B) LIST OF PUBLICATIONS FOR PARTICIPATION IN THE COMPAPTION FOR PROFESOR

CRITERIUM B4 - HABILITATION WORK - SCIENTIFIC PUBLICATIONS IN JOURNALS REFERRED AND INDEXED IN WORLD FAMOUS DATABASES WITH SCIENTIFIC INFORMATION
(WEB OF SCIENCE ИЛИ SCOPUS)

B4.1. Galina Radeva, Anelia Kenarova, Velina Bachvarova, Katrin Flemming, Ivan Popov, Dimitar Vassilev, Sonja Selenska-Pobell, 2013. Bacterial diversity at abandoned uranium mining and milling sites in Bulgaria as revealed by 16S rRNA genetic diversity study. Water, Air & Soil Pollution, DOI 10.1007/s11270-013-1748; (2013):224: Article1748. *IF=1.685*, SJR=0.771, Q2

Abstract. Radionuclide and heavy metal contamination influences the composition and diversity of bacterial communities, thus adversely affecting their ecological role in impacted environments. Bacterial communities from uranium and heavy metal-contaminated soil environments and mine waste piles were analyzed using 16S rRNA gene retrieval. A total of 498 clones were selected, and their 16S rDNA amplicons were analyzed by restriction fragment length polymorphism, which suggested a total of 220 different phylotypes. The phylogenetic analysis revealed Proteobacteria, Acidobacteria, and Bacteroidetes as the most common bacterial taxa for the three sites of interest. Around 20–30 % of the 16S rDNA sequences derived from soil environments were identified as Proteobacteria, which increased up to 76 % (mostly Gammaproteobacteria) in bacterial communities inhabiting the mine waste pile. Acidobacteria, known to be common soil inhabitants, dominated in less contaminated environments, while Bacteroidetes were more abundant in highly contaminated environments regardless of the type of substratum (soil or excavated gravel material). Some of the sequences affiliated with Verrucomicrobia, Actinobacteria, Chloroflexi, Planctomycetes, and Candidate division OP10 were site specific. The relationship between the level of contamination and the rate of bacterial diversity was not linear; however, the bacterial diversity was generally higher in soil environments than in the mine waste pile. It was concluded that the diversity of the bacterial communities sampled was influenced by both the degree of uranium and heavy metal contamination and the site-specific conditions.

B4.2. Anelia Kenarova, Galina Radeva, Ivan Traykov, Silvena Boteva, 2014. Community level physiological profiles of bacterial communities inhabiting uranium mining impacted sites. Ecotoxicology and Environmental Safety, DOI 10.1016/j.ecoenv. 2013.11.012; vol. 100, 226-232. *IF=2.762, SJR=1.11, Q1*

Abstract: Bacterial activity and physiological diversity were characterized in mining and milling impacted soils collected from three abandoned uranium mine sites, Senokos, Buhovo and Sliven, using bacterial dehydrogenase activity and Biolog (EcoPlate) tests. The elemental composition of soils revealed high levels of uranium and heavy metals (sum of technogenic coefficients of contamination; TCCsum) pollution as follows: Sliven (uranium – 374 mg/kg; TCCsum – 23.40) >Buhovo (uranium – 139.20 mg/kg; TCCsum – 3.93) >Senokos (uranium – 23.01 mg/kg; TCCsum – 0.86). The physiological profiles of the bacterial community level were site specific, and indicated intensive utilization of polyols, carbohydrates and carboxylic acids in low and medium polluted environments, and i-erithrytol and 2-hydroxy-benzoic acid in the highly polluted environment of Sliven waste pile. Enzymes which take part in the biodegradation of recalcitrant

substances were more resistant to pollution than these from the pathways of the easily degradable carbon sources. The Shannon index indicated that the physiological diversity of bacteria was site specific but not in line with the levels of pollution. A general tendency of increasing the importance of the number of utilizable substrates to bacterial physiological diversity was observed at less polluted sites, whereas in highly polluted sites the evenness of substrate utilization rate was more significant. Dehydrogenase activity was highest in Senokos upper soil layer and positively correlated ($p<0.01$) with the soil organic matter content. The bacterial activity (EcoPlate) and physiological diversity (Shannon index) correlated significantly and negatively with As, Cu, Zn, Pb and U, and Co, Cr, Ni and Mn, respectively. We concluded that the observed site specific shifts in bacterial communities were complex due to both the environmental peculiarities and the bacterial tolerance to the relevant level of pollution, rather than a strong indication of uranium and heavy metals toxicity

B4.3. Silvena Boteva, Galina Radeva, Ivan Traykov, Anelia Kenarova, 2016, Effects of long-term radionuclide and heavy metal contamination on the activity of microbial communities, inhabiting uranium mining impacted soils, Environmental Science and Pollution Research, 23(6), 5644-5653, DOI 10.1007/s11356-015-5788-5. *IF = 2.741, SJR=0.891, Q1*

