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Analysis and interpretation of community-level physiological profiles in microbial ecology for some locations in Montenegro

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Abstract

The microorganisms play a significant role in aquatic ecosystems and in rivers and lake ecosystems as well. The present study aimed at assessment of impact on chemical residues and radicals and microorganism. For the first time in Montenegro conducted this kind of research. We have been used physiological groups and they profiles using BIOLOG™ Biolog EcoPlate. The investigated microorganisms previously are grown on substrates which are selective. We present on the tables only the 72 hours results because the examination of the results changes mostly before 72 hours or during (0,24, 48). On the two location King's head"-Skadar Like in populatin of protolithic bacteria and Vukovci-River Moraca in lipolytic bacteria the phenotypes is already repaired, but in on the other is in phase reparation.

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Introduction

Physiological groups of microorganisms are the main groups of microorganisms which are serving to secure the transport of matter and energy through aquatic ecosystem (Radonjic *et al*., 2013). Microbial communities show a certain physiological activity, regardless of their systematic affiliation, because they have extracellular enzymes for the degradation of certain substrate.

Different communities of microorganisms can be used to compare and classify sources of carbon used in aquaticecosystems (Garland *et al*., 1991). Carbon is a key factor for governing microbial growth in water, and functional aspect related to substrate utilization that can provide important information beyond that being afforded by taxonomic level investigations or structural investigations based on rRNA or rRNA analysis. (Insam and Goberna, 2004). The functional diversity of microorganisms, particularly those defined by the substrates used for energy metabolism, is integral for our understanding of biogeochemistry (Hooper *et al*., 1996). Indeed, it has been argued that it is diversity at the functional level rather than at the taxonomic level which is crucial for the long-term stability of an ecosystem (Pankhurst *et al*., 1996).

Microbial communities has been researched in January 2015. Sampling was performed from the coast-littoral zone. During the research from November 2012 until January 2015, the localities "Vukovci" on the Morača river as well as "Left" and "Right" arm of Moraca river on the location "Thin cape" (under the bridge) and "Kings head" at the Vranjine Skadar Lake; the first time in Montenegro, observation of microbial community was conducted with the use of BIOLOG™ plates Biolog Eco Plate. What was pointed out were their differences that may arise as a result of the actions of emerging substances in the water. We have investigated the location of the Zeta plain in an area of 30,000 hectares (Fig. 1.) or between kot.42,27815; 19.12354 in the north and 42, 271908; 19.124098, and in the south.

Material and methods

Sampling for microbiological analysis was carried out in the littoral segment of the river and sampled in pre-sterilized glass bottles.

Sampling bottles were washed and dried, then sterilized at 190 °C in a dry sterilizer for one hour. During the sampling, grab sampling procedure, as prescribed by Water Act 27/2007, of Montenegro was followed completely.

Fig. 1. Studied area.

A disposable sterile rubber stopper was carefully removed and the bottle was opened, with one hand holding the cap and the other hand grabbing the water sample, but taking care not to contaminate the cap.

Then, sampling bottle was tightly closed with a sterile cap. Sampling for microbiological analysis was done by quickly submerging prepared bottles, so the contamination of bottle is avoided. Samples were transported to the laboratory in portable fridge.

The microbiological analyses were performed in Hydrobiological Institute of Montenegro, Department of Biology.

The analysis of microbiological parameters were performed according to the standard methods for the analysis of surface waters of the Water Law of Montenegro 27/2007a. For the purpose of analyses the microbiological culture media were used and the ingredients for substrates used in this study are the product of the Institute for Immunology and Virology "Torlak" Belgrade, BioLive-Milano (Italy) and Seminem, Sarajevo (BiH). Substrates were prepared in the same circumstances as the manufacturer and sterilized into an autoclave for 15 to 20 minutes at 121°C under the pressure of 1.5 atmospheres. Sterilization is performed by autoclaving (121°C, 20 min). After sterilization, the final pH value was 7.3. The incubation period lasts for 5 to 7 days at 22 – 26°C.

Nature colonies are overlaid with an appropriate reagent. After a few seconds, the reagent is decanted. Counting only those colonies that showed a characteristic reaction, it appears as enlightened zone. Reagent for proteolytic activity is based on mercuric chloride, which causes the precipitation of the proteins in the medium (in the case of gelatin). Around colonies, which have extracellular protease enzyme and degrade gelatin, occurs settling and milky white surface, but the bright zone around the colony is shown clearly. Its size varies and depends on the activity of the enzyme.

