Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INCREASED ACTIVITY OF SPECIALIZED FUNCTIONAL MICROBIOMES IN HISTORICALLY POLLUTED SOILS AFTER BIOREMEDIATION

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

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

Soil heavy metal pollution is one of the major concerns in urban habitats, due to the associated human and environmental risks. Materials and methods: The aim of this research is to use the DEMSA model to explore the increases in the soil microbial functional profile, based on Biolog Ecoplates results, due to historical pollution with heavy metals. The historical pollution of the 5 sites analyzed induces a high variability in the soil functional microbiomes. The functional intensification is visible in the sum of all activities and the Index of Intensification from each site. At this niche level the Carboxylic and acetic acids guild shows the highest share of activities. Above the intensification level, a guild expansion is observed only in three of the analyzed sites. For these sites, the Index of Expansion is set in the interval of 2.5-6.0, which indicates the presence of multiple functional groups adapted to heavy metals. Historical pollution alters the assemblage of soil general microbial community, by increasing the activity of a small number of very specialized groups.

Key words*: functional increase, intensification matrix, guild expansion, index of alteration.*

INTRODUCTION

Soil pollution with heavy metals in urban areas present an important concern and a high problem for both human and ecosystem health (Binner et al., 2023). In multiple urban areas, historical anthropic activities led to increased levels of heavy metals which had been accumulated in soils and represent a serious threat to human health (Adewumi & Ogundele, 2024). Heavy metals produce a strong destabilizing effect on soil microbial communities, altering their temporal stability and functional profiles, which affect their ecosystem services (Tang et al., 2024). Thus, a more sensitive approach in monitoring and understanding the risks related to heavy metal levels from soils is important in the selection of most suitable methods to remove them from ecosystems (Ahirvar et al., 2023; Shi et al., 2023).

Bioremediation is a viable solution for removing heavy metals from environment, and rebuild plant and microbial communities (Pande et al., 2022; Verma et al., 2021). This process sustains the recovery of soil microbiome and enhance the ecological restoration (Zheng et al., 2024). Constant monitoring of bioremediation is necessary to understand the efficiency of the process, and to forecast vegetation and soil changes (Kuppan et al., 2024; Sales et al., 2020; Bala et al., 2022). These approaches are necessary to constantly increase the heavy metal removal by integrating supplementary solutions (Azhar et al., 2022; Liu et al., 2020; Wang et al., 2022).

Microbial communities are defined by complex assemblages and interactions, based on high species diversity and functions. Based on these traits, the assessment of soil microbiomes and the changes in their activity and community profile, represent a suitable method for the detection of heavy metals impact (Abbaszade et al., 2023; Melloni et al., 2023; Tang et al., 2024).

The aim of this study is to analyze the increasing activities trends in microbial functional profile from 5 historically contaminated soils with heavy metals, after one year of phytoremediation. The proposed objectives are: i) the assessment of microbial functional profile toward an urban standard; ii) the analysis of functional guilds that exceed the activity in urban standard; iii) the assessment of functional alteration and diversity changes in microbial profile.

MATERIALS AND METHODS

Soils from 5 historically polluted sites in Baia Mare (Baia Mare County) were sampled after one year of phytoremediation (Pop et al., 2024). The five sites selected for analysis were: Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP).

Samples were diluted to 10^{-4} and were subjected to inoculation in Biolog Ecoplates, for each sample being selected 3 replications. The entire incubation last 96 hours, with reading performed at 590 nm every 24 h for the detection of changes in microbial activity.

Values of all readings at 96 h, separately for each substrate, were used to calculate the average standard of microbial functional activity. To this average, the +/- standard deviation was applied to set the minimum and maximum potential activity. These three values for each substrate were used as a control in the Detailed Exploration of Microbial Sociological Assemblage (DEMSA) model (Stoian et al., 2022). Based on comparison with the control, all the values that exceeded the average, but were below the maximum were used to build the intensification matrix. All the data that exceeded the maximum of the control were assembled in the expansion matrix. Following Stoian et al. (2022) method, each substrate was considered as a functional group, and each functional groups was associated with a functional guild, based on substrate chemical traits. The functional guilds used in the assessment of pollution impact were Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM).

