Environmental Changes in Lakes Catchments as a Trigger for Rapid Eutrophication – A Prespa Lake Case Study

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1. Introduction

Elucidating upon and/or separating the natural processes of eutrophication from the anthropogenically induced ones in a lake's history have proven to be a formidable task. The nature and patterns of the eutrophication processes, their overall impact on the ecosystem and biota, as well as the possible management practices to be introduced to reverse or slow down the accelerated eutrophication have been in focus only very recently mainly due to imposed EU legislation such as the WFD. Another important and also very demanding task that greatly influences management plans, costs and activities is the detection of the reference conditions for every particular water body. Among various suggested approaches, we have concluded that the best way is to reveal the past changes of the lake's environment by conducting paleo-ecological research (the so called *state change approach*) using core sample analyses of as many parameters as possible and relate them to the biota, algae in particular. In that regard, under the comprehensive River Basin Management Plan developed for the Prespa Lake catchment (the part that belongs to Macedonia), the most wide-ranging 12 month surveillance monitoring has been conducted in order to reveal the present ecological situation and the past changes during the last 10 ka period. The results of these investigations are presented in this chapter.

2. Investigated area

The Prespa Lake has been chosen as a part of the complex Prespa-Ohrid-River Crni Drim system which is thought to be more than 3-5 million years old, and as a pilot project as its catchment is relatively small. Nevertheless, obtaining the research objectives was far from simple, since the Prespa region includes two inter-linked lakes, namely the Micro Prespa and Macro Prespa, surrounded by mountainous ecosystems. These two lakes together form the deep points of an inner-mountainous basin that has no natural surface outflow. Drainage is only provided by means of underground links by which water of the Macro Prespa Lake (approximately 845 m asl) drains towards the west to the approximately 150 metres lower Ohrid Lake. On its northern shore, in the town of Struga, the Ohrid Lake has a natural outlet into the Crni Drim River. The Micro Prespa Lake is shared between Greece and Albania, while the Macro Prespa Lake is shared between Albania, the Republic of

Macedonia and Greece. The Ohrid Lake again belongs partly to the Republic of Macedonia and partly to Albania. The Micro and Macro Prespa Lakes are connected by a small natural channel here referred to as the Isthmus of Koula. Since 1969/70 the water level of the Micro Prespa has been controlled by a regulating weir structure to limit the maximum water level.

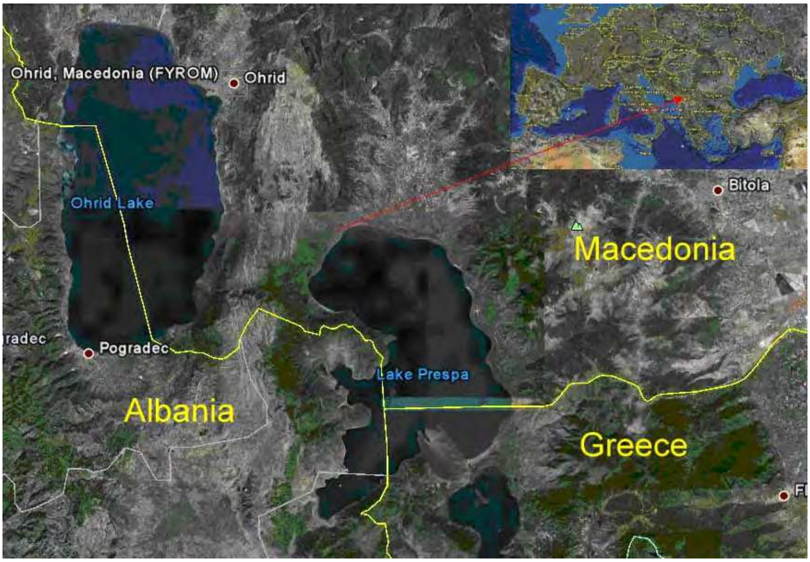


Fig. 1. Google map of the Prespa and Ohrid Lakes system, and the position of the investigated area in the map of Europe.

2.1 Geological and geomorphological features

According to Micevski (2000), the Ohrid-Prespa region is characterised by fairly complex geological-tectonic structures with rocks from the oldest Paleozoic formation to the youngest Neogene and Quaternary sedimentary rocks.

Prespa Valley is surrounded by the mountains of Petrinska Planina, Galichica, Suva Planina, Ivan Planina and Suva Gora. Both mountains and the valley are mainly composed of rocks varying in their age, mineralogical composition and origin. The calcareous rocks are dominant and to a small extent are distributed between magmatic rocks and Grano-Diorites. Syenites are present at the higher elevation areas, but Triassic carbonate rock masses are also present in many areas. Different types of Quaternary sediments, such as alluvial, fluvio-glacial, proluvial, organogenic-marsh and diluvial sediments, are dominant in the valley, especially at the riverbeds. The depth of those sediments varies between 100 and 200 m.

Since the carbonate rocks dominate the geology of Prespa Valley and it is of principal importance for the arguments presented in this chapter, the geomorphological features of Galichica Mountain (located on the North-West side of the valley – Fig. 2) will be presented in more details.

According to its morphometrical characteristics (hypsometry, exposition, inclination), Galichica Mountain exhibits a profound tectonical character (a horst) elevated between two lake basins (Fig. 3). Resulting from its geological composition (almost totally presented by Triassic carbonate rocks), hypsometrical features (only 7.19 km^2 of its surface is above 2.000 m elevation) and climate (or more its fluctuations in the past), the dominant morphogenetic processes that have caused and created modern relief on this mountain are karstic, glacial and periglacial. These processes have been intermingled (supplemented), changed in intensity and duration or fully stopped over the time due to climatic changes.

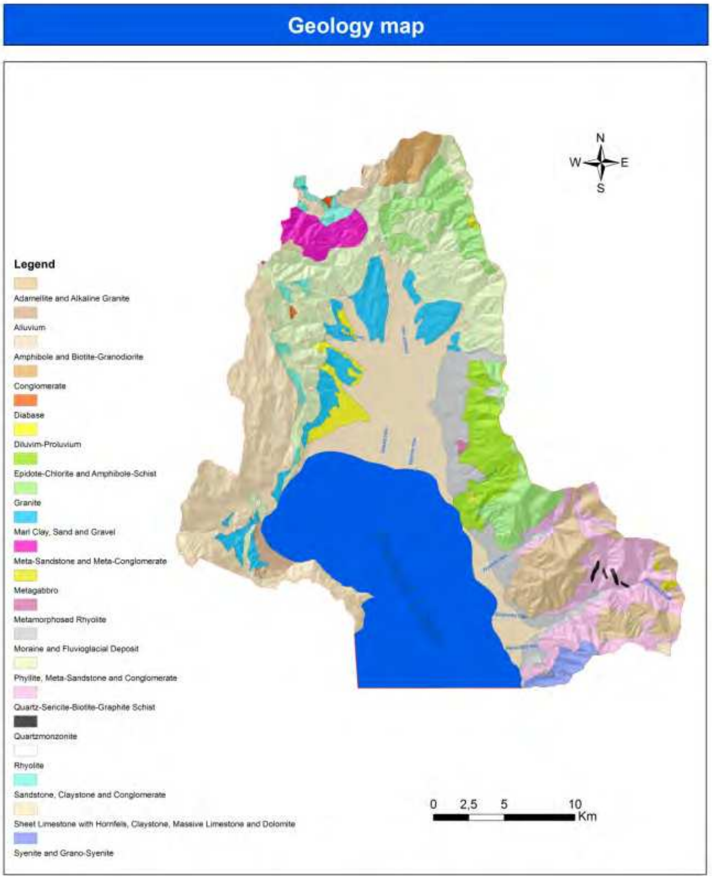


Fig. 2. Geological map of Prespa Lake (Macedonian part only).

Fig. 3. Galichica Mountain – a horst between two lakes, Prespa Lake on the left and Ohrid Lake on the right.

The *karstic features* are the dominant genetic type of relief forms on Galichica Mountain, which is a typical karstic area where the Triassic massive and banked limestone layers lie over the crystal shales. These surfaces have been exposed for a long time to the influence of external factors which have strongly initiated the process of karstification. Micro and macro relief karstic forms, such as *karrens*, numerous karst *sinkholes* and karstic *dry flows*, as well as *karstic fields*, are frequent. From the underground karstic forms, a dozen caves and two chasms have been registered.

Fig. 4. The peak Magaro (2.254 m), the karstic field Lipova Livada and the glacial cirque between them.

Mountain Galichica's altitude and its favourable morpho-plasticity enabled the accumulation of snow and ice during Pleistocene resulting in glacial relief formation. Aside from the two cirques (Fig. 4), a small number of cribs can also be found, but the dominant landscape is formed by the *periglacial* processes resulting in *stony horseshoes, slided blocks, grassy terraces, loose glacial residues* etc. (Fig. 5).

In relation to water transport and balance, such a complex geomorphology based on karstic fundaments has created the only possible type of aquifer in the mountain region – the *fractured type of aquifer* with medium or low water permeability (yield between 0.1 and 5.0 $L*s^{-1}$). The most presented rocks in this aquifer are Paleozoic schists with a low degree of crystallisation (chlorite, sericite, quartz schist and quartzite) Less presented are gneiss, mica-schist and amphibole schist, as well as intrusive rocks (gabbro, granite, syenite, diorite, diabase, quartz-porfire, andesite, etc.).

All those rock masses are rugged with faults, fractures and cracks, which generally cannot accumulate larger reserves of groundwater. These rocks have a very low coefficient of filtration (kf ≤ E-4 $cm*s^{-1}$) and different intensity of fissuring. Local fissuring is very important due to the presence of intense subsurface fissuring and surface to sub-surface weathering (locally over 20 m depth), which influence the hydro-geological characteristics of the rock masses. Often the above mentioned fractures and cracks are closed and filled up with clay material.

Fig. 5. Periglacial landscape on Galichica Mountain.

Similarly the granites, granodiorites and syenites are characterised with low water permeability, with yield of 0.1 – 0.5 L cm*s^{-1} (Pelister, Ilinska mountain, Stogovo, etc.).

Having this kind of natural geomorphology in a lake's catchment means that any significant change in the land cover would result in massive washout of nutrients, minerals and sediments into the lake, due to:

- dominant surface run-off and negligible penetration of the water into the underground aquifers;
- increased erosion (*erosion risk* – Fig. 6) due to abrasive forces on exposed carbonate rocks;
- decreased capacity of soils to accumulate and store excess amounts of nutrients.

2.2 Hydrology

The Prespa catchment area includes two lakes: Micro and Macro Prespa, and permanent or seasonal streams, which discharge into the two lakes. The major contributing rivers to Macro Prespa Lake are Golema, Brajcinska and Kranska Rivers in the Republic of Macedonia and Aghios Germanos River in Greece. There is no major source of surface water input from Albania to Mikro Prespa. Together with the Ohrid Lake, the Prespa Lakes form a unique ensemble of water bodies in the Balkan regions. The Prespa Lakes form the deep points of an inner-mountainous basin that has no natural surface outflow. Drainage is only provided through karstic underground links by which water of the Macro Prespa Lake (approximately 845 m asl) drains westwards towards the Ohrid Lake lying approximately

150 metres below. The Macro Prespa and the Ohrid lakes are separated by the Galichica Mountain, where a surface drainage network is almost absent due to the karstic nature of that mountain chain. The karstic mountains of Mali i Thate (Dry Mountains) in Albania and Galicica in are highly porous, resulting in high capacity for water transport.

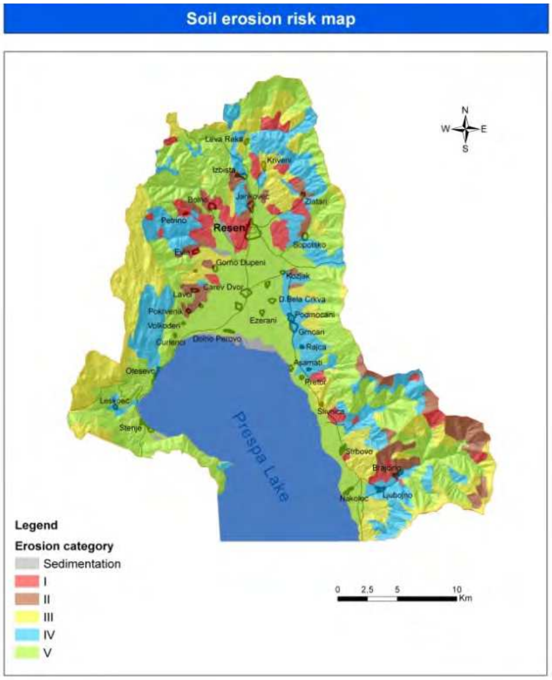


Fig. 6. Soil erosion risk map for the Prespa Lake watershed (Macedonian part only).

The karstic connection of the Prespa Lake and Ohrid Lake is evidenced by spring observations. The Saint Naum spring and Tushemisht springs on the south coast of the Ohrid Lake originate from the Prespa Lake. Lake Ohrid has a surface outflow to the Crni Drim River. Since 1962 the outflows of the Ohrid Lake have been controlled in the City of Struga by means of a weir. Operation of the weir is governed by the needs of downstream hydropower plants and the need to maintain the lake’s water level within optimal levels (maximal water level is 693 m and the minimum water level is 691.65 m).

The Micro and Macro Prespa Lakes are connected in Greece via a small natural channel. From the North and East of the catchment, several small and mostly ephemeral watercourses flow into the Macro Prespa Lake.

The main tributary of Prespa Lake is the Golema River. Two springs which are situated on the slopes of Plakenska and Bigla Mountains form the water courses of the Leva and Bukovska Rivers that join into the Golema River. Elevations of the Golema River springs are at 1440.00 m asl, while it inflows into the lake at 850.00 m asl. Larger tributaries of Golema River flow on the left side of the catchment. These are Krivenska, Celinska and Sopotska. Bolnska River used to inflow into the Golema River in the past, while at present it has become a tributary of Istocka River which inflows into the lake. The total catchment area of the Golema River is F=182.9 km^2, the length of the river is L=26.1 km.

During the last century the Macro Prespa Lake experienced a significant water level fluctuation. After its last peak in June 1963, the level generally dropped to its last low by approximately 8 metres up until 2002 (Fig. 7). This signifies a loss in water volume, with a loss of water surface maybe being even more significant. The major tributary of Macro Prespa Lake, the Golema River, shows high variations of the discharge during that time. It has been noticed that for the series of the minimal discharges it is typical that in some years, during the summer period, the river has very low water discharges or even no water at all. Floods have been registered in 1962 and 1979 with maximal discharge of Q max=33.4 m^3/s and 30.0 m^3/s respectively.

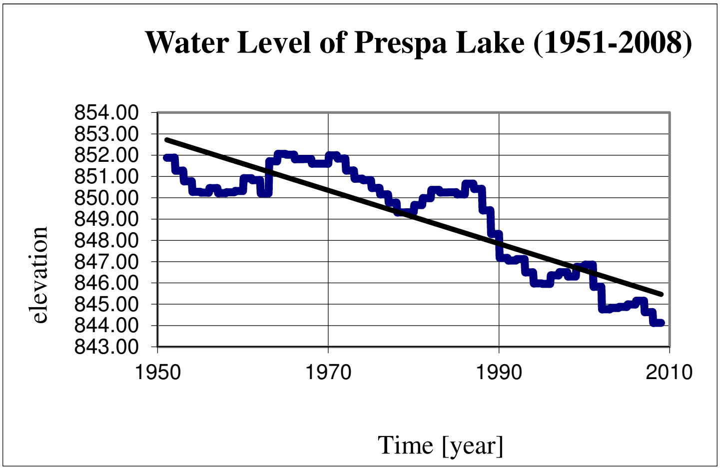


Fig. 7. Water level decrease of Macro Prespa Lake during the 1951-2008 period.

2.3 Soils

Depending on the various pedogenetic factors in the region, there are several soil types presented on the soil map (Fig. 8). Dominant soils in the Prespa Valley are *alluvial soils* (fluvisols) located in the lowest region. These soils are formed over the sediments by the rivers. On the other hand, on the central region of the Ezerani protected area, as well as within the band closest to the lakeshore – *hydric soil* formation is ongoing, which leads to the formation of *gleysols* in different stages of evolution. Around the fluvisols, *colluvial soils* are well developed. These soils are formed above thicker sediments and are being created by the rivers and torrents in the area. On a significant part in the valley and hills on the western side, *chromic luvisols* have been formed and these soils are mainly used for agriculture. On the mountain region various types of *cambisols* have been formed. On the Baba Mt., *eutric* and *district cambisols* are dominant. Natural vegetation adapts to the soils and on the eutric cambisols there are oak stands, whilst on the district cambisols beech compositions stand. R*ankers* (humus-accumulative soils) with various phases of development are formed on the highest altitude in the subalpine and alpine areas. On these soils only grass vegetation is growing.

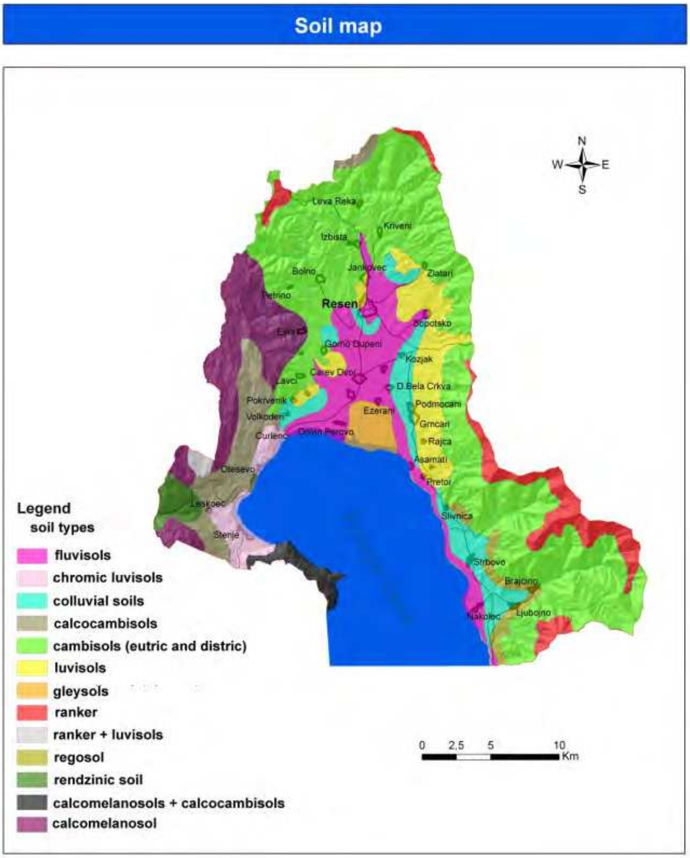


Fig. 8. Soil map of Macro Prespa Lake (Macedonian part only).