Accordingly, it is sometimes necessary (in high numbers and/or high activity) to conduct the review in this group of bacteria, but after 3 – 5 days of incubation, so that the affect of merging zone illumination on active colonies is avoided, as well as having more difficult time counting.

Reagent for proteolytic bacteria
$HgCl_2$ – 15 g;
Concentrated HCl – 20 cm^3;
Distilled water - 100 cm^3.

Note: add half the amount of HCl in water, in that dissolved $HgCl_2$ and add the rest of water.

Lipolytic bacteria
Bacteria that have the ability to decompose the fatty material, i.e. possessing extracellular hydrolytic enzymes lipase collectively referred to as lipolytic bacteria. Foundation for lipolytic bacteria was tributyrin agar
Peptone – 5 g;
Yeast extract – 3 g;
Tributyrin – 3 cm^3;
Agar 15g;

Distilled water – 1000 cm^3. Sterilization is performed by autoclaving (121°C, 20 min). After sterilization, the final pH value was 7.3.

Incubation was performed for 5 – 7 days in an incubator at 22 – 26°C. All colonies show more or less enlightened zone around the area where they were counted, because it is a sign that the bacteria possess extracellular lipase enzyme that breaks down fatty substances.

In these strains the first active marking bacterial colonies can be carried out already after 48 hours or 3 days, with counting at the fifth and seventh day of incubation at the very end. Size of the illumination zone will be different depending on the amount and the activity of the lipase enzyme, produced by a bacterium.

After isolation of microbial community, samples were transported to the laboratory RIGEL srl, Piazza Contessa Carolina Del Maino, 11 - Italy.Community level physiological profiling (CLPP) begins its development of the BIOLOG system in the late 1980. The system was developed to identify bacteria of clinical importance by assessing each bacterium's usage of any of 95 different carbon sources in one microtiter plate. Originally, the principal users of the BIOLOG system were pharmaceutical, biotech, cosmetics, and medical device companies, as well as labs testing for diseases of humans, animals and plants, labs performing environmental monitoring, and companies or organizations involved in production or testing food and drink.(Garland,1997). In the 1990s, environmental researchers realized that useful physiological data concerning "whole" communities could be generated by inoculating mixed microbial assemblages into the BIOLOG plates. Noting the response of the mixed community to the carbon sources communities can also be assessed (Firestone *et al.*, 1997). The Biolog Eco Plate contains 31 of the most useful carbon sources for soil community analysis. These 31 carbon sources are repeated 3 times to give the scientist more replicates of the data. Communities of organisms will give a characteristic reaction pattern called a metabolic fingerprint. These fingerprint reaction patterns rapidly and easily provide a vast amount of information from a single Biolog Micro Plate (Fig.2) The community reaction patterns are typically analyzed at defined time intervals over 2 to 5 days. We present you 72 hous research. The changes in the pattern are compared and analyzed using statistical analysis software which is an integral part of BIOLOG™ Biolog Eco Plate. The most popular method of analysis of the data is via Principle Components Analysis (PCA) by average well color development (AWCD) data, but alternative methods may also offer advantages (Insam and Goberna,2004).The changes observed in the fingerprint pattern provide useful data about the microbial population changes over time.

Average metabolic response (AMR) is method for averaging the mean difference between the O.D. of the C-sourse.

AMR=Σ (O.D.well-O.D.neg)/95;is the optical densit of each carbon sourse-contaning well minus the O.D. of negative control well.

The CMD is calculated by summing the number of positive responses (purple-colored wells, Fig.3) observed after incubation. A thresholds O.D. must be established in advance to understand which purple containing wells and the control well.

Coloration indicates a usage of the carbon source by the microbial community. This threshold is commonly set at O.D.=0,25 (Lehman *et al.*, 1997).

Results

Microbial communities have great potential for temporal or spatial change, and thus represent a powerful tool for understanding community dynamics in both basic and applied ecological contexts.

