



AREA

"ADVANCING RESEARCH IN AGRICULTURAL AND  
FOOD SCIENCES AT FACULTY OF AGRICULTURE,  
UNIVERSITY OF BELGRADE"



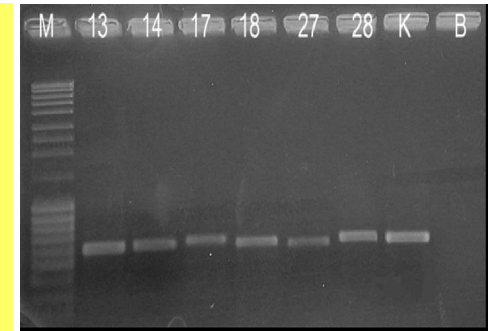
This project has received funding  
from the European Union's  
Seventh Framework Programme for  
research, technological  
development and demonstration  
under grant agreement no 316004

# Molecular Laboratory for Microbial Ecology

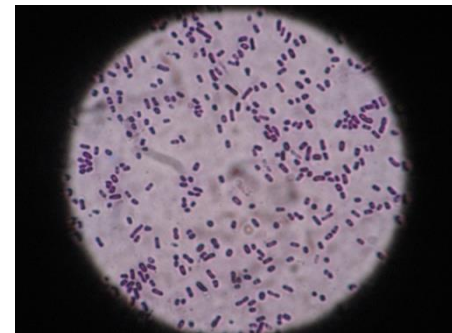
DNA extraction



DNA  
amplification



Quantification of  
PCR products by  
electrophoresis



Purification of  
DNA fragments

Detection of  
target regions in  
from  
environmental  
samples



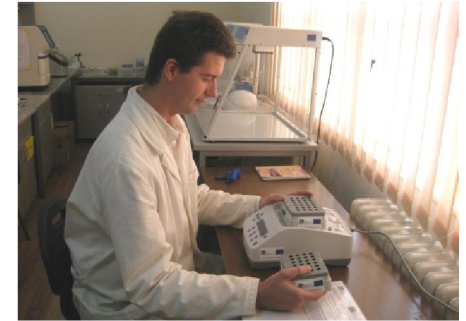
Identification of  
microorganisms  
from  
environmental  
samples



Team leader  
Professor  
Vera Raicevic  
([verar@agrif.bg.ac.rs](mailto:verar@agrif.bg.ac.rs))



Assistant professor  
Blazo Lalevic  
([blazol@agrif.bg.ac.rs](mailto:blazol@agrif.bg.ac.rs))



### Biodiversity of barren soil and bioremediation possibility

Blazo Lalevic<sup>1</sup>, Vera Raicevic<sup>1</sup>, Saud Hamidovic<sup>2</sup>, Zoran Krivoselj<sup>3</sup>, Dragan Kikovic<sup>3</sup>, Josip Colo<sup>2</sup>  
<sup>1</sup>University of Belgrade, Faculty of agriculture, Belgrade, Serbia  
<sup>2</sup>Faculty of agricultural and food sciences, Sarajevo, Bosnia and Herzegovina  
<sup>3</sup>Faculty of natural sciences, Kovelova Mitrovica, Serbia

**Introduction**  
Coal area under barren rock used for steel using electric arc furnace and blast furnace coal combustion acts for 60% of total energy supply. One of the most important areas of research in bioremediation is "Kokari" with annual productivity of brown coal over a whole year. Phytoremediation involves the use of plants to extract, stabilize, or destroy contaminants in soil and water bodies. Areas with mining involve coal a "barrenland" with overabundant material from the past (and more deposited) into the previously mined part. This will lead to the significant soil pollution, as well as to a formation of toxic substances, such as heavy metal ions and organics.  
**Ecological restoration** is one of the methods of recovering degraded or polluted ecosystems. Natural recovery of these environments is a slow process. A bioremediation process accelerates the natural recovery and enhances the biodiversity, as it is important for ecological restoration processes. In these restoration, microbes play important role and their activity can be used as parameter of the quality of barren soil and restoration possibility. Besides, soil microbial activity was connected with different parameters, such as nitrogen. Some steel bodies have developed lichens and broad range of bacterial concentrations. Because the microorganisms and plants are one of the most important parameters of ecological restoration, the present paper assesses the microbial and plant diversity of barren soil in brown coal under field "Kokari" (Bosnia and Herzegovina), in order to determine the restoration potential of selected area.