The procedure was applied to score the most reduced changes in each of the site microbial functional profile, compared to the general microbial activity within the urban area. All data were analyzed in RStudio (R Core Team, 2024), with formulas from packages “psych” (Revelle, 2024) - basic statistics, “agricolae” (de Mendiburu, 2023) - ANOVA and LSD test, and “vegan” (Oksanen et al., 2022) - NMDS ordinations. All functional guilds were analysed for their potential interaction with correlations form the package “Hmisc” (Harrell, 2025).

RESULTS AND DISCUSSIONS

The use of the DEMSA concept in screening the changes of soil functional microbiome from polluted soils provides a detailed perspective on both the diversity of activities and community groups (Stoian et al., 2022; Vidican et al., 2016). Intensification of activities is analyzed as the proliferation of guilds (and groups) within the upper limits of the standard community. In comparison, expansion is analyzed as activities that exceed the maximum limit of the standard community and present a specialized proliferation in functional community.

Microbial functional guilds indicate an intensification of activities and present a large variation between sites (Table 1). The mean of activities extracted in the Intensification matrix showed the greatest values associated with CH and CX guilds. CT site presented a maximum of activity, but the differences compared to next two sites (CR and FR) was not significant. An interesting phenomenon was observed for sites ROMP and URB, where the activity was 20 to 200 times lower. This difference indicates a significant decrease in the microbial population in this guild. In terms of CX activity, the greatest values were observed in FR site, with a similar difference compared to ROMP and URB.

Between FR and CR, a significant difference was detected, which offer a gradient of activities in this functional guilds (Table 1). The gradient show two sites that present a higher activity in FR and CT, a medium activity in CR and very low activities in ROMP and URB. For the P, AA and AM guilds ROMP present no activity, while URB microbiome showed reduced activities. Both P and AA presented similar activities associated with FR conditions, while AM reduced its activity at half. AA presented higher activities in CR and FR compared to P and AM guilds, which indicate a better condition for this type of microbiome.

Based on functional guilds, the sum of recorded activities presented large variations between sites (Table 1). CT sites showed the highest sum of activities, with two absorbance units higher than in FR site. CR site present a 6 absorbance unit reduction of microbial activity, while for ROMP and URB this parameter was under 1 unit. These values are visible in the values of Intensification Index, which scores the site-specific changes in the entire microbiome that exceed the average limits of the activity standard.

Table 1. Microbial functional guilds and diversity in Intensification range of activities

Sites	CH	P	CX	AA	AM
CR	2.27±1.17a	0.56±0.14c	2.75±0.24b	1.67±0.22b	0.18±0.18b
CT	4.03±0.16a	2.06±0.16a	3.79±0.62a	2.77±0.50a	1.11±0.03a
FR	2.46±0.46a	1.29±0.12b	4.37±0.07a	2.66±0.46ab	0.84±0.11a
ROMP	0.02±0.02b		0.05±0.05c		
URB	0.23±0.23b	0.09±0.09d	0.24±0.24c	0.05±0.05c	0±0b
Sites	Sum	Index of	Shannon	Simpson	Pielou
CR	7.45±1.02b	17.9±1.56b	2.56±0.15a	0.91±0.01ab	0.94±0.01a
CT	13.7±0.85a	25.3±1.10a	3.31±0.02a	0.96±0.00a	0.97±0.00a
FR	11.6±0.54a	22.6±0.82a	3.06±0.02a	0.94±0.00a	0.93±0.01a
ROMP	0.16±0.13c	0.76±0.55c	0.25±0.25b	0.49±0.28b	0.23±0.23b
URB	0.62±0.62c	2.05±2.05c	0.55±0.55b	0.92±0.07ab	0.26±0.26b

Note: values ± 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); guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM); Index of - Index of Intensification.

The highest change in the microbiome pattern was near to 25% in CT site (Table 1). This value indicates a shift in over a quarter of functional microbiome, followed by FR site where this index is set to 22%. At opposite, ROMP maintained its functional community near to the standard, which indicate that soil conditions significantly restrict the entire microbial community.

