

Use of Ecoplates in Soil Polluted with Heavy Metals

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REVIEW

Abstract

Global concern revolves around the persistent attributes of harmful non-biodegradable contaminants, especially heavy metals and metalloids, which draws attention to environmental pollution. As regards to soil ecosystem, microorganisms have a significant impact on heavy metal concentrations, either actively or passively. Heavy metal toxicity profoundly impacts the metabolic activities of microbial communities, leading to disruptions in essential cellular processes. Heavy metals interfere with the enzymatic reactions necessary for energy of metabolism, nutrient cycling, and organic matter degradation, resulting in reduced microbial growth and activity. The Biolog Ecoplate method is used as an effective tool for assessing microbial activity. The potential use of Biolog Ecoplate in the study of microbial community structure and diversity in heavy metal-polluted soil was investigated. In order to achieve this goal, multiple keywords combinations were used to filter the most important results in the field. In metal-contaminated soils, the use of Biolog Ecoplate has led to notable changes in microbial community composition, marked by increased populations of resistant and metal-reducing microorganisms.

Keywords: Functional microbial community; heavy metal toxicity; microbial community structure; substrate assessment.

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INTRODUCTION

Soil contamination caused by heavy metals represents a significant global ecological concern (Cd, Pb, Cu, Zn). Among these metals, Cd is recognized as a particularly harmful element due to its remarkable ability to spread within the soil—especially in soils with low Cation Exchange Capacity (CEC) and acidic pH (Xie et al., 2016). They accumulate mostly in smaller organisms and slowly moves through the food chain to higher trophic levels. However, the presence of heavy metals has negative effects on the growth, structure and metabolism of soil microorganisms. This is primarily due to perturbation of their normal functions, protein denaturation and destabilizing pH integrity of the cell membrane (Xie et al., 2016).

Due to their small size, wide distribution, and significant involvement in metabolic processes, microorganisms are considered as the most effective indicators for 15 harmful substances on soil (Bouchez et al., 2016). Heavy metals play important roles within microbial communities, influencing metabolic functions, genetic processes, and activities associated with the carbon and nitrogen cycles (Li et al., 2020). Bioaccessible heavy metal components could penetrate cell membranes, binding to enzyme amino acids displacing vital metal ions from cells. This process could prevent important enzyme activity for cellular metabolism or lead to DNA and protein damage, which could explain how heavy metals impede metabolic functions and genetic processes (Liu et al., 2018).



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Various research studies have highlighted the adverse effects of heavy metals on microbial populations on soil (Bouchez et al., 2016). Heavy metals are recognized for their ability to impair the function of soil enzymes, disrupting the utilization and transformation of C, N, and organic matter. They also reduce both the diversity and abundance of microorganisms. Consequently, this may lead to the prevalence of certain heavy metal-tolerant microorganisms in soil ecosystems (Jarosławiecka et al., 2022), and a modification in the overall community composition. Increased concentrations of heavy metals in soils caused notable effects on both the population and overall functioning of soil microbial communities. Several investigations that use isolation-based methods have demonstrated that the presence of heavy metal pollution led to important changes in the composition of microbial populations (Xie et al., 2016). Hence, from the ecological point of view, it is important to identify microbial metabolic characteristics related to polluted soils (Kuzniar et al., 2018). CLPP method, the BILOG system, is a suitable approach for assessing the functional diversity of microbial populations (Frac et al., 2012; Rutgers et al., 2016; Garland, 1999; Stoian et al., 2022). This approach has proven effective in profiling the functional capacity of soil organisms to utilize distinct carbon substrates (Garland, 1997; Garland and Mills, 1991). It has also been widely applied to assess the functional variety present within microbial communities in soil samples obtained from agricultural and grassland ecosystems.