2.4 Climate

Being close to the Mediterranean seas (Adriatic and Aegean), it could be expected that the influence of the Mediterranean climate on the location of Prespa Valley would be significant. But the high mountains surrounding the valley mark the existing highland properties of the climate. The specific orographic conditions that have an impact on the dynamic factors of the climate, as well as the impact of geographical and local factors, create three different types of climate throughout the whole watershed (Fig. 9). The whole region of the Prespa Lake watershed belongs to the Eco-region 6 – Hellenic Western Balkan.

- Warm and cold sub-Mediterranean climatic area, from 600 to 900 m and from 900 to 1,100 m altitude, respectively,
- Sub-mountainous and mountainous sub-Mediterranean climatic area, from 1,100 m to 1,300 m and from 1,300 m to 1,650 m altitude, respectively,
- Sub-alpine and alpine climatic area from 1,650 m to 2,250 m and above 2,250 m altitude, respectively.

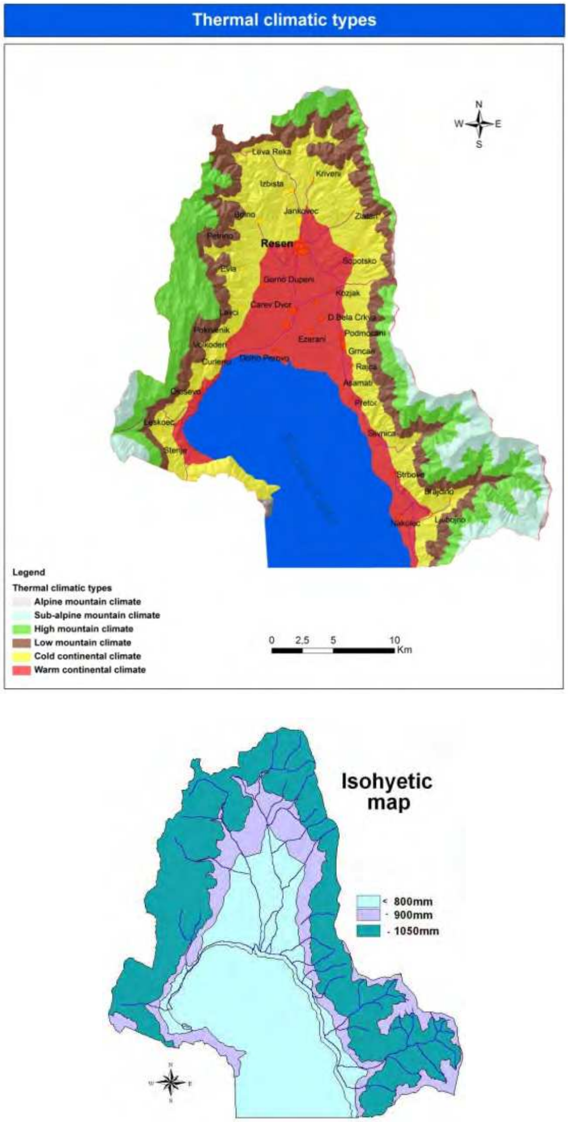


Fig. 9. Climatic types and isohyetic map of Macro Prespa Lake (Macedonian part only).

2.5 Flora and fauna

The vegetation varies from submerged aquatic formations and reed beds to shrublands of junipers and oaks to forests of oak, beech, mixed broadleaves to alpine grassland. In total, there are 1,326 plant species in Prespa, 23 freshwater fish species, 11 amphibian and 21 reptile species, more than 42 mammal species, among which are the brown bear, the wolf, the otter and the chamois, and over 260 bird species.

A shelter for over 90 species of migratory birds, Prespa Lakes are also home to tens of species that have been officially registered as critically endangered or vulnerable. Among them is the Dalmatian Pelican, one of the largest flying birds in the world, who seeks secluded wetlands to build nests and to hatch chicks in what is its largest breeding colony worldwide.

Fig. 10. Dominant reed bed flora and the Dalmatian Pelican (*Pelicanus crispus*) on Macro Prespa Lake.

From the phytocoenological point of view, the presence of endemic plant community *Lemneto-Spirodeletum polyrrhize aldrovandetosum* is the most important.

3 Anthropogenic impacts on the status of surface and ground water bodies

3.1 Estimation of the point source of pollution

In the Municipality of Resen, according to the 2002 census of the Republic of Macedonia, 16,825 people live in 44 inhabited places. In addition there are several tourist centres that are creating additional pressure on the sewage network and water bodies, especially in the summer period: Hotel Pretor, Pretor – 54 guests and 50 summer houses (around 200 persons = 254 people/total); Hotel Kitka, Resen, 40 guests in total; Auto camp Krani, house trailers and tents 3,000 beds, 42 villas 168 beds and 32 bungalows 130 beds = 3,298 people/total; private accommodation in villages Brajčino, D.Dupeni, Pretor, Slivnica, Ljubojno and Stenje = 375 tourists. The calculations for this magnitude of pressure are presented on Tab. 1.

According to these calculations, current load from household sewage (without wastewater treatment) plays a significant role in the pollution of water bodies in Prespa Lake watershed.

On the Macedonian side of Prespa Lake there are several medium-size enterprises from eight industrial branches: food processing, poultry farming, textile, metal processing, wood processing, civil construction, ceramics and the chemical industry.

Based on the environmental data provided by the enterprises themselves, an overall estimation of the point source pollution can be determined as presented in Tab. 2 (values of considerable pollution are coloured in red):

Parameter	*Unit*	*Value*
Inhabitants	person	**20,792**
Q_{water} per capita	l/d***P**eople **E**quivalent	150
BOD_5	g/**PE***d	60
COD	g/PE*d	110
TSS	g/PE*d	70
N (as TKN)	g/PE*d	8.8
P	g/PE*d	1.8
Calculation for Wastewater Quantity and Quality: [1]		
Flow (Q)=(People*Q per capita)/1000	m^3/d	3,118.8
	m³/year	***1,138,362***
BOD_5	kg/d	1,247./5
	kg/year	455,344.8
	mg/l	***400***
COD	kg/d	2,287.1
	kg/year	834,798.8
	mg/l	***733.3***
TSS	kg/d	1,455.4
	kg/year	531,235.6
	mg/l	***466.7***
N	kg/d	183
	kg/year	66,783.9
	mg/l	***58.7***
P	kg/d	37.4
	kg/year	13,660.3
	mg/l	***12***

Table 1. Calculations for 20,792 people (together with 3,967 tourists), based on average loads per person.

[1] Based on German ATV 131A Standard (May, 2000) and German Wastewater Ordinance (2004) for assessing wastewater load, approved by Guidance for the Analysis of Pressures and Impacts in accordance with the WFD (2004).

Indicator:	*SwissLion (Agroplod) doo (5.11.2008) 3rd point (biscuits-napolitana*	*SwissLion (Agroplod) doo (5.11.2008) 2nd point (resana cakes)*	*SwissLion (Agroplod) doo (5.11.2008) 1st point (coffee & peanuts)*	*Algreta AD Resen (14.10.2009) Recipient Golema River*	*CD Frut, Carev Dvor (28.11.2008) Recipient Bolsnica River*	*MDK (II class waters)**	***Total:***
pH value	6.5	6.5	8.7	6.54	6.2	6.5- 6.3	6.88
Total suspended solids TSS (mg/L)	25	30	25	29	53	10 – 30	162
BOD_5 (mg/L)	4.5	6.6	7.3	7.7	5.3	2 – 4	31.4
COD (mg/L)	341	372	341	18.4	9	2.5 – 5	1.081
Nitrates (mg/L)	3	50	3	0.4	1.3	15	57.7
Nitirites (mg/L)	0	0	0	0	0.3	0.5	0.3
NH_4 (mg/L)	0.4	0.150	0	0.19	0.1	0.02	0.84
Fe (mg/L)	/	/	/	>1	0.25	0.3	1.25
Mn (mg/L)	/	/	/	0.315	0.3	0.05	0.615
Al (mg/L)	/	/	/	0.009	/	1-1.5	0.009
Cd (mg/L)	/	/	/	/	0.0005	0.0001	0.0005
Cl_2 (mg/L)	14.9	17.7	82.2	/	0.0025	0.002	114.8
Cr_{total} (mg/L)	/	/	/	/	0.038	0.05	0.038
Cu (mg/L)	/	/	/	/	0.012	0.01	0.012
Ni (mg/L)	/	/	/	/	0.035	0.05	0.035
Zn (mg/L)	/	/	/	/	0.075	0.1	0.075
Turbidity (NTU)	20	10	20	393	/	0.5-1	443
Total N (mg/L)	/	/	/	/	/	0.2 -0.32	
TDS (mg/L)	385	290	580	/	146	500	1,401
Total P (mg/L)	/	/	/	/	/	10 – 25	
Eutrophication Indicators – *Most probable number of thermo-tolerant coliform bacteria /100 ml*	240,000	240,000	240,000	/	/	5 – 50	240,000

Table 2. Calculation of various pollutants per source of pollution.

From a poultry farm, typical emissions to wastewater include: ammonia, uric acid, magnesium, sulphates, total nitrogen (N) and total phosphorus (P), as well as small concentrations of heavy metals (Cu, Cr, Fe, Mn, Ni, Zn, Cd, Hg and Pb).

*According to the Regulation for Classification of Water (Official Gazette of the Republic of Macedonia No. 18-99)

Using these emission factors, total releases of NH_3 from manure in "Swiss Lion Agrar" poultry farm are: 13,600 kg/year. Some 720 mg*L^{-1} of the total nitrogen and total phosphorus concentrations of 100 mg*L^{-1} are released on average per year. The BOD levels are reported to be 1,000 – 5,000 mg*L^{-1} (BREF, 2003).

Process wastewater is a major source of pollutants from textile industries (WHO, 1993). It is typically alkaline and has high BOD, from 700 to 2,000 milligrams per litre, and high chemical oxygen demand (COD), at approximately 2 to 5 times the BOD level. The wastewater also contains chromium, solids, oil and possibly toxic organics, including phenols from dyeing and finishing, and halogenated organics from processes such as bleaching. Dye wastewaters are frequently highly coloured and may contain heavy metals such as copper and chromium. Wool processing may release bacteria and other pathogens as well. Pesticides are sometimes used for the preservation of natural fibres and these are transferred to wastewaters during washing and scouring operations. Pesticides are used for mothproofing, brominated flame retardants are used for synthetic fabrics and isocyanates are used for lamination.

Point sources of pollution in the watercourses arrive from domestic sewage networks, WWTP, sparsely built-up areas, industry, contaminated sites or storm water outfalls. In addition, there are other point source pressures like fish farming.

1. *Domestic wastewater (household sewage) load estimations based on pressure from 20,792 people (without WWT):* BOD_5 – 455,344.8 kg/year; COD - 834,798 kg/year; total suspended solids – 531,235.6 kg/year; N - 66,783.9 kg/year; P – 13,660.3 kg/year. Only 55% of settlements are connected to the domestic wastewater system (data only for Golema River basin). In the whole Prespa watershed percentage is even lower, due to the high amount of sparsely built-up areas.
2. *Industrial pollution*
   On the Macedonian side of Prespa Lake there are several medium-size enterprises from eight (8) industrial branches: food processing, poultry farming, textile, metal processing, wood processing, civil construction, ceramics and the chemical industry, all causing pressure on the water bodies.
   Major pressures are coming from industry facilities "Algreta" (aluminium & zinc foundry), "Hamzali" (ceramic plant), food and beverage industry "CD Fruit", "Swiss Lion Agroplod" (food and beverages) and "Swiss Lion Agrar" (poultry farm). Impacts on the water body include high levels of ammonium, nitrates, phosphorus, aluminium, very high concentrations of Cl_2, high BOD_5 and COD concentrations, increased number of thermo-tolerant coliform bacteria, increase in heavy metals pollution-Fe, Zn, Cr, Cd, very high turbidity, phenols, benzene, halogenated organics, illegal pesticides in high quantities, brominated flame retardants, isocyanates used for lamination, oils and grease. Both "Swiss Lion Agroplod" and "CD Fruit" Carev Dvor are planning to have their small WWTP operational in the near future, but currently they are discharging effluents directly into the water bodies (no pre-treatment). Wastewater coming from industrial facilities only in the town of Resen is estimated to be approximately 69,350 m^3/year. Total annual amount of wastewater from CD Fruit is around 9,000 m^3. There is also pressure from agricultural activities and from sparsely built-up areas and storm water outflows that do not have their own infrastructure.

3.2 Estimation of diffuse sources of pollution

Based on information given by representatives of the Union of Agricultural Associations and the local AES office, by and large fertilisation of apples/fruits among individual growers in the Prespa region is performed in three phases, as follows (Tab. 3):

- autumn basic fertilisation with complex NPK (4:7:28) fertiliser in amounts of 500 to 700 kg per hectare ($kg*ha^{-1}$);
- early spring fertilising with complex NPK (15:15:15) in amounts of 400 to 600 $kg*ha^{-1}$; and
- late spring fertilisation with usage of nitrate fertilisers, such as ammonium nitrate, in amounts of 300 to 400 $kg*ha^{-1}$.

Some farmers limit fertiliser application to only twice per year. Use of organic fertilisers is very rare. Based on these data, the total annual quantity of fertilisers used for apple production in the Golema River basin (for 1,200 ha) equals roughly 1,900 tons. There is no information on fertiliser use for other crop types, but this type of data is not of interest because farmers are not growing anything other than apples and the future trend is in increased apple production. Nevertheless, the presented fertilisation scheme should be regarded as a mere generalisation used for approximation purposes and presented as the total calculated quantity.

In total 920,150 kg of nitrogen as nitrogen fertilisers is applied each season. It is practically impossible to determine to what extent farmers in the region overuse fertilisers. Furthermore, some of the more important general characteristics for the soil types found in the region are that mechanical content of all types with high percentage is sandy and with dominance of grit fractions, which means that the soil types are permeable for water and dissolved mineral matters. Therefore, water from precipitation and/or irrigation can exert a strong impact on the dilution of nitrogen forms from the fertilisers and other materials, which can finally reach the water courses by underground leaching or surface run-off.

System of Fertilisation and Period	Fertiliser Type	Quantity (kg/ha)	Active substances (kg/ha)		
			N	**P_2O_5**	**K_2O**
Basic autumn fertilisation	NPK 4:7:28	700	28	49	196
Early spring fertilisation	NPK 15:15:15	500	75	75	75
Late spring fertilisation	NH_4NO_3 34%	400	136	0.0	0.0
Total		**1600**	**239**	**124**	**271**

Table 3. Practice of fertilisation in private orchards in the Prespa region.

In total about 477 tons of phosphorous in P_2O_5 form is used in the catchment area. As a result of the widely accepted perception of low fertility of the soil with available phosphorous, a lot of P-fertilisers are used. Examples have been reported that farmers who have analysed soil samples in various soil-testing laboratories in the country have been advised not to apply certain nutrients – in particular P and K – for a period of three to four years in order to reach the required balance. Yet again, this cannot be taken as a general rule for the entire region, since there are farmers who, due to limited finances, do not use high quantities of fertilisers. Nevertheless, there is significant proof of overuse of phosphorous and it should be assumed as one of the major risks for pollution and eutrophication of the water from agricultural sources. More than 1,000 tons of potassium oxide is also applied through the fertilisation process in the Prespa region.

There are no exact data available regarding the amount of pesticides used in the Prespa region. Table 4 represents rough data on the use of pesticides in the entire Prespa region, calculations based on average quantities of pesticides used for one hectare of apple orchards per year and wheat production fields.

Pesticide type	Quantity (tons per year)	% of total
Fungicides	38.5	60%
Herbicides	3.2	5%
Insecticides	22.5	35%
Total	64.2	100%

Table 4. Use of pesticides in the Prespa region.

In total 64,000 kg of pesticides are used each year in the Prespa region. The behaviour of pesticides in the soil varies, some are easily soluble and move with water while others are less movable. It is hard to predict the movement of pesticides in general and each active matter together with other components used to produce pesticides should be investigated separately. It is obvious that much lower amount of pesticides is used in comparison with fertiliser use.

Due to the inconsistency of the current solid waste management system in the Municipality of Resen, including Golema River watershed, as well as the low public awareness, significant quantities of mainly organic (waste apples and yard waste), and partly hazardous (pesticide packaging) solid waste generated by the agricultural activity are being disposed of in the river channels and the riparian corridors. This inappropriately disposed waste has considerable negative impact on the surface, ground waters and soil, and especially on the Golema River, hence influencing the Prespa Lake system (Fig. 11).

4. Materials and methods

Aiming to resolve the detected intensive eutrophication of Prespa Lake watershed and to produce a management plan that is going to address this issue, the analyses presented in this chapter have actually been conducted in order to resolve the anthropogenic from natural processes of eutrophication. By focusing on the *reference conditions*, we have been able to detect the intensive human impact dated 1,500 years ago through massive deforestation and subsequent washout of nutrients into the Prespa Lake. This influence in combination with the very recent (only some 100 years) intensive pollution impacts in the watershed have triggered the turnover of the lake from the nitrogen towards phosphorus driven ecosystem and corresponding cyanobacterial toxic 'water blooms'.

Fig. 11. Waste apple dumping in Golema River and persistent foam on the Prespa Lake surface.

Assessment of the ecological status of the water bodies have to be based on the comparison of the level of deviation of current conditions of the biological quality elements in a water body with pristine conditions of the same one which means conditions without any anthropogenic disturbance. The level of deviation in terms of numeric Ecological Quality Ratios (EQR) have to be used for assigning an appropriate ecological class to a water body and describe its ecological status. Due to the usual practice of lack of continuous monitoring data on the biological quality elements which is necessary for description of the trend of the ecological condition of the aquatic ecosystems, the WFD leaves space for two approaches to be used for bridging this gap. A combination of the available historical data with at least a one year period of monthly detailed field monitoring of biological quality parameters gives a firm and reliable starting point in assessment and description of the ecological status of a certain water body. This approach combined with an iterative re-assessment process based on monitoring data of key type-specific biological quality elements to be derived during the river basin management plan implementation will enable a more comprehensive description of the ecological status of the water bodies and more efficient assessment of the effectiveness of the measures and activities implemented aimed at achieving the prescribed environmental objectives.

