42%), which was 8.71% more compared to *L. valdiviana* (24.12). With regard to the crude lipid and both species have 0.95%. The content of NFE is also almost identical in the both species (34.3-34.4). Franca et al. (2009) established in *L. valdiviana* crude protein content of 19.66% in the dry matter, a fiber content of 13.06%. In our species crude fiber content was with 20.2% lower, and protein was higher with 17.5% compared to that studied by Franca et al. (2009). With regard to the raw fiber is observed at a large quantity in *L. valdiviana* (10.42%), and less in *L. minuta* (8.82%). 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.

Table 2. Average concentrations (mg.kg-1) ± standard deviation (SD) (n=3) of metals in water Tvardica, in *L. minuta* and bioconcentration factor (BCF plant/water)

Metal	Water Tvardica average±SD	*L. minuta* average±SD	BCF plant/water
Mn	0.2593±0,09	3 199.61±5.32	12339.4
Zn	1.2450±0.023	99.14±0.45	79.6
Fe	0.8770±0.09	1 306.03±4.33	1489.2
Cu	0.1064±0.05	10.09±2.65	94.8
Ni	0.0612±0.02	0.4798±0.09	7.8
Pb	0.0203±0.014	0.1928±0.08	9.4
Cd	0.0051±0.002	0.0489±0.02	9.5
Cr	0.020±0.01	0.2207±0.07	11

The concentrations of metals in the *L. minuta* followed a downward trend (Table 2): Mn>Fe>Zn>Cu>Ni> Cr>Pb>Cd.
The bioaccumulation capacity of *L. minuta* is shown through its bioaccumulation factors, indicating a decreasing order as follows: $BCF_{plant/water}$: Mn>Fe>Cu >Zn>Cr> Cd>Pb>Ni.
The bioaccumulation capacity of *L. minuta* for Mn is thousand times higher than it is for the other metals.
There is no difference in the order of the Mn and Fe content in a plant compared to the sequence of their bioaccumulation ability.The difference in the order of another metal content in a plant compared to the sequence of their bioaccumulation ability can be seen in Table 2. This difference suggests the different bioaccumulation 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).

Table 3. Average concentrations (mg.kg-1) ± standard deviation (SD) (n=3) of metals in water Nikolaevo, in L. valdiviana and bioconcentration factor (BCF plant/water)

Metal	Water Nikolaevoaverage±SD	*L. valdiviana* average±SD	BCF plant/water
Mn	0.2543±0.07	2 563.00±6.3	10078.6
Zn	1.0082±0.009	97.20±40.2	96.4
Fe	0.6500±0.08	1 342.52±5.0	2065.4
Cu	0.1050±0.04	7.74±2.06	73.7
Ni	0.0635±0.025	0.4552±0.08	7.1
Pb	0.0201±0.01	0.1817±0.05	9
Cd	0.0050±0.002	0.0456±0.02	9.1
Cr	0.025±0.015	0.1835±0.07	7.3

The concentrations of metals in the *L. valdiviana* followed a downward trend (Table 3): Mn>Fe>Zn>Cu >Ni>Cr>Pb>Cd.
The bioaccumulation capacity of *L. valdiviana* is shown through its bioaccumulation factors, indicating a decreasing order as follows: $BCF_{plant/water}$: Mn>Fe>Zn>Cu >Cd>Pb>Cr>Ni.
The bioaccumulation capacity of *L. valdiviana* for Mn is thousand times higher than it is for the other metals.
Manganese has shown a significantly higher bioaccumulation ability (thousand times higher) compared to the other metals in the

tissues of *L. minuta and L. valdiviana*. This established and Kastratović et al.(2015) inexamination to *L. minor* and recorded the average value of the content of Mn in the root 3427 mg.kg^{-1} and 2225 mg.kg^{-1} in leaves.

In our both studied species the accumulation of heavy metals is comparable andwith the most strong bioaccumulation was observed in manganese and iron. These are metals which are necessary for the metabolism and can more easy to be absorbed from surrounding environment and transported to the green parts of the plants (Lasat, 2010).

CONCLUSIONS

In the studied *L. minuta* and *L. valdiviana* most accumulation of manganese was observed. Both species are with very good bioaccumulation of heavy metals and can be used for water treatment. Furthermore, it is found and a high content of protein in *L. minuta* and *L. valdiviana* (26.42% and 24.12%), which makes it possible to use as feed in nutrition different kinds of fish, poultry, swine and other animals.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Faculty of Agriculture, Trakia University, Project №2A/16.

