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USING MULTIVARIATE STATISTICAL ANALYSIS FOR A PRELIMINARY ASSESSMENT OF ECOLOGICAL RISK IN SHALLOW LAKES

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Abstract. Shallow lakes have an important role for the humanity and maintenance of environmental quality. However, shallow aquatic ecosystems are affected by numerous stressors as eutrophication, pollution, invasion of different species, drought, uncontrolled fishery, climate changes. Mentioned stressors often have a synergistic impact that enlarges the consequences and contributes to accelerated degradation of the shallow lake ecosystem. To assess the (ecological or environmental) risk, researchers use different kind of models that can be grouped into two main categories: statistical and dynamic models. By applying multivariate statistical analysis, it is possible to determine the statistical relationship between variables (physico-chemical and/or microbiological parameters) and reveal relations that are not obvious but may be relevant for the research problem. Multivariate statistical analysis could be used for reducing the number of variables in the analysis, but also for understanding the structure of the investigated phenomenon or the relative importance of the analysed factors. In this paper, the main idea was to choose easily measurable parameters that could be used in future prediction of the risk of biomass hyperproduction and microbial risk. Factor analysis (principal components analysis) was used for reducing the number of variables. Data collected from two very shallow Pannonian lakes, Palic and Ludas, located at the north of the Republic of Serbia were used for the analysis. Two parameters were chosen as good descriptors of the lake state and good candidates for future detection of the risk of biomass hyperproduction and microbial risk: chlorophyll a (eutrophication) and enterococci (faecal pollution). The candidates for explanatory variables (predictors) were dissolved oxygen, saturation percentage, pH, electrochemical conductivity, temperature, and total suspended solids. The independent variables were analysed separately with the enterococci and chlorophyll *a* in order to improve the understanding of the connection. In the analysis of a set of variables with enterococci, the first component explains 38% of the variance of the original data set, while the second component explains another 22%. In the analysis of a set of variables with chlorophyll a, the first component explains 45% of variance of data, while the second component explains another 20%. In the formation of the main components, in addition to enterococci, and the chlorophyll a, a significant part in both models, temperature, pH and suspended solids. In this way, the determined three potential predictors among easily measurable parameters, which could be further used in risk modelling.
Keywords: shallow lakes, pollution, eutrophication, risk, principal components analysis

1. INTRODUCTION

Shallow aquatic ecosystems are affected by numerous stressors as eutrophication, pollution, invasion of different species, drought, uncontrolled fishery, climate changes. Given the specific morphology, shallow stagnant or slowly moving waters are especially sensitive to mentioned stressors, which often have a synergistic impact that enlarges the consequences and contributes to accelerated degradation of shallow lake ecosystem. Shallow eutrophic lakes are the subject of continuing research worldwide, e.g. Barton Broad in Great Britain (Madgwick, 1999), Lake Balaton in Hungary (Tatrai et al., 2000), Lake Okeechobee in Florida USA (Havens and Gawlik, 2005), Lake Donghu in China (Xie, 2006), lakes Honda and Nueva in southeast Spain (de Vicente et al., 2006), Lake Veluwe in Netherlands (Ibelings et al., 2007), as well as numerous Danish shallow lakes (Sondergaard et al., 2005). Cultural eutrophication was the main cause of impairment of listed lakes. Despite extensive research in last four to five decades, a lot of questions considering this research area remain unanswered. There is still a lot to learn and understand, regarding interactions that exist between nutrients and ecosystem stability (Smith & Schindler, 2009).

In order to assess the (ecological or environmental) risk, researchers use different kind of models that can be grouped into two main categories: statistical and dynamic models. In aquatic ecosystems analysis, statistical models are usually applied for risk assessment, and dynamic models for assessing the functioning of the system. Both groups have advantages and downsides. It is possible to determine statistical relationship between variables (physico-chemical and/or microbiological parameters), by applying statistical modelling methods, and reveal relations that are not obvious but may be relevant for the research problem. Statistical models could be used for reducing the number of variables in the analysis, but also for understanding the structure of the investigated phenomenon or the relative importance of the analysed factors. On the other hand, (dynamic) models of aquatic ecosystems are diverse in structure and purpose, as distinct and motivation for their development. Their use, as well as application of different methods for risk assessment actually make scientific work applicable in decision-making when it comes to risk management, planning, and preparation of policies, strategies and regulations.

