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COMPARATIVE ANALYSIS OF HEAVY METAL CONTENT IN AQUATIC MACROPHYTES IN THE RESERVOIRS GRUŽA, BUBANJ AND MEMORIAL PARK

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ABSTRACT. The paper presents results of the comparative investigation of heavy metal content in some aquatic macrophytes in three reservoirs in Kragujevac basin. The presented results indicate on conclusion that all investigated elements (Fe, Mn and Cu) were found in the Gruža reservoir in the greatest quantity, and after that in reservoirs the Bubanj and the Memorial Park. These results are expected with respect that Gruža reservoir endures the greatest anthropogenic influence, and also it is located in zone where natural characteristics of ground favour appearance of investigated elements. In acceptance of these elements the most prominent place have *Mentha aquatica* L. (in accumulation of Fe and Mn), *Myriophyllum spicatum* L. (in accumulation of Fe and Mn), *Lemna minor* L. and *Lemna gibba* L. (in accumulation of Mn and Fe), and *Polygonum amphibium* L. especially in accumulation of Cu. Different species of aquatic macrophytes in dependence of their structure, genetic predispositions, life form, morphologic-anatomic-physiological adaptations, conditions of aquatic environment accumulate investigated elements in different concentrations.

INTRODUCTION

Macrophytes as plants that populate different aquatic ecosystems have important role in productive and throphic relations of aquatic ecosystems, so they are one of the most important elements of life of aquatic biotopes (Wetzel, 1983, 1992). Also, they are important as oxygen producers, as substrate, biotope or source of food for aquatic organisms. Aquatic plants represent concurrents to phytoplankton, so they can to prevent appearance of "algal blooming", and on that way they participate in maintenance of stability of dynamic equilibrium of aquatic ecosystems.

Aquatic plants, specially submerged have characteristic of accumulation of chemical elements from water, so the concentration of some of them can be to several thousand times greater in plant tissues in relation to their concentration in external environment. Aquatic macrophytes can accumulate significant quantity of heavy metals in their tissue ($10-10^6$ times greater concentration then in the water). Beside heavy metals, hydrophytes extract different toxic materials, such as pesticides, derivative of phenols and etc. Bioaccumulation of metals by aquatic macrophytes depends on plant species and its organs, as well as numeral abiotic factors, such as temperature, pH, concentration of chemical elements (Lewis, 1995). Aquatic plants due to accumulation of greater quantities of chemical elements contribute to decrease of eutrophication, to circulation of nutrients, stabilization of sediments and control of water quality. Owning abilities for absorption and accumulation of great quantity of chemical elements, aquatic macrophytes have important role as biofilters, bioaccumulators and bioindicators

Aquatic macrophytes are excellent indicators of quality of aquatic environment where they live. Their distributions in the time and space, as well as their content indicate on important characteristics of water environment where they live.

Artificial lakes represent specific ecosystems with numeral differences in comparison to natural lakes, and they were made as multipurposed (production of electric power, water supply, irrigation, sport and recreation, etc.).

For the Gruža reservoir the first results were published by Veljović (1984, 1986, 1990) and sa for the Bubanj by Simić et al. (1994).

In this study, special attention was given to role of aquatic macrophytes as bioindicators, with respect that content of some element in plant tissue is indicator of presence of that element in the aquatic environment, too.

DESCRIPTION OF INVESTIGATED LOCALITIES

The Gruža, the Bubanj and the Memorial Park reservoirs there are located in Kragujevac basin in the Šumadija region of Serbia.

The **Gruža reservoir** (Fig.1) was formed by damming a middle course of the Gruža River, for water supplying of Kragujevac and surrounding settlements. Building of dam is started in 1979, and the reservoir was completely formed in 1985. The reservoir is located on altitude of 238-269 m (Ostojic, 2000). Length of the reservoir is 10 km. This reservoir is very jagged, with greater or smaller inlets. Total volume of water is 64.6 millions of m^3. Maximal depth immediate near the dam is 31 m; oscillation of water level in the reservoir is 3-5 m. The reservoir is supplied with water mainly by atmospheric rainfall, but five smaller right tributaries and upstream part of the Gruža River with small brooks empty directly in it, too.

