

**РЕЗЮМЕТА НА ПУБЛИКАЦИИТЕ НА ДОЦ. Д-Р ГАЛИНА РАДЕВА, с които  
участва в обявения конкурс за АД „Професор“ на английски език**

*Публикациите са подредени и номерирани (B4 или Г7, Г8) както са представени в обобщената таблица за изпълнение на минималните национални изисквания за заемане на академична длъжност „Професор“*

**B4\_1 Radeva G.**, Kenarova A., Bachvarova V., Flemming K., Popov I., Vassilev D., Selenska-Pobell S. 2013. Bacterial Diversity at Abandoned Uranium Mining and Milling Sites in Bulgaria as Revealed by 16S rRNA Genetic Diversity Study. *Water, Air & Soil Pollution*, 224, (11), p. 1748. <https://link.springer.com/article/10.1007/s11270-013-1748-1>, IF= 3.0, Q1  
<https://www.scimagojr.com/journalsearch.php?q=24554&tip=sid&clean=0>

**Abstract** Radionuclide and heavy metal contamination influence 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 Radeva G\*.**, Kenarova A., Bachvarova V., Flemming K., Popov I., Vassilev D., S. Selenska-Pobell S. 2014. Phylogenetic diversity of Archaea and the archaeal ammonia monooxygenase gene in uranium mining-impacted locations in Bulgaria. *Archaea*, vol. 2014, Article ID 196140 doi: 10.1155/2014/196140. IF= 2.709, 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.

**B4\_3** Nikolova R., Gatev, E., Kenarova A., Petkova, M., Dinev N, Baldrian, P., **Radeva, G\***. 2025. Selective Pressure of Heavy Metals on Soil Microbial Taxa Near a Smelting Area, *Toxics*, 13(12), 1025. <https://doi.org/10.3390/toxics13121025> IF=4.1, Q1.

**Abstract** Soil pollution by heavy metals (HMs) poses a major threat to soil quality and human health, with mining and smelting industries identified as key sources. Soils around smelters are often considered polluted hotspots, being generally unsuitable for agricultural activities. Although many studies have identified microbial taxa able to survive in such environments, most have focused on relatively low HM concentrations. The purpose of the study was to assess the ecological risk and to evaluate the diversity and structural shifts in microbial communities, as well as to predict key metabolic pathways associated with HM resistance in soils near Pb–Zn smelter in Bulgaria. The soils ranged from low-risk to disastrous, with cadmium (Cd) identified as the primary contributor to soil toxicity. High-throughput sequencing of 16S rRNA and ITS amplicons revealed widespread dominance of the phyla Proteobacteria, Actinobacteriota and Acidobacteriota, and Ascomycota, with the prevailing classes Acidobacteriae, Chloroflexia, and Eurotiomycetes, indicating their high tolerance to HMs. Functional predictions suggested enrichment of key pathways in the most polluted soils related to HM resistance, including efflux systems and detoxifying enzymes. These results highlight the necessity of integrating soil microbial indicators into agricultural management strategies to ensure safe food production.

**B4\_4** Nikolova, R., Boteva, S., Kenarova, A., Dinev, N., **Radeva, G\***. 2023. Enzyme activities in soils under heavy metal pollution: a case study from the surroundings of a non-ferrous metal plant in Bulgaria. *Biotechnology & Biotechnological Equipment*, 37 (1), doi.org/10.1080/131028the18.2022.2149348, IF=1.762, Q3.