The aim of our research is to conduct a preliminary assessment of lake water quality in order to identify main predictors of biomass hyperproduction and microbial risk. The same idea was successfully applied by Pinto et al. (2012) on peri urban river system. Nevertheless, the application of this idea to eutrophic shallow lakes has new challenges. Before developing the predictive model, it is necessary to carefully analyse and prepare data, which is the aim of this paper. For that purpose, we used principal component analysis, a statistical method that can be applied for analysis and interpretation of complex data sets as well as for determination of pollution sources (Gvozdić et al., 2012). The principal component analysis is successfully applied in water research when manipulating with large datasets, e.g., when it is necessary to identify the parameter that predominantly contributes to the pollution (Okonofua et al. 2019), or developing water quality indices, because the use of statistical techniques makes this kind of analyses less biased and more objective in nature (Tripathi & Singal, 2019).

2 MATERIAL AND METHODS

2.1 Site description

The data analysed in this paper was collected from two very shallow Pannonian lakes, Palic and Ludas, located at the periphery of the Suboticko-Horgoska Pescara (sand area), at the north of the Republic of Serbia (Figure 1). The lakes are shallow Pannonian lakes, created a million years ago, when the wind created pits separated by dunes that were recharged mostly by natural precipitation and several small water streams.

Figure 1. Surroundings of Palic and Ludas Lake (A – area; V – volume; h – average depth)

Both lakes are exceptional, since they are home to diverse habitats (aquatic, swamp, meadow and steppe) with a number of plant and animal species, some of which are strictly protected. They are a part of IBAs site (Important Bird Area). Palic Lake is a part of a natural park, i.e. a protected area of local significance. Ludas Lake belongs to the first category of protection, as a natural area of exceptional significance and it is on a list of Ramsar sites. Both lakes are part of an area with great recreational and touristic value that has eventually undergone cultural eutrophication. Urbanization for over 100 years led to changing their natural characteristics. Reclamation work across the drainage basin of the lakes influenced loss of the most important natural water recharge. Today, the lakes don't have just recreational purpose, but they are also wastewater recipients. Palic Lake receives treated municipal wastewater, in the annual amount that exceeds its volume. Palic and Ludas are connected by the canal that also receives inadequately treated wastewater from different sources (Rudic et al., 2014; Rudic et al., 2018). Human negligence and sewage discharge as a source of nutrient enrichment induced accelerated eutrophication and deteriorated biodiversity of these lakes (Raicevic et al., 2011; Bozic et al., 2013). The consequence of the discharge of treated and untreated wastewater is polluted water with high trophicity and large amount of sediment. The lakes are eutrophic, with high self-cleaning ability, but they are being loaded with excessive amount of nutrients, therefore the nutrient concentrations in water are always high (Raicevic et al., 2012; Rudic et al., 2015). Recently the signs with warning that water is not recommended for swimming have been frequently posted at the coast during the summer, due to high levels of faecal coliforms, although the site is a popular recreation area.

2.2 Sampling and analyses

For the study, water samples were collected during the period November 2013 to March 2015, in total 11 samples per location, on 5 locations (2 at Ludas Lake and 3 at Palic Lake). The selected locations are along the coastline, since these are the places where the population may be in contact with water, and potentially exposed to waterborne diseases. Water samples were collected at 20-50 cm beneath the water surface (Figure 2). Samples were transported in cool containers under 8°C and tested within 12-24 hours of collection. The analyses of collected samples have been done in laboratories of the Department of Ecological Microbiology, Faculty of Agriculture, University of Belgrade and Institute for the Development of Water Resources "Jaroslav Cerni".