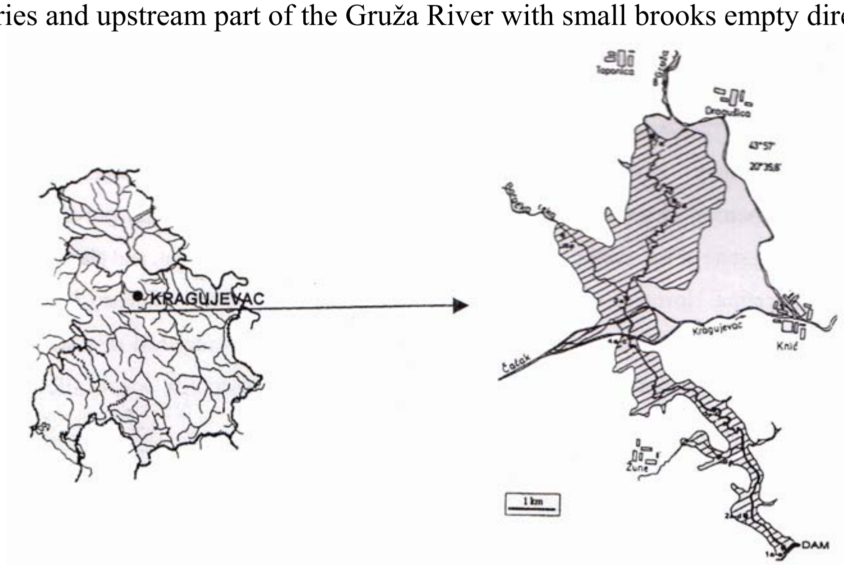

Figure 1. The Gruža reservoir

The **Memorial Park reservoir** (Fig.2) is located in scope of Memorial Park "Kragujevac´s October", at periphery of the Kragujevac. It was formed during the period of 1964-1967. The reservoir is located on Staroselski brook (Susicki brook). Dam is high 19.5 m and long 208.5 m. Length of reservoir is 1300 m, and average width is 175 m. Maximal depth is 14 m. Volume of the reservoir is 950 000 m^3, and surface is 20 ha. This reservoir was built for irrigation of park area, but it do not use for it. It has purpose in esthetic formation of Memorial Park, as object for recreation of citizens of the Kragujevac, sport, fishing, maintaining of sanitation department (Milenković, 2003).

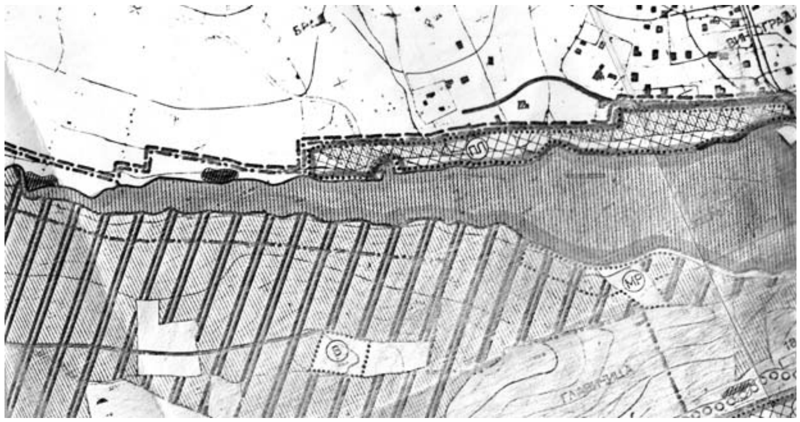

Figure 2. The Memorial Park reservoir

The **Bubanj reservoir** (Fig.3) was formed near asphalt road Kragujevac-Batočina. It originated in alluvial plateau of the Lepenica river, in abandoned cavity where exploitation of soil for factory of bricks was done. Exploitation was performed until 1955, when process of forming reservoir started. Surface of the reservoir is on altitude of 168 m, and the greatest length is in the north-east direction and it is 300 m; the greatest width is 215 m. Surface of the reservoir is 3.7 ha; average depth is 1.5 m (Stepanović, 1974). Recent studies indicate that the width is decreased on 1.27 m (maximal depth is 1.6 m, and minimal is 0.5 m). During summer period, temperature of water goes to 29°C, and during winter the reservoir is frozen. The biggest part of reservoir bottom makes mud, which average thickness is 0.5-0.7 m. The reservoir gets water by rainfall and from drinking fountain Bubanj, as well as from the ground springs.