Assessment of the functional and structural diversity of bacteria directly at the polluted site is important because it allows characterization of a natural bioremediation process (Gałazka et al., 2018). Bioremediation involves the use of living or non-living biomass either to convert hazardous pollutants, such as heavy metals, into less harmful forms, remove toxic elements from the polluted environment, or to decompose organic compounds, eventually converting them into carbon dioxide, water, nitrogen gas, and similar products (Kapahi et al., 2019). A comprehensive evaluation of the ecological hazards associated with Pb and Cd contamination is vital to promote sustainable agricultural progress and minimize potential threats to human well-being (Li et al., 2022). Microorganisms provide cost-effective and efficient biosorption for the removal of pollutants, including non-biodegradable heavy metals, from waste water, either as living or non-living biomass, due to their adaptive mechanisms for metal ion resistance and remediation (Mustaph et al., 2015).

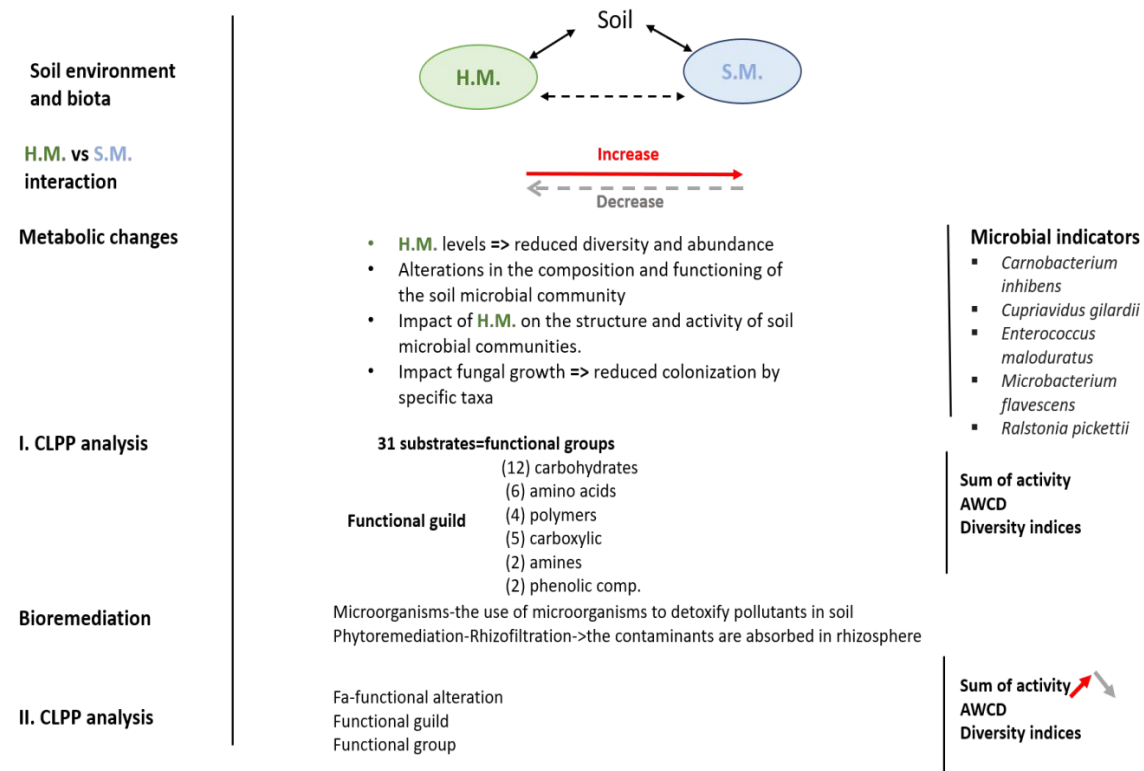


Figure 1. The flow of microbial community level physiological profiling in heavy metal contaminated soils. Legend: H.M.-heavy metals; S.M.-soil metabolism; CLPP- community-level physiological profiling; AWCD-average well color development.

THE USE OF MICROBIAL COMMUNITY PHYSIOLOGICAL PROFILE IN SOIL HEAVY METAL STUDIES

The holistic approach of heavy metals and soil microbiome

Changes in plant productivity and quality, as well as changes in the composition and functioning of the soil microbial community, are consequences of negative impact of heavy metals (Xie et al., 2016). The presence of heavy metal fractions in soils contaminated with Pb, Cd, and Zn altered the richness, diversity, and structure of bacterial communities over extended periods of exposure (Beattie et al., 2018). A significant effect of heavy metal toxicity on microbial communities is the disruption of key metabolic pathways. Heavy metals can interfere with enzymatic reactions essential for processes such as energy production, nutrient cycling, and organic matter decomposition. This disruption can lead to a decrease in microbial biomass, reduced nutrient conversion rates, and altered ecosystem functioning (Kandeler et al., 1996; Stoian et al., 2018).