Diversity indices showed different values that enable the qualitative scoring of the functional microbiome assemblage (Table 1). Shannon-Wiener diversity index (Baliton et al., 2020) showed a diverse community in both CT and FR sites, that indicates a microbial community adapted to the site conditions.

A moderate diversity is visible in CR site, while both ROMP and URB showed very low diversity. Based on this index the sites with low values presented only a fraction of their microbial community that can exceed the area standard, which can be visible in a longer period for the restoration of an intense activity. For the sites with high diversity, the number of functional groups in community is higher and is associated with a coupled activity that can progress faster toward a restoration point.

The Simpson diversity index was used to score both the number of functional microbiomes in each site and their distribution within community (Johnson & Burnet, 2016; Vidican et al., 2016). For ROMP site, the functional microbiome presented a medium heterogeneity, while for the rest of 4 sites this index showed a high heterogeneity (Table 1). This implies a specific localized reaction of soil microbiome that is not replicable in other sites, even in the same area.

There is a large uniformity of functional microbiomes in CT site, and an almost balanced community for CR and FR sites, based on Pielou index. On the other way, the values of this index in ROMP and URB showed unbalanced evenness in community.

The analysis of functional microbiome that exceed the maximum activity of the control was assembled in the Expansion matrix (Table 2). Compared to the Intensification segment of activities, for this community is not present in ROMP and URB sites. All sites presented lower activities that suggest a functional microbiome that is able to proliferate in pollution conditions, but not completely adapted to these. CT site presented the highest activities for CH, P and AA functional guilds, while CX and AM were associated with FR site, both communities presenting a different pattern of consortium. The most visible differences were observed in CX guild, with a significant decrease from FR to CR site. The reduction coefficient for this guild is 2-2.5 times between sites. The sum of activities showed significant differences between CT, FR and CT sites. For the last site, the sum of all activities does not exceed the activity of CH guild in CT site.

In terms of functional modification, the microbiome that exceeds the limits of the standard ecological niche present values in a range between 2.5-6.0 (Table 2). This implies a reduced change in the community assemblage pattern, with the only a reduced potential for evolving toward a highly different pattern.

Diversity analyzed through all three indices shows a moderate community associated with CT conditions, while for FR the value is at the limit between low and moderate (Table 2). CT site presented a very low diversity, which implies a specialized community that can proliferate in this conditions. The higher values for the other two sites indicates a larger functional microbiome that can exceed the standard limits and proliferate in pollution conditions. In terms of pollution effect, Shannon index present a medium to low effect on functional communities. The Simpson index show a medium to high heterogeneity of microbiome, which indicate a future potential development of multiple specialized groups that can use at a higher level soil conditions. Pielou`s index show an almost balanced community, with functional activities that maintain a similar pattern.

Table 2. Microbial functional guilds and diversity in Expansion range of activities

Sites	CH	P	CX	AA	AM
CR	0.33±0.28b		0.20±0.10c	0.44±0.24ab	0.07±0.07ab
CT	1.10±0.25a	0.27±0.18	0.62±0.12b	0.99±0.19a	0.21±0.12ab
FR	0.45±0.28ab	0.08±0.04	1.39±0.19a	0.52±0.29ab	0.26±0.09a
Sites	Sum	Index of	Shannon	Simpson	Pielou
CR	1.06±0.11b	2.56±0.14b	1.19±0.28b	0.64±0.09b	0.90±0.03a
CT	3.21±0.35a	5.90±0.64a	2.28±0.03a	0.87±0.01a	0.90±0.03a
FR	2.73±0.16a	5.31±0.26a	2.00±0.14a	0.83±0.02ab	0.88±0.02a

Note: values ± s.e. followed by different letters present significant differences according to LSD (*p<0.05*). Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR); guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM); Index of - Index of Expansion.

The differences between sites in terms of intensification potential is visible in the position on NMDS ordination graph (Figure 1). From all 5 sites, ROMP and URB show the highest stability, with points placed in the same proximity. The CT, CT and FR sites showed a higher difference in the population of their specific communities. The position of CR, ROMP and URB on the opposite side of guild vectors implies a combined effect of multiple functional groups for shaping the assemblage of intensified community. The rest of the two sites showed a position in the minus quadrats defined by axis 1.

