BOSNA SEMA EDUCATIONAL INSTITUTIONS
SARAJEVO COLLEGE
BOSNIA AND HERZEGOVINA

PROJECT'S NAME

USING BIOINDICATORS IN THE ECOLOGICAL ASSESSMENT OF FRESH WATERS' QUALITY

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SUMMARY

Today, environmental pollution especially water pollution represents the most serious problem in the development and survival of contemporary mankind. Particular problems are present in water pollution, which also includes drinking water. According to the estimation of experts, this can lead to real disaster of mankind very soon. Because of that, some sufficient and effective methods are necessary for the development of preservation ways in human life. One of the ways controlling and preserving water is the establishment of supervision system. Through this system, the amount of pollutants should be observed everyday, as well as estimation of water quality.

With the aim of demonstrating a model for ecological estimation of fresh waters' quality **by using bioindicators** (*cyanobacteria and algae*), structure and dynamics of benthos have been studied at more sites of the river basin of the river Miljacka (central Bosnia and Herzegovina).

The river Miljacka, flowing through Sarajevo, has a critical significance for peoples' lives in Sarajevo. Because, this river has contributed the development of Sarajevo for hundred years. The most part of the river Miljacka is much polluted today. It is still very clean in the upper part and has a high ecological quality, but at the down parts, it is much polluted and very dangerous for peoples' lives and all organisms.

Excellent and objective parameters in estimation of ecological and biological quality of fresh waters are organisms which live in water ecosystems. Particularly, the benthos of running water is the most convenient. In other words, they are the organisms of the river bed. On the basis of the analysis of these organisms, we can estimate the degree of pollution. Saprobiological methods according to Pantel – Buck were applied in phytobenthos analysis. The samples were preserved in 4% formaldehyde. A preparation of diatom was done according to the standard methods – Hustedt's method. Determination of cyanide bacteria and algae classes was performed by using standard keys and light microscope. Determination of indicator values was done according to the Wegl's method. In the end, we got results that showed us different qualities of waters depending on present pollution.

Phytobenthos analysis shows that; the river Miljacka has the second class quality in the upper part of the river course and it has the third class quality in the lower part which is closer to the river Bosnia. Results of saprobiological analysis are also confirmed by the results of chemical analysis of water. A significant concentration of NO_x and PO_x has been established in the second and third class of water quality.

Potential solutions for the water pollution are the development of ecological information system, reconstruction of sewerage system, separation of waste and their purification before getting into the river. Ecologically acceptable substances should be used by households and a system of preserving management should be developed for water as well as for land ecosystems. We can use bioindicators (cyanobacteria and algae) for the determination of water quality.

Because;

- Algae have high rate of reproduction and short life spam that makes them good indicators for short influence.
- As primary producers, algae are directly affected by physical and chemical factors
- Sampling is simple, cheap and has minimal influence of habitats.

Also, some bacteria which are representatives of genera can be successfully used in purification of waste waters in the system of ecological engineering; *Escherihia, Staphyloccocus, Bacillus, Nitrobacter and Nitrozomons.*

PICTURE 1 PICTURE 2

KEYWORDS;

Pollution, water resources, ecological monitoring, chemical analysis, physical analysis, sustainable development, biological communities, ecological assessment…

AIMS OF THE PROJECT;

1-Assessment of biological and ecological qualities of fresh water by using bioindicators (algae).
2-Correlation between physical-chemical analysis of water and structures of living communities.
3-Progress of ecological info system for sustainable usage of water quality.
4-Getting to solution of ecological model for pollution prevention and working on the progress of ecological models on behalf of protecting and rising up the quality of sewage system.

INTRODUCTION

Water is widely spread in living environment. There is approximately 1.6 billion km^3 of water on our planet. 0.5% of that is land waters. They are divided into underground and above ground waters. Rich and developed living world is present in all waters. Hydrobiology is studying life in water. In our land waters, many forms of life important for whole region can be found. Because of this, our waters are very famous and interesting.