**Abstract** Soil enzyme activities are potentially valuable indicators of soil health in cases of heavy metal and metalloid (HM) pollution. However, the extent to which HM pollution affects specific enzyme activities remains unclear. In this study we assessed the level of HM pollution and its effects on soil enzyme activities to select the most reliable biochemical indicators of soil health under HM stress. The initial hypothesis was that enzyme responses would be site-specific,

depending not only on the level of pollution but also on soil abiotic and biotic properties. The Nemerow pollution index (NPI) and the enzyme activity index (EAI) were calculated. EAIs were based on the activities of dehydrogenase (Dha),  $\beta$ -glucosidase (BGI), acid (AcP) and alkaline (AIP) phosphatases, arylsulphatase (Ars) and urease (Ur). NPI indicated slight (1.0) to serious (67.46) soil pollution. The EAI varied from 4.50 to 11.54. The overall functional dissimilarity between soils was around 26%. Cluster and SIMPER analyses both indicated that the activities of Dha, BGI and AIP grouped together with Cu, Zn and Pb, and accounted for 85% of the between-soil functional dissimilarity. The results indicated that there was enzyme functional redundancy between soils. Soil enzymes showed a relatively high capacity to tolerate long-term HM pollution. Dha, BGI and AIP were highly sensitive to environmental fluctuations, including HM concentrations. The long-term HM stress on soil enzyme activities calls for further studies of the soil properties and the time scale involved in the evolution of metal tolerant enzyme producers in HM-impacted soils.

**B4\_5** Aleksova M., Palov D., Dinev N, Boteva, S., Kenarova, A., Dimitrov R., **Radeva G\***. 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), pp. 433–440. DOI:10.7546/CRABS.2020.03.18 IF=0.378, 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<sup>-1</sup>), 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 physicochemical properties and sampling sites. The results showed that the less polluted sites were grouped in a single cluster with the highest number of 16S rRNA 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\_6** Nikolova, R., Kenarova, A., Boteva, S., Dinev, N., **Radeva G\***. 2024. Diversity and structure of soil bacterial communities in the area of non-ferrous metal plant revealed by 16S rRNA gene retrieval. *Comptes Rendus de L'Académie Bulgare des Sciences*, 77 (8), 1260–1268, <https://doi.org/10.7546/CRABS.2024.08.18> IF=0.378, Q3.

**Abstract** The goals of the study were to assess the diversity and structure of the bacterial communities within the soil depth along a gradient of heavy metal (HM) contamination and to identify indigenous bacterial species in the agricultural area of a non-ferrous metal plant KCM 2000 Group (Plovdiv) by using 16S rRNA gene retrieval. 16S rRNA gene clone libraries were

constructed for nine samples, which were collected from two soil depths in June 2020. From each library, up to 100 clones were analysed and grouped into operational taxonomic units (OTUs) by restriction fragment length polymorphism (RFLP). The representatives of the OTUs were sequenced, followed by phylogenetic analysis. The results revealed that phyla Proteobacteria (11.11–71.43%) and Actinomycetota (3.57–33%) were the most abundant. Surface soils (12 phyla and 15 classes) were more diverse than subsurface ones (7 phyla and 12 classes). The lowest diversity at both phylum and class levels was calculated for the moderately contaminated soils from the two studied depths. Thirteen 16S rDNA sequences were identified to a species level, and they belonged to Proteobacteria, Actinomycetota and Firmicutes. This study highlighted that both HM contamination and soil depth caused shifts in diversity and structure of soil bacterial communities.

**Г7\_1** Kenarova A., **Radeva G.**, Traykov I., Boteva S. 2014. Community Level Physiological Profiles of Bacterial Communities Inhabiting Uranium Mining Impacted Sites. *Ecotoxicology and Environmental Safety*, 100, 226-232. <https://doi.org/10.1016/j.ecoenv.2013.11.012>, IF=2.762, 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; TCC sum) pollution as follows: Sliven (uranium – 374 mg/kg; TCC sum – 23.40) 4Buhovo (uranium – 139.20 mg/kg; TCC sum –3.93) 4Senokos (uranium – 23.01 mg/kg; TCC sum – 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

**Г7\_2** Boteva, S., **Radeva, G.**, Traykov, I., Kenarova, A. 2015. 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), 2016, pp.5644-5653. DOI:

10.1007/s11356-015-5788-5. IF=2.741, 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 by the 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 evidence of microbial tolerance and adaptation to 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 were able to reduce the metal stress by intensive phosphatase synthesis. Benefiting from passive environmental remediation and the provision of vital ecosystem services.

