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THE FUNCTIONAL MICROBIAL PROFILE IN HEAVY METAL CONTAMINATED SOILS AFTER ONE AND A HALF YEAR OF BIOREMEDIATION

Loredana CRIȘAN, Vlad STOIAN, Larisa CORCOZ, Bianca POP, Alexandra GHEORGHIȚĂ, Anca PLEȘA, Roxana VIDICAN

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Calea Manastur Street, Cluj-Napoca, Romania

Corresponding author email: vlad.stoian@usamvcluj.ro

Abstract

Bioremediation is a sustainable form of improving the health of soils in heavy metal contaminated urban areas. For the research were selected 5 historically polluted sites from Baia Mare city that were phytoremediated for a period of one and a half year. In all sites, microbial functional profile was analysed in Biolog EcoPlates, a method that enables the detection of microbial heterotrophic communities and their activity in relation to a set of standardized substrates. After a year and a half of bioremediation, the basal activity showed small differences between the microbial functional activity in all 5 analyzed sites. Two sites presented the highest sum of functional activities, with more than 30 units recorded in each. The minimum sum of activity recorded was below 20 units. The same site recorded the highest diversity of the total functional microbiome. The presence of heavy metals is visible in the activation of different functional groups from the total microbial community present in these soils.

Key words: *bioremediation, microbial activity, functional microbiome.*

INTRODUCTION

Soil pollution is a global problem that has already attracted the attention of researchers from various fields, but especially those from the agricultural field, because soil is the main means of agricultural production, which is both source of food for humans and animal feed, and its quality directly influences the quality of life (Ghazaryan et al., 2024), having a significant impact on human health.

Considering the diversity of soil pollutants, a unitary classification using a single criterion could not be achieved, and therefore the sources of contamination and their effects on soil quality, as well as bioremediation methods, must be evaluated separately (Borozan et al., 2021). Most soil pollutants are residues of human activities - biological, domestic and industrial waste, improperly stored waste or manure, which affect the entire soil microbiome and its stability (Briffa et al., 2020; Singha and Chatterjee, 2022). Currently there is a high concern about heavy metal soil pollution, due to their high toxicity, resistance to biodegradation and their ability to accumulate in the long term (Fajardo et al., 2019). For the remediation of these areas, numerous research have been carried out and promising ecological remediation technologies have been developed, which propose the use of plants for the elimination of environmental pollutants (Garbisu et al., 2001).

The current presence of HM into the environment can be associated with the development of mining, metallurgy, industrialization and urbanization, which represent multiple pathways for their accumulation in soils (Ali et al., 2013; Mishra et al., 2023). During the industrial period, in Romania (Baia Mare, Copșa Mică, Zlatna) as in other parts of the world, different levels of soil pollution with heavy metals were recorded in multiple industrial perimeters (Constantinescu, 2008; Haghighizadeh et al., 2024).

Soil microbial community plays an essential role in various fields of human activity, such as agriculture, industry, biotechnology, and health (Malkawi et al., 2024). All biogeochemical cycles that support life on the planet depend on the activity of microorganisms (Brusseau, 2019; Haney et al., 2008). Researchers' interest in soil microbial imbalances is increasing in the context of soil degradation caused by heavy metals (HM) pollution from anthropogenic activities (Campillo-Cora et al., 2025).

The presence of heavy metals consistently affects both bacterial biomass and its activity (Liu et al., 2020). The heavy metals not only impact soil fertility, but also interfere with bacterial communities, leading to a decrease in biodiversity (Pan et al., 2020). Microorganisms are seen as essential factors in maintaining soil fertility (Johns, 2017). Therefore, it is crucial to understand how soil microbial diversity and composition are influenced by different levels of heavy metal contamination (Azarbad et al., 2015).

The context of soil protection and the conservation of microbial resources within this environment is a necessary step toward more resilient ecosystems (Iqbal et al., 2023). The resilience and recovery potential of ecosystems cand be achieved with holistic approaches that stimulate both the microbial communities from soil and the installation of vegetation (Larson et al., 2022; Peddle et al., 2025). Phytoremediation is a modern technique that uses both the plant and their associated microbiome to remove heavy metals from polluted environments with the aim of restoring it to a similar state as the native one (Azubuike et al., 2016; Sarwar et al., 2017; Thijs et al., 2017).