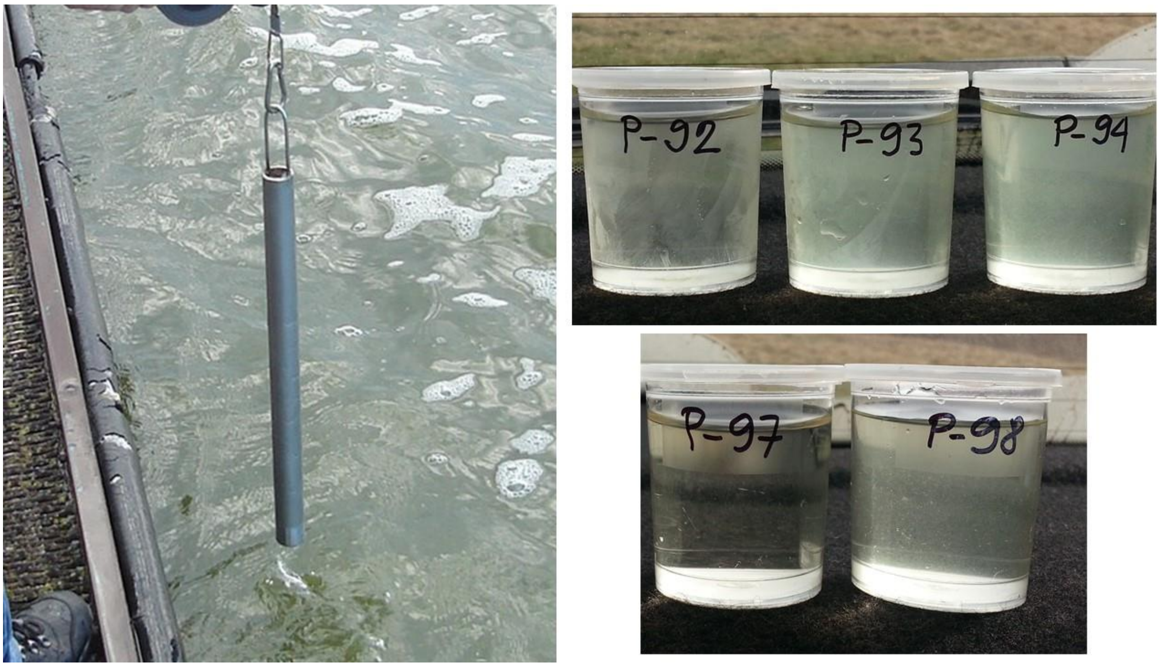

Figure 2. Water sampling (left) and water samples taken in March 2015
(3 from Palic Lake and 2 from Ludas Lake)

Dissolved oxygen, saturation percentage, pH, electrochemical conductivity and temperature were determined in situ, by multi-parameter analyser Consort C6030. Total suspended solids were determined according to the standard method of APHA (2005). Detection of *Enterococcus* was done by the most probable number (MPN) method using three tubes in each dilution. Presumptive test of *Enterococcus* was done using azide–dextroze broth (37°C/48h), and the confirmation by inoculation on the Bile Esculin Agar (37°C/24h).

Results are reported as MPN per 100 mL of water. The determination of chlorophyll *a* concentration was done according to ISO 10260:1992. This method includes collection of algae by filtration, extraction of algal pigments, and spectrometric determination of the chlorophyll *a* concentration in the extract.

2.3 Statistical analysis

The results of the survey carried out in the period 2013-2015 have been used for further processing and it is intended to use them for the development of model for preliminary risk assessment. For data processing and analysis of the results we used IBM® SPSS® v.22 and Microsoft Excel. Factor analysis was performed for selected variables (above), i.e. principal component analysis. Additionally, Pearson correlation was used to make preliminary quantification of association between selected variables.

Principal component analysis represents a research tool that is used to generate hypotheses about the stated problem, in addition to reducing the dimension of the data set. Principal components analysis produces components, so the issues of whether it is truly a factor analysis technique has been raised (Young & Pearce, 2013).

Principal components analysis is used to extract maximum variance from the data set with each component thus reducing a large number of variables into smaller number of components (Tabachnick & Fidell, 2007; Young & Pearce, 2013). The results of this analysis are usually only input for other multivariate analysis methods (Kovačić, 1994). In this paper, principal components analysis was used for reducing the number of variables. Principal component analysis (PCA) is an analytical methodology used commonly in the scientific community as it allows reducing the dimensionality of a data set, while maintaining the characteristics of variables which contribute most to this variation (Garcia et al., 2017).

3 RESULTS AND DISCUSSION

After the survey we obtained a database for 7 parameters, with 55 data elements for each parameter. We used this database for the development and validation of statistical model. We analysed associations between the physicochemical parameters and faecal indicators (enterococci) of water, in order to reveal parameters associated with bacterial occurrence. The candidates for explanatory variables (predictors) were dissolved oxygen, saturation percentage, pH, electrochemical conductivity, temperature, and total suspended solids. The first step towards selecting the appropriate data was to summarize the data features (Table 1) and to determine correlation of response variables and potential predictors (Table 2). When considering skewness and kurtosis (Table 1), data series that have normal distribution are pH, suspended matter and chlorophyll *a*. The lower negative kurtosis value means that the temperature data distribution is slightly flatter than normal although the data is not outside the normal range. The distribution of data for parameters, such as electrical conductivity, dissolved oxygen and enterococci are skewed, so they can be considered non normal.