Abstract: Ore mining and processing have greatly altered ecosystems, often limiting their capacity to provide ecosystem services critical to our survival. The soil environments of two abandoned uranium mines were chosen to analyze the effects of long-term uranium and heavy metal contamination on soil microbial communities using dehydrogenase and phosphatase activities as indicators of metal stress. The levels of soil contamination were low, ranging from 'precaution' to 'moderate', calculated as Nemerow index. Multivariate analyses of enzyme activities revealed the following: (i) spatial pattern of microbial endpoints where the more contaminated soils had higher dehydrogenase and phosphatase activities, (ii) biological grouping of soils depended on both the level of soil contamination and management practice, (iii) significant correlations between both dehydrogenase and alkaline phosphatase activities and soil organic matter and metals (Cd, Co, Cr, and Zn, but not U), and (iv) multiple relationships between the alkaline than the acid phosphatase and the environmental factors. The results showed an evidence of microbial tolerance and adaptation to the soil contamination established during the long-term metal exposure and the key role of soil organic matter in maintaining high microbial enzyme activities and mitigating the metal toxicity. Additionally, the results suggested that the soil microbial communities are able to reduce the metal stress by intensive phosphatase synthesis, benefiting a passive environmental remediation and provision of vital ecosystem services.

B4.4. Michaella Aleksova, Daniel Palov, Nikolai Dinev, Silvena Boteva, Anelia Kenarova, Roumen Dimitrov, Galina Radeva, 2020, Bacterial abundance along a gradient of heavy metal contaminated soils in the region of Zlatitsa-Pirdop valley, Western Bulgaria, Comptes rendus de l'Academie bulgare des Sciences, 73(3), 433-440. *IF=0.378, SJR=0.244, Q2*

Abstract: This study presents the distribution of bacterial abundance in the soil from three sites along the gradient of Cu (53–860 mg kg^{-1}), Zn and Pb, located in the region of Zlatitsa–Pirdop valley, Western Bulgaria. The bacterial abundance was determined by the use of colony forming units and quantitative PCR techniques in five soil samples, collected at 0–20 cm depth. Our results showed that the number of cultivable heterotrophic bacteria and 16S rRNA gene copies decreased in long-term heavy metals contaminated soils. The distribution of 16S rRNA gene copies was mainly affected by the concentration of

heavy metals, as well as by the land use. The principal component analysis was used to visualize the relationships between bacterial abundance, soil physico-chemical properties and sampling sites. The results showed that the less polluted sites were grouped in a single cluster with the highest number of 16S r RNA gene copies, whereas the most polluted site was clustered separately. This study highlights that heavy metal contamination and the land use have significant impact on soil bacteria.

B4.5. Silvena Boteva, Anelia Kenarova, Viktoriya Kancheva, Michaella Aleksova, Roumen Dimitrov, and Galina Radeva, 2021, Long-term heavy metal pollution of soils and its impact on bacterial carbon metabolism, Forestry Ideas, 27(1), issue 21. *SJR-0.171, Q4*

Abstract: Heavy metal pollution of soils may change their chemical and microbiological status. Changes in the function of the decomposer communities may cause disruption in soil nutrient cycling and primary productivity of an ecosystem. In the present study, bacterial capacity to utilize different carbon substrates under heavy metal stress was evaluated by using community level physiological profiling technique and Biolog Ecoplate^{TM} method. Soil samples were taken from the vicinity of mine Chelopech along a Cu gradient and co-pollutants – Zn and Pb. Soil texture was classified as loam, soil pH was defined as acidic, and soils were determined as well nutrient abundant. Soil concentrations of Cu, Zn and Pb varied in the range of 51–860 mg kg^{-1}, 44–180 mg kg^{-1} and 31–175 mg kg^{-1}, respectively. Both, the capacity of impacted bacterial communities to utilize organic carbon substances and bacterial functional diversity decreased under the heavy metal stress. Bacteria from un-polluted soils preferentially utilized carbohydrates and polymers, whereas the heavy metal stressed bacterial communities preferentially used proteinogenic and non-proteinogenic carboxylic acids. The highest levels of adverse impacts were recorded both at seriously polluted soil, and on the utilization of amines and carbohydrates. Local variability of soil properties might modify the effects of heavy metals. It can be concluded that the EcoPlate^{TM} method can be used to evaluate the community functional variability in relation to different levels of heavy metal stress, as statistically significant results have been obtained.