Bearing in mind the above, the development of the Prespa Lake Watershed Management Plan has been fully accompanied with identification, delineation and categorisation of the main surface and ground water bodies in the basin, and included establishment and implementation of a one year surveillance monitoring system of key biological quality elements with monthly field and laboratory analyses on the most important physical, chemical and biological parameters.

Development of a representative monitoring network of sampling sites in the Lake Prespa watershed included five lakes, four rivers and seven ground water (wells) sampling points. The most representative lake sampling points were chosen where continuous pressure from various anthropogenic activities was most expected. The river sampling points were the mouth waters of the four main rivers in the Prespa watershed flowing in the Prespa Lake. The ground water sampling points were chosen to represent the quantitative and chemical status of main ground water bodies, with the aim of providing clues as to the possible influence by the continuous pressure of various anthropogenic activities, as well as a certain correlation between the ground water bodies and surface water bodies. Figure 12 represents the chosen sampling points on the map.

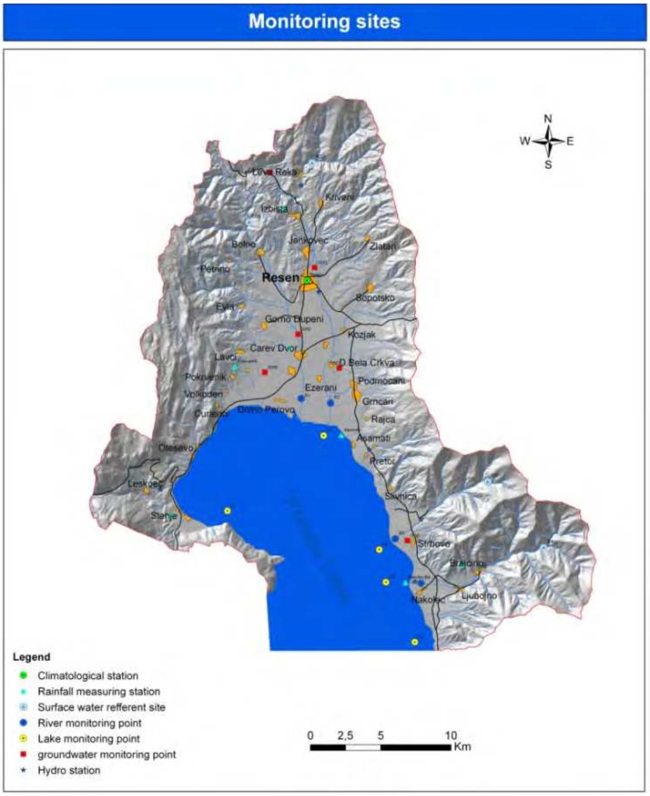


Fig. 12. Sampling points in Macro Prespa Lake watershed.

Five lake sampling sites were chosen as the most representative points for establishment of a relevant monitoring system of biological and physic-chemical quality parameters including: site Stenje (L1) village as a reference site for the deepest part of the lake in that area, a site in the vicinity of the mouth of Golema River (L2) for checking the impact of the river on the lake’s ecosystem, a site in the vicinity of the mouth of the Kranska River (L3), a site in the vicinity of the mouth of the Brajcinska River (L4) and the Dolno Dupeni site (L5) as a reference site of the overall conditions at the border with Greece.

Four major river courses, Istocka, Golema, Kranska and Brajcinska (R1-R4), were permanently monitored during the 12 month sampling period at their mouths in Prespa Lake, in order to determine the overall qualitative and quantitative pressures on the lake's ecosystem. Their reference sites (R1(r)-R4(r)) were checked only once (March 2010) to establish the reference physic-chemical and biological conditions, and delineation of the water bodies.

Table 5 represents the full range of analysed parameters during the 12 month monitoring period and the frequencies used to monitor different environments or media.

Parameter	**Rivers**	**Lakes**	**Sediments**	**Biota**
Phytoplankton		Annually/12x		
Benthic macroinvertebrates	Seasonally/4x	Seasonally/4x		
Fish	Seasonally/2x	Seasonally/2x		
Phytobenthos	Annually/12x	Annually/12x		
Macrophytes	Seasonally/2x	Seasonally/2x		
Flow	Annually/12x			
Depth	Annually/12x	Annually/12x		
Temperature	Annually/12x (profiling)	Annually/12x (profiling)		
Transparency	Annually/12x	Annually/12x		
Suspended solids	Annually/12x	Annually/12x		
Dissolved oxygen	Annually/12x	Annually/12x (profiling)		
pH	Annually/12x	Annually/12x (profiling)		
Conductivity	Annually/12x	Annually/12x (profiling)		
Alkalinity	Annually/12x	Annually/12x		
Ammonium (NH_4)	Annually/12x	Annually/12x		
Nitrate (NO_3)	Annually/12x	Annually/12x		
Nitrite (NO_2)	Annually/12x	Annually/12x		
Total Nitrogen	Annually/12x	Annually/12x	Seasonally/4x	
Inorganic nitrogen	Annually/12x	Annually/12x		
Organic Nitrogen	Annually/12x	Annually/12x		
Ortho-phosphate (PO_4)	Annually/12x	Annually/12x		
Total phosphorus	Annually/12x	Annually/12x	Seasonally/4x	
Sulfate (SO_4)	Annually/12x	Annually/12x		
Calcium (Ca)	Annually/12x	Annually/12x		
Magnesium (Mg)	Annually/12x	Annually/12x		
Chloride	Annually/12x	Annually/12x		

Parameter	Rivers	Lakes	Sediments	Biota
COD	Annually/12x	Annually/12x		
BOD	Annually/12x	Annually/12x		
Cadmium (Cd)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Lead (Pb)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Mercury (Hg)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Nickel (Ni)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Arsenic (As)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Cooper (Cu)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Chromium (Cr)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Zink (Zn)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Iron (Fe)	Seasonally/4x	Seasonally/4x	Seasonally/4x	
Pentachlorobenzene	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Hexachlorobenzene	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
DEPH	Seasonally/4x	Seasonally/4x	Seasonally/4x	
Nonylphenols	Seasonally/4x	Seasonally/4x	Seasonally/4x	
4-tert-Octylphenol	Seasonally/4x	Seasonally/4x	Seasonally/4x	
Naphtalene	Seasonally/4x	Seasonally/4x	Seasonally/4x	
Floranthene	Seasonally/4x	Seasonally/4x	Seasonally/4x	
DDT (6,7)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
DDD (6,7)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
DDE (6,7)	Seasonally/4x	Seasonally/4x	Seasonally/4x	Seasonally/4x
Total Microcystins (cell and free)		Annually/12x	Seasonally/4x	Seasonally/4x

Table 5. Parameters and frequencies analysed during the 12 month monitoring period in Macro Prespa Lake watershed.

4.1 Field sampling

Conducting analysis of physic-chemical parameters, priority substances, phytoplankton and phytobenthos quality parameters require a representative water and biological material sample to be collected, preserved and prepared for laboratory analyses. For consistent collection of materials a sampling manual is prepared. After collection, materials were transported to the Laboratory for Ecology of Algae and Hydrobiology at the Institute of Biology Faculty of Natural Sciences in Skopje for further treatment.

4.2 Laboratory analyses

Table 6 describes the analysed parameters, type of the analyses and the methods used during the 12 month surveillance monitoring in Prespa Lake watershed.

The spectroscopic analyses were conducted on Photometer System Max Direct-LoviBond® (www.tintometar.com) with the use of LoviBond® analytical kits with appropriate analytical range.

Parameter	Type of analysis	Type of method
Flow	Field Measurement	Hydrometrical wing
Depth	Field Measurement	Van Veen bottom sampler
Temperature	Field Measurement	Tintometar Senso Direct 150
Transparency	Field Measurement	Secchi Disk
Suspended solids	Laboratory Analysis	Analytical
Dissolved oxygen	Laboratory Analysis	Oxy meter - Tintometar Senso Direct 150
pH	Field Measurement	pH meter - Tintometar Senso Direct 150
Conductivity	Field Measurement	Conductivity-meter - Tintometar Senso Direct 150
Alkalinity	Laboratory Analysis	Analytical
Ammonium (NH_4)	Laboratory Analysis	Analytical-spectroscopic
Nitrate (NO_3)	Laboratory Analysis	Analytical-spectroscopic
Nitrite (NO_2)	Laboratory Analysis	Analytical-spectroscopic
Total Nitrogen	Laboratory Analysis	Analytical-spectroscopic
Inorganic nitrogen	Laboratory Analysis	Analytical-spectroscopic
Organic Nitrogen	Laboratory Analysis	Analytical-spectroscopic
Ortho-phosphate (PO_4)	Laboratory Analysis	Analytical-spectroscopic
Total phosphorus	Laboratory Analysis	Analytical-spectroscopic
Sulfate (SO_4)	Laboratory Analysis	Analytical-spectroscopic
Calcium (Ca)	Laboratory Analysis	Analytical-spectroscopic
Chloride	Laboratory Analysis	Analytical-spectroscopic
COD	Laboratory Analysis	Analytical-spectroscopic
BOD	Laboratory Analysis	Analytical-spectroscopic
Magnesium (Mg)	Laboratory Analysis	Analytical-AAS[2]
Cadmium (Cd)	Laboratory Analysis	Analytical-AAS
Lead (Pb)	Laboratory Analysis	Analytical-AAS
Mercury (Hg)	Laboratory Analysis	Analytical-AAS
Nickel (Ni)	Laboratory Analysis	Analytical-AAS
Arsenic (As)	Laboratory Analysis	Analytical-AAS
Cooper (Cu)	Laboratory Analysis	Analytical-AAS
Chromium (Cr)	Laboratory Analysis	Analytical-AAS
Zink (Zn)	Laboratory Analysis	Analytical-AAS
Iron (Fe)	Laboratory Analysis	Analytical-AAS
Pentachlorobenzene	Laboratory Analysis	Analytical-GC[3]
Hexachlorobenzene	Laboratory Analysis	Analytical-GC
DEPH	Laboratory Analysis	Analytical-GC
Nonylphenols	Laboratory Analysis	Analytical-GC
4-tert-Octylphenol	Laboratory Analysis	Analytical-GC
Naphtalene	Laboratory Analysis	Analytical-GC

[2] Atomic Absorption Spectrophotometry
[3] Gas Chromatography (GC)

Parameter	Type of analysis	Type of method
Floranthene	Laboratory Analysis	Analytical-GC
DDT (6,7)	Laboratory Analysis	Analytical-GC
DDD (6,7)	Laboratory Analysis	Analytical-GC
DDE (6,7)	Laboratory Analysis	Analytical-GC
Total Microcystins (Cell bound and extracellular)	Laboratory Analysis	Immunological-ELISA[4]

Table 6. Parameters, analyses and methods used during the 12 month surveillance monitoring on chosen sampling sites in Prespa Lake watershed.

The microcystin analyses were conducted with ELISA M956 Microplate Reader Metertech® with the use of Abraxis® Microcystins-DM ELISA 96 Microtiter Plates, product no: 522015 (www.abraxis.com). Analyses of microcystins in sediments used the same method with prior procedure of digestion and pre-treatment of the collected sediments. Analyses of microcystins in biological tissues were conducted by means of their extraction with appropriate procedure and subsequent ELISA analyses.

Heavy metals' analyses were conducted with Atomic Absorption Spectrophotometer Varian 10BQ.

Analyses for priority substances were conducted on Gas Chromatograph Varian.

Determination of chlorophyll pigments was conducted by the trichromatic method (Strickland and Parson, 1968) and measured on Photometer System Max Direct-LoviBond®.

Assessment of phytoplankton and phytobenthos species' composition was performed with microscopic analyses of the collected native and preserved algal material. The microscopic analyses of the collected material were performed on a light microscope Nikon E-800 with a Nikon Coolpix 4500 digital camera.

4.3 Hydrology

The quantities of the waters in the watershed were followed and measured by permanent water quantity stations and occasional (once per month) measurements of the water levels and flows, using the obtained rating curves Q=f(H).

On each watercourse where permanent monitoring is not yet established, direct hydrometrical measurements were performed, in parallel with the water quality samplings, in order to determine the overall flux of pollutants into the lake ecosystem. The hydrometrical measurements were performed according to ISO standards: measurements' ranges are based on ISO 748:1979 and ISO2537:1988, using a hydrometrical wing for the flow measurements. Working methodology was undertaken according to the WMO's "Guide to Hydrological Aspects".

Water velocities measurements were performed by the usual methodology on several verticals in a perpendicular transect. The number of verticals should be uneven depending on the profile width, such as:

[4] Enzyme-Linked Immuno Sorbent Assay (ELISA)

• For profile of 10 m	3 to 5 verticals
• For profile of 10 to 50 m	5 to 7 verticals
• For profile of 50 to 100 m	7 to 9 verticals
• For profile of more than 100 m	9 to 15 verticals

The number of measuring points for water velocity on every vertical is dependent on the depth of that vertical, such as:

• On verticals up to h≤30 cm depth	one point on 0.5 h
• On verticals from h≤30 to >30 <100 cm	three points: 0.2; 0.6 and 0,8h
• On verticals from h≤30 and the bottom	in four points: 0.2; 0.6; 0.8 h and bottom

4.4 Hydrogeology

Monitoring of the ground water level (GWL) of the ground water facilities (boreholes, wells) was executed occasionally (once per month) in the 12 month period (from March 2010 to February 2011). The measuring was performed using an electrical water level meter (type GTS, made in Italy) equipped with lightening and sonorous signal. In the case of the presence of artesian ground water level (which is expected in Pl_3 sediments – GW6), the monitoring was executed by measuring the natural capacity [l/sec] of the well.

4.5 Macrophytes

Monitoring of macrophytes in Lake Prespa was performed in the period from April to May 2010 and from August to September 2010 on five locations along the Macedonian coastline: Stenje, Golema River, Krajnska River, Brajcinska River and Dolno Dupeni.

The macrophytes field survey methods which have been used in the biological monitoring of Lake Prespa were consistent with the Water Framework Directive: a) **characteristic zonation -** determination if all type-specific vegetation zones for this Lake; b) **preparing the lists of species present in a lake (floristic inventories) -** determination of collected plant species using different floras and keys; c) **determination of vegetation density -** which focuses on the overall abundance of macrophytes. Abundance were estimated on a five-point scale (Braun-Blanquet 1964) where 1 = very rare, 2 = infrequent, 3 = common, 4 = frequent and 5 = abundant. The vegetation density is the result of different pressures, such as alteration of the shoreline, artificial water level fluctuations, artificial wave action and the trophic state; d) **mapping of lake's vegetation** (phyto-littoral mapping) - according to this method, the qualitative composition and distribution of aquatic vegetation was investigated.

4.6 Fish

Fish for analyses were collected with various fishing gear in day and night time experimental fishing. A cast net was also used for day time fishing with mesh size of 13 mm, whereas the night time fishing was performed with bleak nets (mesh size from 12 mm and 13 mm), and barbell nets with mesh size of 22 mm, 24 mm, 26 mm and 28 mm. The height of each fishing gear was basically a hundred heights per each mesh and the length was about 50 metres. All nets were set between 3 and 6 pm, fished overnight and lifted between 8 and 10 am in order to ensure that the activity peaks of each fish species was included.

The location of each net was selected according to proposed sampling sites in the project and based upon the past experience of the investigator.

All captured fish were identified, and length and weight was measured. The age and sex composition of the fish population from Lake Prespa were performed with standards ichthyological methods. Analyses of the scales were used for determination of the age structure of the fish populations, as well as length and weight growth. The obtained results were statistically processed.

4.7 Benthic invertebrates

Benthic invertebrates from the Prespa Lake and its main tributaries were collected according to the requirements of the EU Water Framework Directive (WFD) 2000/60/EC.

From the Prespa Lake itself, the collection of bottom fauna samples was performed by several different devices: Ekman grab, sediment corer, triangle bottom dredge and hand net. Macroinvertebrate standard methods applicable to lakes were used (ISO 9391:1995 and ISO 7828:1985). Concerning to the main tributaries of Prespa Lake, benthic invertebrate samples were collected with a Surber sampler or hand-net following standard methodology for collection of bottom fauna (ISO 8265:1988 and ISO 7828:1985). For preservation of biological samples 70% ethyl-alcohol or 4% formaldehyde were used, samples properly labelled and additionally processed in the laboratory.

In the laboratory, the animals were flushed with tap water through a standard sieve (280 μm pore size). Material was divided by groups, for further determination, mainly to the lowest taxonomic level (genus/species). Generally, determination to the species level is recommended because the species level logically provides the most detailed and sound information about autecological demands of a certain animal species. Determination was performed using identification manuals. Based on WFD principles, data informing on the communities' taxonomic composition, abundance, diversity and sensitive taxa were taken into consideration, both for Prespa Lake and its tributaries. Biotic indices that are suitable for Prespa Lake and its watershed monitoring purposes were used.

5. Results of the performed surveillance monitoring in Prespa Lake watershed

According to the historical hydrology data and observations, there are four major tributaries in the Macedonian part of Macro Prespa Lake which can be considered as separate rivers of significance and should be subsequently subdivided into the Istočka, Golema, Kranska and Brajčinska Rivers. Although small in it watershed, Kurbinska River was also taken into consideration because of it relatively significant quantity of water.

All of the other temporal water courses have been proven to be torrent carriers which usually drain forested areas and have little significance in the overall water quality analysis; although they may have a significant role in the water balance of the lake at specific times, as well as in flood hazard management because of their character.

Considering the Prespa Lake itself, there is a littoral plateau of approximately 14-16 metres depth that completely surrounds the lake and two major depressions – one near the village of Stenje (approximately 47 metres depth) and the other in the vicinity of Agios Germanos in Greece (approximately 58 metres depth) that represent the true profundal of the ecosystem. It is therefore a logical solution to delineate the water bodies in the Macedonian part of the Prespa Lake according to sampling sites presented on Figure 12, and to consider the littoral and profundal part of the lake as a one water body.

This delineation of the surface water bodies in the Macedonian part of Macro Prespa Lake has been used as a basis for a 12 month surveillance monitoring programme conducted in the period April 2010 - June 2011.

5.1 Rivers

All rivers in Prespa Lake watershed belong to the same river type (type 1 – siliceous rivers of mid-altitude and size) in the one eco-region 6 (Hellenic Western Balkan). They are mostly mountain type rivers with steep slopes and a stony bottom, normally with well-developed riparian vegetation; only the small part of the watercourses prior to their mouth in Prespa Lake may be slow flowing and on sandy or muddy substrata. In combination with the very small river lengths (and also catchments - <100 km^2), the only important driving force that might impact the natural ecological conditions in the rivers are human activities.