Within the literature database, numerous studies report the successful utilization of the CLPP method to discover metabolically active microorganisms at heavy metal contaminated sites (Margesin et al., 2011).

The implementation of the substrate induced profiling method represents an indirect approach to characterize changes in the carbon metabolism of an entire microbial community during a remediation process (Kuźniar et al., 2018).

The diversity and composition of microbial communities exposed to heavy metals stress are closely related to how these communities assemble. Assessing the relative significance of deterministic and stochastic processes can offer a more lucid comprehension of how microbial communities react to the stress induced by heavy metals (Wang et al., 2021).

In areas contaminated by heavy metals, the prevalence of tolerant microorganisms increased in proportion to the heavy metal gradient (Kozdrój, 2001; Xie et al., 2014). Microbial diversity, functionality, resilience, and community formation have been thoroughly studied as important markers for assessing the influence of varying concentrations of heavy metals on microorganisms (Ding et al., 2022). The microbial diversity term includes genetic diversity, which refers to the distribution and the quantity of genetic information. At the same time, the diversity of bacterial and fungal species in microbial communities and also ecological diversity, which refers to the complexity of interactions, the structure of the microbial community, the number of trophic chains and the number of guilds.

Heavy metals vs soil microbiota interaction

The presence of heavy metals introduces a particular selective stress on the microbial community. As heavy metal concentrations increase, microbial community assembly tends to become more determined by deterministic factors (Li et al., 2022). Toxicity of heavy metals can impact fungal growth, leading to reduced colonization by specific taxa and a higher tendency to exclusion (Li et al., 2022).

Heavy metals are known to be toxic and affect soil microorganisms especially fungi, impacting the diversity, community structure and functions (Song et al., 2018; Wu et al., 2019). In the food web, protists are the predators, actively responding to soil management and nutrient availability, playing a vital role in the co-occurrence networks of the soil microbiome (Yang et al., 2015; Leon et al., 2018; Zhang et al., 2019; Geisen et al., 2018; Xiong et al., 2018; Zhao et al., 2019). Despite the potential variation in the responses of bacteria, fungi and protists to heavy metal contamination, research is limited, particularly in comparing their diversity and community structure, especially under conditions simulating real-world heavy metal contamination (Qi et al., 2022).

Numerous previous studies have established that abiotic factors, such as soil pH, electrical conductivity, and heavy metal concentration, can directly or indirectly impact the soil microbiome (Frossard et al., 2017). Studies have shown that microbiome diversity and community structure undergo changes along heavy metal pollution gradient. Moreover, they revealed a higher prevalence of generalist bacteria compared to fungi and generalist protist, attributed in part to significantly higher biomass of bacteria, which range from two to four orders of magnitude more than protists (Fierer, 2017). In addition, protists exhibit smaller niches than bacteria, indicating that protist communities are less sensitive to dispersal limitations (Wu et al., 2018).

The analysis of metabolic changes

Low concentrations of heavy metals can boost microbial diversity, but high heavy metal levels can lead to diminished diversity and abundance (Ma et al., 2021; Qi et al., 2022). Microbial communities consist of a handful of common species and numerous uncommon ones (Saunders et al., 2016; Sandor et al., 2016). Both these common and rare species exhibit distinct reactions to heavy metal stress. Interactions among microorganisms are vital for their survival in heavy metal-contaminated soil, revealing that the intricacy of microbial networks grows with higher heavy metal concentrations (Chun et al., 2021; Sun et al., 2022). Microbial diversity, function, stability, and

composition have been extensively recognized as important measures for assessing the effects of varying heavy metal concentrations on microorganisms (Li et al., 2022).

The basic of CLPP analysis

The use of indices that quantify the functional dimension of biodiversity is very important for a comprehensive understanding of the interplay between biodiversity, ecosystem performance, and environmental limitations.