CRITERIUM G7 - SCIENTIFIC PUBLICATIONS IN JOURNALS REFERRED AND INDEXED IN WORLD FAMOUS DATABASES WITH SCIENTIFIC INFORMATION (WEB OF SCIENCE или SCOPUS), NOT CONSIDERED TO HABILITATION WORK

G7.1. Boteva S., A. Kenarova, G. Radeva, I. Traykov, V. Bogoev, 2011, Community dynamics of pelagic bacteria in a high mountain Lake Sulzata – Rila Mountain, Bulgaria. Biotech and Biotech Eq., v.25, №4, 2620-2626. IF=0.760, SJR=0.205, Q3

Abstract: This study analyzed the temporal dynamics in the abundance and composition of pelagic bacterial communities inhabiting Sulzata Lake (2 500 m a.s.l.), a lake of glacial origin situated in Rila National Park (Rila Mountain, Bulgaria). Epifluorescence microscopic counting and amplified rDNA restriction analysis (ARDRA) were used to monitor the succession of planktonic bacterial communities. Sulzata Lake was well abundant in bacteria (on average (69.13±81.1) X 10^5 cells ml^{-1}), as their number fluctuated drastically depending on the values of environmental determinants. ARDRA analysis yielded high bacterial diversity resulting in different band patterns. The observed changes in ARDRA profiles were a consequence of strongly fluctuating population sizes under the fluctuation of environmental

determinants causing changes in the rate of dominance, hence in the bacterial diversity (H': 0.69–2.07) and low levels of genetic similarity (0-65%) between the pelagic bacterial communities. The diversity of different ecotypes increases adaptation fitness which guarantees stability and drives the evolution of an ecosystem under extreme and extremely variable conditions.

G7.2. Anelia Kenarova, Marta Encheva, Valentina Chipeva, Nesho Chipev, Petya Hristova, Penka Moncheva, 2013. Physiological diversity of bacterial communities from different soil locations on Livingston Island, South Shetland Archipelago, Antarctica, Polar Biology, DOI 10.1007/s00300-012-1254-8, 36(2), 223-233, vol. 36 (2), 223-233. *IF=2.071, SJR=1.116, Q1*

Abstract: Terrestrial food webs of Antarctica are simple and dominated by microorganisms. Soil bacteria play an important role in nutrient cycling, yet little is known about their capacity to utilize differentcarbon sources and to participate in site nutrient turnover. BiologEcoPlateTM was applied to studythe catabolic activity and physiological diversity of bacteria inhabiting the soil of moss, vascular plants, and fell field habitats from Livingston Island, Antarctica. Additionally, the number of oligotrophic and copiotrophic bacteria was counted by the agar plate method. Results indicated a lack of site-specific distribution of bacterial abundance, in contrast to bacterial catabolic activity and community level physiological profiles. Community level physiological profiles revealed a common capacity of soil bacteria to intensively utilize polyols, which are cryoprotectants widely produced by Antarctic organisms, as well as site-specific phenolic compounds (vegetated habitats),amino acids/amines (moss habitats), carbohydrates and carboxylic acids (fell field habitat). It wasconcluded that the physiology of soil bacteria is habitat specific concerning both the rate of catabolic activity and pattern of carbon source utilization.

G7.3. **Encheva M, Zaharieva N., Kenarova A., Chipev N., Chipeva V., Hristova P., Ivanova I., Moncheva P., 2013, Abundance and activity of soil actinomycetes from Livingston Island, Antarctica. Bulgarian Journal of Agricultural Science, 19(2), 68-71. *IF=0.136*, SJR=0.162, Q3**

Abstract: The soils of Antarctica are severe environments inhabited by well adapted microorganisms, the knowledge of which is scarce. The study on their abundance, diversity and physiology will providethe investigators with new data on the mechanisms of their adaptation and gives new opportunitiesto isolate microorganisms with unique properties for practical use. The aim of the present work was to study bacterial abundance including actinomycetes and physiological diversity of Antarcticsoils, by agar plate method and BIOLOG EcoPlates technique, respectively, and based on this actinomycete strains to be isolated, identified and screened for antimicrobial activity against different test bacteria. The objectives of our investigation were seven Antarctic soils taken from moss, Deschampsia vegetated and fell field habitats of Livingston Island. Some of the soil physicochemical parameters, like pH, moisture and humus, were determined. Bacterial physiological activity and diversity were assessed based on the average color development of EcoPlate wells, and community capacity to utilize different chemical categories of carbon sources,respectively. The results showed that highest physiological diversity and more intensive utilization of polyols, amino acids and phenolic compounds, and high abundance of actinomycetes characterized soil bacteria from habitats with cryptogam (moss) coverage. Most morphologicallydifferent actinomycete strains were isolated from the moss habitat denoted in the study as S6. Theaffiliation of the strains to genus *Streptomyc*es was proved by the PCR amplification of 16S rDNA,using genus-specific primers. The screening of antimicrobial activity of the isolates by diffusion bioassay, using agar plugs showed that the strains synthesized antibacterial substances, active against both Gram-negative and Gram-positive bacteria. We conclude that the soils of moss habitats are favorable environments supporting high bacterial physiological

activity, and high number and diversity of actinomycetes most of which are active producers of antibacterial metabolites.