The small size and rapid growth of microorganisms allow for complex community interactions to be studied much more readily than with plants or animals. It is not just a problem with surface waters claims (Adams *et al.*, 2015) but also water used for irrigation remains unnoticed when the quality level is changing. Mehanned *et al.*, 2014. assessed the importance of some chemical factors on some fecal bacterial communities. For example, temporal dynamics in response to natural successional processes or experimental manipulation can be tracked for many generations within reasonable time frames. Additionally, variation in microbial community structure may have effects on ecosystem processes (e.g. nutrient recycling, decomposition) or the effectiveness of microbial invasions (e.g. growth of pathogens, release of genetically engineered microorganism Radonjić *et al.*, 2013).

Table 1. Result of CMD on the Moraca River and some place on the Skadar Lake for lipolytic bacteria.

C sources/Carbohydrates	Sample/1	Sample/2	Sample/3	Sample/4	Sample/5
D-Celiobiose		+		+	
α-D-Lactose		+			
β-Methyl-D-Glucoside		+		+	
D-Xylose		+	+	+	+
i-Erytritol					
D-Mannitol		+	+	+	+
N-Acetyl-D-Glucosamine		+		+	
C sources/Carboxylic acid	Sample/1	Sample/2	Sample/3	Sample/4	Sample/5
D-Glucosaminic Acid					
D Galactonic Acid γ-Lactone		+			+
D-Galacturonic Acid		+	+	+	+
2-Hydroxy Benzoic Acid		+			
4-Hydroxy Benzoic Acid					+
γ-Hydrohybutyric Acid			+		+
Itaconic Acid			+		+
α-Ketobutyric Acid					
D-Malic Acid	+				
Glycyl-L-Glutamic Acid					
C sources/Phosphorylated chemical	Sample/1	Sample/2	Sample/3	Sample/4	Sample/5
Glucose-1-Phosphate		+		+	
D,L-α-Glycerol Phosphate		+		+	
C sources/Esters	Sample/1	Sample/2	Sample/3	Sample/4	Sample/5
Pyruvic Acid Methyl Ester	+	+	+	+	+
C sources/Amino acid	Sample/1	Sample/2	Sample/3	Sample/4	Sample/5
L-Arginine					+
L-Asparagine			+		+
Phenilalanine					
L-Serine		+	+	+	+
L-Threonine					
C sources/Amines	Sample/1	Sample/2	Sample/3	Sample/4	Sample/5
Phenylenetyl amin					
Putrescine		+	+		+
C sources/Polymers	Sample/1	Sample/2	Sample/3	Sample/4	Sample/5
Tween 40	+				+
Tween 80	+				
α Cyllobiose				+	
Glycogen					

Explenance

Sample/1-Vukovci-River Moraca lipolytic bacteria Sample/2 - Upper-Right" Moraca lypolitic; bacteria;

Sample/3-"Lower-Left" Moraca lypolitic bacteria; Sample/4- King's head"-Skadar Like- lipolytic bacteria;

The presence of high concentration of toxic heavy metals in industrial waste directly leads to contamination of receiving soil and water bodies and has deleterious impact on both human health and aquatic life (Sremacki *et al*., 2015) In the present study samples, from textile mill effluent from different areas of Jessore city, bacteria were analyzed for the identification and characterization which shows low tolerance to Copper, Mercury and Zinc (Ashikuzzaman *et al*., 2015). Understanding how community processes affect ecosystem is a central challenge in ecology, and microbial communities offer a potentially powerful forum for advancing this understanding. Unfortunately, the lack of effective methods for describing microbial communities has restricted our understanding of microbial community dynamics.

Methods based on describing the distribution of individual types of microorganisms are time-consuming and biased due to their reliance on cultural practices. Using Biolog Eco Plate, we obtain 0-time, 24 hour at 48 and 72 h and after 7 days.

We Present only the 72 hours results because the examination of the results changes mostly before 72 hours or during (0,24, 48). Result of CMD, are present on Table1 and Table 2.

Table 2. Result of CMD on the Moraca River and some place on the Skadar Lake for proteolitic bacteria.

