

DYNAMICS OF FUNCTIONAL MICROBIOME IN POLLUTED SOIL AFTER ONE YEAR OF BIOREMEDIATION

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Abstract

Heavy metal pollution is a worldwide major concern. It represents a threat to humans, animals, plant health, and has negative impact on the environment. The Biolog Ecoplate approach was used to observe the daily changes in soil functional microbiome, in 5 sites from Baia mare exposed to historical pollution and after one year of bioremediation. The study has shown that EcoPlates are an effective tool for the assessment of microbial activity in heavy metal contaminated soils in Baia Mare. Microbial activity and diversity decreased with the degree of pollution, highlighting the impact of contamination on the soil microbiome. Within the analysed functional guilds, the values extracted showed a high range of activities. Polymers guild showed the highest activity in CT site, with a similar trend of carbohydrates and the maximum form of activities. Site FR induced the highest activity for carboxylic & acetic acids and amino acids, while the AWCD recorded its maximum.

Key words: heavy metal, pollution impact, microbiome changes, guild activity.

INTRODUCTION

In recent years, soil pollution by heavy metals has become a global concern due to their high toxicity, inability to biodegrade and tendency to accumulate over the long term (Fajardo et al., 2019). Heavy metals not only affect soil fertility, but also alter bacterial communities, causing a significant reduction in biodiversity (Pan et al., 2020). The diversity and number of sensitive soil bacteria may decrease, while resistant bacteria manage to adapt and multiply, leading to the formation of a specific bacterial community structure (Liu et al., 2020). Heavy metal contamination constantly influences bacterial biomass and activity (Kavamura and Esposito., 2010).

Soil is the substrate of terrestrial ecosystems and plays an important role in maintaining their balance. It has multiple functions, that include the sustain of food systems, the regulation of climate and water resources, and provide a habitat for many life forms (Ponge et al., 2015). This resource enable the continuous production of food for an increased population, maintain the biodiversity and biomass yields, along with carbon sequestration (Silver et al., 2021; Tahat et al., 2020). In Europe, there are estimated

approximately 2.5 million sites with potentially contaminated soils, with heavy metals considered some of the most common contaminants (Pb, Cd, Cu, Zn) (Pérez et al., 2018). This leads to significant problem due to their lack of biodegradation, which lead to ecotoxicity and the cumulative behaviour (Fajardo et al., 2019; Pan et al., 2020).

Various research has highlighted that the presence of heavy metals in soil cause adverse effects on the soil microbiome, which lead to compromised soil functions (Xu et al., 2017; Jacquiod et al., 2018; Zhao et al., 2019). Heavy metal pollution generates stress that can influence the characteristics of bacterial communities (Lin et al., 2016).

Microbial communities in soil contribute significantly to nutrient cycling, soil fertility enhancement and carbon sequestration, which are essential for sustaining soil functionality (Fierer et al., 2017).

Toxic stress caused by heavy metals and metalloids significantly affect the diversity and structure of soil microbial communities (Zhao et al., 2019) and alters ecosystem functions (Bai et al., 2020). The metal contaminants impact functional genes and perturb soil ecological functions mediated by specialized

groups (Yin et al., 2015; Liu et al., 2018). Also, the interaction between soil microorganisms and heavy metals influences their oxidation state and lead to immobilization process that link them to organic compounds (Ahmad et al., 2019; Hou et al., 2020). This interaction has a direct impact on the bioavailability and ecotoxicity of metals (Gadd, 2024), and some bacteria can synthesize chelated compounds with siderophores that attach to metals such as Cd, Pb and Zn, leading to the formation of bio precipitates with low bioavailability (Gadd, 2024; Silva et al., 2024).

At opposite, increased species diversity in soil communities act to soil strength and resilience improvements (Shu et al., 2021).

The Biolog method is a viable used technique for analysing the metabolic activity and catabolic potential of bacterial communities (Dror et al., 2020). The Biolog Ecoplate system includes a microplate with 96 wells, each containing 31 different types of carbon substrates (Gryta et al., 2014). Community-level physiological profiling (CLPP) provides a simple and efficient method to assess the natural variation and diversity of soil microbial communities (Gomez and Garland, 2012; Gao et al., 2018). This method is based on the capacity of microorganisms to utilize various organic sources (Epelde et al., 2015; Li et al., 2015) and allows the effects of soil management practices and pollution on microbial communities to be highlighted (Gryta et al., 2014; Epelde et al., 2015; Li et al., 2015). The study of microorganisms resistant to heavy metals and metalloids has proven useful in bioremediation research in contaminated areas (Hu et al., 2021). However, there is currently limited data on the relationship between the diversity, structure and function of soil microbial communities in relation to total or bioavailable metal species (Guo et al., 2017; Tipayno et al., 2018; Zeng et al., 2020).