Traditional measures of biodiversity, such as species richness or various diversity indices such as Shannon's, have traditionally been based on three key assumptions: (i) equality of all species (with relative abundances determining their importance), (ii) equality of all individuals regardless of size, and (iii) accurate assessment of species abundance using suitable tools and uniform units (Magurran, 2005).

How ecoplates work?

In each 96-well plate, there were three replicate wells, each containing 31 distinct individual carbon sources, and one well with water as a reference (blank sample). The carbon substrates were categorized into five groups: carbohydrates (Cs), carboxylic and ketonic acids (CK), amines and amides (AD), amino acids (AA), and polymers (P). To prepare the soil samples, 1 gram of soil was mixed with 99 milliliters of sterile water and shaken for 30 minutes at 4°C. After this, 120 µL of the resulting sample suspension was introduced into each well of the Biolog EcoPlates™ and incubated at 26°C. The utilization of these carbon sources was assessed by monitoring the reduction of tetrazolium violet, a redox indicator dye that transitioned from colorless to purple as a result of microbial metabolic activity (Islam et al., 2011; Stefanowicz, 2006).

Ecoplates-What are they?

Hence, is necessary to conduct research to profile the microbial communities present in environmental samples (Garland and Mills, 1991). It is important to note that rapid analysis of CLPP can serve as a valuable tool to obtain insights on fundamental ecological aspects. Previous research has led to the development of a specific set of substrates for characterizing communities in environmental samples (Garland and Mills, 1991). Carbon sources, which include classified groups such as amines and amides, amino acids, carbohydrates, carboxylic acids and polymers, are part of the activities in which soil microorganisms are involved (Rutgers et al., 2016).

Advantages of using Biolog Ecoplate

Biolog EcoPlate has been specifically designed for performing community analysis and microbial ecology research. Biolog Ecoplate helps to assess microbial community characteristics by introducing a mixture of microorganism cultures or environmental samples, such as soil, water, wastewater, activated sludge, compost, and industrial waste (Gryta et al., 2014). Biolog has proven to be effective in evaluating the functional capacity of soil organisms to metabolize specific carbon substrates and has been widely applied in assessing functional diversity (Garland and Mills, 1991; Garland, 1996). The use of the BIOLÓG system is an indirect method for characterizing alterations in the carbon metabolism in a microbial community throughout a remediation process (Alisi et al., 2009; Miller and Rhoden, 1991).

Bioremediation and soil microbial community

The remediation of soil contaminated with heavy metals is mandatory for environmental conservation and protection living organisms (Gonzalez Henao and Ghneim-Herrera, 2021). In contrast, biological approaches, such as bioremediation (using microorganisms to detoxify pollutants in soils) and phytoremediation (Figure 1) (using plants to extract heavy metals), are considered effective methods for heavy metal remediation (Gonzalez Henao and Ghneim-Herrera, 2021). Microbial bioremediation is cost-effective and environmentally sustainable process that significantly reduces the costs associated with cleaning up heavy metal pollution (Kumar Mishra, 2017).

Using microorganisms and their innate abilities to resist harmful metal ions through processes such as trapping them inside or outside cells, actively expelling them, and reducing metal ions, bioremediation emerges as a highly encouraging method for heavy metals removal or recovery (Ayangbenro and Babalola, 2017). It contrasts with traditional chemical and physical approaches due to its environmental friendly nature, preserving soil integrity, and preventing secondary pollution.

Microbial biosorbents, in particular, are remarkably effective at removing pollutants, although alternatives like bioleaching and phytoremediation also exist (Jarosławiecka and Piotrowska-Seget, 2022).

Numerous scientists are very concerned about the environmental consequences of unaddressed polluted regions (Guo et al., 2017; Zeng et al., 2020; Tipayno et al., 2018). Consequently, numerous investigations have been conducted to improve this outcome. These studies aim to gain a deeper insight into the formation and functioning of microbial communities in heavy metal contaminated areas. This knowledge is essential for refining and designing

the most effective and ideal approach for bioremediation, as demonstrated by a variety of relevant studies (Guo et al., 2017; Zeng et al., 2020; Tipayno et al., 2018). Microbial remediation of heavy metals mainly involves several mechanisms, including biosorption (including precipitation, chemical adsorption, ion exchange, surface precipitation, complexation with organic ligands and redox reactions), biomineralization (which includes bioleaching, a process that mobilizes heavy metal ions from insoluble ores through dissolution or complexation), and bio oxidation (Jin et al., 2018).