Due to the lack of a continual monitoring system in the area, the short timeframe of the project and the need for obtaining the most reliable data relevant for the development of the Management Plan, delineated river water bodies in Prespa Lake watershed were monitored for the basic physical, chemical (including major nutrients, priority substances and heavy metals) and biological parameters. Out of the WFD proposed biological quality elements, the categorization of rivers is chosen to be based on phytobenthos and macroinvertebrates. The reference conditions are also based on these parameters.

Basic physical parameters

The basic physical parameters measured during the established monitoring system in the course of the project (Fig. 13) reflect the overall natural conditions of the selected river water bodies. The only obvious sign of human impact are the values for *conductivity* and *total dissolved solids* which are much higher in Golema River and Istočka River. Values for *pH* are also worth notifying since in July they were also significantly lower than expected in Golema Reka River water bodies (3.92-5.17 respectively), but also in Kurbinska River (3.9!). This finding may be attributed to some specific pollution impact at the moment of sampling, but also to some other origin and therefore deserve investigative monitoring in future.

The values for dissolved oxygen generally decreased in the water bodies under intensive human influence (Golema River), but were still in the realm of a good ecological status; Kurbinska River is again an exception since there is no major human impact (apart of the water abstraction) recorded for this water body, but its DO value was only 5.4 mg $O_2 \cdot L^{-1}$. Nevertheless, the performed monitoring period was far too limited to enable any firm conclusions on the oxygen dynamics or the underlying causes; it should be substantially extended in future.

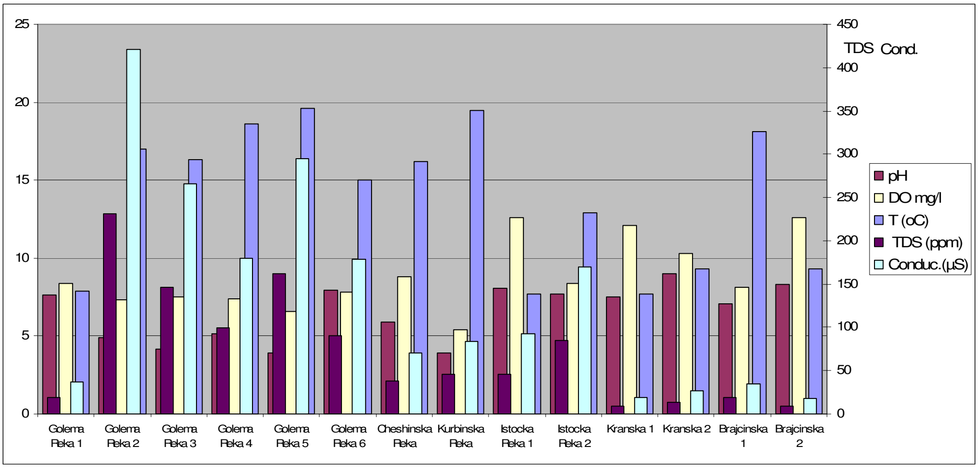


Fig. 13. Basic physical parameters of delineated rivers in Prespa Lake watershed.

Nutrient status

Measured major nutrients in delineated river water bodies in Prespa Lake watershed (Fig.14*)* were mostly based on *nitrogen* and *phosphorus* compounds, with the addition of *sulphates* as an indicator of the direct human influence. All of the examined water bodies show highly increased nutrient composition, especially regarding the *total N* and *P* compounds. In water bodies of the Golema and Istočka Rivers, *ammonia* and especially *sulphates* were very high, but their presence is also remarkable in the rest of the examined rivers. *Nitrites* were present in all examined water bodies, except in Kurbinska River, in low quantities, pointing to continual wastewater pressure. Detected high amounts of nutrients in examined river water bodies points to a complete lack of wastewater treatment, significant diffuse source pollution run-off and vast amounts of waste and agricultural and industrial discharge (refer to Fig. 15 for an estimation of the total annual load of nutrients by different river water bodies). These results might explain, in broad terms, the detected variations of *acidity* in examined waters which were most probably directly connected to the discharged nutrient quantities and the season of fertiliser application; all of the examined nutrients, especially sulphates, have high potential of forming acids which have rapid or prolonged impact on water biota and chemistry. In other words, river water bodies in Prespa Lake watershed have very low acid neutralising capacity caused by prolonged deposition of acidifying chemicals.

Amid the lack of categorisation of surface waters according to nutrients in the Macedonian legislation (only the values for *total N* and *ammonia* are considered, but with wrongly stated units of measurement; *Official Gazette of RM 18/99*), the detected values for *phosphates, sulphates, total N* and *ammonia* (Fig.14) place the lower parts of the examined water bodies highly in the V water quality class. In this respect, Golema River and Istočka River have the maximum detected levels, but even Kranska River and Brajčinska River have been shown to experience intensive pressure from nutrients.

According to results presented in Figure 15, *sulphates* are the major nutrient carried to Prespa Lake mostly by Brajčinska, Istočka and Golema Rivers. Significant amounts of *nitrogen* and *phosphorus* compounds are also recorded in all major water bodies, except Kurbinska River which has been found not to be subject to intensive human impacts regarding nutrients.

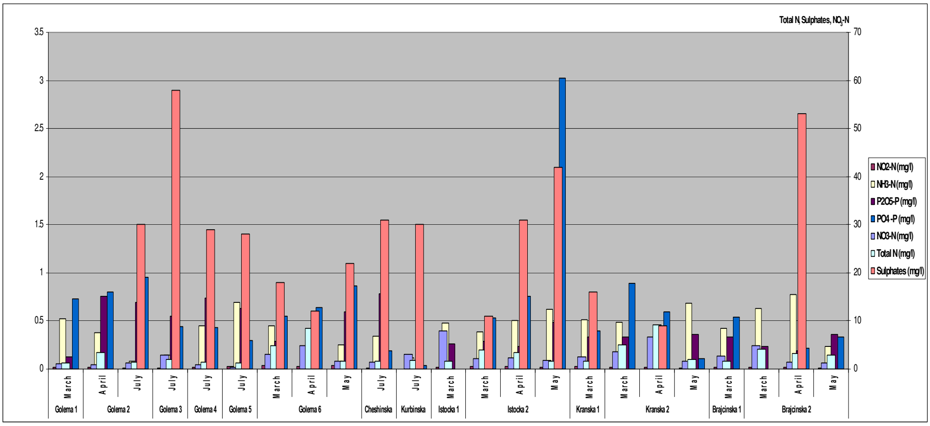


Fig. 14. Detected nutrients in delineated rivers in Prespa Lake watershed.

Conducted surveillance monitoring is far from sufficient to enable reliable quantification of the total nutrient pressure in the Prespa Lake system. Nevertheless, the obtained estimations of cumulative nutrient loads (Fig. 16) are in concordance with the estimations done for the diffuse source of pollution coming from agriculture. Furthermore, these results disprove the hitherto reported estimations (Grupce, 1997; Naumovski et al., 1997) of the phosphorus load in Prespa Lake of 84 tons per year (of which 41 tons per year are coming from natural processes and 43 tons per year are due to anthropogenic activities) by almost double amount (Fig. 16), without including all of the possible emissions (diffuse sources, natural run-off, etc.).

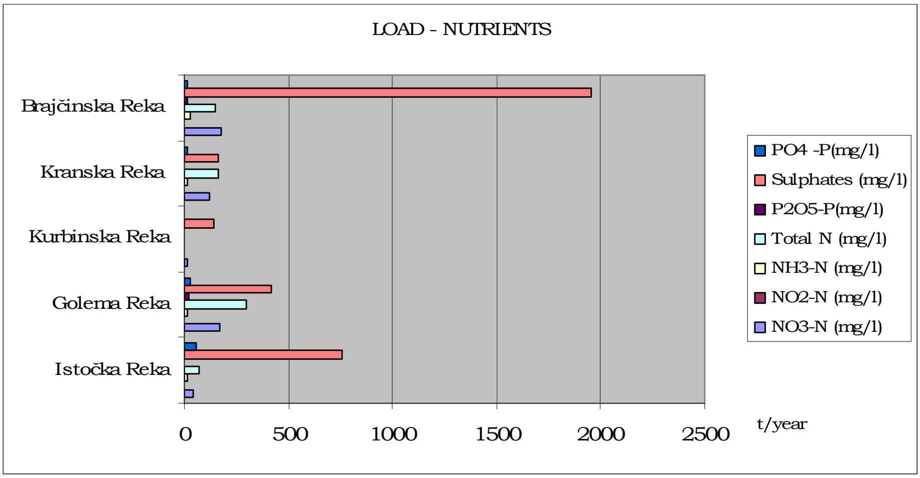


Fig. 15. Estimated total nutrient load detected in investigated rivers of Prespa Lake watershed.

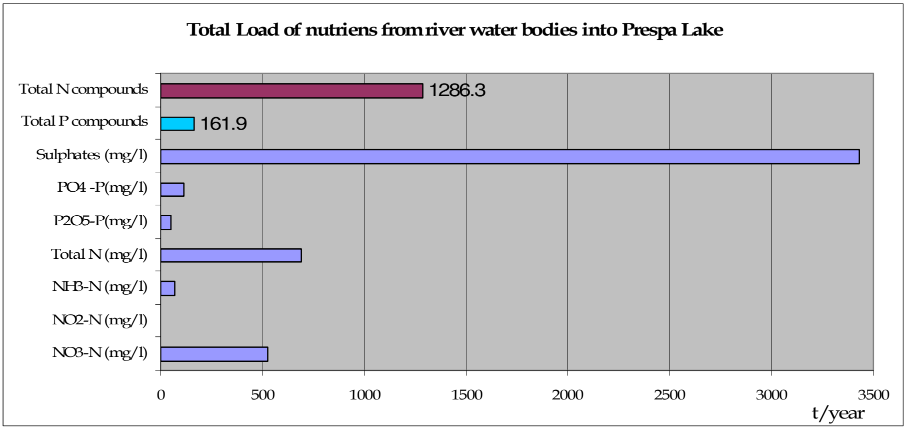


Fig. 16. Estimation of the cumulative load of nutrients coming from all river water bodies into Prespa Lake.

Heavy metals

Three heavy metals dominate the river water bodies of Prespa Lake watershed: *manganese, iron* and *aluminium*. Their recorded concentrations are usually way beyond the permissible levels for natural conditions (III-IV water quality class) and therefore denote an intensive human origin (Fig. 17). Again, Golema and Istočka Rivers express the highest concentration levels, while the lower segment of Kranska River recorded very high *manganese* and slightly lower *iron* concentrations. Upper segments of all river water bodies (the reference conditions) have been found with heavy metals concentrations several times lower than the lower parts, thus reflecting the natural background emissions to the water habitats.

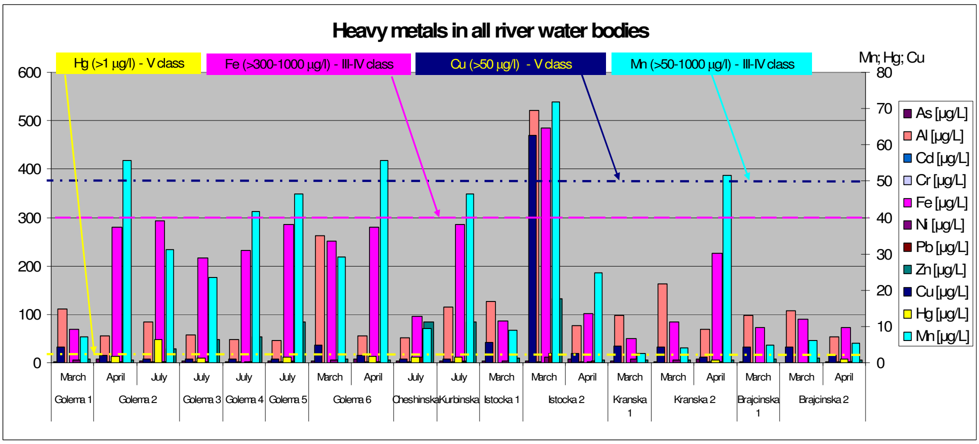


Fig. 17. Detected concentrations of heavy metals in river water bodies of Prespa Lake watershed.

Two other heavy metals, *copper* and *zinc*, have also been detected in significant quantities in the lower river parts of the Prespa Lake watershed, being most pronounced in the Istočka River. Their increased presence is the inevitable result of adverse human impact in the region.

The most severely influenced river water bodies, Golema and Istočka Rivers, are also characterised with a marked presence of *mercury, lead* and *arsenic*. Their toxicity and harmful effects on the environment and humans are well known and do not need any further elaboration.

Figure 18 gives an overview of the total intensity of the heavy metal load detected in the mouth waters of river water bodies prior to entering the Macro Prespa Lake. It is very clear that Golema and Istočka Rivers are the major source for all measured heavy metals, carrying more than 8 tons of *iron* and *aluminium* annually into the lake, but also significant amounts of *manganese, zinc, copper* and *lead* or *mercury*. Rivers Brajčinska and Kranska also add to the overall heavy metal load (Fig. 19), but to a much lesser extent.

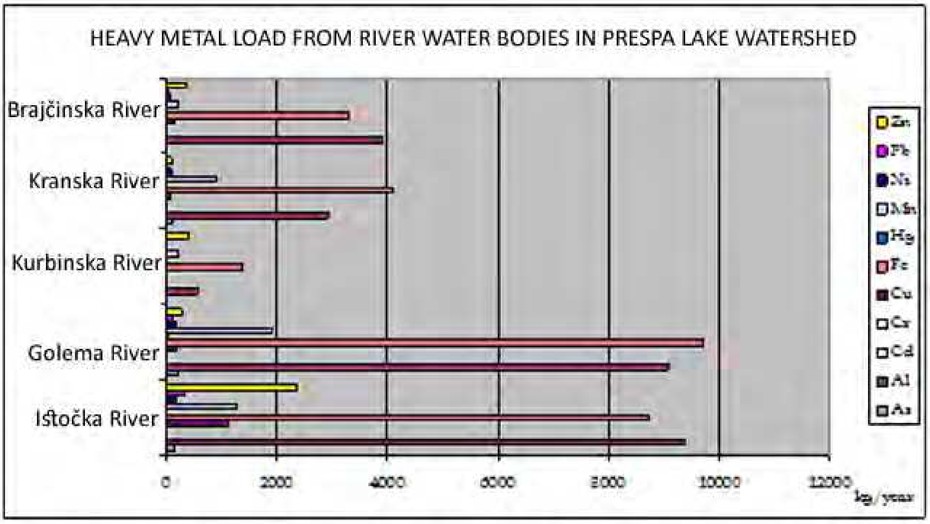


Fig. 18. Estimation of heavy metal load from river water bodies in the Prespa Lake watershed.

In summary, according to calculated estimations, Macro Prespa Lake receives more than 27 tons of *iron* and almost 26 tons of *aluminium* per year, coming from its major tributaries. It is also loaded with 4.6 tons of *manganese*, 3.5 tons of *zinc* and more than 1.5 tons of *copper* per year. Toxic metals are less abundant (563, 504, 132 and 118 kg per year for *arsenic, lead, chromium* and *mercury* respectively), but they do represent a significant load and a dangerous hazard to water biota (through processes of bioaccumulation) and humans. At this point, we do not have any information on the intensity of accumulation of the various heavy metals in water biota, specifically in fish; therefore these investigations will be proven invaluable for the proposed WFD monitoring system to be established in Prespa Lake watershed.

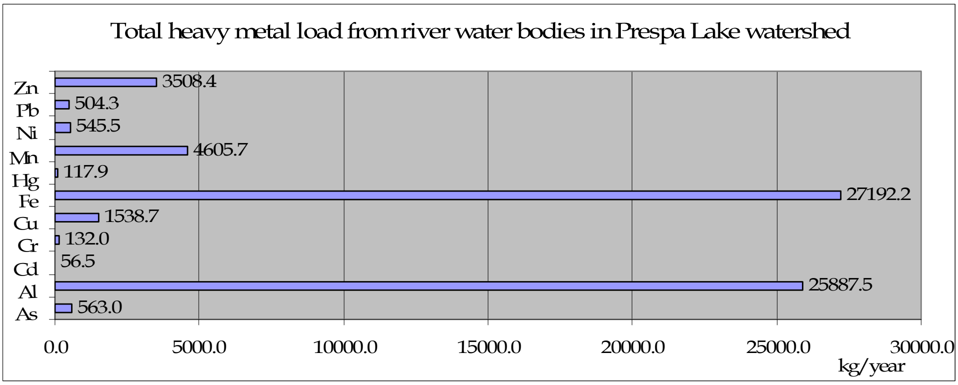


Fig. 19. Estimation of the overall heavy metal load originating from the river water bodies in Prespa Lake watershed.

Priority substances

The presented results for WFD priority substances are the first record of this kind for the Prespa Lake watershed (Fig. 20). Out of the proposed priority substances for the surveillance and operational monitoring purposes (WFD 2008), the comprehensive analyses performed so far in Prespa Lake watershed embrace *Chlorinated aromatic hydrocarbons, Poly-aromatic hydrocarbons (PAHs), Poly-chlorinated biphenyls (PCBs), Organophosphate pesticides, Phenols, Phthalates* and *Organochlorine pesticides*.

A total of 18 priority substances (out of the whole set of 80 analysed chemical compounds) were detected in river water bodies in Prespa Lake watershed (Fig. 16). *Bis(2-Ethylhexyl)phthalate* was present in almost all samples, the highest values recorded in Golema and Brajčinska Rivers. *Dibutilphthalate* was also found in all river water bodies, except Kurbinska River, but in slightly lower concentrations. O*rganochlorine pesticides* were recorded in different concentrations and were dominant in investigated water bodies. *Gama-HCH (Lindan)*, *Alpha HCH* and *Alpha Endosulfan* were the most commonly present in all water bodies, but the very high values for *Heptachlor* in Golema River 6 and especially in Kranska River. In summary, it is very clear that all of the examined river water bodies in Prespa Lake watershed are under prolonged influence of a significant pollution pressure coming from excess utilisation of various pesticides groups, plastic materials and industry. Even the uphill mountain river parts not subjected to any significant (visible) human impact, that should be used as reference conditions in the watershed, are also under obvious pressure; DDE and DDD although banned from utilisation are still present in Golema 1, Kranska 1 and Brajčinska 2 water bodies. These results point out that the surface water bodies in Prespa Lake watershed have been, and still are, subjected to intensive pressure coming from agriculture and irregular waste disposal. Apart from an *in situ* pollution of the surface waters, aero deposition might play a significant role especially in polluting the upstream mountain river segments with pesticides or their residues. Although in domestic legislation only *Heptachlor* and *PCB* are listed in water categorisation tables (both found in concentrations stated for V or III-IV category respectively), other detected substances will also have to be included in future.