The use of EcoPlate procedure for the analysis of phytoremediation effect on soil microbiome is a viable instrument to detect the metabolic changes of microorganisms in relation to the removal of heavy metals (Liu et al., 2020). The method provides a physiological profile for soil microbiome and enable the detection of the most active functional groups, based on their ability to decompose a standardized set of substrates (Stoian et al., 2022; Urbaniak et al., 2024).

The aim of this article was to assess the kinetics and dynamics of the functional microbiome in the soils of 5 sites from Baia Mare that were historically polluted with heavy metals, after one year and a half of phytoremediation. The EcoPlate method was used to assess the details of functional microbiome profile, the activity and structure as a response to phytoremediation.

MATERIALS AND METHODS

The soil samples were taken in 2022 from 5 historically polluted from Baia Mare, on which a phytoremediation procedure was applied for one and a half year prior to sampling (Pop et al., 2024; https://uia-initiative.eu/en/uia-cities/baia-mare). The five sites (CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb and URB-Urb) are located inside the city of Baia Mare (47°39′ N 23°34′ E), located in the northwestern part of Romania. The locations were selected due to the varying levels of soil contamination with heavy metals and the existence of phytoremediation techniques applied.

For the analysis of soil microbiome traits was selected the Biolog EcoPlates technique, due to its ability of functional microbiome detection and the assessment of their activities in relation to a set of standardized substrates (Pop et al., 2024; Stoian et al., 2022). For these analyses, soil samples were diluted to 10^{-4} prior to the incubation in EcoPlates at room temperature. Measurements were taken at 590 nm using a plate reader, for a period of 5 days. Each reading was performed at 24h, until the readings reached a plateau phase with no further increases in readings observed.

The results from EcoPlates were analyzed according to the methodology proposed by Stoian et al., 2022, which separate the values form plate wells in 5 functional guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM). These functional guilds are composed from a different number of functional groups based on their chemical similarity. The functional groups used in the analysis were selected based on their significant change between sites: W – Water (basal community, as a control for the entire set of substrates), CH2 - d-Cellobiose, CH5 - d-Xylose, CH7 - d-Mannitol, CX3 - d-Galacturonic acid, CX5 - 4-Hydroxy benzoic acid, CX7 - Itaconic acid, AA6 - Glycyl-l-glutamic acid, AM1 – Phenylethylamine. Along with these parameters, the recorded sum of all microbial activities (Sum) was used to analyze the differences between the 5 sites in terms of the total microbial community, while Average Well Development Color (AWCD) was used as an average of metabolic activity (Xie et al., 2006). Diversity indices Shannon-Weiner (H) Simpson (S) and Pielou (J) were used to assess the specific functional diversity of soil microbiome in each site (Lan et al., 2019).

The data analysis was performed in RStudio, version 2022.02.3 (R Core Team, 2024), using the "psych" (Revelle, 2019; Corcoz et al., 2022a) and "agricolae" (de Mendiburu, 2020; Corcoz et al., 2022b) packages. Basic statistics were extracted for all the functional groups and guilds, from which means, and their standard errors were used for the detection of specific physiological level. Diversity indices were calculated in the "vegan" package (Oksanen et al., 2022). Least Significant Differences (LSD) test and ANOVA were used to test the entire database for the assessment of significant differences between the 5 sites.

RESULTS AND DISCUSSIONS

In the current context of the rehabilitation of mining regions, environmental legislation and actors involved in this field, phytoremediation is considered a relatively new, low-cost, environmentally friendly and sustainable method. This technique can be applied in affected mining areas to reduce risks and improve the visual impact on the landscape. (Coman et al., 2009).

Certain heavy metals, such as Cu, Zn and Fe, are necessary for the normal development of microorganisms, but become toxic when present in high concentrations. Heavy metals have been shown to influence microbial populations, having negative effects on cell membranes, growth and metabolic activities, leading to decreased soil microbial biomass and diversity. The tolerance of soil microorganisms to heavy metal contamination varies considerably, and the proportion of resistant culturable microorganisms can fluctuate between 10% and nearly 100% (Abdu et al., 2017; Kamal et al., 2010).

After a year and a half of bioremediation, the basal activity showed small differences between the microbial functional activity in all 5 analyzed sites (Table 1). Two sites presented the highest sum of functional activities, with more than 30 units recorded in each. The minimum sum of activity was observed in the Ferneziu site, where this parameter was below 20 units. The same site recorded the highest diversity of the total functional microbiome.