For existence of rivers climatic factors (together with vegetation and geomorphologic conditions) are very important. In our country, above ground running waters are not the same as in the past. In the past, they were richer with water. Riverbeds and canons (Drina, Tara, Una, Neretva, and Cetina) represent that; in the past, large amounts of water were floating through these regions. There are also rivers in our country which are now richer in water, than in the past. This happens due to development of forests and other vegetation.

PICTURE 3 and PICTURE 4

WATER SABROBITY

Distribution and density of plant and animal populations are in function of environmental factors and its changes.

Present and absence of some organisms can be good indicator for the classification of species and level of pollution.

The most famous indicators of water pollution are **saprobes (**Greek. sapros-rotting, bios-life) are organisms that have been adapted to live in polluted water. These ecosystems (polluted water) consist of many bacteria.

Level of pollution in water can be determined by the presents and amount of specific aquatic organisms called indicators- saprobes.

Saprobe system was first used by Kalkwitz and Marson in 1909. From that, biocenosis and indicator organisms are determined in different places of rivers.

Zone of the highest pollution is called polysaprobic zone, and nearly all organisms live here.

Other lower polluted places have alpha and beta mesosaprobic zones where also indicatory organisms present are.

The lowest polluted places are oligosaprobic zones that have its specific biocenosis.

There are also non polluted waters or catarobic waters.

The categorization is done by means of water pollution level (saprobe). Organisms that live in polluted water are saprobes.

Organisms that live in fresh (no polluted) waters in which are no organic compounds are catarobionts.

Later, saprobe system was used and developed by many other scientists.

The most perfect saprobe system was recommended by *Sladecec,* which classifies water into four groups:

1) Catarobic waters
2) Limnosaprobic waters
3) Eusaprobic waters
4) Transsaprobic waters

Catarobic waters are the cleanest waters without any pollution (fresh underground waters, waters used for drinking, waters found at homes). Catarobic waters are not classified into any subgroups.

Limosaprobic waters are less or more polluted underground, aboveground or other waters and they are used in different industries.

They are classified as:

1) Xenosaprobic
2) Oligosaprobic
3) Beta mesosaprobic
4) Alpha mesosaprobic
5) Polysaprobic waters.

Eusaprobic waters are polluted and they contain high amount of organic substances that can be dissolved by biochemical process by the help of microorganisms.

They are classified into subgroups:

1) Isosaprobic
2) Metasaprobic
3) Hipersaprobic
4) Ultrasaprobic waters.

Transsaprobic waters are polluted waters where biochemical process of dissolved substances cannot be done. These waters are the most polluted waters, and consist of toxic substances or other inorganic salts. In these places (waters) organisms cannot live.

They are classified into three subgroups:

1) antisaprobic
2) radiosaprobic
3) criptosaprobic waters

There is also saprobe system that is developed by *Liedmann.* This system also recognizes 4 basic phases of pollution that determines levels of bonity.

For each of these he used specific colors for easier recognition:

1) Fourth level of bonity fits polysaprobic zone, gives red color.
2) Third level of bonity fits alphamesosaprobic zone, gives yellow color.
3) Second level of bonity fits betamesosaprobic zone, gives green color.
4) First level of bonity fits oligosaprobic zone, gives blue color.

Every of these zones have indicatory species where the **algae** take important place**.**

Oligosaprobic waters- the last oxidative process, organic materials are mineralized. Plants and animals which live in fresh or a little bit polluted waters are called as oligosaprobic organisms. Mountain Rivers, mountain brooks and lakes are oligosaprobic waters. These waters are clear and blue with high amount of dissolved oxygen. Also, the number of bacteria is very less.

Most organisms are sensitive to changes in the amount of dissolved oxygen and pH values, in fact on inorganic pollution. Silicate algae are indicators of this zone: *Cyclotella compta, Merdion circulare, Nitzschia linearis, Surirella spiralis etc.*

Betamesosaprobic waters are mostly fresh waters because the amount of dissolved oxygen is still high. A lot of natural lakes and rivers which are far away from industries are betamesosaprobic waters. The number of bacteria in these places is increased. The color of water is green. Indicators of this zone are: *Cyslotella meneghiniana, Melosira varians, Diatoma vulgare, Synedra ulna, Synedra acus, Navicula cuspidate, Pinnularia viridis, Gomphonema olivaceum* etc.