**Material and methods**  
The present study was carried out in the barren coal mine field "Kokari". During the three years (summer of 2011-2013), plant diversity of barren soil was analyzed using the methodology of Swiss-French phytosociological school and determination of plant species was conducted. Chemical and microbiological characteristics of barren soil were performed in summer of 2013. The basic chemical properties of barren soil were analyzed using the standard methodology, organic pollutants (PAHs and PCBs) by GC/MSD method, while hydrocarbons using the GC/MSD method. Diversity of bacteria and substrate level of barren soil were tested and analyzed using the sequencing method onto selective media. Total number of bacteria, cyanobacteria, bacilli, Clostridia sp., sporogonies, fungi and agonomycetes were determined on different agar media. Hydrolytic and phosphatase activity were also measured. Two most abundant strains were purified and identified by 16S and 18S rDNA sequencing and phylogenetic analyses of 16S rDNA.

**RESULTS**

	CO <sub>2</sub>	CH <sub>4</sub>	CO <sub>2</sub> / CH <sub>4</sub>	Org. C	Total N	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
2011	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02
2012	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02
2013	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Family	Plant species in 2013	Fa	Mn	Cu	Zn	Co	Cr	Ni	Pb	
Amaranthaceae	Amaranthus hybridus Waldst.	3.69	268.0	39.59	78.23	0.73	24.95	84.97	167.80	23.80
Chenopodiaceae	Atriplex oblongifolia Waldst. & Kt.									
Convolvulaceae	Convolvulus arvensis L.									
Gramineae	Briza media L.									
Cyperaceae	Cyperus distachyos (L.) Pers.									
Poaceae	Festuca pratensis Huds.	123.05	7.30	3.96	3.98	332.2	20.8	7.61	2.32	
	Festuca ovina (L.) Link.									
	Vulpia myuros (L.) C. C. Gmel.									
	Polygonum aviculare L.									
	Polygonum lapathinum L.									

Depth (cm)	Total number of bacteria	Ammonification	Acetobacter	Ascomycetes	Fungi	Actinomyces	DNA	PHA	ASA
0-20	3.1	2.3	0.8	1.2	0.0	24.3	4.2	6.52	2.0
20-40	2.3	2.5	0.4	1.3	0.0	16.7	2.3	1.12	0.1

Isolate	API	16S rDNA
13k	Bacillus thuringiensis	Bacillus sp.
18k	Pseudomonas sp.	Bacillus sp.

Acknowledgements: The financial support by project of Ministry of Education and Science of the Republic of Serbia No. TR1080, and EU Commission project AREA, contract No. 318004.

Assistant professor  
Igor Kljujev  
([ikljujev@agrif.bg.ac.rs](mailto:ikljujev@agrif.bg.ac.rs))



Assistant  
Jelena Jovicic Petrovic  
([jelenap@agrif.bg.ac.rs](mailto:jelenap@agrif.bg.ac.rs))

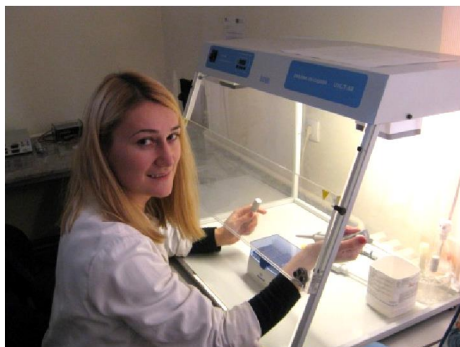
PhD student  
Danka Radic  
([danka.radic@hotmail.com](mailto:danka.radic@hotmail.com))



PhD student  
Jelena Ivkovic  
([petricevicjelena@yahoo.com](mailto:petricevicjelena@yahoo.com))

PhD student  
Ivana Spasojevic  
([ivana.mirovic@gmail.com](mailto:ivana.mirovic@gmail.com))

PhD student  
Bojana Vujovic  
([bojana.burger@gmail.com](mailto:bojana.burger@gmail.com))



PhD student  
Vera Karlicic  
([karlicicvera@gmail.com](mailto:karlicicvera@gmail.com))



Specialist  
Natasa Stosic  
([natasa@agrif.bg.ac.rs](mailto:natasa@agrif.bg.ac.rs))