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Can a benthic diatom community complement chemical analyses and discriminate between disturbed and undisturbed saline wetland habitats? A study of seven soda pans in Serbia

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Abstract

Soda pans are protected in the European Union as Natura 2000 areas in the category of "Pannonic saline steppes and marshes". There are at least six soda pans of reference status in Serbia and only a half have strict legal protection. The number of similar, but disturbed (modified) habitats that could be reconstructed is still unknown. We conducted sampling in five natural and two disturbed soda pans aiming to compare a difference in physical and chemical water properties and benthic diatom communities. In addition, we tried to apply recently developed Diatom Index for Soda Pans (DISP) and Trait-based index (TBI) to test the applicability of taxonomic and trait-based approaches in ecological status assessment of soda pans in the southern part of the Carpathian Basin. In contrast to natural soda pans, lower pH, and nutrients levels were recorded in disturbed pans. A total of 86 taxa of benthic diatoms, including 11 new for diatom flora of Serbia, were identified. A lower species richness and Shannon index was recorded in natural soda pans. *Nitzschia austriaca, N. supralitorea*, *Navicula veneta* and *N. wiesneri* were dominant diatoms in soda pans of the reference status. Both DISP and TBI index indicated lower ecological status of two disturbed pans. We concluded that two indices, supplemented with indicator values for some local diatom species, can be a promising tool for soda pan conservation in this part of the Carpathian Basin.

Keywords: soda pans, diatoms, Diatom Index for Soda Pans (DISP), Trait-based index (TBI)

Introduction

Continental saline waters are unique and extreme environments, inhabited by specific biota, with high conservation value (Williams 2002; Gavrilović et al. 2018). In recent years, one type of these habitats has been attracting the attention of biologists who have tried to develop a system of new biological quality elements or indicators for assessment of its ecological status. Several ecological criteria were used to designate the type of small saline lakes known as soda pans. A bed of these natural or near-natural wetlands is permanently or seasonally covered by saline water (domination of sodium) that supports different sodic wildlife communities (Boros et al. 2013). In a broad sense, especially if we take into account nature protection objectives, soda pans encompass all types of water bodies covered with sodic water (from small area of still water to large, but shallow lakes).

Diatoms, known bioindicators in freshwaters and an important biological quality element of Water Framework Directive (WFD), can also be used as indicators for soda pans since many species show different level of sensitivity to salinity or conductivity, specific anions etc. (Blinn 1993; Ács 2007; Stenger-Kovács et al. 2018). A different approach to the ecological status assessment of saline astatic water bodies has recently been used aiming to test the applicability of benthic diatoms as a biological quality element. It seems that there is regularity in the appearance of these diatom studies, a trend towards a more functional approach. First studies were focused on testing of traditional diversity measures, taxonomic distinctness indices and indicator species analyses (Stenger-Kovács et al. 2014; Stenger-Kovács et al. 2016; Lengyel et al. 2016; Földi et al. 2018), while later investigations dealt with functional diversity indices (Stenger-Kovács et al. 2020), trait-based index (Stenger-Kovács et al. 2018), and functional (trait) diversity (Ács et al. 2019).

The Carpathian Basin is a region rich in different types of sodic waters, including salt marshes and different types of soda lakes (Boros et al., 2013). According to one investigation conducted in the middle of the last century, the number of shallow alkaline ponds in the Pannonian plain in Yugoslavia (corresponding to the northern province Vojvodina in Serbia nowadays) was very high since the study included around 75 shallow alkaline ponds (Petrović 1980). Unfortunately, today most of these waterbodies have been dramatically modified or even completely disappeared (Boros et al. 2014; Vidaković et al. 2019). The intensive agriculture has been the most significant factor of the negative impact on these habitats in Hungary and Serbia (Boros et al. 2013), resulting in reduced salinity/conductivity and disturbed natural hydrological regime of soda pans. Fortunately, a type-specific index (DISP) and Trait-Based Index (TBI) designed to show a significant positive correlation with conductivity in small, shallow, high salinity lakes were recently developed in Hungary by Stenger-Kovács et al. (2018). There is a great similarity in the chemical composition of alkaline wetlands in Serbia and the Hungarian part of the Carpathian Basin (Petrović, 1980). The sodic base type ($Na – HCO_3$) is dominant since more than 50% of all natural soda waters in the region belong to this chemical type (Boros et al. 2013). In our opinion, the similarity of water chemical composition of soda pans and a large overlap between diatom species pools in Serbia and Hungary are very important prerequisites for the application of DISP and TBI indices in the southern part of the Carpathian Basin.