Alphamesosaprobic waters' quality is always decreasing. Canals and rivers near populated places are alphamesosaprobic waters.

Number of bacteria is very big. Water smells very bad. The number of species is decreasing. Indicators of this zone are: *Navicula crytocephala, Stephanodiscus hanzshia palea* etc.

Polysaprobic waters are the waters that are the most polluted. It is because of the big amount of unclean city waters and some industrial wastes.

In these waters, polysaprobic organisms are found. There is no dissolved oxygen and rotting process is found either. Just microorganisms can live here. Silicate algae cannot exist here.

MATERIALS AND METHODS

In our project, the river Miljacka and the source of the river Bosna are the rivers where we did all our investigations. All chemical analysis is done in the center for ecology and natural resources PMF University in Sarajevo. We performed two different working phases for Phytobenthos researches;

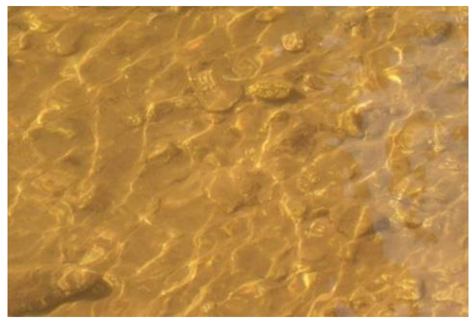
1- Land based researches 2- Laboratory researches

1-Land based researchesL; Phytobentos samples were taken from natural base by standard scrapping method. After that, material was conserved in 4% formaldehyde, marked and stored until analysis. (Pictures 5, 6, 7, 8)

PICTURE 5

PICTURE 6

PICTURE 7



PICTURE 8

2-Laboratory researches;We made include more subphases in which following procedures were done:

A-Determination of macroscopic forms; qualitative presence of some macroscopic forms of algae is established, that is quote in work results. Big inorganic substances are removed (sand, mud, clay).

B-Chemical treatment of samples; description of used method (Hustedt, 1930):

In labeled laboratory glasses about 50 ml of material has been put. The glass was filled with distillated water. After few minutes large mixtures were broken down. Water has been separated from sediment accumulated on the base of laboratory glasses and water with diatoms has been settled 1-2 hours. After that, we took out this water till the level of 1/6 of glass; we mixed the rest of sample with concentrated H_2SO_4, in relation 1:1. Blend contents with glass stick about 20 -30 minutes. In this reaction, heat was released so it has to be done with great caution. H_2SO_4 carbonate organic substance.

$KMnO_4$ solution must be added to the mixture until it gets dark color. When mixture starts to get violet color of **hypermanganate,** process is finished. This reagent is used as strong dehydration mean, for burning rest of carbon and other pitchy things. After 30 minutes, adding concentrated oxalic acid will cause reduction, and color of mixture will disappear. We add oxalic acid until mixture gets yellow color. After color disappearing distillated water is added until the end of the glass and it is left for 24 hours. After that, supernatant is taken and distillated water is added again. Procedure is repeated every 24 hours until mixture gets reaction that is close to pH7. Procedure finishes by pouring out of water and by adding ethanol.

C-Preparation of permanent slide samples; On a clean cover glass we put 2-3 drops of diluted algae suspension and heat it while water vapors and content gets white color. On a microscopic slide put 1-2 drops of canada balsam. Put cover glass on canada balsam with contest turned down by help of pincers. Continue with heating until bubbles appear. After cooling, mark permanent slide samples**.**

D-Determination of forms from silicate algae order has been made by using light microscope. Quantitative presence of all determined forms is established.

E-Information analysis; according to absolute numerous of some species, their relative frequency is established according to Pantle and Buck's scale:

Relative abundance mark	Taxon
1	Rare
2	Frequent
5	Large

Tabular descriptions are made according to localities, where indicator values by Wegl (1983) are added to some species.