Monitoring the bioremediation based of CLPP analysis

Studies revealed that heavy metals have had a notable adverse impact on the functional diversity of microorganisms in CT soil, that increasing heavy metal concentrations led to reduced microbial bioactivity due to different levels of sensitivity to metal toxicity (Piotrowska-Seget and Kozdrój, 2008). Moreover, changes in the physiological profiles of microorganisms when exposed to Cd were exposed. Additionally, a negative correlation between total petroleum hydrocarbons (TPH) and the diversity of functional soil bacteria (CLPP) has been reported. This effect implies a significant alteration in cell proliferation rates as a result of short- or long-term exposure of bacteria to these metals (Piotrowska-Seget and Kozdrój, 2008).

Numerous studies indicate that prolonged exposure to heavy metal contamination reduces biodiversity or disrupts the community structure (Lenart-Boroń et al., 2014). In addition, metal exposure could give rise to resilient microbial populations. In terms of population, we have Gram-positive and Gram-negative bacteria. Some examples of Gram positive are *Bacillus*, *Arthrobacter* and *Corynebacterium*, and Gram negative like *Pseudomonas*, *Alcaligenes*, *Ralstonia* or *Burkholderia* (Lenart-Boroń et al., 2014).

Microorganisms have a major influence on the amount of heavy metals in soil, which can be direct or indirect (Kuźniar et al., 2018).

Soil bacteria are usually characterized by their metabolic diversity. Over 150 distinct bacterial metabolic pathways and approximately 900 different bacterial reactions have been identified (Kuźniar et al., 2018). Therefore, from an ecological point of view, it is essential to assess microbial metabolic composition in contaminated soils. An effective method for assessing functional variety within microbial communities is the CLPP technique, which is also referred to as the BIOLOG system (Frąc et al., 2012; Garland, 1999).

CONCLUSIONS

The ability of microorganisms, including bacteria, fungi and algae, to remove heavy metals from their environment or convert them into less harmful forms is an area of great interest to scientists. This study plays a two vital role in determining the connection between bacterial tolerance to metals and the particular type of metal being analyzed. The research covers both metals, such as Cd and Pb, and metalloids, such as arsenic. The varied metal detoxification methods used by microorganisms present a wide range of possibilities for diverse remediation strategies. *Klebsiella* and *Enterobacter* emerge as promising options for bioremediation and bacteria-assisted phytoremediation in soils contaminated with, Cd and Pb. However, further research is necessary to comprehensively understand the mechanisms behind their resistance to Cd and Pb. For agricultural activated sludge for agricultural waste, ecotoxicity assessment provides essential information on the safety of use of Biolog Ecoplate (Gryta et al., 2014). Moreover, this method is not only effective but also very affordable, fast and easy to prepare. Metabolic profiling is used for the identification and characterization of microorganisms. It is increasingly applied in ecotoxicology research to assess the capabilities of agricultural applications (Gryta et al., 2014).