A few studies targeted microscopic organisms in intermittent soda wetlands in Serbia, but these investigations were sporadic and resulted mainly in species inventories. Recent studies have been mostly focused on benthic diatoms in undisturbed soda pans (Vidaković et al. 2019). A brief summary of past research conducted in natural and degraded soda wetlands is given in Gavrilović et al. (2018). Tóth et al. (2014) reported the difference in zooplankton composition between disturbed and soda pans in natural status in Serbia. In their work, Tóth et al. (2014) chose an early summer as a reference period for the ecological status monitoring since the typical zooplankton community has developed in soda pans by that period. Similarly, in their study designed to develop the trophic diatom index for lakes (TDIL), Stenger-Kovács et al. (2007) started from similar presumptions: (1) a selection of a single preferred substratum with a widespread distribution such as *Phragmites australis*, and (2) a limitation to one season e.g. sampling in spring. The epiphytic community developed on young reed stems in shallow saline wetlands is a pioneer community made up of diatoms that were recruited mostly from a bottom (pans in natural status), or both from bed of pans and freshwater inflow (disturbed, open water bodies). We hypothesized that epiphytic diatom assemblage sampled from young common reed stems (*Phragmites australis*) in spring in modified pans will contain diatoms with different ecological preferences and functional traits compared to soda pans in natural status.

The aims of our studies were: (1) to confirm or report changes in chemical typology and trophic status of seven soda pans, (2) to analyze their benthic diatom communities (epiphytic and epipelic) using traditional diversity measures (species richness and Shannon diversity), and (3) to investigate the relevance of diatoms as ecological indicators in undisturbed and disturbed soda pans in Serbia using two specific diatom indices: Diatom Index for Soda Pans (DISP) and Trait-Based Index (TBI).

Material and methods

Study site and sampling plan

Water and benthic diatom samples were taken between 8 May and 19 June 2019 from seven soda (caustic) pans located in the southern part of the Carpathian Basin (the Vojvodina Region, Serbia) (Fig. 1). The first group of five pans is considered pristine (in natural status) according to Boros et al. (2013) (Table 1). Slano Kopovo and Lake Rusanda (asphalt road separates the whole lake onto one larger – Lake Velika Rusanda, and one smaller – Lake Mala Rusanda) are declared protected areas (Nature park "Rusanda" and Special nature reserve "Slano Kopovo"), whereas Medura and Čoka Kopovo are part of the national ecological network. The second group consisted of two soda pans affected by anthropogenic disturbance and thus, are considered as degraded. Slatina (near to Baranda village) and Velika Slatina (near Sefkerin village) were both subjected to considerable modification of the natural hydrological regime caused by the construction of irrigation channels. There were two deviations from the sampling plan. Firstly, the lack of macrophyte vegetation (including other types of vascular plants on the shore) in Medura prevented us from taking epiphytic diatom samples. Secondly, due to the inaccessibile terrain, the sampling in Slatina was repeated.

Fig. 1 Map of studied soda pans with the location of sampling sites

Table 1 GPS locations of sampling points and details of investigated soda pans

Water sampling and analysis of physical and physico-chemical parameters

Water temperature (T), pH, and conductivity (COND) were measured using Water Multimeter 18.52.01 (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), transparency (TRANS) with a Secchi disc, and dissolved oxygen (DO) with a DO meter HI9147 (Hanna Instruments, Woonsocket, the USA) in the field. Parallel with the *in situ* measurements, samples for water chemical analyses were taken with two plastic bottles (0.5-L and 1-L). Water depth was measured using a folding ruler at each sampling site. The determination of cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) was done by inductively coupled plasma optical emission spectrometry (ICP-OES) using Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, the UK). Ammonia concentration (NH_4^+) in water was measured using 930 Compact IC Flex ion chromatograph (Metrohm, Herisau, Switzerland) following SRPS EN ISO 14911 (2009). The measurements of nitrite (NO_2^-), nitrate (NO_3^-), and phosphate (PO_4^{3-}) was conducted with the same ion chromatograph according to U.S. EPA (1997). Total phosphorus (TP) was measured using UV/Vis spectrophotometer Specord 50 (Analytic Jena, Jena, Germany) following SRPS EN ISO 6878 (2008). Total nitrogen (TN) was determined after the chemical conversion of nitrogen compounds to nitrogen monoxide by use of the catalytic combustion (SRPS EN 12260 2008). The calculation of carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) was based on p– and m–alkalinity that was determined by the titration method (APHA, AWWA & WPCF 1995a). The determination of chloride (Cl^-) was done based on the argentometric method following APHA, AWWA, and WPCF (1995b). Finally, sulphate (SO_4^{2-}) concentration was determined using the gravimetric method with the ignition of residue according to APHA, AWWA, and WPCF (1995c). Salinity was calculated according to formula based on the relationship between conductivity and salinity given by Boros et al. (2013).

Sampling and analysis of benthic diatom community

The epiphytic samples were taken by cutting five 15-cm long submerged reed stems (*Phragmites australis*). Diatoms growing on pan's mud were collected by taking approximately 10 cm^3 of the superficial sediment layer. All samples were placed in plastic bottles and preserved with ethanol (final concentration of 20% by volume for epiphytic and 50% for epipelic samples). Diatom samples were cleaned using a hot concentrated hydrochloric–acid (HCl) and supersaturated solution of potassium permanganate ($KMnO_4$) following the procedure described by Taylor et al. (2007). Finally, treated valves were mounted in Naphrax®. LM micrographs were taken with the Zeiss Axioimager.M1 microscope with DIC optics and AxioVision 4.9 software at 1600× magnification. For determination of diatom abundance at least 400 valves per sample were counted using the Leica DM750 microscope (Leica Microsystems) with objective HI PLAN 100×/1.25 OIL (at 1000× magnification).