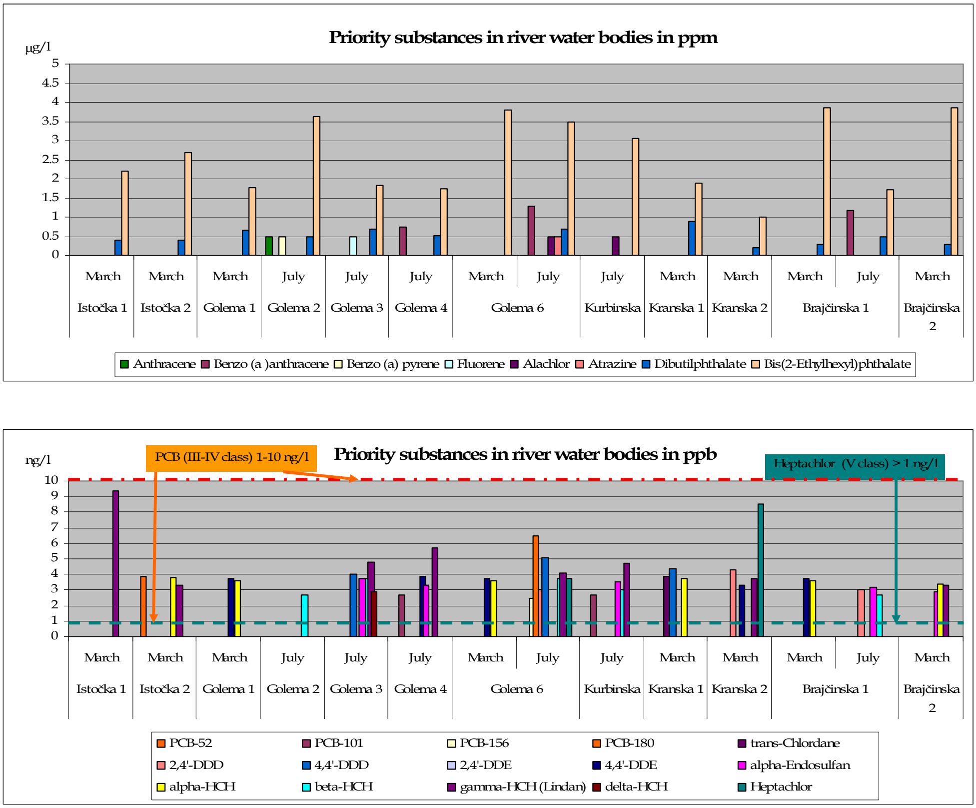


Fig. 20. Detected priority substances (without heavy metals) in river water bodies of Prespa Lake watershed.

The final conclusion on detected priority substances in the river water bodies in Prespa Lake watershed is that numerous different compounds were detected, some of them with very high concentrations (III-IV or V water quality class) and that all of them represent an elevated risk for the environment, water biota and humans. Toxic and already forbidden chemicals like *DDD* or *DDE* are still present in the waters what underlines their constant utilisation. Apart from detected pesticides or their derivatives, detected *phtalathes* as main chemicals in the production of plastics (Fig. 21) directly emphasise irregular solid waste treatment or disposal, and insufficient industrial and domestic wastewater treatment.

It is very critical to note that the spreading of detected priority substances also affects the remote regions not subject to any visible human influence in the Prespa Lake watershed thus endangering the environment and biota in already declared protected areas, like NP “Pelister” and “Galichica”. Their potential for bioaccumulation and prolonged devastating impact should be the focus of the Management Plan and consequent reduction measures.

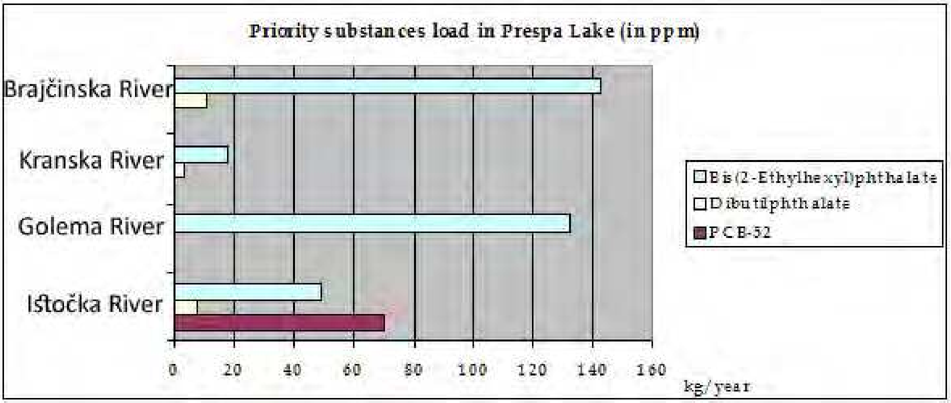


Fig. 21. Priority substances load to Prespa Lake coming from river water bodies in the watershed (in ppm).

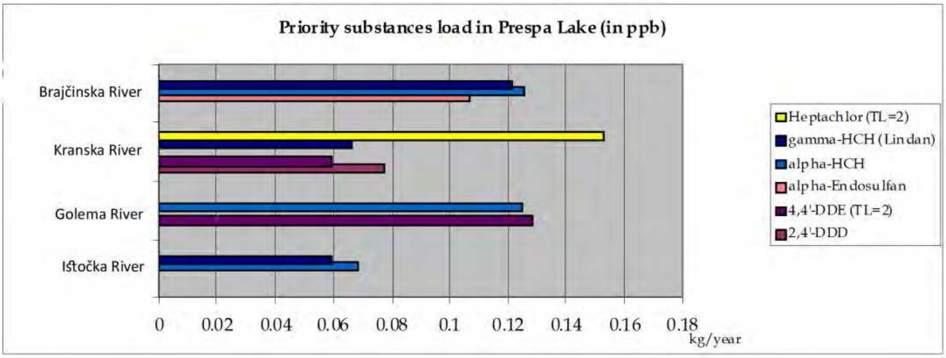
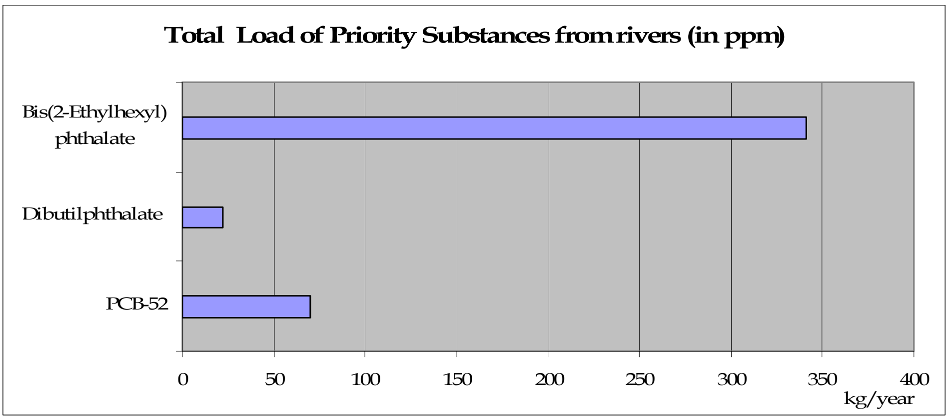


Fig. 22. Priority substances load to Prespa Lake coming from river water bodies in the watershed (in ppb).



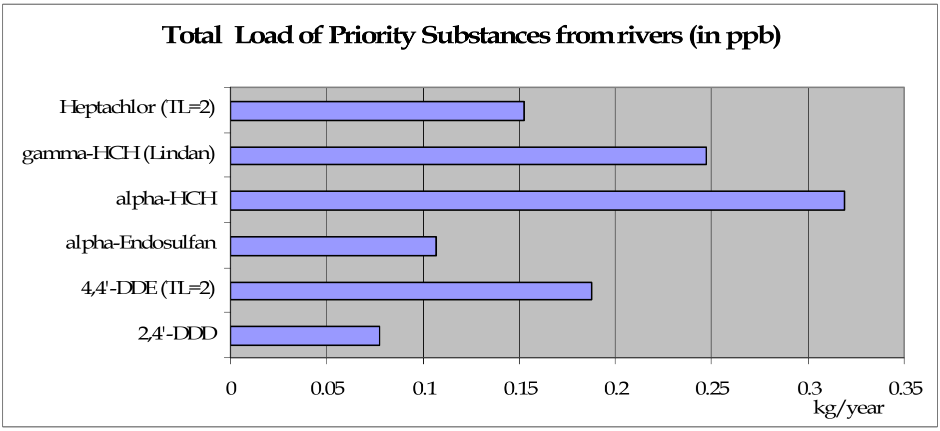


Fig. 23. Estimated total load of priority substances to Prespa Lake coming from river water bodies in the watershed (in ppm and ppb).

Ecological quality elements

Having in mind that there are practically no useful data (apart from the one project report – Krstic, 2007) on WFD ecological quality elements for the rivers in Prespa Lake watershed, the only feasible and promising approach was to use benthic organisms, algae and macrozoobenthos, to evaluate the ecological conditions in rivers. For the same reasons, there was no firm scientific opportunity to run any statistical calculations on the obtained data without significant bias and/or error.

The principal approach used in this situation was to determine the benthic algal and macrozoobenthic assemblages in upper river segments (river water bodies not subject to any significant human influence) to parts of the rivers where increased human interference in water physical or chemical properties was detected (usually lower river stretches after the point or diffuse wastewater input or leaching coming from cultivated land). Using this approach, we were able to detect the benthic assemblages in natural conditions (or reference conditions) for the rivers in Prespa Lake watershed, and also to reveal to the highest possible extent the succession of benthic communities under the detected human pressure. All of the methods for sampling, handling and analysing the samples were performed according to WFD recommended ISO or CEN standards (ISO, 2003; CEN, 2003).

Regarding the WFD proposal for using biotic indices in the process of describing the ecological status of water bodies, our approach was ruled by the following facts and postulates:

i. The biotic indices in relation to water quality monitoring (for both algae and macrozoobenthos) have been developed in different countries based on long-term monitoring data and detected autecological preferences of different taxa. In the case of Prespa Lake and the water bodies in its watershed, neither of those lines of data are available. Even more so since there were numerous diatoms (a group of algae) described new to science in the region (Levkov et al., 2006) for which there are no data in the literature;

ii. Using biotic indices developed for any organism in a region (country, continent) other than the region (river, lake, watershed) in question has already been proven erroneous on multiple occasions (Van Dam et al., 2005; Krstic et al., 2007). This is fundamentally based on differences in the *total capacity* of every ecosystem, numerous (not accountable) sources of variation of the ecological parameters and direct human influence. Since all of the indices are derived via assigning specific numbers to organisms (algae in particular) to be used in formulae calculations, the only scientifically sound approach is to develop a specific index for every water body;
iii. The last (and not least) reason against using bio-indices other than a basic community similarity (or dissimilarity) index is their gross oversimplification of the biological (or genetic) responses of living biota to different environmental conditions. The same organism (if we exclude taxonomical errors as yet another frequently very misleading source) cannot react in the same way in different environments (habitats); therefore, using and comparing biotic indices based on species variations (numbers in the population or different biological treats) between habitats seems (and has been proven) highly inapplicable.

With all the stated limitations in focus, class boundaries among different water quality classes and the reference conditions were based on benthic flora and fauna community structure in which dominant taxa characteristics, and therefore different classes, are recorded. In the case of benthic algae, clear distinction among class boundaries are based on the occurrence of taxa indicative for higher eutrophication or saprobity levels, or mass occurrence of specific cyanobacteria indicating very high pollution. Macrozoobenthos communities were subjected to evaluation according to the Danish Stream Fauna Index (DSFI – Skriver et al., 2000) and EPT richness (Bode et al., 1997), but we still express our reservations regarding the above mentioned restrictions for application of the biological indices. To achieve the best possible level of certainty regarding the biological quality elements in Prespa Lake watershed, the surveillance and investigative monitoring must be significantly extended.

In general, algal assemblages in river water bodies of Prespa Lake watershed are quite distinctly separated along the eutrophication or pollution gradient detected in examined watercourses. Namely, the upper river segments of all rivers are dominated by diatom flora characteristics for clean, slightly acidophilic, oligotrophic waters or **good ecological conditions** composed of *Diatoma hyemalis, Diatoma mesodon, Hannea arcus, Meridion circulare, Meridion circulare var. constricta, Eunotia minor, Achnanthidium jackii, Decussata hexagona, Encyonema mesianum, Krsticiella ohridana, Pinnularia eifelana, Pinnularia sudetica, Psammothidium daonense*. The rocky bottom of these river parts is usually not covered by any visible flora, but occasionally fragments of water mosses, typical chrysophyte (golden algae) *Hydrurus foetidus* colonies or the red alga *Lemanea fluviatilis* which are usually deprived of any rich epiphytic diatom flora. Therefore, this algae assemblage is chosen to be the indicator for the *reference conditions of all rivers* in Prespa Lake watershed.

The second type of algal assemblage is found in lower river stretches prior to or after the major pollution events indicating **moderate ecological conditions**; Golema Reka 2, 3, 4, Češinska, Kurbinska, Kranska 2 and Brajčinska 2. This assemblage is clearly dominated by diatom taxa indicative for higher nutrient concentrations in the water and more intensive decomposition processes, such as: *Melosira varians, Fragilaria capucina, Ulnaria ulna, Achnanthidium lanceolatum, Cocconeis placentula* var. *euglypta, Navicula phyllepta, Navicula cryptotenella, Navicula halophila, Navicula lanceolata, Navicula tripunctata, Navicula cryptocephala, Frustulia vulgaris, Reimeria sinuata, Gomphonema olivaceum, Gomphonema angustatum, Gomphonema micropus, Gomphonema aff. olivaceoides, Encyonema silesiacum, Amphora pediculus, Nitzschia linearis, Nitzschia palea, Surirella minuta*. Usually, the green branched alga *Cladophora glomerata* is also markedly present in these water bodies, bearing a rich epiphytic growth usually by *Cocconeis placentula* var. *euglypta*, but sometimes blue-green cyanobacteria *Heteroleiblenia kossinskajae* or *Pseudoanabaena limnetica* were observed as significant epiphytes.

Finally, the last detected algal assemblage was found in the most severely polluted river bodies of Prespa Lake watershed, like Golema Reka 5, 6, 7, and Istočka 2, and thus denoting **bad or poor ecological conditions**. The typical example of this algal assemblage was found at Golema Reka 5 sampling site where the diatom flora is reduced to as much as only 3 - 4 taxa, like *Nitzschia palea, Navicula cryptotenella* and *Ulnaria ulna* representing more than 98% of all detected cells. Highly decreased algal biodiversity is replaced by a mass development of two cyanobacterial species *Pseudoanabaena limnetica* and *Phormidium limosum* which completely cover the rocks on the bottom.

The WFD requires classification, in terms of ecological status, for all European surface waters. The classification should be based on reference conditions, which are intended to represent minimal anthropogenic impact and observed deviation from these conditions (Andersen et al., 2004). For each surface water body type, type-specific biological reference conditions were established, representing the values of the biological quality elements for that surface water body type at high ecological status.

Among the biological communities, the macrozoobenthos is by far the most frequently used bioindicator group in standard water management (Hering et al., 2004). Numerous biotic index and score systems have used macrozoobenthos in the assessment of running waters (Rosenberg & Resh, 1993). The most represented biotic index or score methods are: taxa richness, number of EPT taxa, Saprobic Index (SI), Biological Monitoring Working Party (BMWP) Score, Average Score Per Taxon (ASPT), Danish Stream Fauna Index (DSFI). All indices were part of the respective national method planned for biological monitoring in the context of the Water Framework Directive (Birk & Hering, 2006).

Thus, in the frame of the project "Development of Prespa Lake Watershed Management Plan" and according to WFD requirements, categorisation of the delineated water bodies in the Prespa Lake watershed based on macrozoobenthos was done. Two metrics (EPT richness and DSFI) in assessment of the ecological health of the rivers were used. These metrics were selected because statistical power to detect a difference between the nutrient enriched and non-impacted sites was >0.99 for total taxa richness and number of EPT taxa. DSFI also had relatively high power >0.95 (Sandin & Johnson, 2000) (Table 7).

In order to assess the ecological conditions of the river water bodies and related macrozoobenthos assemblages, the analyses of collected samples were performed to detect the presence of so called *positive* (like Ephemeroptera, Plecoptera, Trichoptera, Diptera, Gammaridae and even Astacidae) versus *negative* taxa (usually Oligochaeta – Chironomidae or Tubificidae). Pollution sensitive taxa like *Ecdyonurus venosus*, *Baetis alpinus*, *Capnia vidua*, *Brachyptera risi* and *Potamophylax latipennis, Crenobia alpina* and even crayfish *Austropotamobius torrentium* were compared for the percentage abundance within the community, and used to derive the EPT and DSFI indices.

EPT richness	**DSFI value**	**Water quality**
>10	**7**	**high (reference condition)**
6-10	**6**	**good**
2-5	**5**	**moderate**
<2	**4**	**poor**
	1-3	**bad**

Table 7. Water classification based on EPT and DSFI.

5.2 Prespa Lake

Out of the WFD's biology quality elements, phytoplankton, zoobenthos, macrophytes and fish were in the focus of investigations, supported by a full range of physic-chemical analyses including heavy metals and priority substances. Water for the chemical analyses was sampled as a collective sample from the full water column on the site or as sediment, while the basic physical parameters were measured at every sample depth. Special attention was paid to the sampling of plankton for algae and benthic habitats (littoral, sub-littoral and profundal) for the macrozoobenthos analyses.

In order to determine the *reference conditions* for Prespa Lake, analyses of core samples dated 10 ka (500, 1,000, 2,000, 5,000 and 10,000 years respectively; the deepest analysed core sample from approximately 30 metres of the core depth) were performed for the first time regarding the total phosphorus content and diatom composition.

Macrophytes and fish samples from the selected sampling sites on Prespa Lake were collected during June 2010 and according to WFD sampling guides.