Table 1. Dimension and diversity of site-specific functional microbiome after bioremediation

Sites	Water	Sum	AWCD	H	S	J
CR	0.07±0.01a	25.39±4.15ab	0.75±0.13ab	2.82±0.11bc	0.93±0.01ab	0.82±0.03bc
CT	0.05±0.03a	29.11±1.24ab	0.89±0.03ab	2.99±0.05ab	0.94±0.00a	0.88±0.01ab
FR	0.07±0.01a	19.18±2.71b	0.55±0.09b	2.72±0.05c	0.91±0.01b	0.79±0.02c
ROMP	0.08±0.00a	34.87±2.19a	1.05±0.07a	3.06±0.01a	0.95±0.00a	89±0.00a
URB	0.06±0.01a	31.77±5.98a	0.96±0.19a	2.98±0.10ab	0.94±0.01a	0.87±0.03ab
F test	0.65	2.76	2.87	3.44	4.04	3.77
p.val	0.640	0.088	0.081	0.052	0.033	0.040

Note: means ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites: Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP). Diversity indices: H – Shannon, S – Simpson, J – Pielou.

In the water group (basal community), the highest level of microbial activity was observed in the ROMP site with a value of 0.08, and the lowest level in the same group was recorded with a value of 0.05 in CT (Table 1). Compared to these values, CR, FR and URB sites do not show significant differences.

The sum of microbial activities shows significant differences between analysed sites. The lowest functional activity was recorded in FR site, with less than 20 absorbance units Compared to this value, CT, ROMP and URB sites showed an activity with 55-65% higher. CR site present an average activity, with 6 units higher than FR and almost 10 units compared to ROMP. A significant difference was identified in ROMP site which showed a microbial activity with 8.9 units higher than FR. After comparing the results from the CR, CT and URB sites, there are no insignificant differences. Following the analyzed values of the AWCD variable, significant differences between the sites were recorded. The highest average activity was identified in ROMP, followed by URB. On the other hand, the lowest value was identified in FR, which presents significant differences from the maximum value, but also from the CR and CT sites. An increase in microbial activity was observed by comparing the values of the sites analyzed. In the ROMP site, which has the highest activity, and FR, which registers the lowest analyzed value. There are no significant differences between CT and URB locations. CR shows 1-unit lower activity than ROMP.

The elimination of heavy metals from the environment represents a major challenge because their decomposition, as in the case of other pollutants, cannot be achieved by biological or chemical methods (Sharma et al., 2023).

The highest diversity (H index) value was recorded in ROMP. Compared to this value, CT and URB locations do not show significant differences (Table 1). The site with the lowest value recorded in FR shows a significant difference compared to ROMP. Insignificant difference of the activities shows the results in CR, compared to CT and URB.

Heavy metals are known for their ability to reduce or inhibit soil enzymatic activity, disrupt carbon, nitrogen, and organic matter transformation processes, and decrease both biodiversity and soil microbial biomass (Giller et al., 2009). As a result, this may favor the emergence of certain microorganisms resistant to heavy metals in the soil (Giller et al., 2009). Soils polluted with heavy metals restrict plant growth due to their toxicity. In addition, heavy metal toxicity influences the size of microbial populations, their diversity, activity and genetic structure (Ayangbenro & Babalola, 2017).

In the analyzed group of polymers, a significant difference is registered in the ROMP site, which presented an activity 1 unit higher than FR, with the lowest value (Table 2). Compared to ROMP, URB does not show significant differences. Similarly, the values in CR and CT show no significant differences.

In terms of CH guild activity, a significant difference is observed between ROMP site, which presents the highest analyzed value, with 50% compared to FR, which has the lowest activity level and respectively 4 units compared to the CR site. The CT and URB sites are significantly different from each other by 1 unit.

The highest recorded value for CX is presented in the ROMP site, and the lowest activity in FR There is a significant difference between the two locations of 60%. After comparing the results between CT and URB, we notice that there are no significant differences, but it shows a difference of 1 unit higher than CR.

Between the sites analyzed for AA guild, the highest microbial activity was recorded in URB, with a value of 7.81, and the lowest value of 3.82 in FR. There was a significant difference between the two locations. The CR site shows a difference of 2 units from the FR. CT and ROMP values show no significant differences.

In the analyzed group AM, significant difference of 1 unit exists between the maximum value of 1.71 in URB and the minimum value of 0.96 in CT. Compared to the maximum value analyzed, the CR, FR and ROMP sites do not show significant differences.