Calculation of DISP and TBI indices

Diatom Index for Soda Pans (DISP) index was calculated using the formula given by Stenger-Kovács et al. (2018):

$DISP=\Sigma\, a_i*s_i*v_i/\Sigma\, a_i*s$

a_i= relative abundance of the taxon i,

s_i= sensitivity value of the taxon i, and v_i= indicator value of the taxon i

Indicator and sensitivity values for species listed in the work of Stenger-Kovács et al. (2018) were used. The index values vary in the range between 1 and 6; the lower the value the worse the ecological status.

Trait-based index (TBI) index was calculated according to the equation given by Stenger-Kovács et al. (2018):

TBI= log10(*MS1*+MLW2+MLW3+0.003/P+S4+H+L+0.003)+4.5

MS1: relative abundance of small-sized (biovolume <100 μm^3) motile diatoms

MLW2: relative abundance of motile diatoms with the ratio: $2 \leq$ Length/Width < 4

MLW3: relative abundance of motile diatoms with the ratio: $4 \leq$ Length/Width < 6

P: relative abundance of diatoms classified in the planktic group

S4: relative abundance of diatoms with biovolume in the range 600 μm^3 - 1500 μm^3

no matter which ecological guild they belong to

H: relative abundance of diatoms classified in the high profile guild

L: relative abundance of diatoms classified in the low profile guild

The classification of diatom taxa into four ecological guilds and values of the Shannon diversity index was based on information and calculations obtained from the OMNIDIA 6.0.2 database. Biovolume of individual algal cells was calculated following the procedure proposed by Stenger-Kovács et al. (2018). First, linear dimensions of ~20 valves of each individual taxon were measured. Then, biovolume was calculated using geometric approximations of individual algal cells and related equations proposed by Hillebrand et al. (1999). The average values of length, width, and thickness of each diatom species were used for the biovolume calculation.

Statistical analysis

Welch's *t*-test was used to compare means of physical and chemical parameters, DISP and TBI indices between natural and degraded pans. Before analyses, only data related to TP and sum of inorganic nitrogen (NH_4^+, NO_2^-, NO_3^-) were transformed using square-root transformation because standard deviations greatly differed in two groups.

Results

Physical and chemical parameters

According to Hammer's (1986) salinity classification, four studied soda pans (Medura, Lake Mala Rusanda, Lake Velika Rusanda and Velika Slatina) can be characterized as hyposaline (3 - 20 g/L), while three (Slano Kopovo, Čoka Kopovo, and Slatina) fitted into the subsaline category (0.5 - 3.0 g/L). Investigated soda pans can be arranged into several chemical types based on the percentage of the equivalent sum of total cations and anions using a 25% limit (Hammer, 1986). Sodium was a dominant cation in all pans, while bicarbonate-carbonate complex was solely present or in combination with sulfate and chloride (Table 2). The sodic base type (Na – HCO_3) of water was recorded in Medura and Čoka Kopovo. In Slatina, a dominance of sodium and magnesium was observed, so the special sub-type of basic alkaline water was recognized in this pan (Na – Mg – HCO_3). In other four pans, different combinations and order of dominant anions (chloride, sulfate and bicarbonate-carbonate complex) existed in water: Na – Cl – HCO_3 in Slano Kopovo and Velika Slatina, Na – Cl – SO_4 – HCO_3 in Lake Velika Rusanda, and Na – HCO_3 – SO_4 – Cl in Lake Mala Rusanda.

Table 2 Physical and chemical characteristics of water in seven soda pans sampled in 2019

The investigated soda pans are shallow water bodies with high measured pH and DO values. However, two degraded soda pans (Velika Slatina and Slatina) were an exception since in those pans water pH was more neutral, and significantly lower than in natural soda wetlands. When it comes to nutrient content, there were enormous differences, especially in total phosphorus and inorganic nitrogen concentration between natural and modified soda pans (Table 3). Based on measured TP concentrations, all natural soda pans can be considered hypertrophic. For example, total phosphorus in Lake Velika Rusanda was extremely high (23 mg/L), while in Velika Slatina the measured value was around the detection limit. As for the nitrogen species content, ammonia concentration was the highest in Lake Mala Rusanda, nitrite in Velika Rusanda, and nitrate in Slano Kopovo. The lowest measured values of nitrogen compounds were recorded in Velika Slatina.