C sources/Carbohydrates	Sample/6	Sample/7	Sample/8	Sample/9	Sample/10
D-Celiobiose					
α-D-Lactose					
β-Methyl-D-Glucoside					
D-Xylose					+
i-Erytritol					+
D-Mannitol	+	+	+		+
N-Acetyl-D-Glucosamine	+	+	+		
C sources/Carboxylic acid	Sample/6	Sample/7	Sample/8	Sample/9	Sample/10
D-Glucosaminic Acid					
D Galactonic Acid γ-Lactone					+
D-Galacturonic Acid	+	+			
2-Hydroxy Benzoic Acid					
4-Hydroxy Benzoic Acid					
γ-Hydrohybutyric Acid			+		+
Itaconic Acid					+
α-Ketobutyric Acid					
D-Malic Acid					+
Glycyl-L-Glutamic Acid					
C sources/Phosphorylated chemical	Sample/6	Sample/7	Sample/8	Sample/9	Sample/10
Glucose-1-Phosphate					
D,L-α-Glycerol Phosphate	+	+	+		+
C sources/Esters	Sample/6	Sample/7	Sample/8	Sample/9	Sample/10
Pyruvic Acid Methyl Ester	+	+	+	+	+
C sources/Amino acid	Sample/6	Sample/7	Sample/8	Sample/9	Sample/10
L-Arginine					
L-Asparagine	+			+	
Phenilalanine					
L-Serine					+
L-Threonine					+
C sources/Amines	Sample/6	Sample/7	Sample/8	Sample/9	Sample/10
Phenylenetyl amin					
Putrescine					+
C sources/Polymers	Sample/6	Sample/7	Sample/8	Sample/9	Sample/10
Tween 40	+	+	+	+	
Tween 80	+	+	+	+	
α Cyllobiose					
Glycogen					

Sample/6- Vukovci-River Moraca protolithic bacteria; Sample/7- Upper-Right" Moraca protolithic bacteria; Sample/8- Lower-Left" Moraca protolithic bacteria; Sample/9- King's head"-Skadar Like-protolithic bacteria; Sample/10-"Thin cape" protolithic bacteria.

Table 3. Average metabolic response for samples analyzed after 72 hours.

Locations	Average metabolic response for locations during 72 hours
Vukovci-River Moraca lipolytic bacteria	0.369
„Upper-Right" Moraca lypolitic; bacteria	0.757
"Lower-Left" Moraca lypolitic bacteria	0.757
„King's head" Skadar Like- lipolytic bacteria	1.244
"Thin cape" lipolytic bacteria	0.859
Vukovci-River Moraca protolithic bacteria	0.483
"Upper-Right" Moraca protolithic bacteria	0.476
"Lower-Left" Moraca protolithic bacteria	0.560
"King's head"-Skadar Like- protolithic bacteria	0.202
"Thin cape" protolithic bacteria	0.313

Discussion

Sample / 1- Vukovci Moraca lipolytic bacteria and Sample / 9- "King's head" Skadar Lake protolithic bacteria they have the smallest number of positive dome/cupolas. Positive on polymerase Sample / 1, growth in D-Malic Acid medium, until Phenylalanine affects is a evident Sample Code / 9 .All samples for growth used C source carbohydrates and hydrocarbon acids. Also, the used amino acid to.Phosphorylated chemical, are founded in Sample/2 - "Upper-Right" Moraca lypolitic bacteria and Sample/4"King's head" Skadar Lake lypolitic bacteria on both the substrate (Glucose-1-Phosphate and D,L-α-Glycerol Phosphate to). And on one substrate in Sample/6- Vukovci Moraca protolithic bacteria; Sample/7-"Upper-Right"Moraca proteolytic bacteria; Sample/8 "Lower-Left" Moraca protolithic bacteria; Sample/9- "King's head" Skadar Lake protolithic bacteria; Sample/10- "Thin cape" Skadar Lake proteolytic bacteria. Classification and characterization of heterotrophic microbial communities of the basic of passers of community level sole carbon source has been analyzed be (86) The BIOLOG™ EcoPlate, community assay is rapid and, it appears, sensitive enough to conduct the intensive sampling required to examine the mechanisms coupling sours of bacterial substrates to dynamics in the bacteria planktonic community in aquatic system (Radonjić, *et al.*, 2015).