G7.4. Silvena Boteva, Anelia Kenarova, Galina Radeva, Ivan Traykov, Valentin Bogoev, 2013. Community dynamics of pelagic bacteria in the high mountain lake Bubreka – Rila Mountain, Bulgaria. Comptes rendus de l'Academie bulgare des Sciences, vol. 66 (11); 1579-1586. *IF=0.198, SJR=0.205,* **Q2**

Abstract: This study analyzed the temporal dynamics in the abundance and composition of pelagic bacterial communities inhabiting Bubreka Lake (2282 m a.s.l.), a lake of glacial origin situated in Rila National Park (Rila Mountain, Bulgaria). Epifluorescence microscopic counting and amplified rDNA restriction analysis(ARDRA) were used to monitor the succession of planktonic bacterial communities. Average bacterial abundance was $40.9\pm40.8\times10^8$cells l^{-1}, as their number fluctuated dramatically depending on the values of environmental de-terminants. ARDRA analysis yielded time specific bacterial genetic patterns resulting in low number of bands which were unique for each sampling time. In general, bacterial diversity was very low (H': 1.12–1.93) decreasing from July to September.

G7.5. **Galina Radeva, Anelia Kenarova, Velina Bachvarova, Katrin Flemming, Ivan Popov, Dimitar Vassilev and Sonja Selenska-Pobell, 2014. Phylogenetic diversity of archaea and archaeal ammonia monooxygenase gene in uranium mining impacted locations in Bulgaria, Archaea, vol. 2014, Article ID 196140, 10 pages, 2014. doi:10.1155/2014/196140.** *IF=2.709, SJR=1.23,* **Q1**

Abstract: Uranium mining and milling activities adversely affect the microbial populations of impacted sites. The negative effects of uranium on soil bacteria and fungi are well studied, but little is known about the effects of radionuclides and heavy metals on archaea. The composition and diversity of archaeal communities inhabiting the waste pile of the Sliven uranium mine and the soil of the Buhovo uranium mine were investigated using 16S rRNA gene retrieval. A total of 355 archaeal clones were selected, and their 16S rDNA inserts were analysed by restriction fragment length polymorphism (RFLP) discriminating 14 different RFLP types. All evaluated archaeal 16S rRNA gene sequences belong to the 1.1b/Nitrososphaera cluster of Crenarchaeota. The composition of the archaeal community is distinct for each site of interest and dependent on environmental characteristics, including pollution levels. Since the members of 1.1b/Nitrososphaera cluster have been implicated in the nitrogen cycle, the archaeal communities from these sites were probed for the presence of the ammonia monooxygenase gene (amoA). Our data indicate that amoA gene sequences are distributed in a similar manner as in Crenarchaeota, suggesting that archaeal nitrification processes in uranium mining-impacted locations are under the control of the same key factors controlling archaeal diversity.

G7.6. Boteva S. and A. Kenarova, 2017, Oil contamination of sandy and loamy alluvial soils and its impact on indigenous bacteria, Comptes rendus de la'Academie bulgare des Sciences, v. 70 (6), 803-812. *IF=0.251, SJR=0.21, Q2*

Abstract: The aim of the study was to analyse the effects of oil contamination on bacterial communities in soil. In a microcosm experiment, alluvial soils with different textures (sandy and loamy) and crude oil with different specific densities were used. The experiment was based on the enumeration of bacterial heterotrophic populations and oil degraders through the use of conventional plating techniques. Additionally, the dehydrogenase activity of soil bacteria was followed throughout the experiment which lasted seven months. Soil amendment (5%) with crude oil caused slight increase in the total number of heterotrophic bacteria, especially in sandy soils. More evident were the stimulation effects of the contaminant on oil degraders and dehydrogenase activity - more than 2000 times increase in the number of oil degraders and more than 35 times increase in dehydrogenase activity. The in-time patterns of changes in bacterial parameters were specific depending on soil texture (heterotrophic bacteria and dehydrogenase activity) and oil density (oil degraders). The rate of oil degradation correlated significantly with the number of oil degraders, manifesting the shifts in soil bacterial communities under the power of oil inputs. Results obtained in the present study indicated that soil bacteria were able to adapt to the crude oil in concentration of 5% and to take part in the natural bioremediation and recovery of soil.

G7.7. Silvena Boteva, Anelia Kenarova, 2017, Impact of methane concentration and temperature on the activity of a methanotrophic strain isolated from a municipal landfill, Comptes rendus de l'Academie bulgare des Sciences, Vol 70, No9, pp.1271-1278. *IF=0.251, SJR=0.21, Q2*