**Г7\_3** 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*, SE 3, pp. 31-39. SJR=0.144, 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<sup>-1</sup>) 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<sub>3</sub>-N), 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.

**Г7\_4** Nikolova R., Petkova M., Dinev, N., Kenarova, A., Boteva, S., Berov D, **Radeva G.\*** 2022. Correlation between bacterial abundance, soil properties and heavy metal contamination in the area of non-ferrous metal processing plant, Southern Bulgaria. *BioRisk* 17, pp. 19–30.DOI:

10.3897/biorisk.17.77458. SJR=0.297, Q2

**Abstract** In the present study, the correlation between bacterial abundance and soil physicochemical properties along the heavy metal contamination gradient in the area of non-ferrous metal processing plant was assessed. Our results showed that bacterial abundance (number of heterotrophic bacteria and number of 16S rRNA gene copies) decreased with 45–56% (CFU) and 54–87% (16S rRNA gene) along the Zn, Pb and Cd contamination gradient. The total bacterial abundance (16S rRNA gene) increased exponentially in contrast to the abundance of heterotrophic bacteria. The reduction of bacterial abundance in heavily contaminated soil indicated that the soil properties (soil pH, total organic carbon, inorganic ions, soil texture) could modify the effects of heavy metals and the response of microorganisms to that stress in long-term contaminated soils.

**Г7\_5** Nikolova, R., Kenarova, A., Petkova, M., Dimitrova, G., **Radeva, G.\*** 2026. Impact of heavy metal pollution on soil bacterial carbon metabolism. *Comptes Rendus de l'Académie bulgare des sciences*, 79 (1) p.28-36, <https://doi.org/10.7546/CRABS.2026.01.04> . IF=0.378, Q3

**Abstract** The aim of the study was to evaluate the long-term impact of heavy metals on soil bacterial metabolism by assessing their capacity to utilize a set of carbon sources. The study area is in the vicinity of the Pb/Zn smelter KCM 2000, South-Central Bulgaria, with considerable Pb, Zn, Cd, Cu, and As pollution. The Biolog Ecoplate approach was used to evaluate the bacterial metabolic activity, expressed by the average well colour development; the bacterial metabolic fingerprints expressed by community-level physiological profiling, and Shannon bacterial functional diversity. The results revealed reduced metabolic activity recorded for bacterial communities inhabiting the heavily polluted soils. The most preferred carbon groups utilized by the bacterial communities were the polymers, followed by carbohydrates and amino acids. Local variability of soil properties, such as inorganic ions, total organic carbon, pH, soil moisture, and soil texture, could modify the effects of heavy metals. This study highlighted that Biolog Ecoplate™ is a suitable approach for assessing bacterial functional shifts in relation to long-term soil pollution stress.

**Г7\_6** Boteva S., Kenarova A., Kancheva V., Aleksova M., Dimitrov R., **Radeva, G.** 2021. Long-term heavy metal pollution of soils and its impact on bacterial carbon metabolism. *Forestry Ideas*, 27, No 1 (61): 245–255 SJR=0.162, Q4.

**Abstract** Heavy metal pollution of soils may change their chemical and microbiological status. Changes in the function of 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™ method. Soil samples were taken from the vicinity of mine Chelopech along 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<sup>-1</sup>, 44–180

mg·kg<sup>-1</sup> and 31–175 mg·kg<sup>-1</sup>, 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™ method can be used to evaluate community functional variability in relation to different levels of heavy metal stress, as statistically significant results have been obtained.

**Г7\_7** Aleksova, M., Kenarova, A., Boteva, S., **Radeva, G.** 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. DOI:10.7546/CRABS.2019.10.08 IF=0.343, 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.