Table 2. Site-specific effect of bioremediation on functional guilds activities

Sites	P	CH	CX	AA	AM
CR	3.78±1.05a	6.37±0.23bc	8.73±1.99ab	5.21±1.45ab	1.31±0.29a
CT	3.40±0.20a	8.92±0.37ab	9.30±1.54ab	6.54±0.70a	0.96±0.58a
FR	3.32±0.87a	5.09±0.71c	5.40±1.31b	3.82±0.85b	1.55±0.60a
ROMP	4.35±0.65a	10.07±0.98a	11.86±1.68a	6.90±0.08a	1.68±0.16a
URB	4.01±1.07a	9.06±1.31a	9.17±2.61ab	7.81±0.38a	1.71±0.80a
F test	0.27	6.46	1.5	3.54	0.34
p.val	0.893	0.008	0.273	0.048	0.846

Note: means ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites: Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP).

For CH2 functional group the highest activity was recorded in the CT site, with a value of 1.56, and the lowest activity in CR, presenting a significant difference (Table 3.). The results between FR and CR show no significant differences. There was also no significant difference in activity between ROMP and URB sites. In the CH5 functional group, there was a significant difference between the CT site with the highest value of 0.98 and the CR site with the lowest value of 0.07. FR and URB sites show no significant differences between them. ROMP showed a 0.91 increase in activity over CR.

After analyzing the CH7 group, the ROMP site shows an activity 2 units higher than FR resulting in significant differences between them. URB with the value of 2.17, does not show significant differences compared to ROMP. CR and CT sites show higher activity than FR.

The highest functional activity of CX3 was recorded in ROMP with a value of 2.25 and the lowest activity was recorded in FR with a value of 0.15 (Table 3). The URB and CR sites do not show significant differences between them. Functional activity in the CT site was 1 unit higher than in FR. A significant difference of 1 unit was observed between ROMP and FR sites for CX5 group. CR and URB sites show no significant differences. Compared to these values, a decrease in microbial activity was observed in CT.

The analysis of CX7 functional group values shows significant differences. The highest value was recorded in ROMP with more than 91% compared to CT, which has the lowest activity. FR and URB sites show no significant differences between them. Compared to these values, a decrease in CR activity was observed.

The highest activity of AA6 functional microbiome was recorded in the URB site with a value of 0.81, and the lowest activity was recorded in the CR site with a value of 0.14. Compared to these values, CR shows 64% lower activity than CT. There are no significant differences between the values of the FR and ROMP sites.

Table 3. Differences between the most significant activities of functional groups after bioremediation

Sites	CH2	CH5	CH7	CX3	CX5	CX7	AA6
CR	0.82±0.11b	0.07±0.01b	1.13±0.62ab	1.45±0.70ab	1.72±0.24a	1.18±0.56ab	0.14±0.07b
CT	1.56±0.09a	0.98±0.14a	1.47±0.45ab	1.99±0.06a	1.02±0.56ab	0.07±0.00b	0.78±0.07a
FR	0.83±0.06b	0.43±0.15ab	0.65±0.58b	0.15±0.08b	0.23±0.17b	1.85±0.14a	0.26±0.16ab
ROMP	1.05±0.08b	0.96±0.41a	2.21±0.13a	2.25±0.05a	1.73±0.22a	1.92±0.18a	0.20±0.05b
URB	1.02±0.13b	0.31±0.24ab	2.17±0.06a	1.52±0.72ab	1.60±0.51a	1.38±0.63a	0.81±0.34a
F test	9.78	3.07	2.38	3.2	2.96	3.69	3.44
p.val	0.002	0.068	0.121	0.062	0.075	0.043	0.051

Note: means ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites: Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP).

CONCLUSIONS

The presence of heavy metals was visible in the activation of different functional groups from the total microbial community present in these soils. The basal community showed reduced activities within all analysed sites but performed well in specific functional groups and guilds.

The maximum sum of activities was recorded in Romplumb and Urbis sites, with more than 31 absorbance units each.

Carbohydrates and Carboxylic & acetic acids functional guilds showed the highest metabolic activities in Colonia Topitorilor, Romplumb and Urbis sites.

Amino acids guild showed the lowest activity in Ferneziu site, while Amines/amides guild had an activity bellow 1 unit in Colonia Topitorilor site.

The most significant activities of functional groups were recorded in Romplumb site (CH7 and CX3).

Ferneziu site presented the lowest activities for CH2, CH5, CH7, CX3 and CX5 compared to the other 4 sites analysed.