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

REFERENCES

1. Anedda E, Farrell ML, Morris D, Burgess CM. Evaluating the impact of heavy metals on antimicrobial resistance in the primary food production environment: A scoping review. *Environmental Pollution*. 2023; 320:121035.
2. Ayangbenro AS, Babalola OO. A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *International journal of environmental research and public health*. 2017;14(1):94.
3. Beattie RE, Henke W, Campa MF, Hazen TC, McAliley LR, Campbell JH. Variation in microbial community structure correlates with heavy-metal contamination in soils decades after mining ceased. *Soil Biology and Biochemistry*. 2018; 126:57-63.
4. Bouchez T, Blieux AL, Dequiedt S, Domaizon I, Dufresne A, Ferreira S, Godon JJ, Hellal J, Joulain C, Quaiser A, Martin-Laurent F. Molecular microbiology methods for environmental diagnosis. *Environmental Chemistry Letters*. 2016;14:423-41.
5. Chiara A, Rosario M, Flavia T. Bioremediation of diesel oil in a cocontaminated soil by bioaugmentation with a microbial formula tailored with native strains elected for heavy metals resistance. *Science of the Total*.
6. Chun SJ, Kim YJ, Cui Y, Nam KH. Ecological network analysis reveals distinctive microbial modules associated with heavy metal contamination of abandoned mine soils in Korea. *Environmental Pollution*. 2021 Nov 15; 289:117851.
7. Ding J, Travers SK, Eldridge DJ. Microbial communities are associated with indicators of soil surface condition across a continental gradient. *Geoderma*. 2022; 405:115439. *Environment*. 2009;407(8):3024-32.
8. Fierer N. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*. 2017; 15(10):579-90.
9. Frąc M, Oszust K, Lipiec J. Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. *Sensors*. 2012; 12(3):3253-68.
10. Frąc M, Oszust K, Lipiec J. Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. *Sensors*. 2012; 12(3):3253-68.
11. Frossard A, Hartmann M, Frey B. Tolerance of the forest soil microbiome to increasing mercury concentrations. *Soil Biology and Biochemistry*. 2017;105:162-76.
12. Gałazka A, Grządziel J, Gałazka R, Ukalska-Jaruga A, Strzelecka J, Smreczak B. Genetic and functional diversity of bacterial microbiome in soils with long term impacts of petroleum hydrocarbons. *Frontiers in Microbiology*. 2018; 9:1923.
13. Garland JL, Mills AL. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and environmental microbiology*. 1991; 57(8):2351-9.
14. Garland JL. Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS microbiology ecology*. 1997; 24(4):289-300.
15. Garland JL. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. *Soil Biology and Biochemistry*. 1996;28(2):213-21.
16. Garland JL. Potential and limitations of BIOLOG for microbial community analysis. In *Microbial biosystems: new frontiers. Proceedings of the 8th International Symposium on Microbial Ecology 1999*; 1-7. Halifax, NS: Atlantic Canada Society for Microbial Ecology.
17. Geisen S, Mitchell EA, Adl S, Bonkowski M, Dunthorn M, Ekelund F, Fernández LD, Jousset A, Krashevskaya V, Singer D, Spiegel FW. Soil protists: a fertile frontier in soil biology research. *FEMS Microbiology Reviews*. 2018; 42(3):293-323.
18. Gryta A, Frąc M, Oszust K. The application of the Biolog EcoPlate approach in ecotoxicological evaluation of dairy sewage sludge. *Applied biochemistry and biotechnology*. 2014; 174:1434-43.
19. Guo H, Nasir M, Lv J, Dai Y, Gao J. Understanding the variation of microbial community in heavy metals contaminated soil using high throughput sequencing. *Ecotoxicology and environmental safety*. 2017; 144:300-6.
20. Islam MR, Singh Chauhan P, Kim Y, Kim M, Sa T. Community level functional diversity and enzyme activities in paddy soils under different long-term fertilizer management practices. *Biology and Fertility of Soils*. 2011; 47:599-604.
21. Jarosławiecka AK, Piotrowska-Seget Z. The effect of heavy metals on microbial communities in industrial soil in the area of Piekary Śląskie and Bukowno (Poland). *Microbiology Research*. 2022; 13(3):626-42.
22. Jin Y, Luan Y, Ning Y, Wang L. Effects and mechanisms of microbial remediation of heavy metals in soil: a critical review. *Applied Sciences*. 2018; 8(8):1336.
23. Kandeler F, Kampichler C, Horak O. Influence of heavy metals on the functional diversity of soil microbial communities. *Biology and fertility of soils*. 1996; 23:299-306.