REFERENCES

Abou el-Kheir W., Gahiza I., Farid A., Tarek T.,Doaa H., 2007. Assessment of the Efficiency of Duckweed (Lemnagibba) in Wastewater Treatment. International journal of agriculture & biology, 9 (5):681–687.

Chang J.S., Yoon H., Kim K., 2009. Heavy metal and arsenic accumulating fern species as potential ecological indicators in As-contaminated abandoned mines. Ecological Indicators, 9, 1275–1279.

Chaudhary E., Sharma P., 2014.Assessment of Heavy Metal Removal Efficiency of Lemna minor. International journal of innovative research and development, 3(6): 176-178.

Drost W., Matzke M,. Backhaus T., 2007. Heavy metal toxicity to Lemna minor: studies on the time dependence of growth inhibition and the recovery after exposure. Chemosphere, 67, 36-43.

Franca G., Filho J., Pereira G., Faria G., Melo F, Santos J., 2009.Valornutricional de Lemna valdiviana Phil (Araceae) submetida a diferentes concentration de fertilization com excremento de aves. Biotemas, 22 (3): 19-26.

Greger M., 1999. Metal availability and bioconcentration in plants, in:M.N.V. Prasad, J. Hagemeyer (Eds.), Heavy Metal Stress inPlants — From Molecules to Ecosystems, Springer, Berlin, 1–27.

Hawker D., Connell D., 1991. An evaluation between bioconcentration factor and aqueous solubility. Chemosphere,23, 231-241.

Kastratović V., Jaćimović Z., Đurović D., Bigović M., Krivokapić S. 2015. Lemna minor L. as bioindicator of heavy metal pollutionin Skadar Lake (Montenegro). Kragujevac J. Sci.,37, 123-134.

Landolt E., Kandeler R., 1987. The family of Lemnaceae - a monographic study, Vol. 2: Phytochemistry, physiology, application and bibliography., Vol. 4 in Biosystematic investigations in the family of duckweeds (Lemnaceae). Geobotanischen Instutites der ETH, Stiftung Rubel, Zurich.

Lasat M.M., 2010. Phytoextraction of metals from contaminated soil: A review of plant/soil/metal interaction and assessment of pertinent agronomic issues. Journal of Hazardous Substance Research, 2, 1-25.

Lehman P., Silk W., Knight A., 1981. Protein and Nitrate Content of Lemna Sp. as a Function of Developmental Stage and Incubation Temperature. Plant Physiol., 68, 127-132.

Lemon G.D., Posluszny U., Husband B.C., 2001. Potential and realized rates of vegetative reproduction in Spirodelapolyrhiza, Lemna minor, and Wolffia borealis.Aquat Bot., 70 (1): 79–87. doi:10.1016/S0304-3770(00)00131-5.

Olajire A., Altenburgerb R., Kqsterb E., Brack W., 2005. Chemical and ecotoxicological assessment of polycyclic aromatic hydrocarbon—contaminated sediments of the Niger Delta, Southern Nigeria.Sci Tot Environ, 340, 123–136.

Prasad M., Malec P., Waloszek A., Bojko M., Strzałka K., 2001. Physiological responses of Lemnatrisulca L. (duckweed) to cadmium and copper bioaccumulation. Plant Science, 161, 881–889. DOI: 10.1016/S0168-9452(01)00478-2.

Van Hoeck A.,Horemans N., Monsieurs P., Cao H., Vandenhove H.,Blust R,. 2015. The first draft genome of the aquatic model plant Lemna minor opens the route for future stress physiology research and biotechnological applications. Biotechnol Biofuels, 8, 188.

Vinodhini R., Narayanan M., 2009. The Impact of toxic heavy metals on the hematological parameters in common Carp (Cyprinuscarpio L.). Iran. J. Environ. Health. Sci. Eng., 6(1): 23-28.

Yadav S.K., Juwarkar A., Kumar G., Thawale P., Singh S., Chakrabarti T., 2009. Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by Jatrophacurcas L.: Impact of dairy sludge and biofertilizer. Bioresource Technology, 100, 4616–4622.

Zhao J., Davis L., Verpoorte R., 2005.Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol. Adv., 23, 283–333.