Using following formula, according to the indicator value and relative frequency of indicatory species, we came to saprobe index (S) on some researched localities:

$$S = \frac{\sum^{n}(s_i a_i)}{\sum^{n}(a_i)}$$

S - Saprobe index
s_i -indicatory value of species
a_i -relative frequency

According to saprobe index, saprobe degree is evaluated to following table:

Saprobe degree	Saprobe index	Class of bonity
Oligosaprobic	1,0-1,5	I
Oligo-betamesosaprobic	1,51-1,8	I-II
Beta mesosaprobic	1,81-2,3	II
Beta to alphamesosaprobic	2,31-2,7	II-III
Apha mesosaprobic	2,71-3,2	III
Apha to polisaprobic	3,21-3,5	III-IV
Polisaprobic	3,51-4	IV

RESULTS AND DISCUSSION

1-PHYSICAL AND CHEMICAL ANALYSIS OF WATER

KOZIJA CUPRIJA LOCALITY

Description of locality: At the bottom of the river there are stones. Water is low level, does not have any smell, colorless. It has a little sediment. There is no vegetation around. (Pictures 9, 10)

Nitrates NO_3	2, 58 mg/l
Ammonium NH4-N	0, 083 mg/l
Phosphates PO4-P	0, 795 mg/l
pH	8

PICTURE 9

PICTURE 10

BENTBASA LOCALITY

Description of locality: Stones at the bottom. There is a little sediment, water colorless. There is a little vegetation – 5%. (Pictures 11, 12)

Nitrates NO_3	1, 83 mg/l
Ammonium NH4-N	0,084 mg/l
Phosphates PO4-P	0,247 mg/l
pH	8

PICTURE 11

PICTURE 12

OTOKA LOCALITY

Description of locality: Stones are at the bottom of river. Water is not clean. There are a lot of waste materials- trashes in and around the river. Water smells very bad. There is sediment. There is vegetation. (Pictures 13, 14)

Nitrates NO_3	2, 67 mg/l
Ammonium NH4-N	0,073 mg/l
Phosphates PO4-P	1, 17 mg/l
pH	8

PICTURE 13

PICTURE 14

VRELO BOSNE LOCALITY

Description of locality: Water is fresh and clean. Does not have any color. Stones are at the bottom. (Pictures 15, 16)

Nitrates NO_3	2, 34 mg/l
Ammonium NH4-N	0,071 mg/l
Phosphates PO4-P	less than 0,004 mg/l, is under apparatus's scale, so apparatus didn't mark value
pH	7

PICTURE 15

PICTURE 16

2-ALGAEOLOGIC (SAPROBIOLOGIC) QUALITY ANALYSIS OF WATER

LOCALITY 1; Kozija Ćuprija

On observed locality, 17 taxons are established and all of them are known as indicatory value. The highest frequency degree has following species: *Diatoma vulgare*, *Gomphonema olivaceum*, *Navicula radiosa, N. Viridula* and others.
According to indicatory value of presence species, established value of saprobe index is 2, actually fits beta mezosaprobic degree. Consequently, according to qualitative-quantitative analysis of water, observed locality has water that is II class of bonity. (TABLE 1)

Table1:

LOCALITY	**Kozija Ćuprija**	**Indicatory value of species/taxon**
SCIENTIFIC NAME OF SPECIES	**Relative abundance**	
Phylum Heterokontophyta		
Class Bacillariophyceae		
Achnanthes sp.	1	2
Cocconeis pediculus	1	1.7
Cocconeis placentula	1	1.6
Cymbella sinuata	1	1.5
Cymbella sp.	1	1.7
Diatoma vulgare	3	2.2
Gomphonema olivaceum	3	2
Navicula gracilis	1	1.7
Navicula radiosa	3	2
Navicula rhynchocephala	1	2.7
Navicula viridula	3	2.6
Nitzschia lineris	1	1.5
Nitzschia palea	1	2.7
Rhopalodia gibba	1	1.4
Surirella ovata	1	2
Synedra ulna	1	2
Phylum Chlorophyta		
Cladophora sp.	3	2.3
Saprobe index(WEGL)	**2**	
Bonity class	**II**	