Basic physical parameters

The basic physical parameters detected in the waters of Prespa Lake (Fig.24) revealed some interesting features of this unique ecosystem. For example, recorded temperatures show a normal and gradual increase towards warmer months, but there was no sharp and rapid decrease in one water layer (thermocline) during the warmest month (July 2010) although the water temperatures between the deepest and the shallowest parts differ by more than 10^{o}C. This may be a result of a very turbulent climate in the sampling period with constant mixing of the water layers or as a consequence of intensive discharge of the deep water sources (sub-lacustrine water sources) again related to the rainy season. High deep water temperatures of 14-15^{o}C also indicate the full intensity of thermal insulation and possible full scale mixing of the entire water column during storms, which results in a constant nutrient supply of the epilimnion layer.

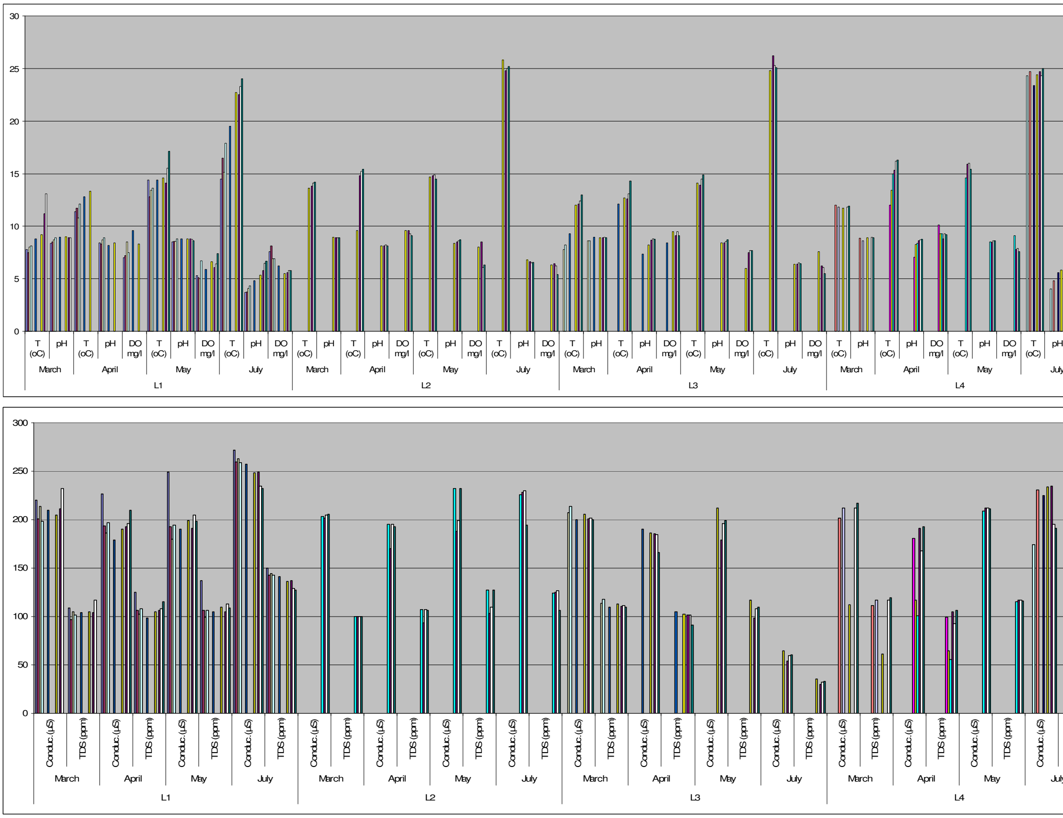


Fig. 24. Basic physical parameters detected at Prespa Lake sampling sites. The above findings are additionally co
oxygen results. Deep water layers had showed relatively high DO values in the summer months, their values eve
water layers. Maximal DO values were recorded at around 3-4 m depth layers where the phytoplankton was in
These results are sharply opposite to frequent statements of oxygen depletion in the deep water layers of Prespa
values during our investigations were 5-5.3 mg*L^{-1} at the deepest part of the L1 sampling site in May 2010.

Another peculiarity recorded in these investigations was a very low pH reaction of the deep waters (beyond 10 m on L1 and L4) in July 2010, ranging as low as 3.7! This condition can arise if decomposition (bacterial) of organic material releases carbon dioxide and thus increases the amount of dissolved carbon dioxide; an increase in carbon dioxide decreases pH. Organic acids, often expressed as dissolved organic carbon (DOC), also decrease pH (Strumm & Morgan, 1981). Both of these possibilities point to the increased pressure on the lake in the spring-early summer period, which was also recorded in the data for the rivers in Prespa Lake watershed (Fig. 13).

Conductivity and TDS results obtained for the Prespa Lake (Fig. 20) sampling sites are in the realm of natural conditions for this type of lake (for example, comparison to Dojran Lake which shows conductivity values at around 800 – Krstić, 2011). Nevertheless, there is a slight increase of conductivity and corresponding TDS values in the deep water layers in July 2010, thus supporting the above statements of release of charged ions (either by microbial activity or human input) in this period.

Nutrient status

Detected nutrient levels in Prespa Lake sampling sites (Fig. 24) fully reflect the overall conditions already established for the watershed. The lake is dominated by sulphates, the same as the rivers in the watershed (Fig.14), but there is also a marked presence of total N basically due to elevated concentrations of nitrates and ammonia. Regarding ammonia, the whole investigated area was found to be in the III-IV category class as stated in the domestic legislation, while with regard to the total presence of nitrates Prespa Lake has to be declared as a Nitrate Vulnerable Zone as stated in EU legislation[5].

With respect to *phosphorus* content in the waters of Prespa Lake the situation is even worse; detected *total phosphorus concentrations* (based on the sum of detected values for P_2O_5-P and PO_4-P) place the lake in realm of hyper-eutrophic conditions, both regarding domestic and EU legislation.

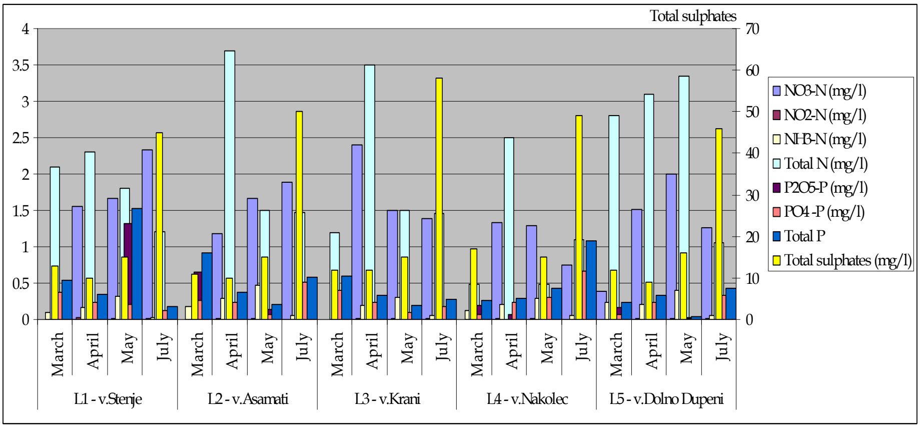


Fig. 24. Detected nutrient levels in Prespa Lake.

[5] Nitrates Directive (91/676/EEC).

Heavy metals

Contrary to presented results for the river water bodies (Fig. 13), the concentrations of heavy metals detected in the waters of selected Prespa Lake sampling sites (Fig.25) point to *copper, iron* and *zinc* as dominant metals. For *copper* the detected values were almost entirely in III-IV category, but the increased presence of the other two metals in detected concentrations also confirms the prolonged input.

There is also a marked presence of the toxic *mercury* and *arsenic* in Prespa Lake waters. Their increased detected concentrations in July 2010 clearly support the argument for intensified human activities in the spring period; *mercury* appears at the L2 sampling site as a result of the Golema River influence and is detected almost in all samples with concentrations high above the V water quality class. Its presence is striking on L5 (v.Dolno Dupeni) with almost eight times increased concentration compared to the 1 mg*L^{-1} level for V quality class. *Arsenic* is also present in all sampled waters of Prespa Lake, but in much lower concentrations than *mercury*. It rose to III-IV water quality range only in L4 (v.Nakolec – mouth waters of river Brajčinska) in July 2010. Nevertheless, its accumulation and persistence in Prespa Lake waters is evident.

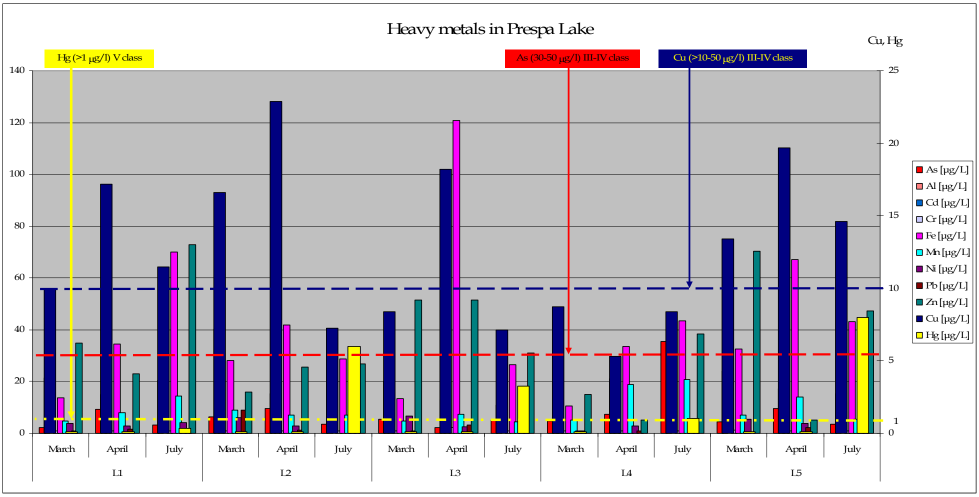


Fig. 25. Detected concentrations of heavy metals in the water of selected sampling sites of Prespa Lake.

Priority substances

Prespa Lake waters were also found to contain 20 priority substances out of more than 70 analysed during March and July, 2010. The same applied for the river water bodies, with *Bis(2-Ethylhexyl)phthalate* and *Dibutilphthalate* dominating in the samples of Prespa Lake waters. But, there was also a marked presence of *Benzo (a) pyrene, Benzo (a) anthracene* and *Naphthalene* (Fig. 26).

Considering the presence of pesticides or their residues in the Prespa Lake waters (Fig.27), *Gama HCH (or Lindan)* is dominantly found on all sampling sites followed by *Heptachlor* which is usually in concentrations high above the permissible 1 ng*L-1. It is interesting to notice that the L2 sampling site in the vicinity of the mouth waters of Golema River was found with the lowest number of detected priority substances, contrary to the expected and detected pressures coming from this water body. The other sampling sites along the North-East coast of the lake (L3-L5) and the deepest part on L1 sampling site had a much higher number of detected priority substances and maximal values of separate chemicals. These findings corroborate the proposed intensive mixing of the Prespa Lake waters with significant underwater currents that are spreading the pollution impact to a much wider area.

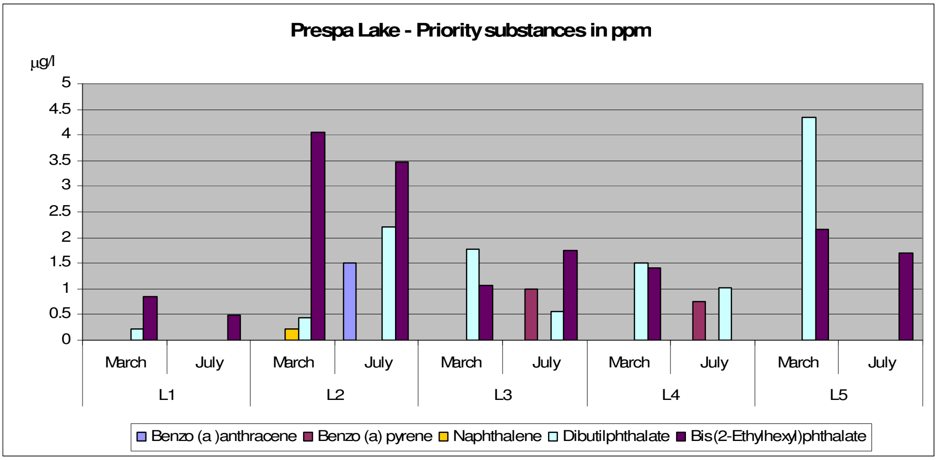


Fig. 26. Priority substances (in ppm) detected in waters of selected Prespa Lake sampling sites.

Priority substances detected in Prespa Lake pose a significant hazard to biota and humans.

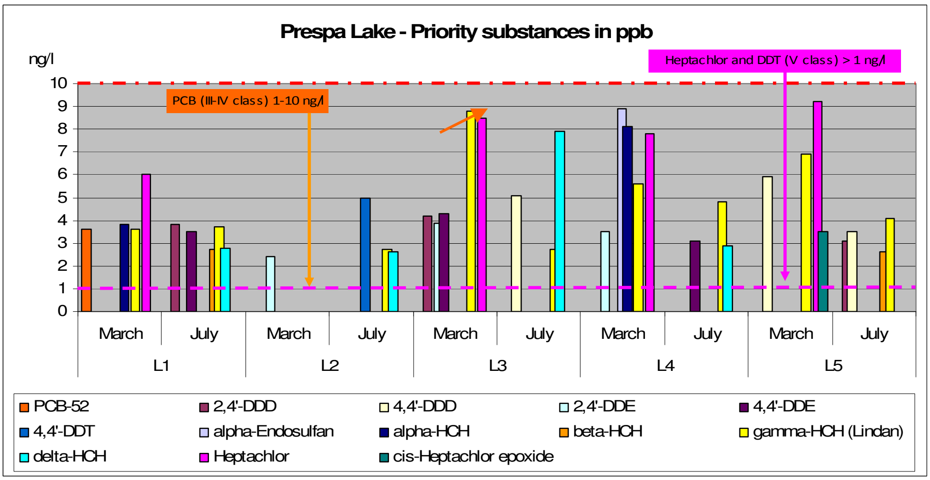


Fig. 27. Priority substances (in ppb) detected in the waters of selected Prespa Lake sampling sites.

By comparing the obtained results on priority substances for the river water bodies and Prespa Lake sampling sites (Fig. 28 and 29) interesting correlations could be formulated. Substances detected in high concentrations in the rivers, like *Bis(2-Ethylhexyl)phthalate* or *gamma-HCH (Lindan)* remain high in the lake's waters as well. Although others that were not recorded in very high concentrations in rivers, such as *Dibutilphthalate* or *Heptachlor*, show much higher concentrations in the lake, while *PCB's* tend to disappear from the lake's waters. These findings shed light on the very complicated and unpredictable pathways the detected priority substances have in the Prespa Lake ecosystem and point to the fundamental necessity to monitor and reveal their final destiny and impact they pose to the ecosystem, biota and human health.

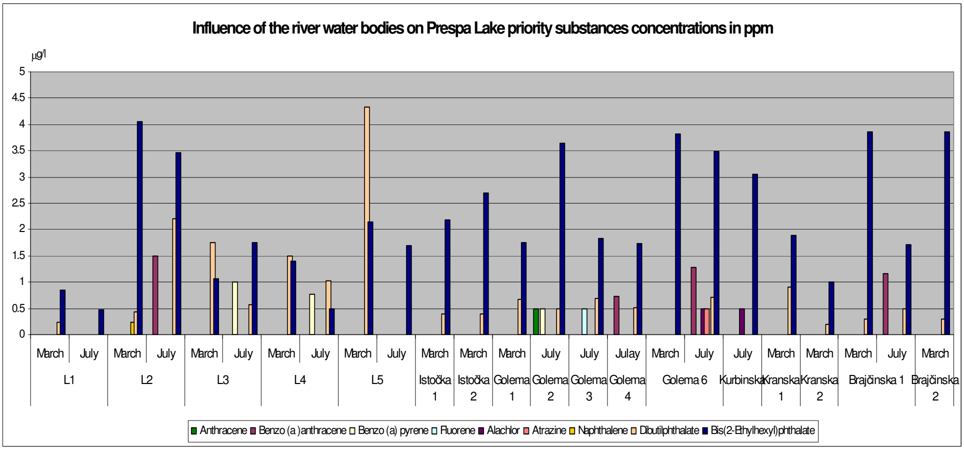


Fig. 28. The influence of river ecosystems on Prespa Lake regarding priority substances (in ppm).

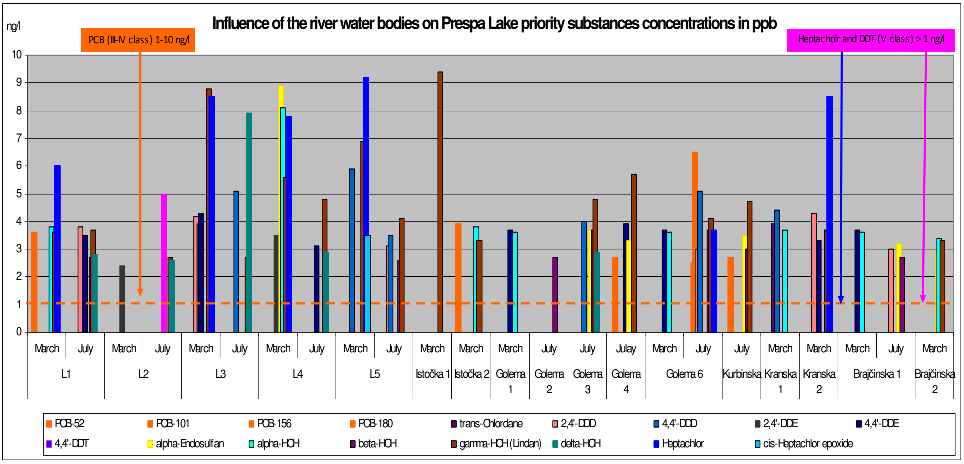


Fig. 29. The influence of river ecosystems on Prespa Lake regarding priority substances (in ppb).

The final analysis of the priority substances in Prespa Lake watershed was performed on sediment samples from the selected sampling sites in the lake (Fig. 30). At this point, only *gamma-HCH (Lindan)* was detected in the sediments of all sampling sites while the results for the L4 sampling site revealed sedimentation of the greatest number of analysed substances. Further monitoring of priority substances in Prespa Lake watershed should include far more frequent samplings and other media (like biota) in order to obtain overall conclusions about their patterns and role in the ecosystem.