REFERENCES

Abdu, N., Abdullahi, A. A., & Abdulkadir, A. (2017). Heavy metals and soil microbes. *Environmental chemistry letters*, *15*(1), 65–84.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals concepts and applications. *Chemosphere*, *91*(7), 869–881.

Ayangbenro, A. S., & Babalola, O. O. (2017). A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *International journal of environmental research and public health*, *14*(1), 94.

Azarbad, H., Niklińska, M., Laskowski, R., van Straalen, N. M., van Gestel, C. A., Zhou, J., ... & Röling, W. F. (2015). Microbial community composition and functions are resilient to metal pollution along two forest soil gradients. *FEMS Microbiology Ecology*, *91*(1), 1–11.

Azubuike, C. C., Chikere, C. B., & Okpokwasili, G. C. (2016). Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. *World Journal of Microbiology and Biotechnology*, *32*, 1–18.

Borozan, A. B., Bordean, D. M., Poiana, M. A., Alexa, E., Caba, I. L., Pîrvulescu, L., ... & Petcu, C. D. (2021). Soil pollution with heavy metals and bioremediation methods. *AgroLife Scientific Journal*, *10*(1), 54–66.

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*, *6*(9).

Brusseau, M. L. (2019). Ecosystems and ecosystem services. In *Environmental and pollution science* (pp. 89-102). Academic Press.

Campillo-Cora, C., Rodríguez-Seijo, A., Pérez-Rodríguez, P., Fernández-Calvino, D., & Santás-Miguel, V. (2025). Effect of heavy metal pollution on soil microorganisms: Influence of soil physicochemical properties. A systematic review. *European Journal of Soil Biology*, *124*, 103706.

Coman, M., Oros, V., Miloiu, E., Taro, G., & Pop, R. (2009). Posibilităţi de Remediere a unor Situri Miniere Contaminate din România. *ProEnvironment Promediu*, *2*(4).

Constantinescu, L. (2008). Soil pollution with heavy metals in critical areas. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture*, *65*(2).

Corcoz, L., Păcurar, F., Pop-Moldovan, V., Vaida, I., Pleșa, A., Stoian, V., & Vidican, R. (2022a). Long-term fertilization alters mycorrhizal colonization strategy in the roots of agrostis capillaris. *Agriculture*, *12*(6), 847.

Corcoz, L., Păcurar, F., Vaida, I., Pleșa, A., Moldovan, C., Stoian, V., & Vidican, R. (2022b). Deciphering the colonization strategies in roots of long-term fertilized festuca rubra. *Agronomy*, *12*(3), 650.

de Mendiburu, F. (2023). agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-7, https://CRAN.R-project.org/package=agricolae

Fajardo, C., Costa, G., Nande, M., Botías, P., García-Cantalejo, J., & Martín, M. (2019). Pb, Cd, and Zn soil contamination: monitoring functional and structural impacts on the microbiome. *Applied Soil Ecology*, *135*, 56–64.

Garbisu, C., & Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource technology*, *77*(3), 229-236.

Giller, K. E., Witter, E., & McGrath, S. P. (2009). Heavy metals and soil microbes. *Soil Biology and Biochemistry*, *41*(10), 2031–2037.

Ghazaryan, K., Agrawal, S., Margaryan, G., Harutyunyan, A., Rajput, P., Movsesyan, H., ... & Singh, A. (2024). Soil pollution: an agricultural and environmental problem with nanotechnological remediation opportunities and challenges. *Discover Sustainability*, *5*(1), 1–33.

Haney, R. L., Brinton, W. H., & Evans, E. (2008). Estimating soil carbon, nitrogen, and phosphorus mineralization from short-term carbon dioxide respiration. *Communications in soil science and plant analysis*, *39*(17-18), 2706–2720.

Haghighizadeh, A., Rajabi, O., Nezarat, A., Hajyani, Z., Haghmohammadi, M., Hedayatikhah, S., ... & Beni, A. A. (2024). Comprehensive analysis of heavy metal soil contamination in mining Environments: Impacts, monitoring Techniques, and remediation strategies. *Arabian Journal of Chemistry*, 105777.

Iqbal, B., Li, G., Alabbosh, K. F., Hussain, H., Khan, I., Tariq, M., ... & Ahmad, N. (2023). Advancing environmental sustainability through microbial reprogramming in growth improvement, stress alleviation, and phytoremediation. *Plant stress*, *10*, 100283.

Johns, C. (2017). Living soils: the role of microorganisms in soil health.