LOCALITY 2; Bentbaša

On observed locality, 18 taxons are established and all of them are known as indicatory value. The highest frequency degree has following species: *Cocconeis pediculus*, *Diatoma vulgare*, *Gomphonema olivaceum*, *Navicula radiosa, N. Viridula, Cladophora sp.* and others.
According to indicatory value of presence species, established value of saprobe index is 2, actually fits beta mesosaprobic degree. Consequently, according to qualitative-quantitative analysis of water, observed locality has water that is II class of bonity. (TABLE 2)

Table 2:

LOCALITY	**Bentbaša**	**Indicatory value of species/taxon**
SCIENTIFIC NAME OF SPECIES	**Relative abundance**	
Phylum Bacteriophyta		
Sphaerotilus sp.	1	3.4
Phylum Heterokontophyta		
Class Bacillariophyceae		
Achnanthes sp.	1	2
Cocconeis pediculus	5	1.7
Cocconeis placentula	1	1.6
Cymbella sinuata	1	1.5
Cymbella sp.	1	1.7
Diatoma vulgare	3	2.2
Gomphonema olivaceum	3	2
Navicula gracilis	1	1.7
Navicula radiosa	3	2
Navicula rhynchocephala	1	2.7
Navicula viridula	3	2.6
Nitzschia lineris	1	1.5
Nitzschia palea	1	2.7
Rhopalodia gibba	1	1.4
Surirella ovata	1	2
Synedra ulna	1	2
Phylum Chlorophyta		
Cladophora sp.	5	2.3
Saprobe index (WEGL)	**2**	
Bonity class	**II**	

LOCALITY 3; Otoka

On observed locality, 8 taxons are established and all of them are known as indicatory value. The highest frequency degree has bacteria and animals that lived in ancient, eventually number of taxons from algae group is decreasing.
According to indicatory value of presence species, established value of saprobe index is 2, 8 actually fits alpha mesosaprobic degree. Consequently, according to qualitative-quantitative analysis of water, observed locality has water that is III class of bonity. (TABLE 3)

Table 3:

LOCALITY	**Otoka**	**Indicatory value of species/taxon**
SCIENTIFIC NAME OF SPECIES	**Relative abundance**	
Phylum Bacteriophyta		
Sphaerotilus sp.	5	3.4
Phylum Heterocontophyta		
Class Bacillariophyceae		
Gomphonema parvulum	1	2.1
Cyclotella Meneghiniana	3	2.6
Cymbella tumida	1	2.2
Nitzschia palea	3	2.7
Navicula sp.	1	2
Cladophora sp.	3	2.3
Protozoa		
Vorticella sp.	5	3.1
Saprobe index(WEGL)	**2.8**	
Bonity class	**III**	