Species richness, Shannon diversity, and occurrence of characteristic diatoms for soda pans

In seven studied soda pans a total of 86 taxa of benthic diatoms were recorded (Supplementary table 1). The genus *Nitzschia* had the highest number of identified taxa (24), followed by genera *Navicula* and *Craticula* with 8 taxa each. Only *Nitzschia frustulum* was recorded in all soda pans. In addition, 11 species new for Serbian diatom flora were recorded: *Achnanthidium macrocephalum* (Hustedt) Round & Bukhtiyarova, *Amphora indistincta* Levkov, *Craticula accomodiformis* Lange-Bertalot, *C. nonambigua* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito, *Denticula subtilis* Grunow, *Gomphonema insigniforme* E.Reichardt & Lange-Bertalot, *Halamphora dominici* Ács & Levkov, *H. kevei* Levkov, *Luticola pulchra* (McCall) Levkov, Metzeltin & A.Pavlov, *Nitzschia austriaca* Hustedt, *N. rosenstockii* Lange-Bertalot (Fig. 2, Supplementary table 1)

Fig. 2 Light microscopy (LM) micrographs. 1-3 *Craticula nonambigua*; 4 *C. accomodiformis*; 5, 6 *Gomphonema insigniforme*; 7-12 *Denticula subtilis*; 13-15 *Nitzschia austriaca*; 16, 17 *N. rosenstockii*;

18-21 *N. supralitorea*; 22-24 *Navicula wiesneri*; 25-27 *N. veneta*; 28-30 *Luticola pulchra*; 31, 32 *Halamphora dominici*; 33, 34 *H. kevei*; 35-37 *Amphora indistincta*; 38, 39 *Achnanthidium macrocephalum*. Scale bar = 10 µm

The total number of diatom species in both, epiphytic and epipelic communities, differed considerably between natural and degraded soda pans. The highest species richness (31) was observed in Slatina in the epipelic diatom community, while the highest number of epiphytic diatoms (24) was recorded in Velika Slatina. By contrast, the epipelic diatom community was generally poorly developed in natural soda pans. For example, in Lake Velika Rusanda only *Surirella brebissonii* was found on the lake's bottom.

Although the diversity index (*H'*) of diatom samples was higher in degraded pans (2.83-3.49), there were also some natural soda pans with rather high *H'* values (Supplementary table 1). For example, Shannon diversity values of diatom epiphytic communities found in Lake Mala Rusanda and Slano Kopovo were 2.81 and 2.45, respectively. On the other hand, in spring 2019 Lake Velika Rusanda supported a small number of species, two taxa accounted for >95% of total abundance resulting in the lowest Shannon diversity (1.15).

In studied soda waters, 26 diatom taxa reached 5% threshold of the relative abundance in at least one sample and thus can be considered as dominant (Supplementary table 1). In natural soda pans, motile, small-sized brackish-fresh and fresh-brackish diatoms were prominent in the epiphytic diatom community. A small sigmoid species *Nitzschia austriaca* was identified in four soda pans in natural status. In Slano Kopovo and Lake Mala Rusanda this diatom markedly dominated over other species with relative abundances of 46% and 27%, respectively. In Čoka Kopovo, *N. austriaca* was the second most abundant diatom (after *Halamphora kevei* with 55%), since a third of all counted valves belonged to this species. Similarly, *N. supralitorea* was characteristic and dominant diatom found in benthic communities of all "pristine" soda pans with the exception of Čoka Kopovo. Although it was identified in less than half of investigated soda pans, *Nitzschia thermaloides* was a diatom with the highest relative abundance, 70.3% of total diatoms in Lake Velika Rusanda. The genus *Navicula* was represented by several taxa, but only two species were characteristic and dominant in soda pans. *Navicula veneta* was a prominent epiphytic diatom in Slano Kopovo and Lake Mala Rusanda, while *N. wiesneri* inhabited the sediment surface in Medura.

In soda pans affected by anthropogenic disturbance, both epiphytic and epipelic diatom communities were well developed. In Velika Slatina, brackish-fresh species *Nitzschia inconspicua* was the most numerous epiphytic diatom, followed by fresh-brackish *N. paleacea*. In Slatina, the community developed on reed encompassed as many as ten *Nitzschia* species, but diatoms of genus *Navicula* (*N. wendlingii*) and *Tabularia* (*T. fasciculata*) prevailed. Moreover, different *Gomphonema* species were abundant in both degraded pans, whereas *Achnanthidium minutissimum* was a prominent feature of the epiphytic community only in Slatina. Specific abiotic factors acting on the bottom of the pan led to the establishment of the diatom community consisted of species with, at first glance, different ecological preferences. For example, populations of *Hantzschia amphioxys* and co-dominant *Denticula subtilis* were especially large in epipelic diatom community of hyposaline pan Velika Slatina, whereas in the same pan a freshwater diatom *Nitzschia palea* var. *debilis* was abundant as well. In subsaline pan Slatina, *Navicula wendlingii* was a dominant diatom on mud substrate followed by the above-mentioned species *Nitzschia palea* var. *debilis*.