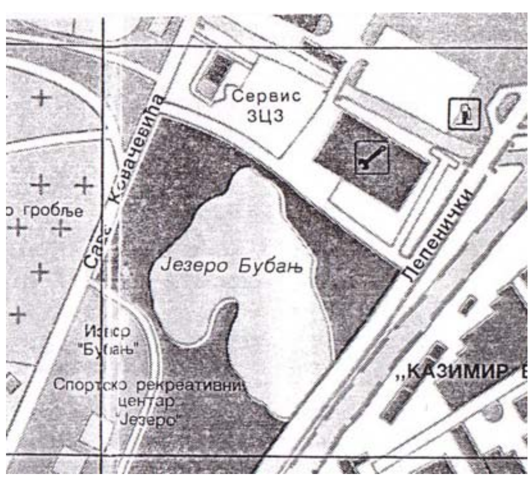

Figure 3. The Bubanj reservoir

MATERIAL AND METHODS

Work in field was carried out during spring and summer of 2004. Sample of aquatic plants were taken from places with the greatest their number and covering. The plants were collected under favorable weather conditions by adequate equipment.

Determination of plant material was performed in laboratory of Institute of biology and ecology (Faculty of Science) in Kragujevac. Determination was carried out using binocular loupe and adequate literature: Javorka & Chapody (1975) and The Flora of Republic Serbia (Josifovic, 1970-1980). Life forms are given according to the Rauncke principle as elaborated by Mueller-Dumbois and Ellenberg (1974) and partially modified by Stevanović (1992) in the Flora of Serbia.

Determined plant material was washed in distilled water until removal all impurity, and after that it is dried in dryer, 24 hours, on temperature of 105°C and than it is prepared for chemical analysis according to standard procedure, which applies for water and aquatic plants.

After washing and drying on 105°C to constant mass, certain quantity of the plant material (approximately 2 g of sample with accuracy of ± 0.1 mg) was measured on analytic scale by method of differentiation in mass. Measured sample was transferred in balloon according to Kjeldal and perfused with concentrated HNO_3. Reaction mixture was heated carefully by flame, until the solution became dry. Treatment was repeated until clearing up of the solution, and stopping of releasing of nitric vapors. After that, sample was cooled, and content in the Kjeldal′s dish was perfused with 6 ml of concentrated $HClO_4$ and than heated. Heating was stopped at solution volume of approximately 3 ml in Kjedal′s dish and when solution has became clear and achromatic. After cooling of solution, distillated water was added. After that, content from Kjeldal′s dish was filtrated through quantitative sieve in normal dish of 50 ml volume. On this way prepared solutions were used for determination of heavy metals in plant material by atomic absorption spectrometer (Perkin-Elmer, model 3300/96).

RESULTS AND DISSCUSION

In this study, investigations were done on certain plants, from next reservoir: the Gruža, the Memorial Park and the Bubanj. They showed that concentrations of the heavy metals vary in dependence of plant species and reservoir.

In study, three heavy metals (Fe, Mn and Cu) were investigated in tissues of next plants: *Myriophyllum spicatum* (rooted, submerged), *Polygonum amphibium* (rooted, floating), *Lemna minor* (unrooted, floating), *Alisma plantago aquatica* (emerged), *Menta aquatica* (emerged, Stevanović, 1992).

Elements such as Cu, Mn, Zn, Co, Fe and others that belong to microelements, can be found in plants in very small concentrations in cells and tissues, but without them normal functions of cells and organism are not possible.

In plant cells, the Fe has role in formation of chlorophyll and acceptance of anions from substrate. The Fe is included in structure of great number of enzymes that participate in processes of oxido-reduction in live cells, thanks to ability of easy receiving and releasing of electrons. Citohroms contain the Fe linked for chem, and chem is present in some oxidizes, peroxidazes and catalazes. Some transporters have in their inside Fe, but they do not have porfirinic core, as ferodoxin and proteins with Fe-S group. Also, the Fe is activator of some enzymes that participate in synthesis of porfirinic ring. In cell of leaf, excess of Fe is often bind to one phosphoprotein and make crystals of fitoferitin. For absorption of Fe by plants fero (Fe^{+2}) or feri (Fe^{+3}) ions are very important. Increase of acidity influences on increase of concentration of ferry ions. Fe ions do not accept directly from the solution, but from complex with organic compounds (chelats and siderophors) where they are connected by coordinated bonds. In siderophors feri ion is linked and by that its accessibility is increased some hundred times. The most known symptom of lacking of the Fe is interruption of chlorophyll synthesis, and because of that chlorozes arise at plants. The Fe is weakly mobile, so the chloroze first appears in young leafs (Nešković, Konjević and Ćulafić, 2003).