**Г7\_8** Aleksova, M., Kenarova, A., Boteva, S., **Radeva, G.** 2019. Induced Bacterial Antibiotic Resistance under the Application of Fungicide Azoxystrobin. *Comptes Rendus de l'academie Bulgare des sciences*, 72(11), 1592-1598. DOI:10.7546/CRABS.2019.11.18 IF=0.343, 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<sup>-1</sup>). 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.

**Г7\_9** Boteva, S.B, Kenarova, A.E, Petkova, M.R, Georgieva, S.S, Chanev, C.D, **Radeva, G.S.** 2022. Soil enzyme activities after application of fungicide Quadris<sup>R</sup> at increasing concentration rates. *Plant, Soil and Environment* 68(8):382-392. doi: 10.17221/127/2022-PSE. IF=2.6, Q2.

**Abstract.** The study aimed to assess the effects of fungicide QuadrisR on activities of soil enzymes contributed to soil nutrient turnover. A batch laboratory experiment with QuadrisR-amended (0 mg/kg ds (dry soil) – 35.00 mg/kg ds) loamy sand soil was conducted, and shifts in soil physical environments and enzyme activities (beta-glucosidase, urease, acid and alkaline phosphatases, arylsulfatase and dehydrogenase) were evaluated on experimental days 1, 30, 60, 90 and 120. The results indicated that QuadrisR changed both soil properties and enzyme activities. The most sensitive environmental parameter to fungicide input was soil pH. The most susceptible to QuadrisR enzymes were dehydrogenase and arylsulfatase, and the most resistant – urease. The mean overall dehydrogenase activity decreased by 33%, whereas the profile of arylsulfatase activity tended to a permanent decrease over time. The general pattern of enzyme responses to QuadrisR was an immediate-early (days 1 – 30) decline of enzyme activities after fungicide application, except that of arylsulfatase. Beta-glucosidase manifested a temporal profile of steady-state stimulation under the lowest (2.90 mg/kg ds) and low sensitivity to the higher (14.65 mg/kg ds and 35.00 mg/kg ds) fungicide concentrations.

**Г7-10** Aleksova M, Kenarova A., Boteva, S., Georgieva, S., Chanev Ch., **Radeva G.** 2021. Effects of increasing concentrations of fungicide Quadris<sup>R</sup> on bacterial functional profiling in loamy sand soil. *Archives of Microbiology*, 203(7), pp. 4385–4396. DOI: 10.1007/s00203-021-02423-2. IF=2.552, Q2

**Abstract:** A mesocosm experiment was conducted to assess the side effects of the fungicide Quadris on soil bacterial functioning. Quadris<sup>R</sup> was applied to a loamy sand soil at increasing concentrations (0.0-35.0 mg kg<sup>-1</sup> 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 in the field

recommended fungicide concentration (2.90 mg kg<sup>-1</sup> 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<sup>-1</sup> 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<sup>R</sup> affects bacterial catabolic profiles in loamy sand soils through soil acidification and alters the soil nutrient pool. The study also revealed that CLPP and EcoPlate are useful practical tools for testing the fungicide ecotoxicity.

**Г7\_11** 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*, SE 3, pp. 185-194. SJR=0.144, 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 study aimed 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<sup>R</sup>. A laboratory study was carried out for 120 days on soil mesocosms, amended with Az in concentrations from 0.00 mg kg<sup>-1</sup> to 35.00 mg kg<sup>-1</sup>. 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 Quadris<sup>R</sup> altered soil enzyme system for more than four months, which might reflect the speed of organic matter turnover in soil, especially that of organophosphates.

