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Preliminary research on the water quality of the Topoljski Dunavac: application of the Lemna test

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Introduction

Topoljski Dunavac is a former branch of the Danube River located in the watershed of the Drava and Danube basin (Baranja) with an area of 247-280 ha, depending on the water level. Today, Topoljski Dunavac is connected to the Danube by the watergate and has the characteristics of stagnant water. Topoljski Dunavac is located in the area of Natura 2000 ecological network, important for birds "Podunavlje and lower Podravlje" (HR1000016) and habitat types "The Danube north of Kopački rit" (HR2001309). Duckweeds are very sensitive to changes in the concentrations of nutrients and toxicants due to their rapid growth and high ability to accumulate them (Varga et al., 2019; Radić et al., 2010). This study aimed to determine the influence of the present nutrients and possible contaminants in the water of Topoljski Dunavac on the growth of duckweed (*Lemna minor* L.) in the Lemna test.

Methods

Water was sampled at one site on the Topoljski Dunavac (Fig 1A). Measurements of physicochemical properties of water were performed on-site and in the laboratory once a month during 2015 and in January and February 2016. Water samples for analysis of pesticides (HRN EN ISO), as well as the Lemna-test, were taken in May, September, and November 2015 and in February 2016. Lemna-test was conducted as a standard growth inhibition test with volume modification to 6-well microplates (Fig 1B). Growth was determined by measuring frond number (FN) and fresh weight (FW, biomass). Pigments were extracted in acetone and measured spectrophotometrically. The concentrations of Chl-*a*, Chl-*b*, and Car were calculated by Lichtentaler (1987). Lipid peroxidation was determined by estimating the MDA content using the method described by Heath and Packer (1968). Chlorophyll fluorescence *a* (Strasser et al. 2004) will be used to characterize the state of the photosynthetic apparatus.

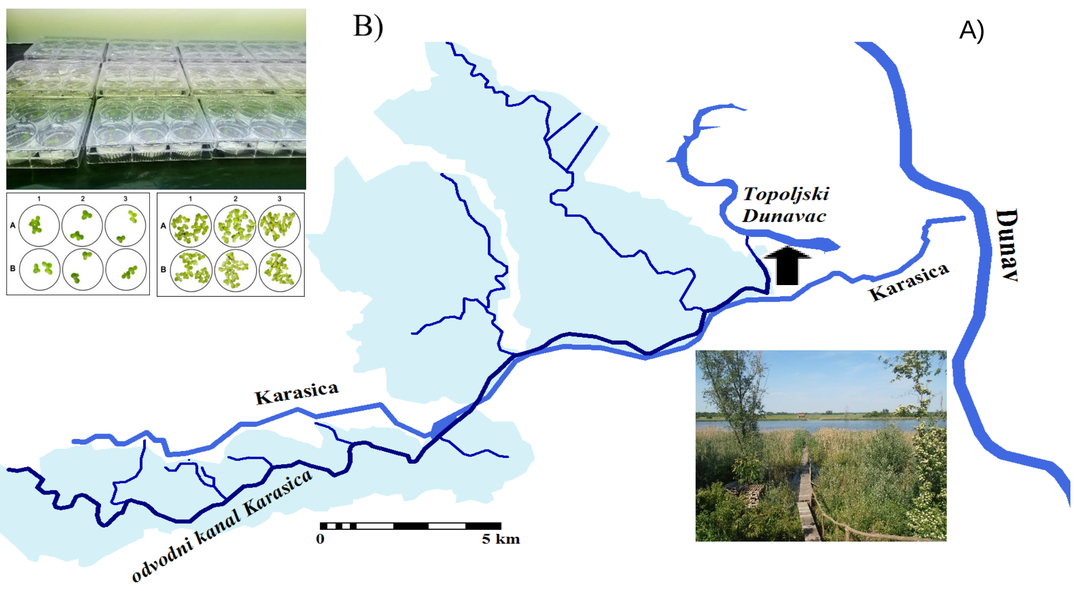

Fig 1. A) The researched site at Topoljski Dunavc; B) Cultivation of *L. min*or in 6 well plates during the experiment.

Results

The water quality of the Topoljski Dunavac depended primarily on seasonal changes in climatic parameters. Specific pollutants, especially organochlorine pesticides in November (0.083 µg/L), probably originated from the surrounding agricultural land. The highest amount of nutrients measured in the winter, and their decrease in the spring, were probably a consequence of the phytoplankton development (Fig 3) and the development of macrophytic vegetation (Table 1). In May 2015, the values of KPK-Cr and TOC (2B) were a consequence of the decomposition of organic matter in the waters of the Topoljski Dunavac. Due to reduced nutrient levels in May, the amount of protein level in *L. minor* decreased (1.2 mg protein/g FW), while at the same time, the concentration of MDA, as the key indicator of oxidative stress, increased (Fig 4C). On the other hand, during September, sufficient nutrients were present in the water to support the highest increase (0.23 ± 0.03) of *L. minor*, and at the same time, no significant accumulation of MDA occurred, indicating no toxic effect of water samples. Photosynthetic efficiency index determined in May (0.101 ± 0.01) and November (0.539 ± 0.15), compared to the control conditions (Steinberg medium, 0.861 ± 0.53), showed a significantly lower vitality of plants grown in the water from Topoljski Dunavac.