The greatest average value of Fe concentration was observed in reservoir the Gruža and after that in reservoirs the Bubanj and the Memorial Park (Fig.4). The greatest content of the Fe was found at plant *Mentha aquatica* from the Gruža reservoir, and at *Myriophyllum spicatum* in the Bubanj and the Memorial Park reservoirs (Fig.4). Increased content of the Fe in the Gruža reservoir occurred at plants *Myriophyllum spicatum* and *Lemna gibba*, and in Memorial Park at plants *Mentha aquatica* and *Polygonum amphibium* (Fig.4). In the Bubanj reservoir (Fig.4), the Fe was more present at *Mentha aquatica* and *Lemna minor*.

For the Mn some row of roles in oxido-reduction processes is suggested. This is element which is part of ferments, and especially it has important catalytic function at some of ferments, such as enolase, hydrogenase, phosphoglucomutase, carboxylase, etc. The Mn is stimulator of plant growth, and two-valence Mn activates many enzymes in synthesis of fat and nucleic acids. It is part for complex for releasing of oxygen in chloroplasts. Increased content of the Mn influences decrease in acceptance of N, P, K, Ca, but because of economic using of noted elements there is not change in yield of plants. In case of lacking of Mn, disorganization of lamellar system of chloroplast occurs, and because of chlorotic and necrotic specks appear in mesophyle of a leaf (Nešković, Konjević and Ćulafić, 2003). The Mn in excess leads to appearance of chloroze due to lacking of Fe, because of antagonism of these two elements.

The greatest average values of Mn concentration is found in the Gruža reservoir (Fig.5), and after that in the Bubanj and the Memorial Park reservoirs (Fig.5). The *Mentha aquatica* was plants that has the greatest acceptance of Mn on all localities. At the Gruža reservoir, *Lemna gibba* and *Myriophyllum spicatum* have more important acceptance of the Mn, but in the Memorial Park reservoir that role have *Myriophyllum spicatum* and *Alisma plantago aquatica*. At the Bubanj reservoir *Lemna minor* and *Myriophyllum spicatum* have more important acceptance of Mn.

The Cu is important element in synthesis of proteins, but also as elements which is part of oxidative ferments, such as poliphenol-oxidases, ascorinoxidases, tyrosinase and cytochrom-oxidase. It was found that almost all Cu is placed in leaves, and especially it is located in chloroplasts, because the Cu has stabilization influence on chlorophyll (it protects it from destruction). Plant needs Cu in very small quantity, but without it occurs typical symptoms such as chlorozes and necroses of leaves, decreased lignifications and decreased secretion of resin at some plants. At young plants stem dies, and apexes growth in form of letter S, and young leaves have rugged surface (Nešković, Konjević and Ćulafić, 2003).

In the Gruža reservoir, the Cu has the greatest average concentration (Fig.7), while it decreases in the Bubanj reservoir (Fig.7). Average concentration of the Cu is almost inappreciable in the Memorial Park reservoir. In the Gruža reservoir, the Cu is most concentrated in plant *Polygonum amphibium*, while in other observed species the Cu was found only in traces. In the Bubanj reservoir, *Polygonum amphibium* has the greatest ability of acceptance of Cu, in comparison to other investigated species. In the Memorial Park reservoir, *Mentha aquatica* concentrates Cu in the greatest quantity, and only *Lemna minor* is more significantly protruded in acceptance of Cu.

From all presented results it can be concluded that all mentioned elements were found in the Gruža reservoir. The Gruža reservoir belongs to euthrophic lakes, and waters are loaded with organic materials, and phosphates and nitrates. They have greater variations of soluted oxygen, smaller limpidity and electro conductivity (Ostojic, 2000). The reservoir is surrounded with stable arable surfaces, from which fertilizers rinse out in the reservoir, and also with numeral settlements, slaughter-house, and factory for production of fungi. Also, the bridge on very frequent traffic way Kragujevac-Čačak extend over it; many boats float in its water, and also, there is urinal-fecal pollution because of using this reservoir in sport and recreative purposes (swimmers, fishermen etc.). Especially should be noted presence of "algal blooming", for which prevention cuprum-sulphate applies and because of that rational expectation and explanation of high average concentration of Cu exists, in comparison to the Bubanj and the Memorial Park reservoirs. The Bubanj reservoir is near to center of the city, and directly by road Kragujevac-Batočina. Also, in the water of this reservoir fecal canalization of near-by restaurant empties; in the vicinity of the reservoir is industrial zone of the city. The reservoir is used for fishing; along its coasts is promenade and park for recreation and rest of citizens of the Kragujevac. All these influences caused that this reservoir is in state of degradation and strong eutrophication, so the results of increased concentration of investigated elements was expected. The Memory Park reservoir is located on periphery of the city and it was formed on favorable site (in its surrounding there are not arable surfaces, industry, and frequent traffic) and it serves as decorative place, for recreation, fishing, maintaining of sanitation department, etc. Also, this reservoir tolerates anthropogenic influences that led to eutrophication, but it is obvious that this influence is the smallest in comparison to other observing reservoirs, which the obtained results confirm.