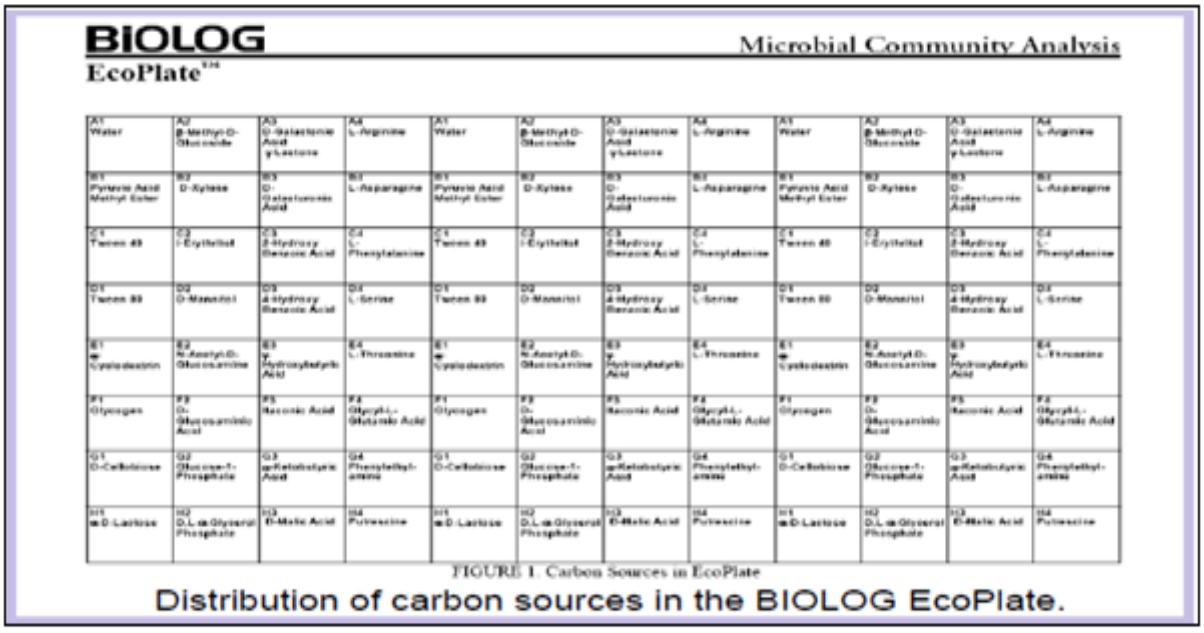


Fig. 2. Biolog Eco Plate.

Methods of optical density transformation and analytical procedures had little effect on the quality of information derived from principal component analysis. Except for a difference in resolution both physicochemical parameters and community level physiological profiles were suitable for identifying different stages of compost maturity. The data suggested that community level physiological profiles may be a promising tool for evaluation of compost maturity (Gucket *et al.*,1996).

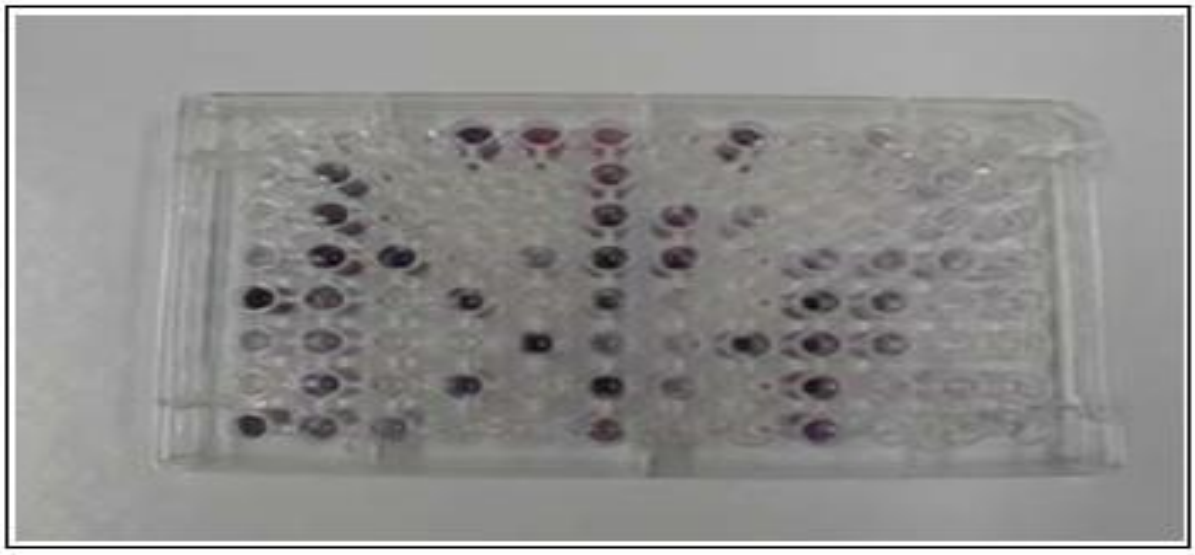
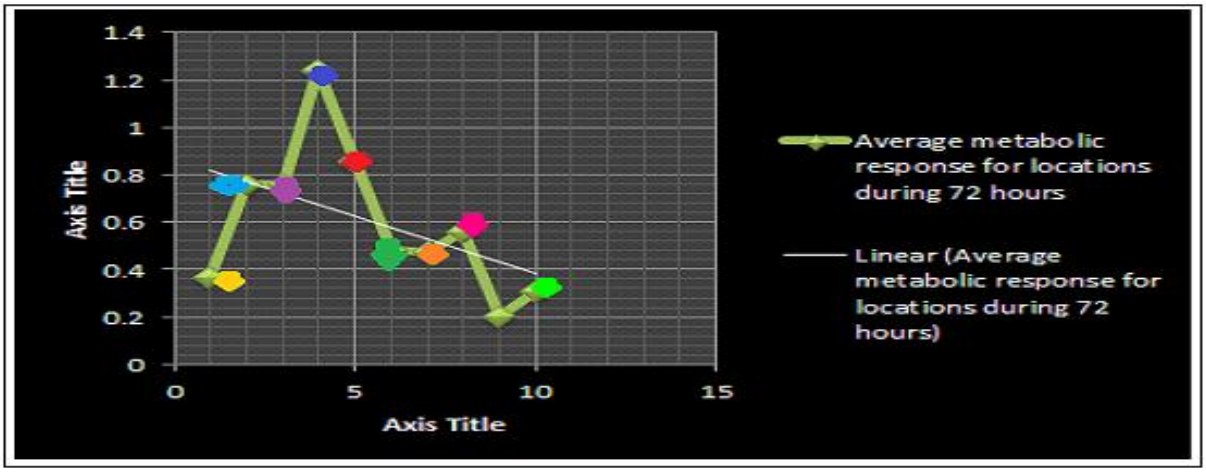