The aim of this research was to assess the effect of bioremediation on the functional microbiome from historical polluted soils with heavy metals. The objectives were designed as an in-depth gradient of functional microbiome analysis. The first objective assess the microbial functional guild activity as shaped by one year of bioremediation. The second objective show the impact of bioremediation on

site-specific microbial functional groups. The third objective analyse the stability and similarity of functional groups and guilds assemblages under the impact of bioremediation.

MATERIALS AND METHODS

The Biolog Ecoplate approach was used to observe the daily changes in soil functional microbiome, in 5 sites from Baia Mare exposed to historical pollution and after one year of bioremediation that are identified by an abbreviation: CR-Craica, CT-Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb and URB-Urbis.

Soil samples were collected in 2023 from an ongoing experiment initiated in 2019 across the five mentioned sites, that are located inside the town of Baia Mare (47°39' N 23°34' E) in North-West Romania, covering a total area of 7.3 hectares of brownfields.

The locations exhibit varying degrees of heavy metal soil contamination due to human-caused pollution, primarily stemming from mining, metallurgical activities, and urban development.

For EcoPlate examinations, soil samples and protocols adhered to the approach outlined by Weber et al., 2009, arranging all substrates according to their chemical resemblance. The solution introduced into EcoPlates underwent dilution up to 10^{-4} , and measurements were taken at 590 nm using a plate reader. The entire procedure spanned five days, reaching the plateau phase wherein no further increments were noted in the readings.

Functional groups and guilds used in the description of microbial metabolic activity follow the proposal of Stoian et al., 2022. Based on chemical similarity, an EcoPlate can provide information on 5 different large guilds: Carbohydrates (CH); Polymers (P); Carboxylic & acetic acids (CX); Amino acids (AA) and Amines/amides (AM) to which is added the SUM, as total metabolic activity, Water – as basal activity which can be used as a control and the Average well color development (AWCD).

All data were analysed in RStudio (r Core Team, 2024), with formulas from packages “psych” (Revelle, 2024) for basic statistics.

ANOVA and LSD test were performed in “agricolae” package (de Mendiburu, 2023) to identify the differences between sites and all data were additionally explored in NMDS ordination (“vegan” package, Oksanen et al., 2022).

RESULTS AND DISCUSSIONS

Functional group analysis shows significant differences between sites, reflecting notable variations in metabolic activity. CH1 group have recorded the highest value (1.47) in the Craica site and the lowest in the Romplumb site (0.67), indicating a major contrast between the two locations. Similarly, CH2 group, had a high value (1.93) in the Craica site, while the lowest value remains in the Romplumb site (0.81). For CH3 functional microbiome the conditions in, Colonia topitorilor site stimulated the activity, while all the rest of the sites showed values of activity 3-4 times lower (Table 1).

The functional microbiome of CH4, had the highest value in the Colonia Topitorilor site and

in the Romplumb site the lowest, and interesting aspect being the high activity recorded in ferneziu site. CH5 group activity show a separation between the sites, with activities that decrease gradually and only two sites that show values under 1.00. CH6, shows the highest value in the Colonia Topitorilor site and Romplumb the lowest, suggesting a possible environmental influences. The microbiome associated with CH7 presented the highest value in the Craica site (2.56), followed by Ferneziu site, both with significant higher activities compared to Romplub site. CH8 shows a different variation trend, with Ferneziu site showing the highest activity value and both Romplumb and Urbis sites with significantly lower values. For both CH9 and CH10 functional groups, the highest value was recorded in the Colonia Topitorilor site, while Romplumb site remains with a similar low activity, an observation that highlight a reduced metabolic efficiency, due to environmental conditions.