Ecological status assessment using Diatom Index for Soda Pans (DISP) and Trait-Based Index (TBI)

The application of traditional, species-based index in five natural and two degraded saline wetlands in Serbia revealed statistically significant differences in their ecological status (Table 3). The lowest DISP values (around 3.0) were recorded in two degraded pans, Velika Slatina and Slatina (Fig. 3). At the same time, the proportion of taxa with known indicator and sensitivity values was the lowest in these two pans, 66.4% and 53.7%, respectively. Similar to DISP index, TBI index indicated the lower ecological status of two degraded saline habitats in contrast to the majority of pans in natural status (Table 3). However, as far as TBI index is concerned, the results of Welch's *t*–test showed that there was no difference between two groups of pans (Table 3)..

Fig. 3 Values of DISP index in investigated saline habitats

Fig. 4 Values of TBI index in investigated saline habitats

Table 3. The average values of the selected physical and chemical parameters, DISP and TBI indices in natural and degraded soda pans

Discussion

As a result of a disturbance, a natural soda pan can lose its typical chemical features (e.g. high conductivity and high pH) or specific structure of living communities (for example low diversity), or ultimately both. In our study, two disturbed soda pans (Slatina and Velika Slatina) were characterized by altered water chemical parameters, higher species richness and Shannon diversity as well as a clear presence of freshwater diatom taxa. Our results are in accordance with the findings of Boros et al. (2013) who placed Slatina (near Baranda) in the category of a modified (artificially stabilized) open waterbody. On the other hand, in their study focused on zooplankton composition of soda pans in the Carpathian basin, Tóth et al. (2014) reported the presence of the primary indicator species for soda pans in reference status, crustacean *Arctodiaptomus spinosus*, in several soda habitats in Serbia, including Slatina near Baranda. It is possible that this soda pan was degraded in the last decade (Tóth et al. conducted their study in 2009, and there were no similar studies since that time), but this also could mean that some organisms can survive despite clear habitat disturbance. For example, during their study of three Central European reconstructed soda pans Lengyel et al. (2016) reported that one of the pans had constantly low conductivity, higher diatom diversity, and species richness, but zooplankton and zoobenthos composition were similar to a pristine saline lake. Second pan Velika Slatina, although hyposaline, can be considered modified since a deep channel is constructed along its bed. First algological studies of this soda pan date from the beginning of 21st century and demonstrated the transition from the epiphytic diatom assemblages dominated by *Navicula slesvicensis* (in spring 2003, conductivity 2215 $\mu S\ cm^{-1}$) to the community with the highest frequency of *Cocconeis placentula* (in summer 2006, conductivity 700 $\mu S\ cm^{-1}$) (Krizmanić 2009). *N. slesvicensis* is the species relatively abundant in slightly brackish waters, such as inland salt springs (Lange-Bertalot 2001), while *Cocconeis placentula* is a fresh-brackish species (Van Dam et al. 1994) with wide ecological tolerance (Stenger-Kovács and Lengyel 2015).

Our results of the major ion composition in natural soda pans revealed the high percentage of sodium and bicarbonate-carbonate ions in total cations and anions, respectively. During the large–scale investigation aiming to explore the chemical characteristics of the astatic soda pans in the Carpathian Basin, Boros et al. (2014) reported the basic soda (Na-HCO_3) as the most frequent chemical type of soda waters. In our study, Medura and Čoka Kopovo belonged to this sodic base type. In other soda pans (Slano Kopovo, Lake Velika Rusanda, and Lake Mala Rusanda) we confirmed the presence and order of other dominant anions (chloride or/and sulfate) reported by Boros et al. (2014).

Typical features of soda lakes are stable high-pH environment (pH above 9) and dominance of carbonate species, HCO_3^- + CO_3^{2-} (Boros and Kolpakova 2018). The chemical processes that generate alkalinity in brine waters are complex, but it is clear that they are related to the lack of Ca^{2+} and Mg^{2+} in the surrounding land (Grant 2006). These alkaline earth metals bind to CO_3^{2-} and precipitate (e.g. as $CaCO_3$ or calcite), but if the concentration of Ca^{2+} and Mg^{2+} is low then CO_3^{2-} remains in the solution developing alkaline environment (Grant 2006). In contrast to the natural soda pans, the water in Velika Slatina was circumneutral (pH~7.6), amount of Ca^{2+} and Mg^{2+} was much higher and chloride dominated over carbonate. Our finding complied with the results of Blinn (1993) who reported the lowest average pH (8.2±0.5) in Na-Cl dominated saline lakes. In Slatina, measured pH value was also near-neutral (pH~7.6), but since a complete chemical analysis of water is lacking the relationship between pH and ion content remained unclear. We recorded higher concentrations of inorganic nitrogen and phosphorus in undisturbed soda pans. In contrast to freshwater habitats where an excessive amount of phosphorus and nitrogen is undesirable, natural astatic soda pans are often hypertrophic due to the nutrient load by the waterfowl (Stenger-Kovács et al. 2018). By contrast, modified saline pans Slatina and Velika Slatina are not closed, but open basins with higher depth and regulated water level, so it is likely that running water diluted or flushed at least a part of the nutrients.