**Г7\_12** Petkova, M., Kenarova, A., Boteva, S., Georgieva, S., Chanev, Ch., **Radeva, G.** 2022. Differences in bacterial functional profiles from loamy sand and clay loam textured soils under fungicide Quadris<sup>R</sup> impact. *BioRisk* 17, pp. 31–43. doi: 10.3897/biorisk.17.76939. SJR=0.297, Q3

**Abstract** The non-target effect of the fungicide Quadris<sup>R</sup> on the bacterial community from grassland loamy sand (LS) and cropland clay loam (CL) soils with unknown history of fungicide

usage was investigated. QuadrisR was applied to soil mesocosms at 0.0 mg kg<sup>-1</sup> (Az0), 2.90 mg kg<sup>-1</sup> (Az1), 14.65 mg kg<sup>-1</sup> (Az2) and 35.0 mg kg<sup>-1</sup> (Az3) calculated towards the active ingredient azoxystrobin (Az). Response of bacterial communities to QuadrisR was investigated during a 120-day incubation experiment, evaluating the shifts in bacterial catabolic profiles by the community-level physiological profiling (CLPP) technique and BiologEcoPlates<sup>TM</sup> method. QuadrisR decreased the overall catabolic activity (AWCD) of soil bacterial communities and the rate of decrease was independent of soil type and fungicide concentration. Fungicide affected negatively the utilisation of amines and positively that of amino acids in both soil types, whereas the effects on other carbon guilds (carbohydrates, carboxylic acids and polymers) corresponded closely to the respective soil type and fungicide concentration. Results indicated the presence of non-target effects of QuadrisR on bacterial functioning; hence, it is important to address the fungicide side-effects on soil health.

**Г7\_13** Petkova, M., Nankova, N., Kancheva, V., Boteva, S., Kenarova, A., **Radeva, G\***. 2023. Distribution of microbial abundance in long-term copper contaminated soils from Topolnitsa-Pirdop valley, Southern Bulgaria. In: Chankova S, Danova K, Beltcheva M, Radeva G, Petrova V, Vassilev K (Eds) Actual problems of Ecology. BioRisk 20: 23-35. <https://doi.org/10.3897/biorisk.20.97795>, SJR=0.254, Q2

**Abstract** This study presents the distribution of bacterial and fungal abundances in long-term copper (Cu) contaminated soils in Topolnitsa-Pirdop valley – a highly industrialized zone with a number of mines and processing plants for copper and other non-ferrous metals. The bacterial (16S rRNA gene copies) and fungal (ITS rRNA gene copies) were estimated using quantitative PCR technique in five topsoils, differently Cu contaminated (ranging from 28.05 to 198.9 mg kg<sup>-1</sup>). Bacterial abundance varied in a range of 1.68 × 10<sup>11</sup> to 3.24 × 10<sup>11</sup> 16S rRNA genes, whereas fungi amounted from 1.95 × 10<sup>8</sup> to 6.71 × 10<sup>8</sup> ITS rRNA genes. Fungal and bacterial abundances were significantly (fungi) and insignificantly (bacteria) influenced by Cu contamination. The fungal/bacterial ratio related negatively with soil Cu, which shifted microbial communities' structure towards bacterial dominance. Since the ratio between bacteria and fungi are vital in explaining many soil functions, the calculated changes in this ratio indicated deterioration in soil quality, being of primary importance for plant production.

**Г8\_1 Radeva, G.,\*** Dimitrova, G., Petkova, M., Nikolova, R., Gatev, E., Dinev, N., Hristova, M., Hristova, V. 2026. Impact of Heavy Metal Pollution on Microbial Functional Diversity in Soils Near the Topolnitsa River, Southern Bulgaria. Book chapter: in “Modern Ecology: Theoretical and Applied View”, edited by Prof. S. Chankova, Publ. Farrago, ISBN: 978-619-206-308-5, p.17-27.