Abstract: The aim of the study was to analyse the effects of methane concentration and temperature on methanotrophic bacteria MM1, isolated from municipal landfill soil cover. In a microcosm experiment, series of glass bottles, inoculated with cell suspension of MM1, were cultured under sterile methane-air atmosphere for four days varying methane (substrate) concentration and environmental temperature. The effects of increasing CH_4 concentration and temperature on the rate of CH_4 oxidation were evaluated by the accumulation of formaldehyde per unit of bacterial biomass. The highest rate of CH_4 oxidation was measured for the microcosm having headspace gas saturation of 30% CH_4. The MM1 showed high CH_4 oxidation rates in the temperature range from 15°C to 45°C, and very low at 5°C. The growth rate increased with increasing methane concentrations and reached its maximum at 30% CH4. The lowest bacterial growth rate was measured at 5°C ($\mu = 0.036\ h^{-1}$, corresponding to 8 h 22 min). When rising the temperature with 10°C the bacterial growth increased up to $\mu = 0.094\ h^{-1}$, and further rise in the temperature did not have significant effect on μ. Results indicated that the studied methanotrophic bacterial strain was able to oxidize CH_4 under wide range of methane concentrations and temperatures. These properties determine the capacity of MM1 as a potential agent for bioremediation programmes applying bacteria into landfill soil cover in order to reduce CH_4 emissions.

G7.8. **Anelia Kenarova, Rossen Tzonev, Silvena Boteva, Valentin Bogoev, Marian Nikolov, Kalina Pachedjieva, Ivan Traykov, Daniela Simeonovska-Nikolova, Krastio Dimitrov, Vladimir Stefanov, Hristina Bakardjieva, Tsveta Dimitrova and Georgi Nachev, 2017, The Framework of the maintenance ecosystem services provided by agroecosystems on the territory of Bulgaria, World Multidisciplinary Earth Sciences Symposium (WMESS 2017), IOP Conference Series: Earth and Environmental Science 95 042011 doi:10.1088/1755-1315/95/4/042011. *SJR=0.149***

Abstract: Over the past decade, efforts to value and protect ecosystem services have been promoted by many as the last, best hope for making conservation mainstream. Here, we present the results from the evaluation of the maintence ecosystem services, provided by the agroecosystems in Bulgaria. The evaluation was conducted on a range of national, european and international database following the methodology of MAES and classification system of CICES adapted by the Bulgarian Ministry of Environment and Water. During the study, 213857 agroecosystems were evaluated reffering to their capacity to maintain pollinators, natural pest control, soil quality and atmospheric carbon sequestration. The capacity of agroecosystems to provide the above mentioned ecosystem services was ranged from moderate to very high, tending the mountainous agroecosystems to be more beneficial than that located in the plains and lowlands. The intensive management of the latter in order to achive perceived efficiencies in the production of agriculture goods reduces their importance for local and regional ecological processes.

G7.9. Michaella Alexova, Anelia Kenarova, Silvena Boteva, Galina Radeva, 2019, Azoxystrobin impact on a selection of soil bacterial resistance to aminoglycoside antibiotics, Comptes rendus de l'Academie bulgare des Sciences, 72(10), 1359-1365. *IF=0.343, SJR=0.218, Q2*

Abstract: Fungicides have application in agriculture and still effectively eliminate fungal pathogens of crops. However, fungicides may dissipate to various elements of the environment, such as a soil, and may exert a selective pressure on soil microorganisms, leading to an increase in the prevalence of resistant to antibiotics forms. Considering this problem, the aim of this study was to investigate the power of fungicide azoxystrobin (Az) to select resistant to aminoglycosides (streptomycin, kanamycin and gentamycin) soil bacterial communities. The investigation was performed in soil mesocosms (loamy sand (LS) and clay loam (CL) soils) contaminated with increasing Az doses (0.28–28.93 mg/kg). The selective power of fungicide was evaluated by short-term growing test of soil bacteria in the presence of single aminoglycoside antibiotic (AmGA), and calculating the effective dose, which reduces bacterial growth by 50% (EC50). The results demonstrated Az selection of resistant to AmGAs bacteria even at the lowest (field recommended) fungicide dose. The rate of antibiotic resistance selection was dependent on soil properties, Az dose and time of exposure. The selective power of Az was more effective in LSs than CLs, where the stimulated antibiotic resistance to streptomycin was four times higher than the background one. The results of the study demonstrated that application of Az for crop protection might be a possible route for antibiotic resistance transmission to humans.

G7.10. Michaella Aleksova, Anelia Kenarova, Silvena Boteva, Galina Radeva, 2019, Induced bacterial antibiotic resistance under the application of fungicide azoxystrobin, Comptes rendus de l'Academie bulgare des Sciences, 72(11), 1592-1598. *IF=0.343, SJR=0.218, Q2*

Abstract: In the present study, azoxystrobin was used as a model fungicide to elucidate the capacity of fungicides to induce antibiotic resistance and contribute to the diversification of the resistome expansion routes. The effects of azoxystrobin on soil bacterial resistance to streptomycin, tetracycline, ampicillin and chloramphenicol were tested in loamy sand and clay loam soils, contaminated with increasing fungicide doses within 90 days long-term mesocosm experiment. The levels of azoxystrobin impact were evaluated calculating the effective dose, which inhibits bacterial growth by 50% on the first day and every month later after azoxystrobin application. The results revealed that azoxystrobin stimulated selection of antibiotic resistance in both soil types, and it was established even at the lowest applied dose (0.28 mg kg^{-1}). The mean rate of increase of antibiotic resistance was 2.0- (clay loam soil) and 4.3- (loamy sand soil)

fold than the background. Soil properties and exposure time, but not the applied azoxystrobin doses, were differentiated as major factors modelling the manifestation of antibiotic resistance. A trend of recovery of bacterial antibiotic susceptibility was detected after 90 days of azoxystrobin application in clay loam soil, but not in loamy sand soil. This study highlights the fungicide effects on soil resistome establishment in a context of applied dose, soil properties and exposure time.