The results show that in acceptance of the investigated elements next plants are prominent: *Mentha aquatica, Myriophyllum spicatum*, *Lemna minor* and *Lemna gibba* (in acceptance of Fe and Mn) and *Polygonum amphibium* (especially in acceptance of Cu). *Mentha aquatica* is immersed plant with very well developed rhizomes, much branched stem covered densely with leafs, which enable to it absorption of mentioned elements in greater quantity, and also deposition in its tissues. *Myriophyllum spicatus* is submerged plant that absorbs elements from water environment by whole its surface, and because it root, possibilities for greater acceptance the elements from mud exists. *Polygonum amphibium* (rooted, floating) and *Lemna minor* and *Lemna gibba* (unrooted, floating) have possibility to accept the elements from surface parts of water by their whole surface, and *Polygonum amphibium* (it has the greatest values of bioaccumulation of Cu, so it can be considered as indicator of its presence) from the deeper parts, with respect that it root.

Accumulation of a ion in tissues and organs of aquatic plants depends on concentration of that ion in water environment, physical-chemical characteristics of that pollutant, antagonism and synergism of ions, temperature and pH of water, as well as physiological and biochemical properties of plants (permeability of cell membrane and enzymatic activities during absorption.

Elements entered by xylem juice, which accept from cell and which are weakly mobile (Cu, Mn, Zn) or totally immobile in phloem (Ca, Fe) retain in organ to whom they reached, so there is not any later redistribution. Because of that, we could find also these metals in the older leafs.

During evolution, plants developed defensive mechanism that make possible their survival in contaminated environment by adaptation on greater and greater quantitative and concentration of harmful materials in environment. Biochemical base for tolerance against heavy metals was discovered first at animals and fungi, and after that at plants. Plant produce polypeptides that contain repetitive sequences glutamil-cisteinil linked with glicin. These polypeptides are discovered at many plants that are exposed to heavy metals, and they are commonly known as phytochelatins. Phytochelatins bind metal ions and so make complexes, which are excluded from physiological processes. Synthesis of phytochelats is induced by presence of metals in nutritive substratum.

Aquatic macrophytes are good bioaccumulators of heavy metals and biofilters that accept elements from water and accumulate them. Different species of aquatic macrophytes in dependence of their structure, genetic predispositions, and conditions of water environment accumulate the elements in different concentration.

References

[1] JAVORKA, S., CSAPODY, V. (1975): *Iconographia Florae Partis Austro-Orientalis Europae Centralis,* Academiai Kiado, Budapest.

[2] JOSIFOVIĆ, M. (ed) (1970-1980): *Flora SR Srbije I-X.* SANU, Beograd,

[3] LEWIS, M. A. (1995): Use of fresh water plants for phytotoxicity testing: a review. *Envon. Pollution* **87**: 319-336.

[4] MILENKOVIĆ, A. (2003): *Uporedna analiza sadržaja teških metala u tkivu akvatičnih makrofita akumulacija the Gruža i Spomen park*, diplomski rad; Kragujevac.

[5] MUELLER-DOMBOIS, D., ELLENBERG, H. (1974): *Aims and Methods of Vegetation Ecology.* John Wiley and Sons. New York.

[6] NEŠKOVIĆ, M., KONJEVIĆ, R., ĆULAFIĆ, LJ. (2003): *Fiziologija biljaka*, Beograd.

[7] OSTOJIĆ, A. (2000): *Uporedno ekološka studija zooplanktona akumulacija Grošnica i Gruža,* Doktorska disertacija, Univerzitet u Beogradu, 186pp.

[8] STEPANOVIĆ, Ž. (1974): *Hidrološke karakteristike Kragujevačke kotline, sa posebnim osvrtom na snabdevanje Kragujevca vodom*, Doktorska disertacija; SO Kragujevac

[9] STEVANOVIĆ, V. (1992): *Klasifikacija živothi formi flore Srbije* - In Sarić, M.ed. Flora Srbije, I (2.ed.): 39-46, SANU, Beograd.