Table 1. Descriptive statistics for chosen water quality parameters

		Temperature	pH	Electrical conducti-vity	Dissolved oxygen	Suspended matter	Chlorophyll *a*	Enterococci
Mean		16.68	8.68	974.8	8.39	33.79	264.2	285.8
Confidence interval for mean 95%	Lower bound	14.71	8.57	908.5	7.92	28.62	212.1	161.1
	Upper bound	18.65	8.80	1 041.2	8.87	38.96	316.4	410.4
Median		15.80	8.82	877.0	8.20	31.80	235.7	67.7
Variance		53.14	0.18	60 283.5	3.03	365.71	36 481.9	192 414.6
Standard deviation		7.29	0.43	245.5	1.74	19.12	191.0	438.7
Minimum		6	7.69	569	6.2	1	2.66	1
Maximum		31.3	9.32	1 800	15.7	77	901.6	1 732.9
Skewness		0.06	-0.60	1.50	2.29	0.14	0.84	1.81
Kurtosis		-1.43	-0.73	1.83	6.34	-0.39	0.94	2.35

Through an analysis of the correlation matrix shown in Table 2, it was possible to verify the association between the variables. The resulting correlation coefficients (Table 2) indicate very weak to strong correlation between the variables. Correlation between chlorophyll *a* and pH can be described as moderate, while the correlation between chlorophyll *a* and total suspended solids is strong. Chlorophyll *a*, as well as enterococci, displays weak correlation with electrical conductivity and oxygen, which indicates their low suitability for risk model development. Enterococci show weak correlation with other physico-chemical parameters. The strong correlation between enterococci and chlorophyll *a* is obvious (Table 2), possibly because of increased turbidity caused by phytoplankton growth, which creates UV protection for bacteria (Kay et al., 1994).

Table 2. Correlation matrix of chosen water quality parameters

	Temperature	pH	Electrical conductivity	Dissolved oxygen	Suspended matter	Enterococci	Chlorophyll *a*
Temperature	1						
pH	0,366	1					
Electrical conductivity	-0,077	-0,262	1				
Dissolved oxygen	-0,042	0,014	0,287	1			
Suspended matter	0,176	0,621	-0,520	0,070	1		
Enterococci	0,213	0,313	-0,070	0,046	0,303	1	
Chlorophyll *a*	0,240	0,592	-0,347	0,162	0,740	0,605	1
Significant correlation $P < 0.05$							

In order to reduce the number of parameters for developing predictive model, a factor analysis is applied using the SPSS software package. Therefore, factor analysis with principal components extraction is used to focus the analysis on a manageable subset of the predictors. The independent variables were analysed separately with the enterococci and chlorophyll *a* in order to improve the understanding of their relations with chosen physico-chemical parameters. Since there are to separate issues that are related to investigated shallow lakes, data that are to be used for the assessment of risk from faecal pollution (sanitary aspect) and hyperproduction of biomass (advanced eutrophication) were examined separately.

3.1 Data analysis regarding microbial risk

Microbial risk considers the health risk associated with recreational waters, while the risk of biomass hyperproduction considers high biological productivity common for shallow lakes or ponds. Two parameters were chosen as good descriptors of the lake state and good candidates for future detection of the risk of biomass hyperproduction and microbial risk: chlorophyll *a* (eutrophication) and enterococci (faecal pollution).

Establishing the relation of each physico-chemical parameter with microbial pollution was achieved by subjecting the test parameters to statistical analysis using principal component analysis. Eigenvalues represent the total amount of variance that can be explained by a given principal component (Table 3). The analysis of relations between the variables shows that the first component accounted for 38% of the variance of the original data set, while the second component accounted for 22%. Hence, first two components explain 60% of variance of the dataset, which we assume to be satisfactory (Table 3).

Table 3. Total variance explained (regarding microbial risk)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2,292	38,207	38,207	2,292	38,207	38,207
2	1,260	21,001	59,208	1,260	21,001	59,208
3	0,989	16,477	75,685			
4	0,782	13,031	88,716			
5	0,461	7,682	96,398			
6	0,216	3,602	100,000			
Extraction Method: Principal Component Analysis.						