G7.11. Silvena Boteva, Rossen Tzonev, Anelia Kenarova, Valentin Bogoev, 2020, Agriculture landscape development and its subsequent impact in terms of common agricultural policy - the case of Southwest planning region in Bulgaria, Bulgarian Journal of Agricultural Science, *26 (6),* **1209-1216.** *SJR =0.191, Q3*

Abstract: The purpose of the current study is to analyze the impact of Common Agricultural Policy (CAP) implementation in Southwestern planning region (SWPR) in the Republic of Bulgaria (BG). The analysis showed two opposite tendencies: decreasing of agricultural holdings number and increasing of utilized agricultural area (UAA) per holding, which is a clear indicator of land consolidation and UAA concentration in fewer agricultural holdings. Although results indicated delay in this trend for Blagoevgrad District, this is a prerequisite for an extensive livestock production which allows the conservation and maintenance of pastures and meadows, hence improving the provided ecosystem services and supporting biodiversity conservation.

G7.12. Boteva, S.B., Kenarova, A.E., Georgieva, S.S., Chanev, C.D., Aleksova, M.R., Radeva, G.S. 2020. The resistance and resilience of soil enzymes after the application of fungicide azoxystrobin to loamy sand soil. Ecologia Balkanica, 12 (3), pp. 185-194. *SJR-0.134, Q4*

Abstract: The use of fungicides in crop protection effectively eliminates fungal pathogens of plants. However, they may cause changes in soil microorganisms concerning microbial ability to mediate soil functions. The aim of the study was to evaluate the changes in soil environment, and soil enzyme resistance and resilience (beta-glucosidase, urease, acid and alkaline phosphatases and arylsulphatase) in a response to the increasing concentrations of azoxystrobin (Az), applied under the trade form Quadris[R]. A laboratory study was carried out for 120 days on soil mesocosms, amended with Az in concentrations from 0.00 mg kg^{-1} to 35.00 mg kg^{-1}. Az soil amendment caused changes in soil physico-chemical properties and microbial activity. Microbial responses immediately (day 1) after Az application, showed that more resistant to the fungicide were urease, beta-glucosidase and arylsulphatase in the opposite to the acid phosphatase, which demonstrated high sensitivity to the chemical stress. One month later, the resistance of beta-glucosidase, urease and acid phosphatase decreased even more compared to day 1, the resistance of alkaline phosphatase remained unchangeable, whereas the resistance of arylsulphatase slightly increased. The calculated resilience on day 120 manifested that enzymes were not able to recover within four months after fungicide application to soils. Pearson correlation analysis demonstrated significant linear relationships between Az soil residues and enzyme resistance/resilience. Our results highlighted that the application of QuadrisR altered soil enzyme system for more than four months, which might reflect the speed of organic matter turnover in soil, especially that of organophosphates.

G7.13. Palov, D.D., Aleksova, M.R., Nikolova, R.N., Dinev, N.S., Kenarova, A.E., Boteva, S.B., Dimitrov, R.A., Radeva, G.S. 2020. Relationships between soil microbial activity,

bacterial diversity and abiotic factors along the heavy metal contamination gradient. Ecologia Balkanica, 12 (3), pp. 31-39. *SJR- 0.134, Q4*

Abstract: In this study, the relationships between soil abiotic factors, heavy metals content and soil microbial activity, bacterial abundance, bacterial genotype richness and diversity were analysed in three sites along a Cu gradient (from 53 to 860 mg kg $^{-1}$) and co-contaminants Zn and Pb, located in the region of Zlatitsa-Pirdop valley, Western Bulgaria. Long-term heavy metal contamination had a significant negative effect on soil microbial activity and our results showed that the dehydrogenase activity (DHA) decreased along the contamination gradient with up to 79% compared to the uncontaminated sample. The principal component analysis (PCA) showed that DHA correlated significantly and positively with total bacterial abundance (16S rRNA gene copies) and nitrate ions (NO_3), and negatively with soil pH, heavy metals and their bioavailable forms. Bacterial genotype diversity was mainly influenced by abiotic factors such as soil organic matter and sand fraction of the studied sites.