[10] TOPUZOVIĆ, M., PAVLOVIĆ, D. (2003): The vascular flora of the Gruža reservoir in the Šumadija region of Serbia. *Kragujevac J. Sci.* **25**:107-120.

[11] VELJOVIĆ, V., MARKOVIĆ, A. (1984): *Ekološke karakteristike akumulacionog jezera na Gruži.*- III Kongres ekologa Jugoslavije. Radovi i rezimea, Knj.1: 141-146.

[12] VELJOVIĆ, V., MARKOVIĆ, A., VUKOMANOVIĆ, V. (1986): *Dijagnostički značaj pojave makrofitske flore i vegetacije u novoformiranim akumulacionim jezerima,* Konferencija »Zaštita voda 86«, Kragujevac, Zbornik radova, 82-86.

[13] VELJOVIĆ, V., MARKOVIĆ, A., OGNJANOVIĆ, R. (1990): Vegetation of the river system Gruža with particular regard on the impoundment area Gruža*. Bilten Društva ekologa Bosne i Hercegovine.* Ser. **B**, **5:** 125-129.

[14] WETZEL, R.G. (1983): *Lymnology,* W.B. Saunders Co., Philadelphia, PA: 767 pp.

[15] WETZEL, R.G. (1992): Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic matter in freshwater ecosystems*. Hydrobiologia* **229**: 181-198.