Kamal, S., Prasad, R., & Varma, A. (2010). Soil microbial diversity in relation to heavy metals. *Soil heavy metals*, 31–63.

Lan, X., Du, H., Peng, W., Liu, Y., Fang, Z., & Song, T. (2019). Functional diversity of the soil culturable microbial community in eucalyptus plantations of different ages in Guangxi, South China. *Forests*, *10*(12), 1083.

Larson, J. L., Venette, R. C., & Larson, D. L. (2022). Restoration for resilience: The role of plant–microbial interactions and seed provenance in ecological restoration. *Natural Areas Journal*, *42*(2), 152–159.

Liu, H., Wang, C., Xie, Y., Luo, Y., Sheng, M., Xu, F., & Xu, H. (2020). Ecological responses of soil microbial abundance and diversity to cadmium and soil properties in farmland around an enterprise-intensive region. *Journal of hazardous materials*, *392*, 122478.

Malkawi, H. I., & Kapiel, T. Y. S. (2024). Microbial biotechnology: a key tool for addressing climate change and food insecurity. *European Journal of Biology and Biotechnology*, *5*(2), 1–15.

Mishra, S., Singh, G., Gupta, A., & Tiwari, R. K. (2023). Heavy metal/metalloid contamination: their sources in environment and accumulation in food chain. In *Heavy metal toxicity: environmental concerns, remediation and opportunities* (pp. 19-47). Singapore: Springer Nature Singapore.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn. D,, Ouellette, M., Ribeiro Cunha, E., Smith T, Stier A, Ter Braak C, Weedon J (2022). _vegan: Community Ecology Package_. R package version 2.6-4,https://CRAN.R-project.org/package=vegan.

Pan, X., Zhang, S., Zhong, Q., Gong, G., Wang, G., Guo, X., & Xu, X. (2020). Effects of soil chemical properties and fractions of Pb, Cd, and Zn on bacterial and fungal communities. *Science of the Total Environment*, *715*, 136904.

Peddle, S. D., Hodgson, R. J., Borrett, R. J., Brachmann, S., Davies, T. C., Erickson, T. E., ... & Breed, M. F. (2025). Practical applications of soil microbiota to improve ecosystem restoration: current knowledge and future directions. *Biological Reviews*, *100*(1), 1–18.

Pop, B., Vidican, R., Corcoz, L., & Stoian, V. (2024). The effect of historical pollution on microbial functional kinetics in bioremediated soils from Baia Mare. *Scientific Papers. Series A. Agronomy*, *67*(2).

R Core Team (2024). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Revelle, W. (2024). psych: Procedures for psychological, psychometric, and personality research. Northwestern University, Evanston, Illinois. *R package version*, *2.4.3* https://CRAN.R-project.org/package=psych

Sarwar, N., Imran, M., Shaheen, M. R., Ishaque, W., Kamran, M. A., Matloob, A., ... & Hussain, S. (2017). Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. *Chemosphere, 171*, 710–721.

Sharma, J. K., Kumar, N., Singh, N. P., & Santal, A. R. (2023). Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. *Frontiers in Plant Science*, *14*, 1076876.

Singha, S., & Chatterjee, S. (2022). Soil pollution by industrial effluents, solid wastes and reclamation strategies by microorganisms. In *Soil Health and Environmental Sustainability: Application of Geospatial Technology* (pp. 471-488). Cham: Springer International Publishing.

Stoian, V., Vidican, R., Florin, P., Corcoz, L., Pop-Moldovan, V., Vaida, I., ... & Pleșa, A. (2022). Exploration of soil functional microbiomes - A concept proposal for long-term fertilized grasslands. *Plants*, *11*(9), 1253.

Thijs, S., Sillen, W., Weyens, N., & Vangronsveld, J. (2017). Phytoremediation: state-of-the-art and a key role for the plant microbiome in future trends and research prospects. *International journal of phytoremediation*, *19*(1), 23–38.

Urbaniak, M., Mierzejewska-Sinner, E., Bednarek, A., Krauze, K., & Włodarczyk-Marciniak, R. (2024). Microbial response to Nature-Based Solutions in urban soils: A comprehensive analysis using Biolog® EcoPlates™. *Science of the Total Environment*, *928*, 172360.

Xie, Y., Fan, J., Zhu, W., Amombo, E., Lou, Y., Chen, L., & Fu, J. (2016). Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. *Frontiers in plant science*, *7*, 755.

*** https://uia-initiative.eu/en/uia-cities/baia-mare