G7.14. Aleksova, M., Kenarova, A., Boteva, S., Georgieva S., Chanev Ch., Radeva G. 2021. Effects of increasing concentrations of fungicide Quadris^R on bacterial functional profiling in loamy sand soil. Archives of Microbiology, 203(7), 4385-4396. *IF=1.884, SJR=0.648, Q3*

Abstract: A mesocosm experiment was conducted to assess the side effects of the fungicide Quadris^R on soil bacterial functioning. Quadris^R was applied to a loamy sand soil at increasing concentrations (0.0–35.0 mg kg^{-1} dry soil) calculated according to its active ingredient azoxystrobin (Az). Soil sampling was carried out from the 1st to the 120th day of soil incubation to determine the changes occurred in bacterial catabolism using the technique of community-level physiological profiling (CLPP) via Biolog EcoPlates™. It was found that the field recommended fungicide concentration (2.90 mg kg^{-1} dry soil) altered mostly the low-available Biolog carbon sources (< 0.50 optical density (OD)), whereas the fungicide higher concentrations (14.65 and 35.00 mg kg^{-1} dry soil) were effective also on medium (0.50–1.00 OD) and highly (> 1.00 OD) utilizable ones. Pearson correlation analysis revealed that the main environmental factors correlated with the utilization rates of Biolog carbon sources (CSs) were soil nutrients and pH. No linear relationships were found between Az soil residues and the use of CSs. We concluded that Quadris^R affects bacterial catabolic profiles in loamy sand soils through soil acidification and altering soil nutrient pool. The study also revealed that CLPP and EcoPlate™ are useful practical tools for testing the fungicide ecotoxicity.

G7.15. Anelia Kenarova, Silvena Boteva, 2023. Fungicides in agriculture and their side effects on soil enzyme activity: A review, Bulgarian Journal of Agricultural Science, vol. 1, in press, *SJR=0.248 (2020), Q3*

Abstract: A wide variety of fungicides are globally applied for effective elimination of fungal pathogens in agriculture. The constant increase in their production and use in the years rises a concern about the environmental effects that they can cause. More attention is paid on fungicides such as mancozeb, azoxystrobin, chlorotalonil, carbendazim, tebuconazole and captan due to their widespread application. The studies are focused on the analysis of parameters that could be rapid, sensitive and informative for the fungicides' impact on living organisms. Such parameter is the activity of soil microbial enzymes since their function is responsible for the soil health and fertility. Studies show that dehydrogenase, phosphatase and urease are the most commonly used enzymes due to their role in key metabolic processes, while invertase, β-glucosidase and cellulase were analyzed to a lesser extent. Most of the

fungicides are reported to reduce the soil enzymes' activity while others manifest positive or controversial effects which is determined not only by the fungicide chemical composition but also by its dose, exposure time, and/or soil properties. The aim of the review is to summarize the results and outline the trends of fungicide impacts on soil enzymes that take part in the soil nutrient cycling.

PUBLICATIONS WITHOUT Q и SJR, WHICH ARE NOT INCLUDED IN THE PUBLICATION LIST FOR LAW CRITERIA FULLFILLING, BEING INCLUDED IN THE SCIENTIFIC CONTRIBUTION DISSCUSSION.

G0.1. **Stoyanova T. L., Traykov I T., Kenarova A. E., Bogoev V. M., Yaneva I.Y., 2012, The impact of abandoned uranium mine Senokos on Luda River, Pirin Mountain, Bulgaria, The Fifth International Scientific Conference BALWOIS 2012, Ohrid, Republic of Macedonia, 28th May - 2nd June 2012**

Abstract: The pollution of river ecosystems from abandoned uranium mines is a major environmental problem in many parts of the world. Uranium mining impacts the local environment and the pollution may persist for many years after the site is abandoned. In Bulgaria, uranium mining and the processing of the ore ceased at the beginning of the 1990s. This study investigates the impact of abandoned uranium mine Senokos on Luda River (north-western Pirin Mountain, Bulgaria). Water and sediment samples were collected in October 2009 and in July 2010 and were analyzed for heavy metals (Cr, Cu, Pb, Fe, Zn,Ni, Mn) and radionuclides (natural uranium, total α- and β-activity). Bacterial dehydrogenase activity and their community level physiological profiles were used as biological indicators. Most of the heavy metals, natural uranium, total α-activity, total β-activity in the water and sediments were above the concentration at the reference site. In the sediments, the highest contents of natural uranium, total α- activity and total β-activity were measured at site two, located 1km below the mine. Sediment bacteria under the mine respond to its impact in terms of several times reduction in absolute number, dehydrogenase activity and capacity to utilize different carbon sources (community level physiological profile), no matter the season and water discharge. Downstream, the properties of sediment bacterial communities recover partly, and the rate of recovery is seasonally dependent. The benthic communities at the reference site and the site below the mine are characterised with high values of Margalefs' diversity index. A slight change in the environmental conditions determined the low values of this index at site three, located under the Rakitna Village.