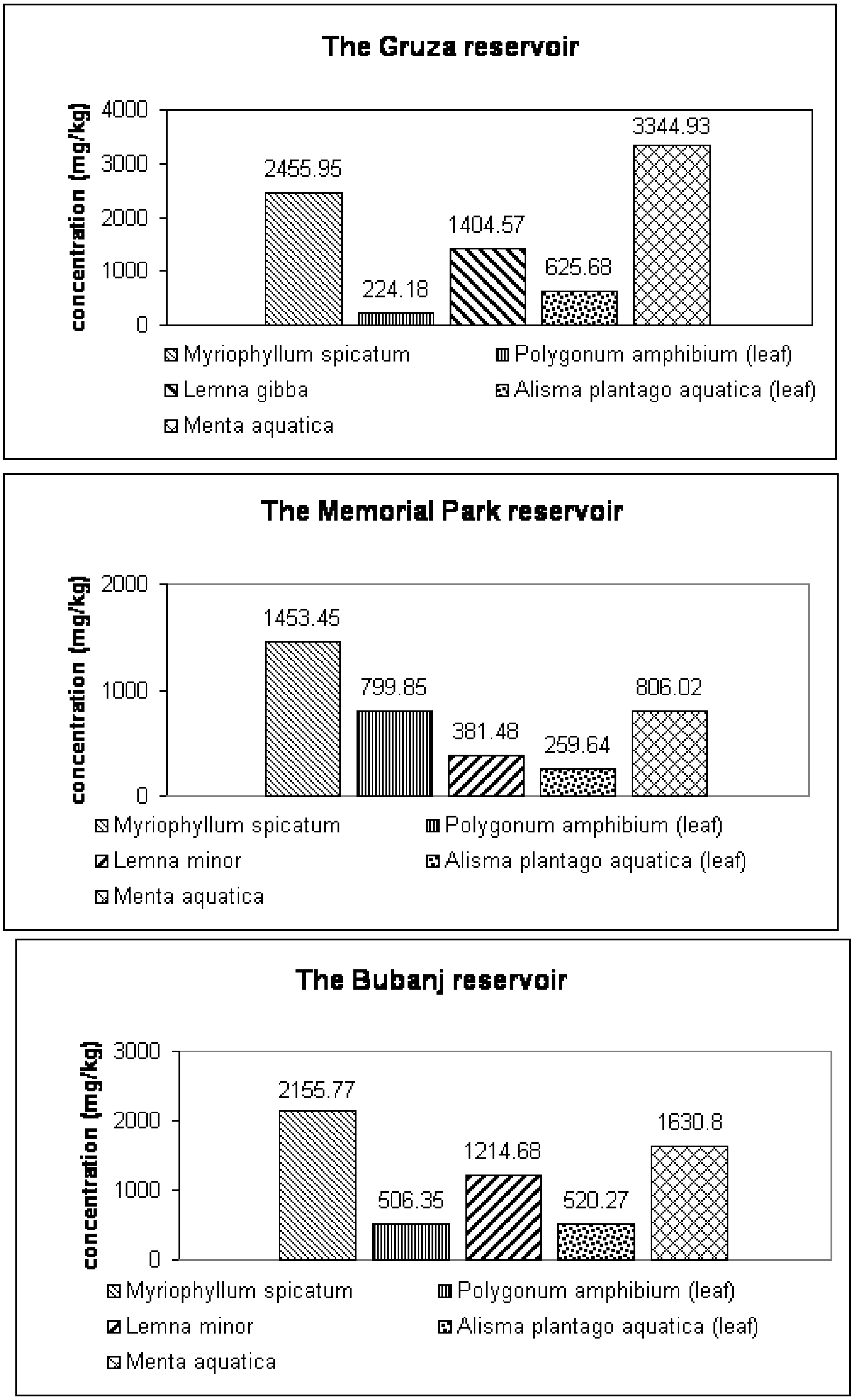

Figure 4. Concentration of Fe (mg/kg of dryed matery) in macrophytes of the Gruža, the Memorial Park and the Bubanj reservoirs

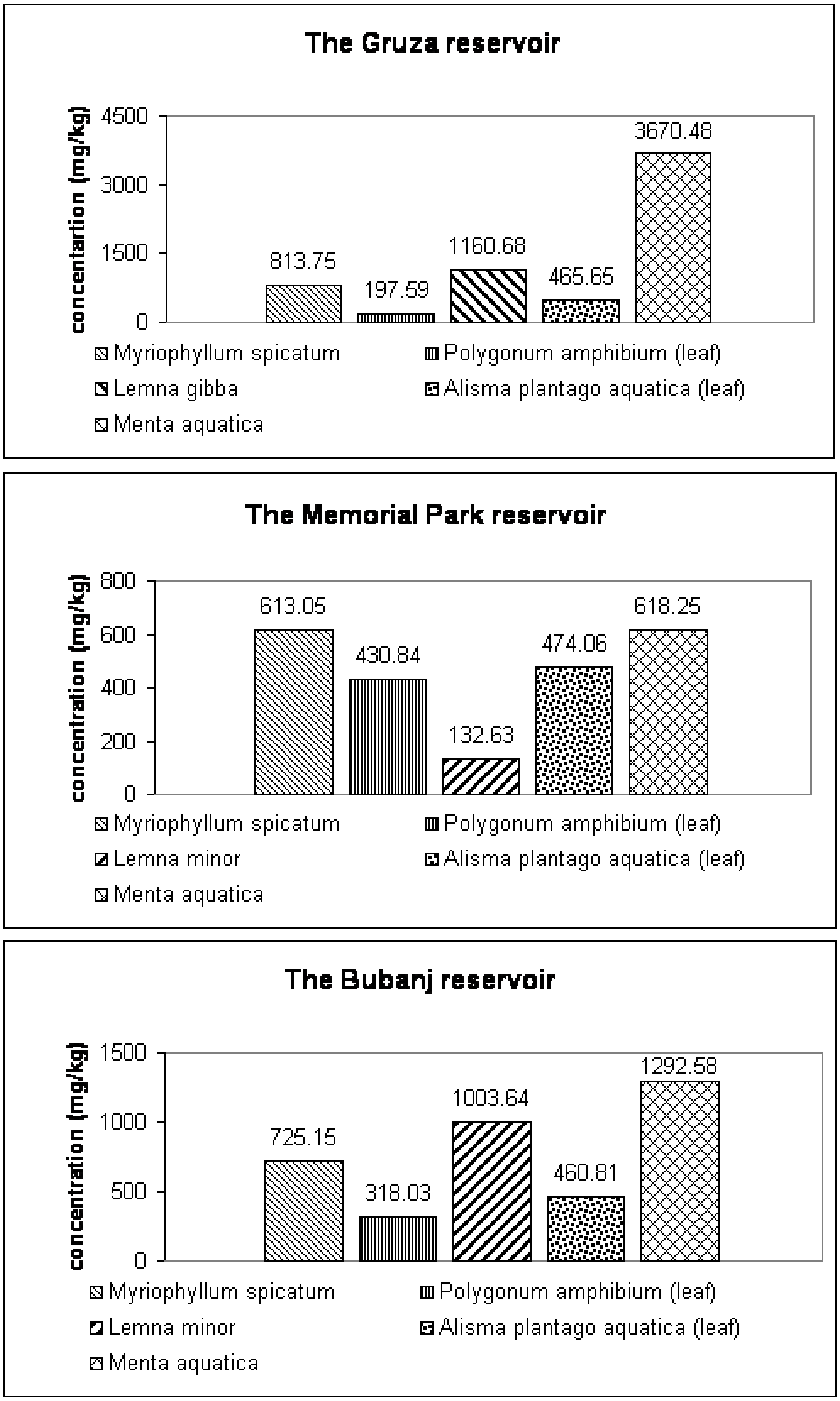

Figure 5. Concentration of Mn (mg/kg of dryed matery) in macrophytes
of the Gruža, the Memorial Park and the Bubanj reservoirs