REFERENCE LOCALITY – STANDARD LOCALITY

LOCALITY 4; Vrelo Bosne

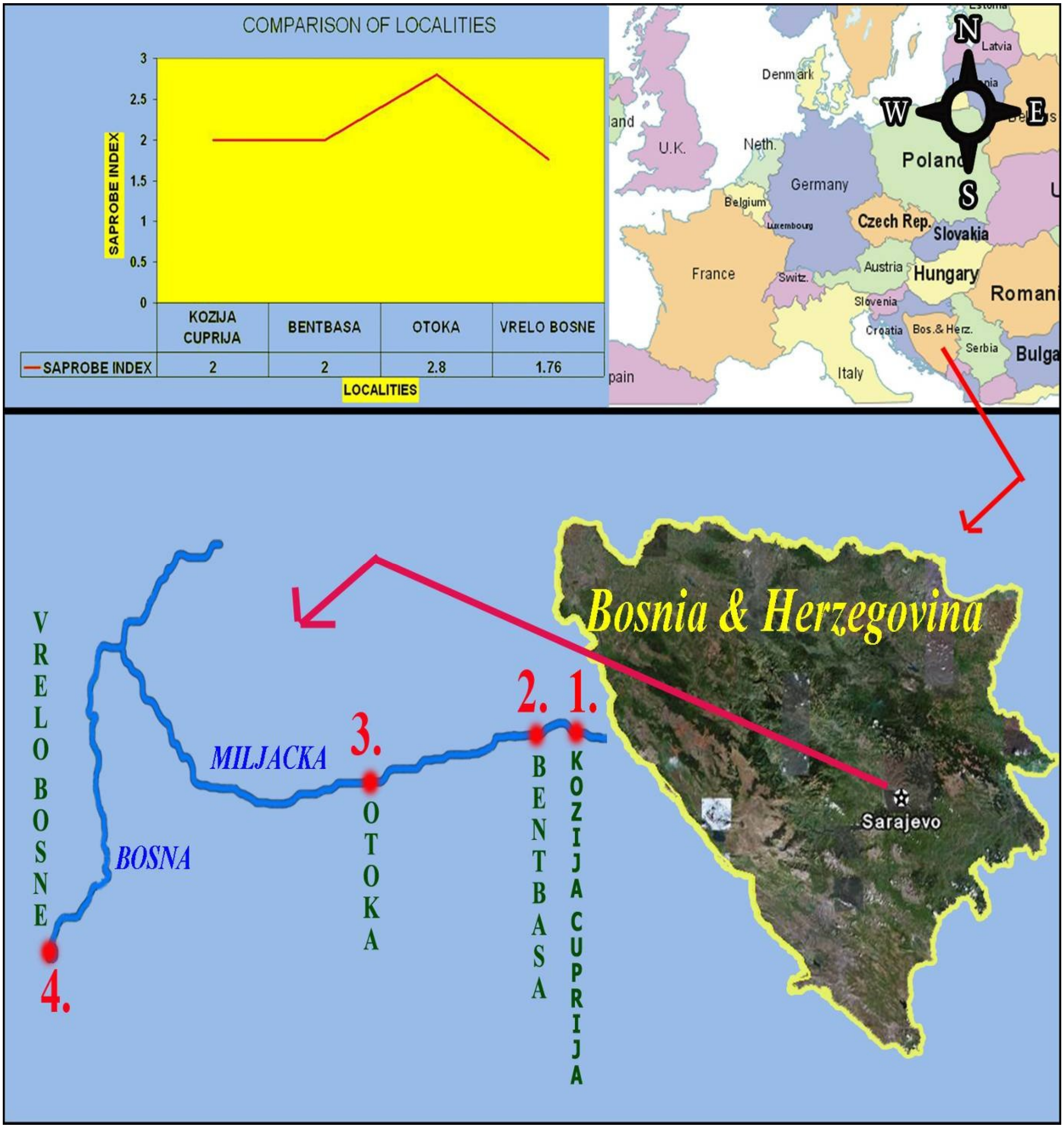
On observed locality, 35 taxons are established and 34 of them are known as indicatory value. The highest frequency degree has species *Cocconeis placentula* (5).

According to indicatory value of presence species, established value of saprobe index is 1, 76 actually fits oligo-betamesosaprobic degree. Consequently, according to qualitative-quantitative analysis of water, observed locality has water that is I-II class of bonity. (TABLE 4)

Table 4:

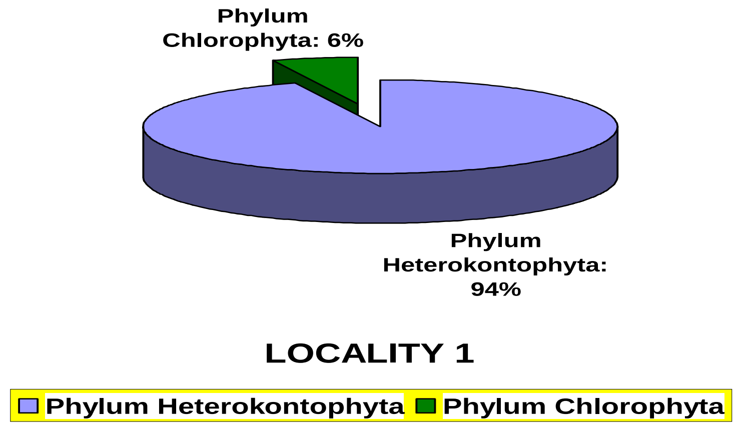
LOCALITY	**Vrelo Bosne**	**Indicatory value of species/genus**
SCIENTIFIC NAME OF SPECIES	**Relative frequency**	
Cyanophyta		
Oscillatoria sp.	1	2,3
Bacillariophyta		
Achnanthes linearis W. Smith.	1	1,5
Amphora ovalis Kutz.	3	1,7
Cocconeis pediculus Ehr.	3	1,7
Cocconeis placentula Ehr.	5	1,6
Cyclotella comensis	1	1,2
Cyclotella sp.	1	1,5
Cymatopleura elliptica (Breb.) W. Sm.	1	1,8
Cymatopleura solea (Breb.) W.Sm.	1	2,2
Cymbela affinis Kutz.	1	1,3
Cymbella ventricosa Kutz.	3	2
Denticula elegans Kutz.	1	1
Diatoma elongatum (Lyngb.) Ag.	1	1,6
Diatoma hiemale (Roth.) Heib.	1	1,2
Diatoma vulgare Bory.	1	2,2
Diploneis ovalis (Hisle.) Cl.	1	1,4
Eunotia sp.	1	1,2
Fragilaria construens	1	1,6
Gomphonema acuminatum Ehr.	3	1,7
Gomphonema olivaceum Kűtz.	1	2
Gomphonema parvulum (Kűtz.) Grun.	1	2,1
Gomphonema sp.	1	2,2
Gyrosigma acuminatum (Kűtz.) Rab.	1	2,2
Hantzschia amphioxys (Ehr.) Grun.	1	2,7
Melosira varians Ag.	1	2
Meridion circulare Ag.	1	1,1
Navicula lanceolata	1	-
Navicula radiosa Kűtz.	3	2
Navicula rhynchocephala	1	2,7
Navicula sp. I	1	2
Nitzschia linearis W. Sm.	1	1,5
Pinnularia viridis (Nitzsch.) Ehr.	1	1,7
Rhoicosphenia curvata (Kűtz.) Rab.	1	2
Surirella sp.	1	1,8
Synedra ulna (Nitzsch.) Ehr.	1	2
Saprobe index	**1,76**	
Bonity class	**I-II**	

After all these researches, we did comparison between Miljacka and the river Bosna, (with the source of the river Bosna), and we understand that, Miljacka river is much polluted than the river Bosna. There are many species living in the polluted water and graphs 1, 2, 3 and 4 given below show us their percentages in the different localities. Saprobe index graph given below in the picture 17 shows the level of pollution in different localities.