G0.2. **Boteva S, Bankova E, Kenarova A, Tishkov S, Traykov I, 2015, Bacterial abundance and activity in oil polluted and restored artificial wetlands, Annuaire de l'Université de Sofia "St. Kliment Ohridski", Faculte de Biologie, 100(4), 332-343**

Abstract: The oil industry has a great environmental risk throughout ship accidents and the impact of waste generated during the oil refining or production of petrochemicals andtheir derivatives. The waste waters of petrochemical industry commonly contain gross amounts of oil and suspended solids. After the purification processes, in some cases the wastewaters are discharged into artificial wetlands for sedimentation and biodegradationof refractory petrochemicals and

organic matter. This paper studied the dynamics of heterotrophic and oil-degrading bacteria in a polluted and a restored artificial wetland of petrochemical plant concerning the local specificity of wetlands and the seasonal changes. The oil polluted wetland was characterized both with higher water and lower sediment abundances of heterotrophs comparing to that of restored wetland. The number of oil-degrading bacteria was relatively similar in the two environments, except that in the sediments of restored wetland, which exceeded up to several times the number of oil-degrading bacteria elsewhere. The share of oil-degrading bacteria in the community of heterotrophs was higher (0.63% vs. 0.04%) in the water column and in the sediments (3.01% vs. 0.009%) of the restored wetland compared to the polluted one. The total activity of water and sediment heterotrophic bacteria was not significantly different between the wetlands with average value ranging from 4.5±2.0 µg O l^{-1} to 5.3±1.3 µg O l^{-1}. In a contrast, the relative bacterial activity, calculated per cell of heterotrophs, differed significantly between the water of polluted vs. restored wetland (81.8±182 x 10^{-4} µg O l^{-1} vs. 30.3±34.4 x 10^{-4} µg Ol^{-1}), and between their sediments (0.07±0.1 x 10^{-4} µg O l^{-1} vs. 0.04±0.05 x 10^{-4} µg Ol^{-1}). The ANOVA analysis indicated a significant contribution of water temperature in the variance of heterotrophs' abundance and activity, and water temperature and wetland local conditions, both contributing in the variance of oil-degrading bacteria abundance.

CRITERIUM G8 – PUBLISHED BOOK CHAPTER OR BOOK

G8.1. Kenarova A and S. Boteva, 2015, Chapter 13 - Functional Diversity of Microorganisms in Heavy Metal Polluted Soils, In: (Sherameti I and Varma A, eds) Heavy Metal Contamination of Soils: Monitoring and Remediation", series "Soil Biology", Springer, v. 44, pp 245-257, ISBN: 978-3-319-14526-6

Abstract: In this chapter, we summarize the results from studies designed to assess the impacts of heavy metal pollution on the physiology of soil microorganisms based on a variety of commercially available assays (Biolog and MicroResp) of community substrate use. The results and conclusions from these studies are contradictory, depending on the metal concentrations and speciation, local environmental characteristics, and finally the different interpretations by the authors of the actual levels of pollution. In general, low and moderate levels (according to the Nemerow index) of metal pollution do not affect carbon use ability and functional diversity of the impacted microbial communities, as opposed to high metal pollution levels where significant adverse effects are recorded as functional responses of microbial communities to metal stress. Microbial functional responses to metal stress were observed as reduced catabolic activity and functional diversity, preferential community shifts from one carbon substrate use to another, and/or increased pollution-induced community tolerance. Finally, the microbial responses are summarized in the context of the modifying effects of the local environment on metal toxicity.

G8.2. Moncheva P., Chipeva V., Encheva-Malinova M., Kenarova A., 2015, Soil Microbial Life of Livingston Island, Antarctica. In: Ch. Pimpirev and N Chipev (eds) Bulgarian Antarctic Research: A Synthesis, St Kl. Ohridski Univ Press, ISBN 978-954-07-3939-7, pp.274 – 296

Introduction: The terrestrial environments of Antarctica are dynamic and variable with low temperatures, low moisture availability, frequent freeze-thaw cycles, scarce vegetation cover, and limited organic matter. They exhibit low complexity food web structures which are dominated by microorganisms. Most of the

energy and materials assimilated by primary production become detritus because of the absence of herbivores, and soil bacteria play a crucial role in its turnover. To enable themselves to survive and grow in cold environments, bacteria have developed a complex range of changes to all of their cellular components, including their membranes, energy-generating systems, protein synthesis machinery, biodegradative enzymes, and the components responsible for nutrient uptake. All the changes can be described in terms of adaptive alterations in the protein and lipids of the bacterial cell.

Livingston Island is a part of the South Shetland Islands, Western Antarctica lying between Greenwich Island and Snow Islands, known for the scientific expeditions that are held there. Livingston Island, like the whole Antarctica, is a relatively less studied area which provokes the interest of scientists because of the possibility new species of microorganisms to be discovered having biosynthetic potential for valuable metabolites.