### **Abstract**

**Aim:** The aim is to assess the impact of heavy metal pollution on soil microbial metabolic activity and functional diversity, as well as to evaluate the ecological risk in the Topolnitsa River region using the Biolog EcoPlate system. Materials and methods: Five samples (0 – 20 cm) polluted with

Cu and co-contaminants Zn, Pb, Cd, and As were collected from the area near the Topolnitsa River and its tributaries: sediment S1 and soil samples S2 – S5. The total bacterial catabolic activity was assessed using average well color development (AWCD) and community-level physiological profiling (CLPP). The relationships between soil parameters, heavy metal concentrations, AWCD, and diversity indices (Shannon (H'), Pielou (E'), and substrate richness (SR)) were analyzed using Pearson's correlation test (Past 4.13). Soil pollution levels were assessed using the PI (Pollution Index) and NPI (Nemerow Pollution Index).

**Main results:** According to NPI, the soils were classified as clean (S1, S2, S3) and lightly polluted (S4, S5), and based on PI(Cu), they were categorized as low (S1, S2), moderate (S3), and strong (S4, S5) pollution. The AWCD values reflected the microbial community's metabolic activity across the soils, with relatively high activity in S2, S1, and S5. The most easily utilized carbon groups were as follows: AA (amino acids) > CA (carboxylic acids) > POL (polymers) > AM (amines and amides) > CH (carbohydrates). Pearson's correlation showed a strong positive correlation between Zn and As, and between Cd and Pb. A perfect correlation was found between H' and E', indicating a close relationship between microbial diversity and community balance. Additionally, soil moisture was positively associated with SR and P2O5.

**Conclusion:** This study demonstrated that HM pollution affects microbial metabolism by decreasing the utilization of carbon sources. The findings confirm that Biolog ECO is an effective method for evaluating ecological risks in ecosystems influenced by industry. Additionally, microbial profiling and activity could serve as indirect indicators for assessing ecological risk in this area.

**Г7\_14** Boteva, S., Kenarova, A., **Radeva, G.**, Taykov, I, Bogoev, V. 2011. Community dynamics of pelagic bacteria in the high mountain Lake Sulzata-Rila mountain, Bulgaria. *Biootechnology & Biotechnol. Equipment*; 25 (4) 2620-26. <https://doi.org/10.5504/BBEQ.2011.0063> , IF=0.76, SJR<sub>2011</sub>=0.205, Q3

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**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  $(69.13 \pm 81.1) \times 10^5$  cells ml<sup>-1</sup>), 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.

**Г7\_15** Aleksova, M., Velkova, L., Dolashka, P., **Radeva, G\***. 2021. Antibacterial activity of bioactive fractions from mucus and hemolymph of different snails species and crab. Bulgarian Chemical Communications, 53, SI A, pp. 022 – 026. DOI: 10.34049/bcc.53.A.0006 . , SJR=0.17, Q4.

**Abstract** This study aims to evaluate the antibacterial activity of six samples contained biologically active fractions isolated from various molluscs species (garden and marine snails) and one representative of the arthropods (*Carcinus aestuarii*, also known as Mediterranean green crab). The minimum inhibitory concentration (MIC) values for *Escherichia coli* and *Brevundimonas diminuta* were in ranges of 145-682.5 µg/ml and 290-431.5 µg/ml, respectively. The dissociated hemocyanin from *Carcinus aestuarii* (CaH) showed the strongest MIC (145-290 µg/ml) and effective concentrations (EC50 values 75.46-112.2 µg/ml) against all Gram-negative bacterial strains. Two of the tested samples – protein fraction from *Helix aspersa* mucus with Mw above 20 kDa and peptide fraction from *Helix lucorum* hemolymph with Mw below 10 kDa, demonstrated selective antibacterial activity against *E. coli* or *B. diminuta*, respectively. The results in this study showed that the bioactive fractions isolated from mucus and hemolymph of different snail species and isoforms of *C. aestuarii* hemocyanin could be considered as new natural antibacterial agents with potential biomedical application. Further studies are needed to confirm the antibacterial activity with a wider range of bacterial strains.