Fig. 3. Example of BIOLOG Eco Plate with changed media on a microtiter plate.

Composting is a self-heating process resulting from microorganism activation, and based on the temperature, it can be divided into four phases: middle-low temperature, high temperature, temperature decline, and stabilization (Min Hwan Oh *et al.*, 2014), but the color change was measured at 595 nm every 24 hours. Uniformity of optical density in all samples with a peak of 1,244 pushed by the sample 5. This kind of metabolic response confirms that the micro-organisms in all simples are modified phenotype population average density 10^6 cells per sample. This phenotype is the result of response micro communities on chemical residues and radicals, as well as emergent substances in water whose presence on the investigated locations are repeatedly proven on earlier research (Radonjić, *et al.*, 2015).



Graf. 2. Average metabolic response for samples analyzed after 72 hours.

Linearity are closest locations 2, 3, 7 and 9 (Graf.2). Their values ranged in that range (OD_0,313-OD_0,757-Table 3.). Minimum metabolic response is given at locations 9 and 1, and this matches with average metabolic response and with the results of the metabolic diversity of microbial communities. The different researchers have studied the rate and extent of color formation in each well can be monitored at 490 nm and recorded by the OMNILOG instrument (Bochner, 2003), a specialized instrumentation provided by Biolog. Kinetic response curves can be generated for each well and used for cellular phenotype comparisons.

Alternatively, color change can be recorded spectrophotometrically (Atanasova and Druzhinina, 2010), or by visual observations (Bochner *et al*., 2001). While the color reaction is most convenient for bacteria, the growth response of filamentous fungi can be recorded as change in the optical density at 750 nm (OD750) (Tanzer *et al*., 2003; Druzhinina *et al*., 2006; Seidl *et al*., 2006). Measurements of growth can also be conducted at 590 nm (Blumenstein *et al*., 2015), which yield results that are comparable to 750 nm. This can be interpreted to mean that, unlike other sites where the phenotypes already repaired, in the samples 1 and 9 micro-organisms are in phase reparation.

Conclusion

Phenotype of micro communities of lipolityc and proteolitic bacteria, on investigation location are product chemical residues and radicals as well. On King's head"-Skadar Like in populatin protolithic bacteria and location Vukovci-River Moraca in lipolytic bacteria the phenotypes is already repaired, but in on the other is in phase reparation.

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