Two traditional diversity measures (species richness and Shannon diversity) revealed a difference in the diatom community structure between natural and disturbed saline pans. Species richness and Shannon diversity were the highest in two pans that channel water at least in some part of the year. Our results comply with the finding of Lengyel et al. (2016) who reported a low species richness (13) and diversity (mean 1.7) in the reference (natural) pans. Based on classification given by Van Dam et al. (1994) most diatoms belong to fresh-brackish and brackish-fresh taxa, but in two modified pans, particularly in epipelic community, freshwater taxa were common and contributed more to the value of the diversity index.

A density of diatom populations developed on the sediment surface (mud) in four large soda pans in natural status (Slano Kopovo, Čoka Kopovo, Lake Mala Rusanda, Lake Velika Rusanda) was very low in spring and thus, the number of counted valves was far less than 400. Our results are in contrast with the study of Lengyel et al. (2016) who did not find a difference between the substrate types in soda pans. We think that in Lake Velika Rusanda and other investigated large soda pans, abiotic factors in benthos were severe in spring (e.g. very low transparency) due to the heavy storms that caused intensive filling and mixing of the water column 1-3 weeks before sampling. As a result of a two-year study of diatom community in Lake Velika Rusanda, Vidaković et al. (2019) also found that the diatom assemblage on mud existed mainly in the summer in this lake. On the other hand, in Medura and two disturbed soda lakes, the epipelic community was well developed. Soda pan Medura was sampled before heavy rains and, at the time of sampling, it was a small, very shallow depression with a transparent water column above the whole water bed. Similarly, Velika Slatina and Slatina although much deeper pans, due to the artificially created high banks were not so prone to water mixing caused by wind.

Nitzschia austriaca, *N. supralitorea*, *Navicula wiesneri* and *N. veneta* were among dominant diatoms in soda pans of reference status. We found *N. austriaca* to be dominant in three soda pans (Slano Kopovo, Čoka Kopovo, and Lake Mala Rusanda). These pans were alkaline (pH 8.21-9.05) and hypertrophic waterbodies (TP 0.56–3.26 mg/L) with low transparency (0.5-5.5 cm) and conductivity that varied from 813 to 4040 µS/cm. Our findings comply with the study conducted in Central European astatic soda pans by Ács et al. (2017) who reported the presence of this small sigmoid species in alkaline, hypertrophic and turbid soda waters with an average conductivity of 3800 µS/cm. Földi et al. (2018) also showed that *N. austriaca* prefers "turbid" soda pans and can indicate their “good” ecological status. The same authors also found that species *N. supralitorea* and *Navicula wiesneri* were significantly related to the "good" status of "turbid" soda pans, while *Navicula veneta* was an indicator of "good" status in "transitional" group of soda pans with "relatively large water surface and narrow macrophyte belt" (Földi et al., 2018). In our study, the highest relative abundance of *N. veneta* was recorded in Slano Kopovo, a soda pan with a large surface area (~120 ha).

Unlike soda pans in "good" status, disturbed saline wetlands were not characterized by the domination of typical sodic water species. In chloride-rich hyposaline soda pan Velika Slatina, *Nitzschia paleacea* and *N. inconspicua* dominated in the epiphytic community, while *Hantzschia amphioxys* and *Denticula subtilis* were the most abundant in the community developed on the sediment surface. *N. paleacea* was recorded in waters with moderate to high conductivity, characterized by elevated chloride concentration (Stenger-Kovács et al. 2014). *Nitzschia inconspicua* occurred with higher frequency in moderate and slightly electrolyte-rich freshwaters (Stenger-Kovács and Lengyel 2015). According to Lange-Bertalot et al. (2017), *Hantzschia amphioxys* is a fresh-brackish species that inhabits circumneutral waters and *Denticula subtilis* is a brackish-fresh species found mainly on wet and moist places. In subsaline soda pan Slatina, *Navicula wendlingii* occurred with the highest frequency on both substrates, followed by *Tabularia fasciculata* and *Nitzschia palea* var. *debilis* in epiphytic and epipelic communities, respectively. *Navicula wendlingii* is a recently described diatom species, so its ecology is poorly known. It was found recently in the lower part of the River Elbe in the zone impacted by tides (Van de Vijver and Lange-Bertalot 2009). *Tabularia fasciculata* is an euryhaline diatom occurring in wide range of habitats, from marine to freshwaters. Bak and Szlauer-Lukaszewska (2012) reported the presence of this species in brackish, slightly alkaline waters influenced by freshwater of river Odra. *Nitzschia palea* var. *debilis*, was characterized by Van Dam et al. (1994) as freshwater species mostly found in pH neutral waters. In Slatina, this species was identified in samples with lower conductivity (828 μS/cm) and pH around 7 (7.67).