Both P and AM guild vectors are oriented toward the centroid of CT group, the vectors indicating these two guilds as potentially responsible for the future changes within the community (Figure 1). The two guilds have a medium position in the community, which makes them good candidates for reorganizing the entire community by an increase in their activity.

An interesting case was observed based on the position of FR plots on ordination (Figure 1). One functional community presents a similar change pattern with the centroid of CT sites. The position near to P vector indicates for this point a higher importance of P guild in the transformation of functional community. The rest of the two plots showed divergent directions of evolution. One plot is oriented toward CR community, outside the influence of a specific guild. For this site there is a potential change due to multiple group interaction. The third plot is placed on the SUM vector direction, which indicates an increase in the global functional activity.

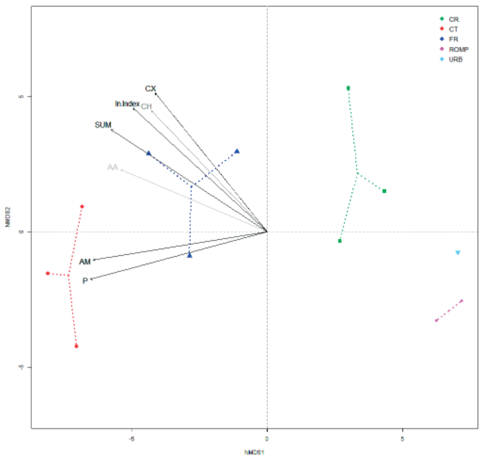

Figure 1. NMDS projection of microbial communities in Intensified niche

Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP); In - Index of intensification; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM)

The Index of intensification vector is placed near to this site and indicates a highly potential change within the community that will evolve into a different pattern of microbiota (Figure 1). AA and CH vectors have a lower impact on the development of functional community. Their position indicates an additional effect of the two guilds for the future shape of microbial assemblage.

The projection of sites that showed an expansion potential in the functional microbiome indicates three different microbial community structure (Figure 2). The most stable and similar community is observed in

CR site, with points placed near to the center of the ordination and with a reduced space between them. The second site in terms of similarity and stability of community is FR, with a point in the ++ quadrat and two points in the +– quadrat. The first point shows a direction of evolution toward CX and AM vectors, indicated the impact of these two guilds in the future development of the community. The last two points showed a divergent orientation and no correlation with any of the guild vectors. This indicates a different pattern of functional activities and a microbiome that will evolve due to the interactions between multiple functional groups.

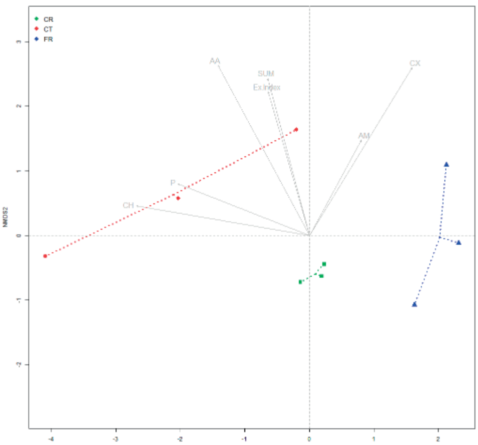

Figure 2. NMDS projection of microbial communities in Expansion niche

Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR); In - Index of Expansion; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM)

The CT points show an interesting projection on the ordination. Two points are placed at a large distance, while the third point is positioned in the middle of this distance (Figure 2). This last point has a microbiome associated with the P and CH guild activity, that are responsible for the development of the community. The most interesting case is represented by the point from CT site that is placed in the upper part of the ordination. Its position is near the vectors represented by the sum of activity and the index of expansion, both suggesting an alteration of the normal shape of community due to an increase in the sum of activities. This point is also placed between AA and CX(+AA) vectors, even at a larger distance, which indicate the role of these guilds in the assemblage of future and reorganized community.