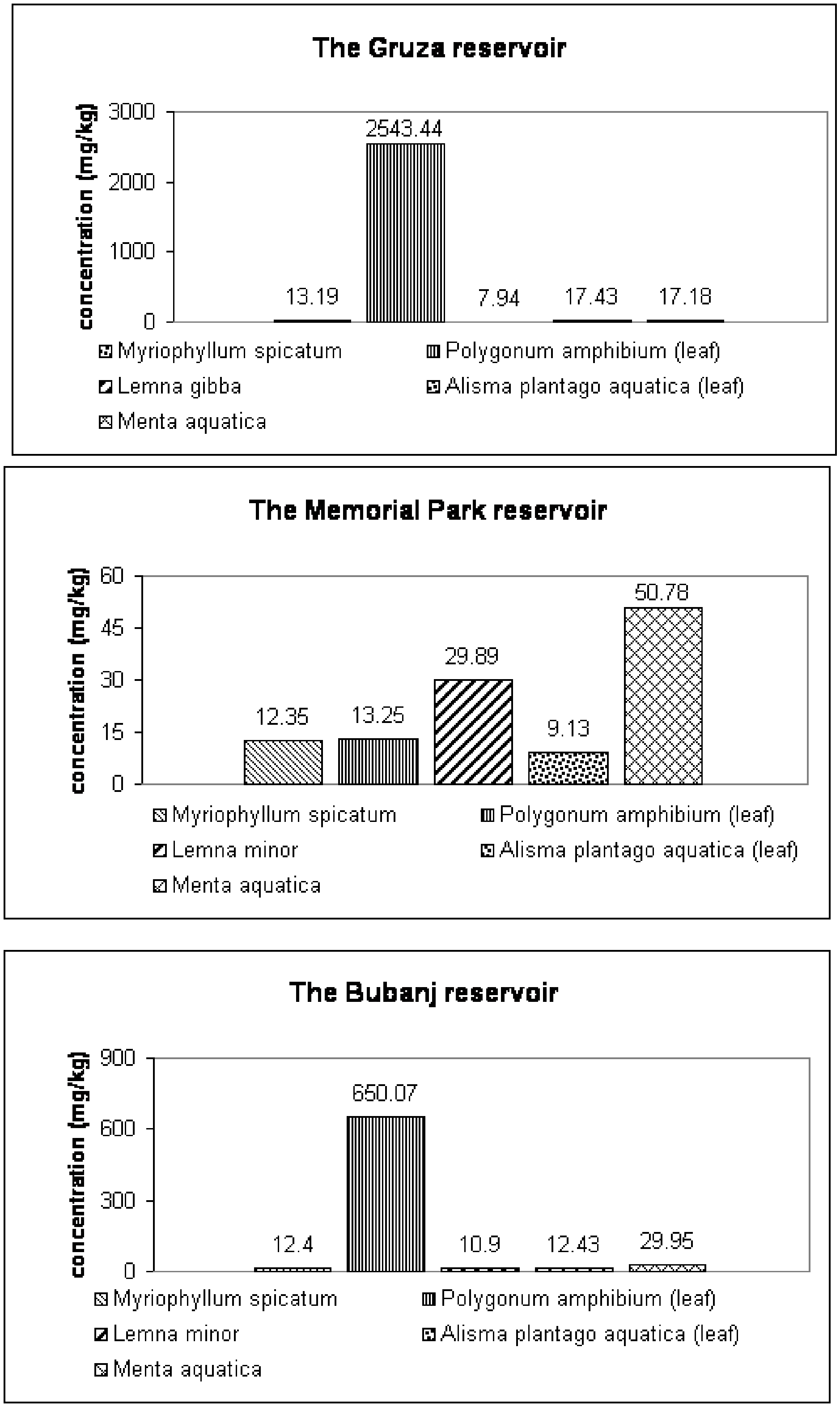

Figure 6. Concentration of Cu (mg/kg of dryed matery) in macrophytes