Similar to species richness and Shannon diversity, a species-based DISP index showed that two modified panss scored lower in ecological status assessment compared to five natural soda pans. However, there are at least two major shortcomings that should be addressed for a successful application of the DISP index in Serbia. Firstly, indicator and sensitivity values for around 25% of the diatom species found in investigated saline pans in Serbia (with >5% relative abundance in each sample) are unknown, since these species are not listed in species pool for index calculation given by Stenger-Kovács et al. (2018). For example, one of these species is *Navicula wendlingii* recorded with high relative abundance (from 5.5-40.1%) in three saline wetlands in Serbia. Secondly, it is possible that indicator values for some diatom species might differ in different parts of the Carpathian Basin. For instance, Stenger-Kovács et al. (2018) reported that conductivity optima for species *Nitzschia thermaloides* fall below 1999 μS cm^{-1}, and gave the indicator value 1 to this species. In contrast, we found *N. thermaloides* with the highest relative abundance (70.3%) in Lake Velika Rusanda which was, at the same time, the soda pan with the highest measured conductivity (14000 µS cm^{-1}). If this species was assigned indicator value 6 (for species with optima >6000 µS cm^{-1}) then Lake Velika Rusanda would have had higher ecological status (5.86 instead of 3.56). In their study of different brackish environments inhabited by diverse diatom taxa, Heudre et al. (2020) reported the presence of *N. thermaloides* in Roanne River at Lenoncourt, at the site with very high conductivity (8183 µS cm^{-1}).

In our study we also used TBI index to complement the ecological status assessment of saline habitats based on traditional, species-based approach. The average value of TBI indices was lower in the group of disturbed soda wetlands compared to the group of natural soda pans, but this difference was not statistically significant. The TBI index is calculated as a ratio of traits (diatom groups) that indicate good or excellent ecological conditions (MS1, MLW2, MLW3) and traits that are related to degraded ecological status (P, S4, H and L) (Stenger-Kovács et al. 2018). In natural soda pans, functional groups of diatoms connected with high conductivity prevailed, while other groups were rare (with relative abundance <0.5%) or absent. In Slano Kopovo and Lake Mala Rusanda, motile diatom species with small biovolume (MS1) were most frequently observed, while in Medura and Čoka Kopovo motile diatoms with more elongated valves (MLW3) made up around half of valves in benthic diatom community. Typical representatives of the MS1 group were *N. supralitorea* (for instance, in Medura) and *N. austriaca* (in Slano Kopovo), while among dominant diatoms in MLW3 the following species were found: *Navicula wendlingii*, *N. veneta* and *Nitzschia pusilla* (in Lake Mala Rusanda). Unexpectedly, in the natural soda pan Lake Velika Rusanda, the TBI index had the lowest value, and thus decreased the average value of TBI indices in the group of natural soda wetlands. This can be explained by the fact that *N. thermaloides*, the most abundant taxon in this soda pan was classified into the S4 group of diatoms that prefer waters with low conductivity and high pH. The presence of this ecological group was not consistent with our *in situ* measurements since in this lake the highest conductivity was recorded. This indicates the need for further studies of *N. thermaloides* autecological properties.

In contrast, diatoms with "undesirable" functional traits reached relative abundance between one-fifth and one-third of the total abundance in two disturbed soda pans. In Velika Slatina, species classified in low (L) and high (H) profile ecological guilds constituted around 20% of all diatoms, while in Slatina, H group solely made up a one-fifth of total diatom abundance. A group of attached diatoms with short stature (L) was represented by *Achnanthidium minutissimum* and *Amphora indistincta*. Former species is considered to be a cosmopolitan, widespread diatom in benthic samples, and has been reported from alkaline to acidic waters (Rimet et al. 2009; Potapova and Hamilton 2007). Stenger-Kovács and Lengyel (2015) reported the presence of *A. minutissimum* in several soda pans in Hungary and Lengyel et al. (2016) showed that the presence of this species in Legény-tó (soda pan) was associated with the change of the natural hydrological regime and connection with the canal. Latter species that was recently described, *Amphora indistincta* was found in moderately polluted freshwaters and was also observed in soda pans of the Carpathian Basin (Stenger-Kovács and Lengyel 2015). Diatoms classified in H group, *Gomphonema parvulum* and *Tabularia fasciculata*, were observed only in the epiphytic community developed in one disturbed pan. According to the classification given by Van Dam et al. (1994), a short-stalked diatom *G. parvulum* is a fresh-brackish species. We found this species in Slatina, a subsaline wetland with the highest Shannon diversity and low measured conductivity of 828 µS/cm (salinity = 0.66 mg/L). Our results comply with Rimet et al. (2009) who stated that *G. parvulum* is a successful competitor for light and space in well-developed algal biofilms.

In conclusion, the results of our study showed that modification of soda pan's natural hydrological regime can cause dramatic alteration, not only in abiotic factors, but also in the structure of pristine biological communities. To some extent, these changes may still be reversible, if we prevent a construction of new channels or roads across saline wetlands. Without freshwater input, saline environments may be restored and inhabited by organisms found in adjacent pans or "salt islands with their metapopulations". The Hungarian approach based on *ex lege* protection of saline habitats and application of new diatom indices for soda pans proved to be successful and should be implemented in other countries, including Serbia.