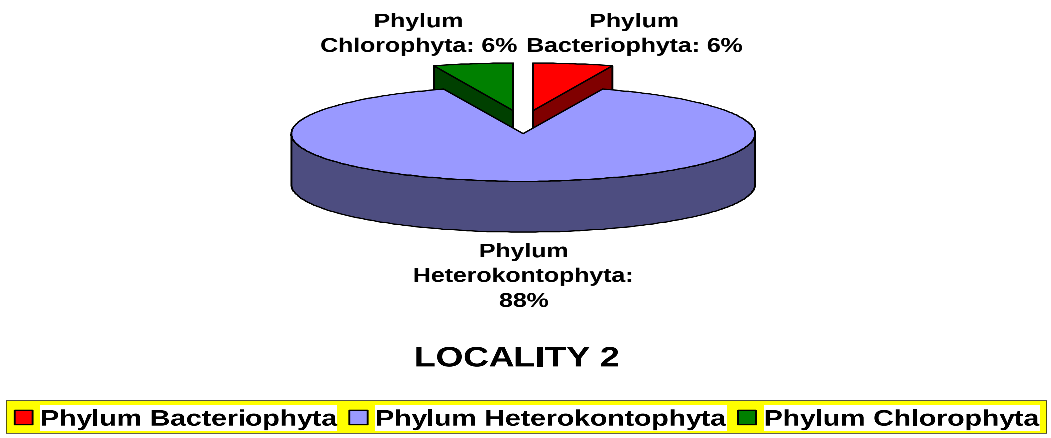


PICTURE 17

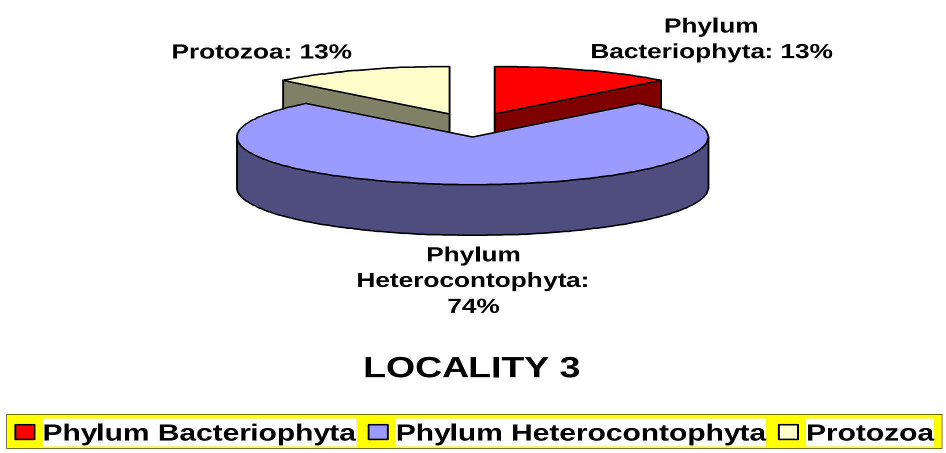
GRAPH 1



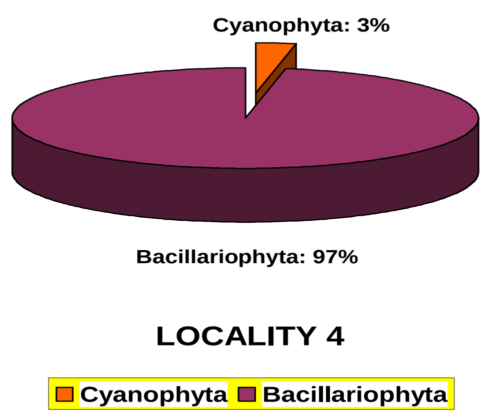
GRAPH 2



GRAPH 3



GRAPH 4



CONCLUSION AND SOLUTION

CONCLUSION

1. Composition of bioindicators (algae and cyanobacteria) in the researched water current, extremely illustratively shows the quality of waters and depends on the type and intensity of pollution by sewage.

2. The best indicators are the species of **silicate algae** which have narrower ecological range of tolerance in relation to pollution , that is water quality.

3. Water quality, on the basis of saprobic index significantly varies on logitudinal profile in the upper part of researched water. The highest values have been found in the upper part of water current, and the lowest after flowing city waste waters.

4. Physical -chemical analysis is in positive correlation with biological analysis. The quantity of nitrate, ammonia and phosphate increases while pollution increases. The same properties are shown by pH waters.

SOLUTION

1- For solving the pollution in waters in urban environments, it is necessary to develop and establish ecological information system in all water in order to monitor (permanent observing of physical-chemical and biological-ecological parameters). Then, it is also necessary to change the models of water using in household and industry, to use ecologically more acceptable (ecologically more biodegradable) substances in household and industry, to decrease significantly the use of detergents and to rationalize the use of drinking waters in non-biological purposes.

2- Substantial researches point to the great ecological purification possibilities of waste waters by using cyanobacteria, algae and bacteria. In their metabolism, polluting substances (nitrate and phosphate) are used for their own respiration. (For example, species which live in the most polluted waters)

3- These researches indicate the possibility of using the following species in technical purification process of waste waters. These species are: *Euglena, Vaucheria, Sphaerotilus sp., Cyclotella Meneghiniana, Nitzshia palea.* These are also bacteria which are representatives of genera; *Escherihia, Staphyloccocus, Bacyllus, Nitrobacter and Nitrozomons* and they can be successfully used in purification of waste waters in the system of ecological engineering.

4- The researches open the new possibilities in biotechnology of sewage and ecological models in controlling of water ecosystems as well as land ones.

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