At guild level, groups showed a significantly different importance in the projection of their communities on NMDS ordination (Table 3). The highest significant in the shape of intensification communities is associated with CX2 and AA6 functional groups. Compared to these groups, numerous ones showed a distinct importance for the development of soil functional microbiomes. Each functional guild present two distinct groups, except AM guild - where each functional group have a different level of impact. Twelve functional groups presented a significant impact for the development of their communities, most of them in the carbohydrates guild.

Table 3. Microbial functional groups associated with Intensification and Expansion in the range of activities

Niche segment	Group	Sig	Group	Sig	Group	Sig
Intensification	P1	*	CH3	*	CX1	*
	P3	**	CH4	*	CX2	***
	P4	**	CH5	*	CX3	**
	AA2	*	CH6	*	CX4	*
	AA3	*	CH8	**	CX7	*
	AA4	**	CH9	**	CX8	**
	AA5	**	CH10	*	AM1	**
	AA6	***			AM2	*
Expansion	AA3	**	CH3	*	CX4	*
	AA4	**	CH6	*	CX8	*
	AA5	*				
	AA6	*				

Note: groups assessed with * showed a significant impact in NMDS ordination (*p<0.05**; *p<0.01***; *p<0.001* ***). Legend: functional groups – P1 – Tween 40, P3 – α-Cyclodextrin, P4 – Glycogen, CH3 – α-d-Lactose, CH4 – β-Methyl-d-glucoside, CH5 – d-Xylose, CH6 – i-Erythritol, CH8 – N-Acetyl-d-glucosamine, CH9 – Glucose-1-phosphate, CH10 – d,l-α-Glycerol phosphate, CX1 – d-Glucosaminic acid, CX2 – d-Galactonic acid γ-lactone, CX3 – d-Galacturonic acid, CX4 – 2-Hydroxy benzoic acid, CX7 – Itaconic acid, CX8 – α-Keto butyric acid, AA2 – l-Asparagine, AA3 – l-Phenylalanine, AA4 – l-Serine, AA5 – l-Threonine, AA6 – Glycyl-l-glutamic acid, AM1 – Phenylethylamine, AM2 – Putrescine.

In terms of expansion, only a reduced number of functional groups can act significantly in the development of new community groups. From these, two functional groups show significant importance for each of CH and CX guilds (Table 3). An interesting case was observed for AA guilds, where 4 functional groups showed different levels of significance. Both P and AM associated groups have a reduced singular impact in the development of soil microbiome.

At community level the interactions between functional guilds are responsible for the development of a specific assemblage and diversity (Table 4). All the correlations between parameters and indices in the intensification niche are significant ($p<0.05$). This indicate a coupled activity and positive influence between functional guilds. This type of interaction is characteristic to polluted soils where the number of microorganism is reduced and their potential niche is not completely overlapped. Also, this type of community present functional microbiomes that are more generalist that specialized and the community maintain its diversity. All the functional guilds are responsible for the growth of the total activity, and between them are visible positive interactions. The highest level of association between two guilds is 0.90 (AA and CX), a value that indicate a coupled activity. CX and AA guilds are responsible for the highest alteration of microbial assemblages, their activities favoring a shift in the community. An important value is the 0.99 correlation between the sum of activity and the index of alteration, which sustain the constant change of microbial community shape as the sum of activities increase. These two parameters are responsible for the increase of diversity (H) and the development of a heterogenous microbiome (S). Heterogeneity is achieved in the community based on the Shannon index increase along with the alteration observed in the microbiota. Both CX and AA guilds are responsible for the sustain of heterogeneous cluster, and lead to an increase in the Simpson index as their activity increase.