However, there are several challenges along the path of a successful ecological status assessment of soda pans. Interannual, seasonal, or even daily fluctuations of master variables (e.g conductivity) caused by natural forces complicate a definition of reference conditions in soda pans (Földi et al. 2018). Our results showed that changes in only one week in a sampling schedule, before and after a heavy storm, can mean a sharp difference in hydrological phases and thus affect the results of benthic diatom community analysis. In addition to hydrological alterations and negative impact of freshwaters on soda pans, there are other pressures as well, such as nutrient and pesticide pollution. Thus, future conservation practice should focus on developing tools that are sensitive enough to detect the presence of these negative compounds in saline habitats.

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Table 1

Name of pan	**Status[1]**	**Area (ha)**	**Altitude (m)**	**Diatom samples**	**GPS Coordinates**	
					epiphyton	**epipelon**
Medura	N	5.8	94	2	N45°59'32.64" E19°08'02.90"	N45°59'30.54" E19°07'55.08"
Slano Kopovo	N	119.3	77	2	N45°37'49.12" E20°12'02.52"	N45°37'49.12" E20°12'02.52"
Čoka Kopovo	N	8.5	78	2	N45°57'18.30" E20°11'58.14"	N45°57'18.30" E20°11'58.14"
Mala Rusanda	N	23	78	2	N45°30'49.68" E20°18'22.80"	N45°30'53.33" E20°18'20.91"
Velika Rusanda	N	148	77	2	N45°31'35.20" E20°18'06.49"	N45°31'35.20" E20°18'06.49"
Velika Slatina	D	-	76	2	N45°03'10.34" E20°29'36.15"	N45°03'10.34" E20°29'36.15"
Slatina	D	-	79	2	N45°04'14.22" E20°28'23.25"	N45°04'14.22" E20°28'23.25"

[1]Status: N - natural; D - degraded.

Table 2

Parameter	Unit	Soda pans						
		Medura	Slano Kopovo	Čoka Kopovo	Mala Rusanda	Velika Rusanda	Velika Slatina	Slatina
Water Depth	cm	34.0	14.5	20.5	19.0	30.0	65.0	–
Temperature	°C	17.0	25.9	25.7	30.9	32.7	28.7	30.4
Transparency	cm	–	0.50	5.0	5.5	12.5	–	–
pH		9.21	8.81	8.21	9.05	9.60	7.63	7.67
Conductivity	µS/cm	8900.00	2700.00	813.00	4040.00	14300.00	4509.00	828.30
Dissolved oxygen	mg/L	11.00	9.30	7.20	7.10	7.20	3.90	6.70
Potassium	mg/L	43.07	7.22	7.12	61.13	140.80	3.26	4.26
Sodium	mg/L	1994.0	612.5	163.5	776.0	2908.0	585.8	102.8
Calcium	mg/L	20.35	27.63	34.28	6.77	6.20	129.80	33.35
Magnesium	mg/L	66.23	22.99	4.36	10.88	8.22	99.27	28.58
Carbonate	mg/L	1096.2	72.6	127.2	150.6	1315.2	30.0	76.2
Bicarbonate	mg/L	1795.8	430.7	306.8	687.5	47.6	601.5	367.2
Ammonia	mg/L	0.84	2.94	1.35	3.63	1.71	0.71	0.29
Nitrite	mg/L	<0.020	0.121	<0.020	0.067	0.390	<0.02	<0.020
Nitrate	mg/L	1.50	4.70	0.80	0.80	<0.5	<0.5	<0.5
Total nitrogen	N mg/L	5.1	3.4	1.4	3.2	1.5	<1.0	–
Phosphate	P mg/L	3.40	1.40	0.49	3.09	21.00	<0.02	<0.02
Total phosphorus	P mg/L	3.40	1.42	0.56	3.26	23.00	0.02	0.03
Chloride	mg/L	750.9	548.9	63.7	493.4	2161.0	1062.0	75.4
Sulphate	mg/L	1152.7	205.0	8.1	701.2	2401.0	10.3	5.7

Table 3. The average values of the selected physical and chemical parameters, DISP and TBI indices in natural and degraded soda pans

Parameter	|Natural	Degraded
pH	8.98 ± 0.23 [a]	7.65 ± 0.02 [b]
DO (mg/L)	8.36 ± 0.78 [a]	5.30 ± 1.40 [a]
TP (P mg/L)	6.328 ± 4.202 [a]	0.025 ± 0.005 [b]
NH_4^++NO_2^-+NO_3^-(N mg/L)	2.02 ± 0.49 [a]	0.45 ± 0.16 [b]
DISP index	4.44 ± 0.30 [a]	3.07 ± 0.20 [b]
TBI index	7.36 ± 1.04 [a]	4.85 ± 0.05 [a]

The data are expressed as the mean ± SE.

Different letters denote significant difference (Welch's *t*-test, $p \leq 0.05$)