24. Kapahi M, Sachdeva S. Bioremediation options for heavy metal pollution. *Journal of health and pollution*. 2019; 9(24):191203.
25. Kozdrój J. Microbial reaction to soil contamination with Cd (II) at different temperatures. *Microbiological research*. 2001; 155(4):285-90.
26. Kumar Mishra G. Microbes in heavy metal remediation: a review on current trends and patents. *Recent patents on biotechnology*. 2017; 11(3):188-96.
27. Kuźniar A, Banach A, Stępniewska Z, Frąc M, Oszust K, Gryta A, Kłos M, Wolińska A. Community-level physiological profiles of microorganisms inhabiting soil contaminated with heavy metals. *International Agrophysics*. 2018; 32(1):101-9.
28. Lenart-Boroń A, Boroń P. The effect of industrial heavy metal pollution on microbial abundance and diversity in soils—a review. *IntechOpen*; 2014.
29. Leon CG, Moraga R, Valenzuela C, Gugliandolo C, Lo Giudice A, Papale M, Vilo C, Dong Q, Smith CT, Rossello Mora R, Yañez J. Effect of the natural arsenic gradient on the diversity and arsenic resistance of bacterial communities of the sediments of Camarones River (Atacama Desert, Chile). *PloS One*. 2018; 13(5):e0195080.
30. Li C, Quan Q, Gan Y, Dong J, Fang J, Wang L, Liu J. Effects of heavy metals on microbial communities in sediments and establishment of bioindicators based on microbial taxa and function for environmental monitoring and management. *Science of the Total Environment*. 2020; 749:141555.
31. Li D, Chen J, Zhang X, Shi W, Li J. Structural and functional characteristics of soil microbial communities in response to different ecological risk levels of heavy metals. *Frontiers in Microbiology*. 2022; 13:1072389.
32. Liu J, Chen X, Shu HY, Lin XR, Zhou QX, Bramryd T, Shu WS, Huang LN. Microbial community structure and function in sediments from e-waste contaminated rivers at Guiyu area of China. *Environmental Pollution*. 2018; 235:171-9.
33. Ma J, Ullah S, Niu A, Liao Z, Qin Q, Xu S, Lin C. Heavy metal pollution increases CH₄ and decreases CO₂ emissions due to soil microbial changes in a mangrove wetland: Microcosm experiment and field examination. *Chemosphere*. 2021; 269:128735.
34. Magurran AE. Measuring biological diversity. *Current Biology*. 2021 Oct; 31(19):R1174-7.
35. Margesin R, Płaza GA, Kasenbacher S. Characterization of bacterial communities at heavy-metal-contaminated sites. *Chemosphere*. 2011; 82(11):1583-8.
36. Miller JM, Rhoden DL. Preliminary evaluation of Biolog, a carbon source utilization method for bacterial identification. *Journal of clinical microbiology*. 1991; 29(6):1143-7.
37. Mustapha MU, Halimoon N. Microorganisms and biosorption of heavy metals in the environment: a review paper. *J. Microb. Biochem. Technol*. 2015; 7(5):253-6.
38. Phan K, Murillo M. Standardization of Methods for Characterizing the Physiological Profiles of Aquatic Microbial Communities using EcoPlates.
39. Piotrowska-Seget Z, Kozdrój J. Changes in culturable bacterial community of soil treated with high dosages of Cu or Cd. *Plant Soil Environ*. 2008; 54:520-8.
40. Qi Q, Hu C, Lin J, Wang X, Tang C, Dai Z, Xu J. Contamination with multiple heavy metals decreases microbial diversity and favors generalists as the keystones in microbial occurrence networks. *Environmental Pollution*. 2022; 306:119406.
41. Qi R, Xue N, Wang S, Zhou X, Zhao L, Song W, Yang Y. Heavy metal (loid) s shape the soil bacterial community and functional genes of desert grassland in a gold mining area in the semi-arid region. *Environmental Research*. 2022; 214:113749.
42. Rutgers M, Wouterse M, Drost SM, Breure AM, Mulder C, Stone D, Creamer RE, Winding A, Bloem J. Monitoring soil bacteria with community-level physiological profiles using Biolog™ ECO-plates in the Netherlands and Europe. *Applied Soil Ecology*. 2016; 97:23-35.
43. Rutgers M, Wouterse M, Drost SM, Breure AM, Mulder C, Stone D, Creamer RE, Winding A, Bloem J. Monitoring soil bacteria with community-level physiological profiles using Biolog™ ECO-plates in the Netherlands and Europe. *Applied Soil Ecology*. 2016; 97:23-35.
44. Sandor V, Vidican R, Stoian V, Sandor M. Influences of soil texture, biota and fertilizers on community level physiological profile.
45. Saunders AM, Albertsen M, Vollertsen J, Nielsen PH. The activated sludge ecosystem contains a core community of abundant organisms. *The ISME journal*. 2016; 10(1):11-20.
46. Song J, Shen Q, Wang L, Qiu G, Shi J, Xu J, Brookes PC, Liu X. Effects of Cd, Cu, Zn and their combined action on microbial biomass and bacterial community structure. *Environmental pollution*. 2018; 243:510-8.
47. Stefanowicz A. The Biolog plates technique as a tool in ecological studies of microbial communities. *Polish Journal of Environmental Studies*. 2006; 15(5).