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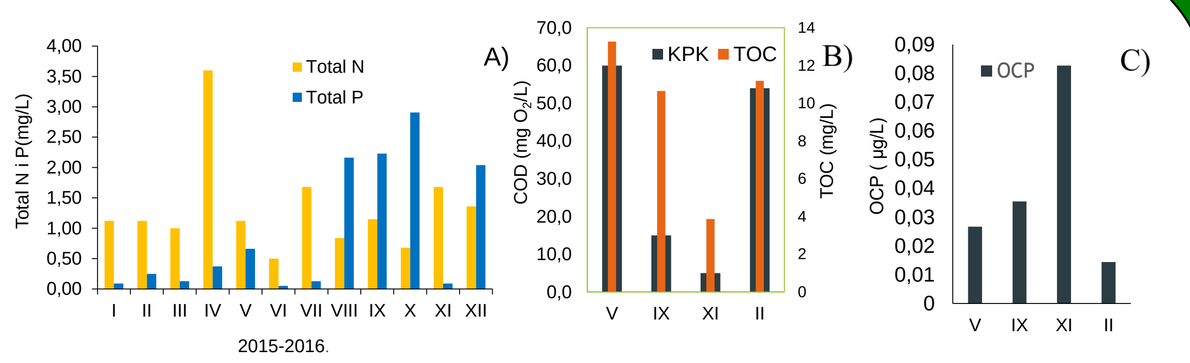


Fig 2. A) Concentration of total nitrogen and phosphorus; B) chemical oxygen demand (COD) and total organic carbon (TOC); C) organochlorine pesticides (OCP) in the water samples from Topoljski Dunavac (March to December 2015, and January and February 2016.)

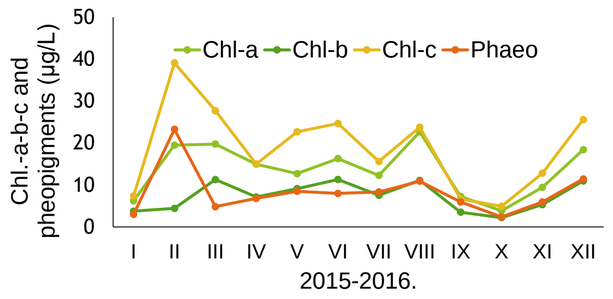


Fig 3. Concentration of chlorophyll *a*, *b*, and *c* and pheopigments of phytoplankton in the waters of Topoljski Dunavac (March-December 2015, and January-February 2016).

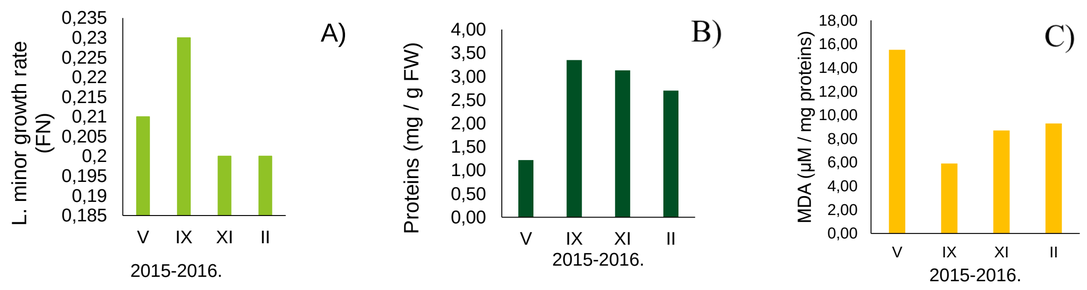


Fig 4. A) Growth rate of *L. minor*; B) protein concentration (mg/g FW); C) malondialdehyde concentration (MDA, µM/mg protein) of *L. minor* grown in water samples from Topoljski Dunavac.

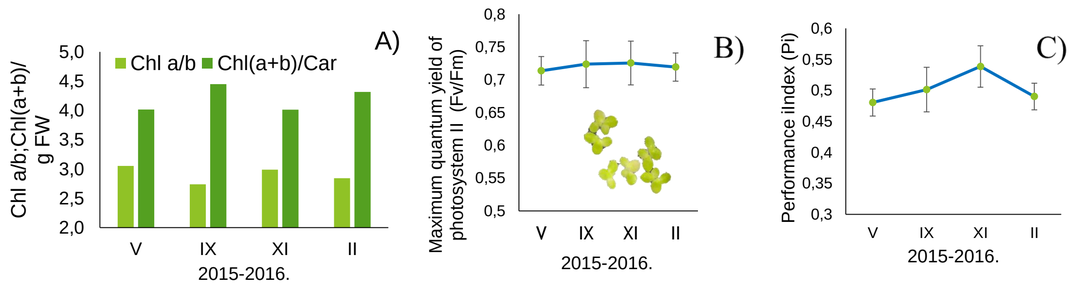
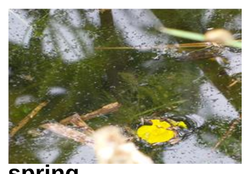


Fig 5. A) Chl *a*/*b* and Chl (*a*+*b*)/Car ratio of *L. minor*; B) Maximum quantum yield of photosystem II (Fv/Fm); C) Performance index (PI_{abs} relative units) *L. minor*

Table 1 Macrophytes in Topoljski Dunavac during spring and summer 2015.

Spring	Summer
Ceratophyllum demersum L.	*Ceratophyllum demersum* L.
Nymphoides peltata (Gmel.) O. Kuntze	*Nymphoides peltata* (Gmel.) O. Kuntze
Salvinia natans (L.) All.	*Myriophyllum spicatum L.*
Utriculariaria neglecta Lehm.	*Najas marina* L.
Phragmites communis Trin.	*Phragmites communis* Trin.
	Schoenoplectus lacuster (L.) Palla
	Typha angustifolia L.


spring


summer

Conclusion

The growth of duckweeds in the Lemna-test depended on the nutrients present in the Topoljski Dunavac. An insufficient amount of nutrients, especially nitrogen, and the presence of pollutants caused stress in *L. minor* and poorer plant vitality. Seasonal changes in climatic parameters and anthropogenic activity significantly influenced water quality in Topoljski Dunavc.

References

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