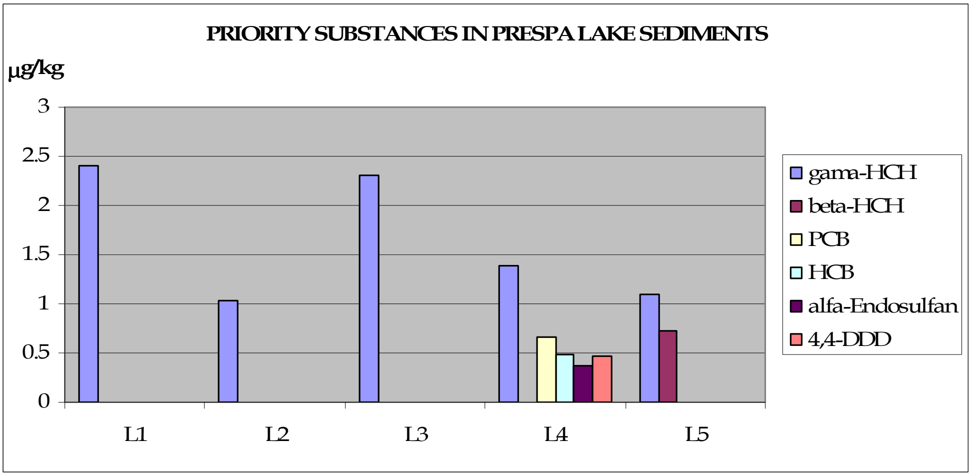


Fig. 30. Results on priority substances obtained by analysis of sediments in Prespa Lake sampling sites.

Ecological quality elements

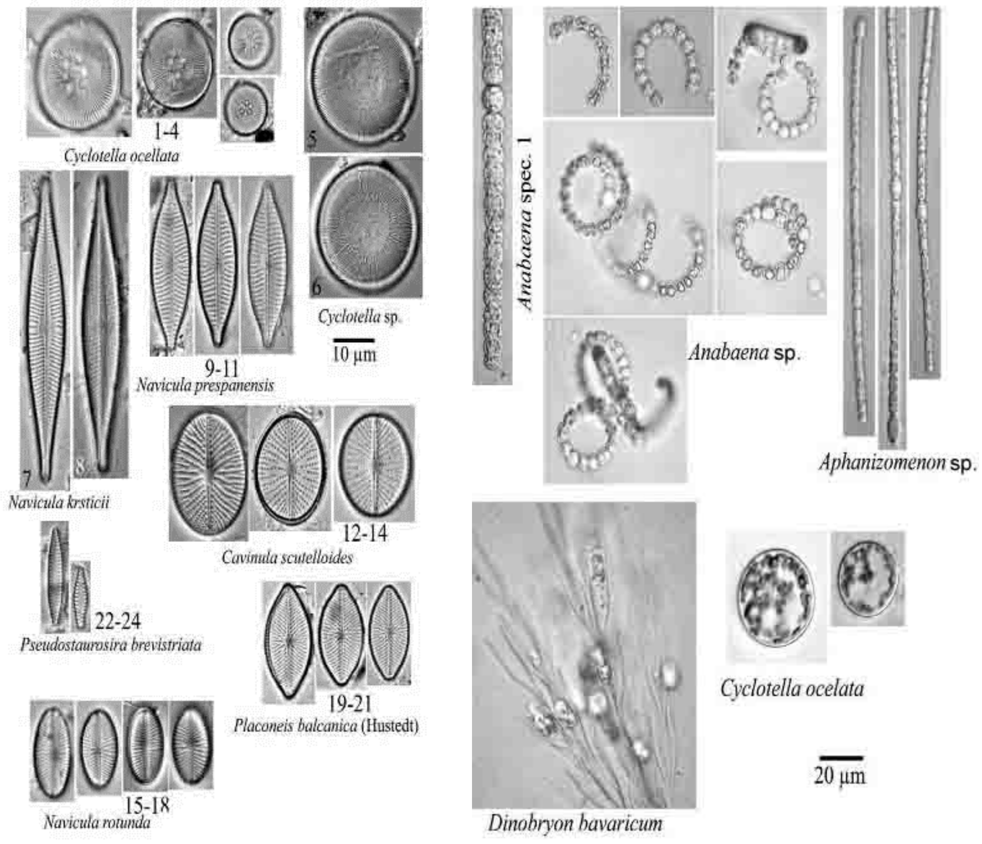
Investigations of biological quality elements in selected Prespa Lake sampling sites (L1-L5) were performed on all selected WFD's Ecological Quality Elements[6] - *phytoplankton, phytobenthos, invertebrate fauna, macrophytes* and *fish.*

Benthic diatom communities in Prespa Lake have been recently well documented (Levkov et al., 2006) and again confirmed with the performed investigations. Nevertheless, their ecological preferences are more elusive since very limited investigations in that context have been performed so far on the lake. The greatest difference in diatom composition between Eastern and Western coast of Prespa Lake was observed in the littoral zone. The bottom of the West coast (from Stenje village to Perovo village) is covered with organic sediment. Beside dominant species *Cavinula scutelloides, Navicula rotunda, N. subrotundata* and *Amphora pediculus*, characteristic species from genera *Aneumastus* and *Sellaphora* are frequent in the benthic communities. The Eastern coast (D. Dupeni village to Pretor) is mainly covered by sand or a mixture of sand and organic sediment. In this region, several *Navicula* sensu stricto taxa are sub-dominant. It is supposed that distribution of these species is influenced by substrate. A few species so far known only from Lake Prespa could be found on both sides.

[6] WFD-Guidance document No.7: Monitoring under the WFD, 2003.

Diatom assemblages on larger depths are dominated by *C. ocellata* complex, as well as species with heavily silicified valves as *Diploneis mauleri*, *Campylodiscus noricus*, *Cymatopleura elliptica* and *Navicula hasta*.

Phytoplankton in Prespa Lake is much more uniform. It is usually composed of planktonic diatoms, like the *Cyclotella ocellata* complex, with very rare presence of algae belonging to other taxonomic groups, like the chrysophyte *Dinobryon bavaricum* during the winter months (November-April). But, there is a rapid change in the dominance during the summer months when strong development (more than 90% of dominance) of potentially toxic cyanobacteria like *Anabaena* sp. or *Aphanizomenon* sp. are usually observed, and clearly documented during the latest investigations in the frame of this project (Fig. 31).



[A] [B]

Fig. 31. Dominant algae in Prespa Lake. [A] Benthic diatoms, [B] Dominant blue-green cyanobacteria, chrysophyte *Dinobryon bavaricum* colony and a diatom *Cyclotella ocellata* in plankton.

In contrast to the extensive use of benthic invertebrates in river monitoring, ecological assessment in lakes has instead focused mainly on the response of open-water phytoplankton (usually measured as concentrations of chlorophyll *a*) to nutrient (mainly phosphorus) enrichment (OECD, 1982) and, to a lesser extent, that of profundal or sub-littoral communities (Dinsmore et al., 1993). While the WFD has proposed a need for monitoring of littoral communities in lakes, their use in regional monitoring of lakes in Europe has been very limited. The lack of incorporation of littoral invertebrates into lake monitoring programmes reflects a traditional and common view that the structural heterogeneity of lake littoral areas, and associated variable distribution of benthic macroinvertebrates, negates the feasibility of their use in ecological assessment (Downes et al., 1993). Many anthropogenic impacts that affect rivers (Boon, 1992) however, also affect lakes, and would be expected to drive changes in the littoral macroinvertebrate community.

In the frame of this project, according to WFD requirements, categorisation of the Macro Prespa Lake based on macrozoobenthos was done. Macroinvertebrates from five sampling sites and different depth regions were collected. Detailed analyses on composition, abundance, diversity of benthic invertebrate fauna and relative contribution of sensitive and tolerant invertebrate taxa were performed. Based on the recommendation by the Swedish Environmental Protection Agency, two indices of bottom fauna were used for ecological assessment of Prespa Lake: the Benthic Quality Index (BQI) based on profundal fauna and the Shannon Diversity index (H′) based on littoral fauna. The corresponding water classification is given in Table 8.

Benthic Quality Index – BQI (Profundal fauna)	**Shannon Diversity Index – H′ (Littoral fauna)**	**Water quality**
>4	>3.00	high
3-4	2.33-3.00	good
2-3	1.65-2.33	moderate
1-2	0.97-1.65	poor
<1	<0.97	bad

Table 8. Water classification based on BQI and H′ for bottom fauna.

In Lake Prespa, the macrophyte vegetation shows relatively high species diversity in different parts of the littoral region. A high number of species is recorded at localities Golema River – 24, Asamati - 23 and v. Stenje – 21, while macrophyte species number is quite lower at Brajcino - 13 and Dolno Dupeni - 12. Recorded differences in the number of macrophyte species are most probably a result of different ecological conditions present at investigated localities, especially regarding nutrients. Namely, the presence of a higher species number at localities Golema River, Asamati and v.Stenje implies a very intensive anthropogenic influence. These areas of the littoral region have an increased presence of organic and inorganic material, what enables intensive growth and development of more diverse macrophyte vegetation.

Due to a decrease of the Prespa Lake water level in the last decade, marsh vegetation (reed and other emerged plants) progressively expanded around the lake (previously submerged littoral). Obtained results show that the dominant emerged plant in all investigated localities was *Phragmites australis* (with a density of 5 according to five-point scale). The reed forms a natural discontinuous belt around the lake comprised of numerous dense complexes. Other representatives of emergent vegetation were present in the belt of reed and in particular localities, where they formed almost pure associations. *Phalaris arundinacea*, *Typha latifolia*, *Typha angustifilia* were present with a density of 2, while *Shoenoplectus lacustris*, *Scirpus sylvaticus*, *Heleocharis pallustris* and *Cyperus longus* were present with a density of 1. Nevertheless, to obtain detailed information about changes in composition and spatial disposition of the vegetation, aquatic and marsh vegetation, long-term investigations are needed.

The fish population of the Prespa Lake is composed of 23 species of which 11 are autochthonous: *Alburnoides prespensis, Alburnus belvica, Anguilla anguilla, Barbus prespensis, Chondrostoma prespense, Cobitis meridionalis, Cyprinis carpio, Pelasgus prespensis, Rutilus prespensis, Salmo peristericus* and *Squalius prespensis*.

In the previous period 12 allochthonous species were introduced in Lake Prespa: *Carassius gibelio, Ctenopharyngodon idella, Gambusia holbrooki, Hypophthalmichthys molitrix, Lepomis gibbosus, Oncorhynchus mykiss, Parabramis pekinensis, Pseudorasbora parva, Rhodeus amarus, Salmo letnica, Silurus glanis* and *Tinca tinca*.

Alburnus belvica and *Rutilus prespensis* were caught in the greatest number of specimens at all investigated localities from Lake Prespa. On the contrary, *Anguilla anguilla*, *Chondrostoma prespense*, *Cobitis meridionalis*, *Pelasgus prespensis* and *Salmo peristericus* were not caught at any of investigated localities. Also, in the catches from all investigated localities *Cyprinis carpio* was present, but in very low numbers.

Populations of some of the introduced species are so reduced that they are very rarely present in representative and experimental fishing, like *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Oncorhynchus mykiss*, *Parabramis pekinensis*, *Salmo letnica*, *Silurus glanis* and *Tinca tinca*. Others like *Carassius gibelio*, *Gambusia holbrooki*, *Lepomis gibbosus*, *Pseudorasbora parva* and *Rhodeus amarus* are present in the catches but in very low numbers.

6. Reference conditions

6.1 Reference conditions for rivers

Although past data for rivers in Prespa Lake watershed are very scarce, the *reference conditions* were more or less easy to determine due to: a) all rivers belong to the same river type; b) they have very short and rapid flows prior to inflow in Prespa Lake; c) their source waters belong to two different parks of nature where they are significantly protected from any important human activities; d) even with a limited number of samplings, water chemistry and biology were easily distinguished from the rest of the river water courses where human impact was much more severe.

Therefore, a relatively solid starting proposal for the reference conditions of rivers in Prespa Lake watershed would be very close to conditions found in water bodies Kranska 1 and Brajčinska 1, which are: natural water courses with good hydraulic contact with surroundings, rich riparian vegetation, clear water with very low conductivity (<100), slightly acidic, low in nutrients which are easily biodegradable, and with variable natural flora and fauna in and around the water courses (Tab. 9).

Reference conditions for the rivers in Prespa Lake watershed	
Parameter (units)	Value
*Dissolved oxygen (mg*L^{-1})*	>9
*Conductivity (μS*cm^{-1})*	<50
pH	6-7
*NH_x-N (mg*L^{-1})*	<0.05
*NO_x-N (mg*L^{-1})*	<0.6
*Total N (mg*L^{-})*	<1.0
*PO_4-P (mg*L^{-1})*	<0.020
*Total P (mg*L^{-1})*	<0.030
*Toxic heavy metals and priority substances (μg*L^{-1})*	<0.001
Dominant algae	Diatoms: *Meridion circulare, Meridion circulare var. constricta, Diatoma hyemalis, Diatoma mesodon, Eunotia spp., Staurosirella pinnata, Hannea arcus, Psammothidium daonense, Amphipleura pellucida, Decussata hexagona, Luticola nivalis, Diadesmis perpusila, Krsticiella ohridana, Pinnularia sudetica.* Red algae: *Lemanea fluviatilis.*
Dominant benthic invertebrates	*Heptagenia sulphurea, Baetis rhodani, Baetis alpinus, Baetis fuscatus, Baetis vernus, Potamophylax latipennis, Capnia vidua, Brachyptera risi, Nemoura cinerea, Austropotamobius torrentium, Astacus astacus*
DSFI index – invertebrates	≥7

Table 9. Reference conditions for rivers in Prespa lake watershed.

6.2 Reference conditions for Prespa Lake

Establishing the reference conditions for Prespa Lake (or any other lake) is a far more difficult task to perform. If the only reasonable and justified principle regarding every water ecosystem as a separate entity (the state-changed approach opposite to spatial state classification – Moss et al., 1997) is applied, than Prespa Lake cannot be compared for its reference parameters to any other lake (even with Lake Ohrid to which Prespa Lake is the major water source). This is even more important if the very turbulent and variable past of Prespa Lake is taken into account. Namely, the lake was formed by three rivers (underwater flows which are still detectable in the lake) which were constrained by karstic masses blocking their way to Ohrid Lake. Only then did the Prespa Lake ecosystem start to develop with a very variable surface area and volume in the past; there are numerous human constructions (buildings, roads) recorded at the lake’s bottom today. All of these characteristics describe Prespa Lake as a very large water source body, intensively mixed by numerous sub-lacustrine sources of water and with very unstable water mass basically depending on climate, hydrologic regime and human activities. It is also a system in which there is a constant mixing of the water column, either by winds or powerful underwater currents and sources, which also means a constant supply of nutrients in the water column.

In a water body such as this, and combined with the classical lack of continual monitoring (especially regarding biology) data, establishing of reference conditions has been proven a formidable task. Nevertheless, we have succeeded in acquiring core samples dated from 10 ka BP and to briefly analyse for the first time the basic chemical (major cations, heavy metals, total N and P content) and biological (diatom assemblages) parameters in the core layers dated from 0.5, 1, 2, 5 and 10 ka BP respectively.

Regarding the concentrations of major cations and heavy metals obtained from the analyses of Prespa Lake core samples (Fig. 32), Prespa Lake is dominated by *aluminium* and *iron* throughout the analysed period. On the other hand, *calcium* has increased more than three times in the same period, as well as *sodium* in the last 500 years, while *potassium* by 30%. These are clear signs of human alterations of the natural conditions.

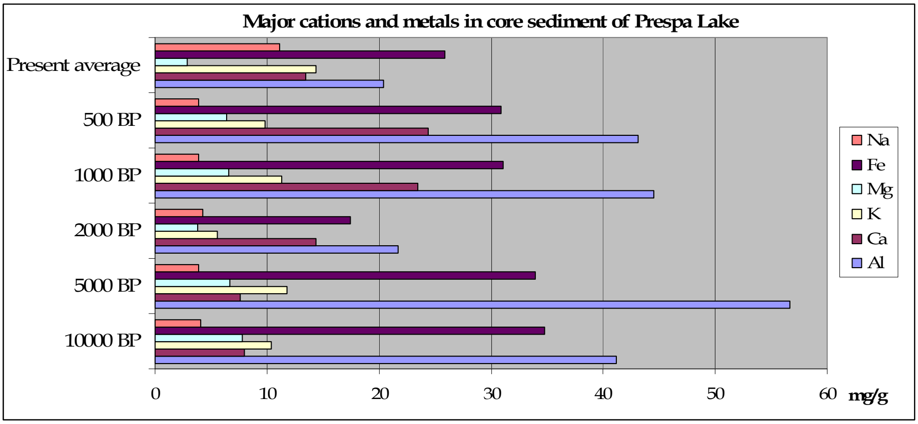


Fig. 32. Major cations and heavy metals in core samples of Prespa Lake.

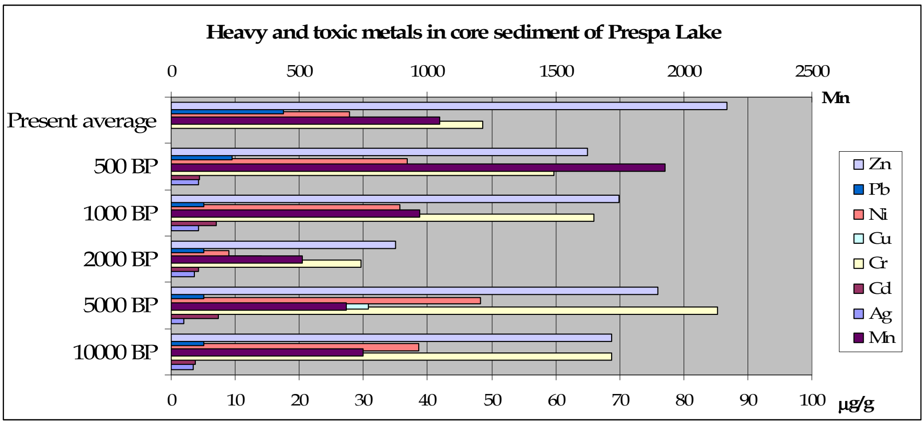


Fig. 33. Heavy and toxic metals in core samples of Prespa Lake.

Regarding heavy and toxic metals (Fig. 33) the greatest increase is recorded in concentrations of *zinc* and *manganese*, but *lead* is also showing a steady increase and a sudden peak in present times. These results are also corroborative of the obvious increase of human impact due to waste input in the lake's sediments in the past 500 years.