Both components included items with both negative and positive loadings (Table 4). Correlation of suspended matter and pH with component 1 is very strong (Table 4). The other parameters are strongly correlated with component 1, except dissolved oxygen. The second component factor is most likely to correlate with electrical conductivity and dissolved oxygen.

Table 4. Results of PCA displayed as component matrix (microbial risk)

	Component	
	1	2
Temperature	0,548	0,143
pH	0,809	0,208
Electrical conductivity	-0,578	0,654
Dissolved oxygen	-0,043	0,812
Suspended matter	0,857	-0,004
Enterococci	0,517	0,330

3.2 Data analysis regarding biomass hyperproduction

The results obtained from the PCA for this set of parameters (enterococci excluded, and chlorophyll *a* included), shown in Table 5, indicate that there are two (2) components with eigenvalues greater than 1. These components contribute mostly to the variation in the physico-chemical properties of the water samples, similar to previous case. In the analysis of a set of variables with chlorophyll *a*, the first component accounted for 45%, while the second component accounted for 20%. Hence, first two components explain 65% of variance of the dataset, which we assume to be satisfactory (Table 5).

Table 5. Total variance explained (regarding biomass hyperproduction)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2,695	44,917	44,917	2,695	44,917	44,917
2	1,222	20,374	65,291	1,222	20,374	65,291
3	0,981	16,353	81,645			
4	0,534	8,900	90,545			
5	0,351	5,852	96,397			
6	0,216	3,603	100,000			
Extraction Method: Principal Component Analysis.						

Component number 1 included one item with negative loadings, i.e. electrical conductivity (Table 6). Correlation of chlorophyll *a*, suspended matter and pH with component 1 is very strong (Table 6). The other parameters are strongly correlated with component 1, except dissolved oxygen. The second component factor is most likely to correlate with electrical conductivity and dissolved oxygen.

Table 6. Results of PCA displayed as component matrix (biomass hyperproduction)

	Component	
	1	2
Temperature	0,413	0,028
pH	0,805	0,117
Electrical conductivity	-0,594	0,573
Dissolved oxygen	0,009	0,909
Suspended matter	0,892	0,036
Chlorophyll *a*	0,852	0,228

Applied methodology discovered relations that were not obvious, but they existed between water quality parameters of investigated shallow lakes. High concentration of suspended matter is one of the main causes of turbidity in many watercourses, and in combination with bacteria they form floccules which are deposited on the sediment at the bottom (Hansen et al., 2008). As a result of small water column of investigated lakes, wind blowing causes water mixing and turbidity becomes almost permanent. Turbidity is also a consequence of phytoplankton growth. Distribution of sunlight, controlled by turbidity, and also by high content of suspended matter, affects concentration of bacteria in water, which explains the correlation of chlorophyll *a* and enterococci in Palic and Ludas lakes.

Change in pH can affect the growth of algae in several ways: changing the availability of carbon, trace elements and basic nutrients, while the extreme pH values have a direct physiological effect (Chen & Durbin, 1994). The survival of pathogens in the environment is also affected by factors, such as pH, aeration, temperature, nutrient content, predation, growth rate, etc. In particular, the survival of enterococci is better at lower temperatures, in finer sediment, neutral pH, and in an environment that is not exposed to the sunlight (Byappanahalli et al., 2012).

The conducted analyses of easily measurable parameters along with the enterococci and chlorophyll *a* didn’t explain the cause and effect relationship, but emphasized their connection. In this way, three possible predictors (suspended matter, pH and temperature), which have a potential to indicate risk of microbial contamination and eutrophication of the lake water are determined.

4 CONCLUSIONS

Principal components analysis was applied to determine what underlying structure exists for measures on the following variables: temperature, pH, electrical conductivity, dissolved oxygen, total suspended solids (potential predictors) on one side and enterococci and chlorophyll *a* on the other side. The results of PCA showed that two components accounted for about 60-65% of the variance in the data sets. In this way, three potential predictors are determined among easily measurable parameters, which could be further used in risk modelling. Results of conducted analyses may be regarded as a basis for further research. Applied methodology discovered relations that were not obvious, but they existed between water quality parameters of investigated shallow lakes. Conclusions will serve as a base for establishing a predictive model of risk assessment for investigated lakes, although for higher precision and accuracy, additional monitoring and analyses (data) are needed.

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