Table 4. Interrelations between microbial guilds and indices in Intensification and Expansion niche

									Intensification
	P	CH	CX	AA	AM	Sum	In	H	S
P		*0.77*	*0.80*	*0.85*	*0.88*	*0.90*	*0.87*	*0.84*	*0.70*
CH	0.49		*0.81*	*0.73*	*0.71*	*0.90*	*0.89*	*0.86*	*0.72*
CX	0.16	0.37		*0.90*	*0.80*	*0.96*	*0.96*	*0.94*	*0.82*
AA	0.37	0.48	0.48		*0.79*	*0.93*	*0.93*	*0.89*	*0.76*
AM	0.11	0.40	*0.66*	0.34		*0.87*	*0.81*	*0.78*	*0.63*
Sum	0.50	*0.78*	*0.79*	*0.79*	*0.65*		*0.99*	*0.95*	*0.81*
In	0.49	*0.76*	*0.79*	*0.79*	*0.64*	*1.00*		*0.98*	*0.87*
H	*0.53*	*0.78*	*0.76*	*0.65*	*0.68*	*0.95*	*0.96*		*0.94*
S	0.38	*0.59*	*0.65*	*0.69*	*0.59*	*0.82*	*0.87*	*0.90*	
Expansion									

Note: values in *italic* present significant correlations ($p<0.05$). Legend In - Index of alteration; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM).

In the Expansion niche (Table 4) the interrelations between guilds and parameters present lower values. There are no significant positive interrelations between functional guilds, this indicating a specialized niche and a complete different metabolism for each group. In the expansion niche are present the most adapted microorganism and their activity is oriented toward a separated area and resources. None of the functional guilds contribute to a powerful increase in the sum of activities or to a significant shift in the community groups. Both the sum of activities and the index of alteration are completely dependent. This indicates a restricted microbial community, that can proliferate in polluted soils and will evolve toward a more diverse population. These microorganisms can serve as a core for the development of a successional diverse community. Any activity recorded in functional guilds produce an increase in the diversity of the entire microbiome, leading to a more heterogeneous assemblage.

CONCLUSIONS

An increase in the activity, higher than 7.45 absorbance units, associated with the intensification segment of ecological niche produces a functional alteration of microbiomes in a range of 17.9-25.3%.

Activities in the microbial communities that exceed maximum levels are associated with low functional diversities and heterogeneous community assemblages.

Intensification is visible in multiple scenarios for the future development of communities and their functional assemblages, based on the specific reaction in most proliferative guilds.

Expansion in site Craica show a functional stability, while for Colonia Topitorilor was observed a divergent pattern of evolution.

A number of 23 functional groups can present intensified activities, while for the expansion profile only 8 of them present the ability of proliferation.

For both Intensification and Expansion segments of ecological niche an increase in the sum of activities leads to a proportional increase in the profile of functional communities and a constant reshaping phenomenon.

REFERENCES

Abbaszade, G., Toumi, M., Farkas, R., Vajna, B., Krett, G., Dobosy, P., Szabó, C., & Tóth, E. (2023). Exploring the relationship between metal(loid) contamination rate, physicochemical conditions, and microbial community dynamics in industrially contaminated urban soils. *Science of The Total Environment*, *897*, 166094. https://doi.org/10.1016/j.scitotenv.2023.166094

Adewumi, A. J., & Ogundele, O. D. (2024). Hidden hazards in urban soils: A meta-analysis review of global heavy metal contamination (2010-2022), sources and its Ecological and health consequences. *Sustainable Environment*, *10*(1), 2293239. https://doi.org/10.1080/27658511.2023.2293239

Azhar, U., Ahmad, H., Shafqat, H., Babar, M., Shahzad Munir, H. M., Sagir, M., Arif, M., Hassan, A., Rachmadona, N., Rajendran, S., Mubashir, M., & Khoo, K. S. (2022). Remediation techniques for elimination of heavy metal pollutants from soil: A review. *Environmental Research*, *214*, 113918. https://doi.org/10.1016/j.envres.2022.113918

Bala, S., Garg, D., Thirumalesh, B. V., Sharma, M., Sridhar, K., Inbaraj, B. S., & Tripathi, M. (2022). Recent strategies for bioremediation of emerging pollutants: a review for a green and sustainable environment. *Toxics*, *10*(8), 484.