The results obtained for the total P content in the present day sediments of Prespa Lake (Fig. 34) are quite interesting. It can be concluded that the phosphorus in Prespa Lake has the crucial role in the overall eco-physiology of the system. It is not deposited at a regular pace and it is also not used in a predictive manner; a significant increase of phosphorous input has also been recorded during the summer months.

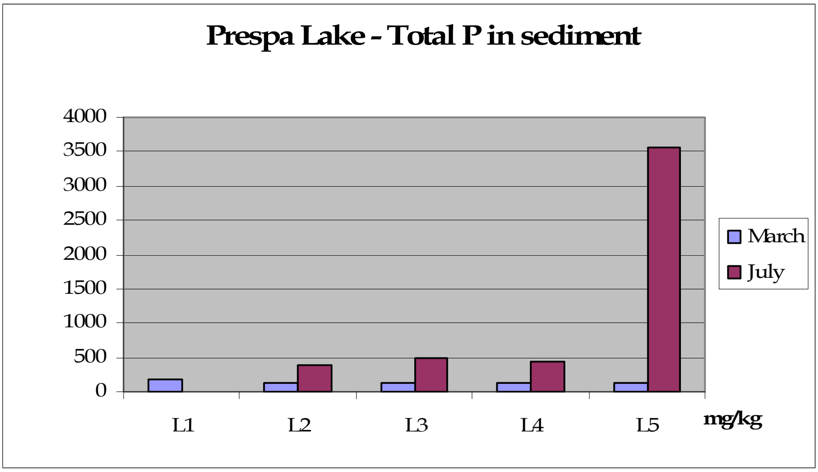


Fig. 34. Total P content measured in recent sediments at the sampling sites of Prespa Lake.

Compared to the results obtained from the analyses of the core samples (Fig. 35), the phosphorus in Prespa Lake reveals other important features. Firstly, it has been deposited in the recent sediments in significantly higher quantities (almost three times higher) than recorded in the core samples. Secondly, its predominance over nitrogen has been taking place in the last 500 years. Thirdly, Prespa Lake has never been a nitrogen limiting lake, since the values for total nitrogen are almost constant throughout the analysed period. Therefore, the principal nutrient that is driving the observed changes in the lake's plankton communities (cyanobacterial 'water blooms') is phosphorus. Observed occurrence of the cyanobacterial 'water blooms' at L5 sampling site (village Dolno Dupeni) and the results for the phosphorus deposition at the same area of the lake is more than just a coincidence which deserves much more attention in the future.

There are very few organisms, or their remains, that are well preserved in lacustrine sediments and can be easily retrieved for observations. Having siliceous cell walls, diatoms are probably the ultimate choice (Krstic et al., 2007) for both monitoring of the recent and paleo environments, since they quickly and constantly change their assemblages according to environmental conditions and their specific autecological preferences (Stoermer & Smoll, 1999).

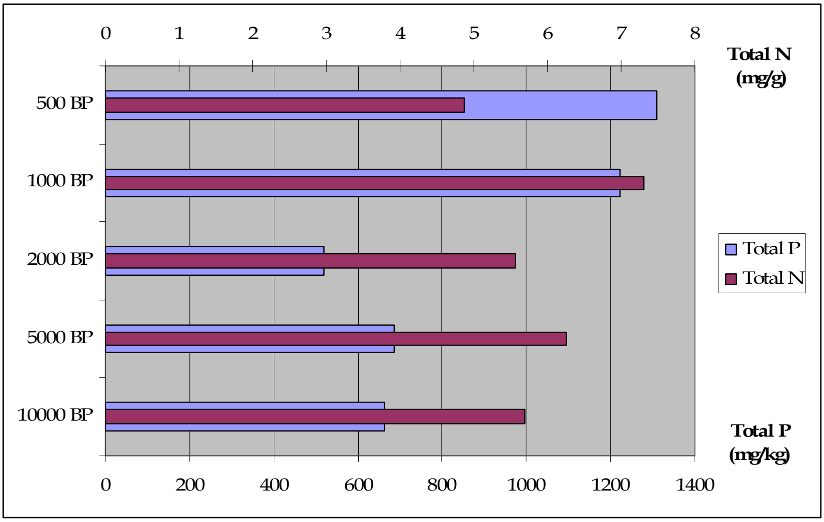


Fig. 35. Total P and total N Prespa Lake core sediments.

By analysing the diatom assemblages in different core layers of Prespa Lake presented on Figure 36, in order to reveal possible changes in dominant planktonic or benthic taxa and thus deduce the corresponding changes of environmental conditions forced by human activities, the following observations can be formulated:

- Diatom assemblages along the 10 ka core of Prespa Lake are surprisingly uniform. Only very slight changes in dominance of specific taxa can be observed; typically dominant throughout the core are *Cyclotella ocellata, Stephanodiscus rotula, Diploneis mauleri* and *Camplylodiscus noricus*.
- Diatom flora of Prespa Lake is very rich in taxa as recorded before (Levkov et al., 2006). But, the overall composition of taxa in the communities point to an ecosystem which is naturally rich in nutrients and enables development of diverse microflora which is reflecting the basic **mesotrophic state** (according to present state of knowledge regarding diatom nutrient preferences and autecology) of the environment at least up to 10.000 years BP.
- The only observed important occurrence of a diatom form that can be conclusive for a significant increase of nutrients in the ecosystem is the appearance of *Aulacoseira* spp. (especially *Aulacoseira granulata*) in the sediments approximately 1000 BP, and persisting in the communities to the present. This unique, but very subtle, change in diatom taxa dominance can be connected to the recorded high increase of phosphorus concentration in the Prespa Lake sediments presented on Fig. 35. For comparison, the *Aulacoseira* taxa determined in Prespa Lake can be found in co-dominance with various cyanobacterial taxa (which are usually regarded as potentially toxic) in the plankton of highly eutrophic lakes like Dojran Lake in Macedonia (Fig. 37 -Krstic et al., in prep). Since we cannot see the cells (or their remains) of other algae in the core layers, by deduction from the present knowledge we can conclude that Prespa Lake has become eutrophic, at least during the most productive periods, due to an increase of

phosphorus and possibly other nutrients not yet analysed in the core samples. The presented time frame corroborates the strong possibility that human activities have played the crucial role in increasing of the trophic status of Prespa Lake.

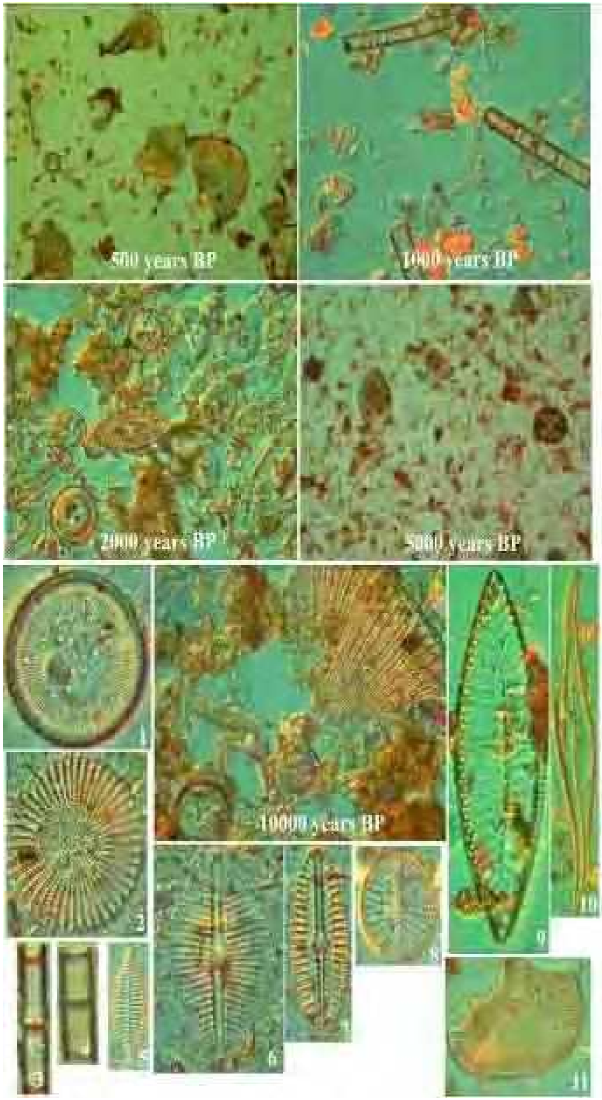


Fig. 36. Comparative presentation of diatom assemblages retrieved from 0.5-10 ka BP core samples of Prespa Lake and some of the most dominant and characteristic taxa in the investigated core samples: *1. Cyclotella ocellata, 2. Stephanodiscus rotula, 3. Aulacoseira granulata, 4. Aulacoseira ambigua, 5. Karayevia clevei var.balcanica* f.*rostrata, 6. Diploneis ostracodarum, 7. Diploneis mauleri, 8. Cavinula scutelloides, 9. Surirella bifrons, 10. Gyrosigma macedonicum, 11. Camplylodiscus noricus.*

Fig. 37. Plankton sample from Lake Dojran (August 2010) dominated by *Aulacoseira granulata* and at least three *Microcystis* taxa; circular filaments belong to *Lynbya contorta.*

The final support for the overall conclusion that the Prespa Lake has completed the turnover to a highly eutrophic system came from the analyses of plankton communities during the summer months (Fig. 38). Only two cyanobacteria forms have produced a typical 'water bloom' from May until September, *Anabaena affinis* and *Anabaena contorta*, which have fully replaced the usual plankton dominance of diatoms belonging to genus *Cyclotella.* Consequently, ELISA tests for cyanotoxins (*microcystins*) in the lake's waters have revealed significant presence of these toxins in the summer months (Fig. 39).

Fig. 38. 'Water bloom' caused by *Anabaena affinis* and *Anabaena contorta* in Prespa Lake waters.

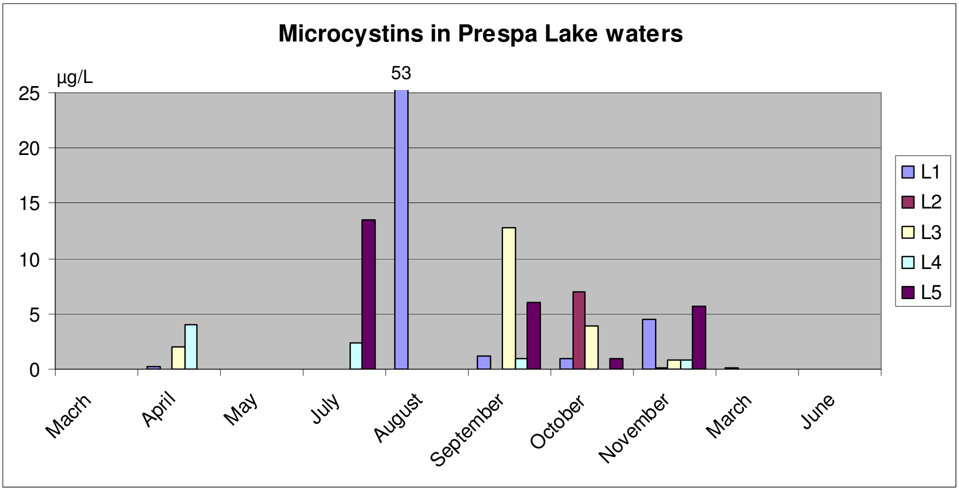


Fig. 39. Cyanotoxins-microcystins in Prespa Lake waters during the 12 month investigation period.

After all presented analyses and elaborations, the reference conditions for the Macro Prespa Lake ecosystem as one water body are presented in Table 10. Presented values for the most important parameters are targeted on the boundary between good and moderate water quality status for Prespa Lake which was surpassed at least a century ago. Having in mind the very high pressure of a variety of pollutants and human influences elaborated in this report, the targeted reference conditions may seem out of reach. But, if the ongoing situation continues, a total turnover of Prespa Lake towards highly eutrophic ecosystem should be expected in a very near future. In that case, the overall status of the Prespa-Ohrid-Crni Drim River system will be jeopardised and much more difficult to control, let alone brought to a good water quality status.

7. Discussion on the global significance of environmental alterations in lakes catchments

The people and their societies are integrated parts of the biosphere. They are dependent on its function and support, but at the same time they are shaping the biosphere globally with marked geological consequences (Steffen et al., 2011). This issue is much broader than the climate change *per se* (Folke et al., 2011). A key challenge for humanity in this new situation is to understand its role in the 'Earth System', start accounting for and governing natural capital and actively shape development in tune with the biosphere (Jansson et al., 1994; Rockström et al., 2009).

It is usually emphasised that during the last couple of generations we have witnessed an amazing expansion of human activities into a converging globalised society, enhancing the material standard of living for a large proportion of people on Earth (Rosling, 2010). This expansion has been quite pronounced since the 1950s, which predominantly benefitted the industrialised world, has pushed humanity into a new geological era, the *Anthropocene*, and generated the bulk of the global environmental changes with potential thresholds and tipping points, currently challenging the future wellbeing of the human population on Earth (Steffen et al., 2007).

Reference conditions for Prespa Lake	
Parameter (units)	Value
Dissolved oxygen ($mg*L^{-1}$)	6-7 (surface); >4 (bottom)
Conductivity ($\mu S*cm^{-1}$)	200-300
pH	7-8
NH_x-N ($mg*L^{-1}$)	<0.05
NO_x-N ($mg*L^{-1}$)	<1.0
Total N ($mg*L^{-1}$)	<3.0
PO_4-P ($mg*L^{-1}$)	<0.005
Total P ($mg*L^{-1}$)	0.015-0.025
Chlorophyll* a *($\mu g*L^{-1}$)	<3.8
Secchi depth (m)	>5
Dominant algae	Diatoms, Chrysophytes, Green coccoid algae, Xanthophytes, Charophytes. No cyanobacteria or 'water blooms' by any algal group.
Dominant benthic invertebrates	Snails, Clamps, Dragon flies, Mayflies, Caddis flies, Leeches, Sponge, Amphipods, Decapods. No Chironomids or Tubificids indicators for eutrophic conditions
BQI index **Diversity index H**	>3 2.33-3.00

Table 10. Reference conditions for Prespa Lake.

The work presented in this chapter enabled a slightly different point of view which broadens the scope of human alterations of nature significantly beyond the last few generations. Namely, if a prolonged civilisation was present in a certain area, like Lake Prespa in our case, in which the geological conditions are fragile enough to critically influence the recipient waters (the lake in our case), than the anthropogenic history of impact is traceable. Unlike soils, water ecosystems can and will accumulate excess chemical compounds very rapidly, but will also rapidly release them in a given situation. This increasing of the 'total capacity' (Svirčev et al., 2010) of a water ecosystem inevitably alters the natural environmental balance and forces the accelerated eutrophication or the rapid aging of the ecosystem. In the case of Prespa Lake, the total deforestations (or the clear cuts –

Fig. 40) have been confirmed many times over in the recent past, but this is the first time they have been detected one millennium ago (Fig. 35). The change from the N driven system towards the P dominated one can only be attributed to intensive P leaching and washout of the karst based geology repeatedly deprived of significant vegetation cover. Human activities in the last 500 years have only added to the already initiated change of the Prespa Lake system.

Strategies for development that ignore the dynamics of the broader social–ecological system may push people into vulnerable situations and persistent traps, and undermine the capacity to sustain human wellbeing in the long-term. Science has responsibility to provide a better understanding of the challenges facing humanity and to explore pathways toward a sustainable world. Global and regional scale integrated assessments, inclusive, transparent, and founded on an understanding of social–ecological interactions play a central role in building momentum for 'Planetary Stewardship' (Folke et al., 2011).

Fig. 40. Clear forest cuts on Bigla Mountain, Prespa Lake watershed (contemporary practice by foresters).

8. Conclusions

The surface waters of Prespa Lake watershed are found to be under intensive human pressure. This pressure is expressed through various physical impacts like alterations of the water courses and water abstraction, chemical pollution originating from untreated wastewaters or agriculture, and deterioration of natural biodiversity by introduction of alien species or over fishing. The intensity and duration of the negative human impacts on Prespa

Lake's natural environment have resulted in severe deterioration of the water quality in almost all water bodies, except the elevated stretches of the rivers way beyond the immediate human activities, if medium range aero deposition is regarded as negligible.

Apart from the performed investigations and obtained results, one of the major contributions to the conducted analyses was via the method used to separate the natural background of nutrient emissions from the anthropogenic influences that need interventions. In the case of Prespa Lake, humans started to alter the environmental properties in its catchment more than 1000 years ago by intensive forest clearings that have resulted in accelerated phosphorus leaching. This process has become even more intensified in the past 100-150 years through untreated wastewaters inflow into the system and intensive agriculture. The final observed outcomes have been the full turnover of the dominant algae in the plankton towards cyanobacterial 'water blooms' during summer periods which have also proven to be toxic for microcystins.

In order to prevent further deterioration of the water quality in the watershed, substantial efforts have to be made and many water pollution prevention measures implemented. Even if these activities are fully implemented and operational, the timeframe for full recovery of the ecosystem may be prolonged, since the accumulated quantities of harmful substances are in the range of highly elevated levels. Nevertheless, if no measures are initiated and implemented in the area, the overall environmental quality in Prespa Lake watershed will be much more degraded in the near future. This is especially important for the Prespa Lake itself since it has already started to show clear signs of becoming eutrophic throughout the year with even more frequent and toxic cyanobacterial 'blooms'. If the turnover towards fully eutrophic system is completed, the activities to restore and improve its water quality in that situation will be much more difficult or even impossible, thus rendering Prespa Lake unsafe and unusable for future generations.

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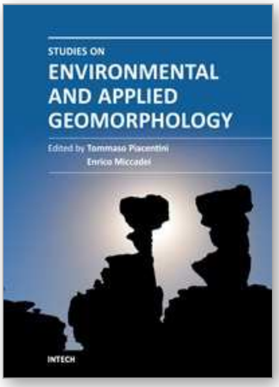

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This book includes several geomorphological studies up-to-date, incorporating different disciplines and methodologies, always focused on methods, tools and general issues of environmental and applied geomorphology. In designing the book the integration of multiple methodological fields (geomorphological mapping, remote sensing, meteorological and climate analysis, vegetation and biogeomorphological investigations, geographic information systems GIS, land management methods), study areas, countries and continents (Europe, America, Asia, Africa) are considered.

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