48. Stoian V, Vidican R, Florin P, Corcoz L, Pop-Moldovan V, Vaida I, Vâtcă SD, Stoian VA, Pleșa A. Exploration of soil functional microbiomes—A concept proposal for long-term fertilized grasslands. *Plants*. 2022; 11(9):1253.
49. Stoian V, Vidican R, Rotar I, Păcurar F, Crișan I. Integration of microorganisms into the flows of grassland and forest ecosystems. *Romanian Journal of Grassland and Forage Crops*. 2018; (17):43-53.
50. Sun C, Wu P, Wang G, Kong X. Heavy metals contained within a Pb-Zn waste heap exhibit selective association with microbial modules as revealed by network analysis. *Bulletin of Environmental Contamination and Toxicology*. 2022; 109(6):1067-74.
51. Tipayno SC, Truu J, Samaddar S, Truu M, Preem JK, Oopkaup K, Espenberg M, Chatterjee P, Kang Y, Kim K, Sa T. The bacterial community structure and functional profile in the heavy metal contaminated paddy soils, surrounding a nonferrous smelter in South Korea. *Ecology and Evolution*. 2018; 8(12):6157-68.
52. Wang Y, Lu G, Yu H, Du X, He Q, Yao S, Zhao L, Huang C, Wen X, Deng Y. Meadow degradation increases spatial turnover rates of the fungal community through both niche selection and dispersal limitation. *Science of the Total Environment*. 2021; 798:149362.
53. Wu Q, Du Y, Huang Z, Gu J, Leung JY, Mai B, Xiao T, Liu W, Fu J. Vertical profile of soil/sediment pollution and microbial community change by e-waste recycling operation. *Science of the total environment*. 2019; 669:1001-10.
54. Wu W, Lu HP, Sastri A, Yeh YC, Gong GC, Chou WC, Hsieh CH. Contrasting the relative importance of species sorting and dispersal limitation in shaping marine bacterial versus protist communities. *The ISME Journal*. 2018; 12(2):485-94.
55. Xie Y, Fan J, Zhu W, Amombo E, Lou Y, Chen L, Fu J. Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. *Frontiers in plant science*. 2016; 7:755.
56. Xie Y, Luo H, Hu L, Sun X, Lou Y, Fu J. Classification of genetic variation for cadmium tolerance in Bermudagrass [*Cynodon dactylon* (L.) Pers.] using physiological traits and molecular markers. *Ecotoxicology*. 2014; 2:1030-43.
57. Xiong W, Jousset A, Guo S, Karlsson I, Zhao Q, Wu H, Kowalchuk GA, Shen Q, Li R, Geisen S. Soil protist communities form a dynamic hub in the soil microbiome. *The ISME journal*. 2018; 12(2):634-8.
58. Yang Y, Song Y, Scheller HV, Ghosh A, Ban Y, Chen H, Tang M. Community structure of arbuscular mycorrhizal fungi associated with *Robinia pseudoacacia* in uncontaminated and heavy metal contaminated soils. *Soil Biology and Biochemistry*. 2015; 86:146-58.
59. Zeng XY, Li SW, Leng Y, Kang XH. Structural and functional responses of bacterial and fungal communities to multiple heavy metal exposure in arid loess. *Science of the total environment*. 2020; 723:138081.
60. Zhao ZB, He JZ, Geisen S, Han LL, Wang JT, Shen JP, Wei WX, Fang YT, Li PP, Zhang LM. Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. *Microbiome*. 2019; 7:1-6.