Baliton, R., Landicho, L., Cabahug, R. E., Paelmo, R. F., Laruan, K., Rodriguez, R., Visco, R. G., & Castillo, A. K. A. (2020). Ecological services of agroforestry systems in selected upland farming communities in the Philippines. *Biodiversitas Journal of Biological Diversity*, *21*(2), Article 2. https://doi.org/10.13057/biodiv/d210237

Binner, H., Sullivan, T., Jansen, M. A. K., & McNamara, M. E. (2023). Metals in urban soils of Europe: A systematic review. *Science of The Total Environment*, *854*, 158734. https://doi.org/10.1016/j.scitotenv.2022.158734

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

Harrell, Jr F. (2025). _Hmisc: Harrell Miscellaneous_. R package version 5.2-2, https://CRAN.R-project.org/package=Hmisc

Johnson, K. V.-A., & Burnet, P. W. J. (2016). Microbiome: Should we diversify from diversity? *Gut Microbes*, *7*(6), 455–458. https://doi.org/10.1080/19490976.2016.1241933

Kuppan, N., Padman, M., Mahadeva, M., Srinivasan, S., & Devarajan, R. (2024). A comprehensive review of sustainable bioremediation techniques: Eco friendly solutions for waste and pollution management. *Waste Management Bulletin*.

Liu, S., Yang, B., Liang, Y., Xiao, Y., & Fang, J. (2020). Prospect of phytoremediation combined with other approaches for remediation of heavy metal-polluted soils. *Environmental Science and Pollution Research*, *27*(14), 16069–16085. https://doi.org/10.1007/s11356-020-08282-6

Melloni, E. G. P., Melloni, R., Pastor-Jáuregui, R., Aguilar-Garrido, A., & Martín-Peinado, F. J. (2023). Microbiological indicators as sensitive indicators in the assessment of areas contaminated by heavy metals. *Soil Research*, *61*(7), 663–673. https://doi.org/10.1071/SR23012

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., Fitz John, 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.

Pande, V., Pandey, S. C., Sati, D., Bhatt, P., & Samant, M. (2022). Microbial interventions in bioremediation of heavy metal contaminants in agroecosystem. *Frontiers in microbiology, 13*, 824084.

Prasad Ahirvar, B., Das, P., Srivastava, V., & Kumar, M. (2023). Perspectives of heavy metal pollution indices for soil, sediment, and water pollution evaluation: An insight. *Total Environment Research Themes*, *6*, 100039. https://doi.org/10.1016/j.totert.2023.100039

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

Sales da Silva, I. G., Gomes de Almeida, F. C., Padilha da Rocha e Silva, N. M., Casazza, A. A., Converti, A., & Asfora Sarubbo, L. (2020). Soil bioremediation: Overview of technologies and trends. *Energies*, *13*(18), 4664.

Shi, T., He, L., Wang, R., Li, Z., Hu, Z., & Wu, G. (2023). Digital mapping of heavy metals in urban soils: A review and research challenges. *Catena*, *228*, 107183. https://doi.org/10.1016/j.catena.2023.107183

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.

Tang, S., Wang, C., Song, J., Ihenetu, S. C., & Li, G. (2024). Advances in Studies on Heavy Metals in Urban Soil: A Bibliometric Analysis. *Sustainability*, *16*(2), Article 2. https://doi.org/10.3390/su16020860

Verma, S., Bhatt, P., Verma, A., Mudila, H., Prasher, P., & Rene, E. R. (2021). Microbial technologies for heavy metal remediation: effect of process conditions and current practices. *Clean Technologies and Environmental Policy*, 1–23.

Vidican, R., Stoian, V., & Sandor, M. (2017). *Fertilization and Pesticides as Elements of Pressure on Microbial Communities* (pp. 229–241).

Wang, Z., Luo, P., Zha, X., Xu, C., Kang, S., Zhou, M., Nover, D., & Wang, Y. (2022). Overview assessment of risk evaluation and treatment technologies for heavy metal pollution of water and soil. *Journal of Cleaner Production*, *379*, 134043. https://doi.org/10.1016/j.jclepro.2022.134043

Zheng, X., Lin, H., Du, D., Li, G., Alam, O., Cheng, Z., Liu, X., Jiang, S., & Li, J. (2024). Remediation of heavy metals polluted soil environment: A critical review on biological approaches. *Ecotoxicology and Environmental Safety*, *284*, 116883. https://doi.org/10.1016/j.ecoenv.2024.116883

CROP
SCIENCES