Table 1. Site-specific differences in metabolic activity of carbohydrates functional groups

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
5_CR	1.47±	1.93±	0.51±	0.92±	1.23±	0.74±	2.56±	1.53±	0.87±	0.61±
	0.14a	0.39a	0.02c	0.26bc	0.27bc	0.19b	0.14a	0.35ab	0.33abc	0.07b
5_CT	1.40±	2.22±	2.01±	1.50±	1.41±	1.63±	1.94±	1.93±	1.41±	0.98±
	0.05a	0.21a	0.21a	0.07a	0.01a	0.06a	0.08ab	0.03a	0.11a	0.08a
5_FR	1.23±	1.61±	1.11±	1.31±	1.20±	0.95±	2.40±	2.08±	1.19±	0.77±
	0.05a	0.22ab	0.23b	0.20ab	0.06ab	0.18b	0.12a	0.42a	0.19ab	0.09ab
5_ROMP	0.67±	0.81±	0.44±	0.34±	0.50±	0.71±	0.78±	0.53±	0.37±	0.36±
	0.12b	0.18b	0.09c	0.02d	0.01d	0.16b	0.17c	0.08c	0.03c	0.03c
5_URB	0.80±	1.09±	0.68±	0.73±	0.90±	0.65±	1.23±	0.91±	0.62±	0.62±
	0.07b	0.16b	0.06bc	0.12cd	0.11cd	0.10b	0.59bc	0.14bc	0.06bc	0.04b

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p < 0.05$ LSD test. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb. N-Acetyl-d-glucosamine - CH8; Pyruvic acid methyl ester - CH1; d l-α-Glycerol phosphate - CH10; d-Cellobiose - CH2; d-Xylose - CH5; β-Methyl-d-glucoside - CH4; i-Erythritol - CH6; d-Mannitol - CH7; α-d-Lactose - CH3; Glucose-1-phosphate - CH9.

Substrate analysis reveals differences between Carboxylic acids groups, with significant fluctuation in metabolic activity between te analysed sites (Table 2). The functional microbiome of CX1 and CX2 presented high activities in Ferneziu site, as an opposite to Romplumb site where were recorded the lowest ones, indicating a clear contrast between locations. Concerning CX3, Ferneziu (2.20) and Romplumb (0.70) show the largest differences, Urbis location presenting a similar low value. For CX4 group, in Ferneziu site was

recorded an average activity significantly higher than the one recorded in Romplumb, scored with the lowest value (0.37). For CX5 microbiome the separation between sites is clear, with the highest value in the Craica site, while both Urbis and Romplumb showed almost two times lower activities.

The analysis of CX6 group shows a pronounced contrast between Ferneziu (2.97) and Romplumb (0.81) activities, similar to CX7, where Ferneziu reaches the highest value. For both CX8 and CX9 groups, the Colonia

Topitorilor offers good conditions for proliferation, compared to Romplumb site. An interesting aspect was observed for CX9 group, where the activity recorded in Craica and Ferneziu sites was similar to the one recorded in Colonia Topitorilor, revealing the generalist microbiome associated with this substrate. The general trend indicates Ferneziu as a site that sustains high functional activities, while the low activities from Romplumb location reflect the restrictions in metabolic potential of microbiome. The metabolic activity of soil microorganisms is influenced by the presence of heavy metals, leading to changes in their chemical behaviour, bioavailability and mobility in the environment (Selvi et al., 2019). The microbial diversity in soils is associated with distinct metabolic functions, along with bacterial ability to convert toxic metals into stable organic compounds or their chemical reduction (Wang and Chen, 2006) that sustain potential proliferation of these specialized microbial groups.

Microorganisms play an important role in biogeochemical cycles and their influence on the environment (Zhang et al., 2023; Ma et al., 2021). Under conditions of heavy metal pollution, the complexity of these biogeochemical cycles is influenced by the expression of functional genes involved in microbial carbon, nitrogen, phosphorus and sulfur processes, as well as genes that enable microorganisms to resist to heavy metals, which are essential for their metabolic adaptation (Li et al., 2021).

Regarding the results recorded in the five analysed sites, metabolic variability indicates that metabolism was an adaptive one where the highest activities were recorded, or sensitive to environmental constraints based on the lowest values. Moreover, environmental factors have a direct influence on metabolic expression, and the metabolic differences observed may represent key mechanisms by which organisms adapt to changing conditions (Prabhakaran et al., 2016).

Table 2. Site-specific differences in metabolic activity of carboxylic acids functional groups

	CX1	CX2	CX3	CX4	CX5	CX6	CX7	CX8	CX9
5_CR	1.90± 0.12a	0.79± 0.17b	1.84± 0.16a	0.69± 0.03bc	2.07± 0.07a	2.53± 0.11ab	1.39± 0.61b	0.72± 0.04b	1.49± 0.36a
5_CT	1.83± 0.07a	1.53± 0.04a	1.59± 0.16a	1.71± 0.06a	1.91± 0.19a	2.26± 0.19b	2.36± 0.06a	1.08± 0.06a	1.63± 0.22a
5_FR	1.89± 0.09a	1.73± 0.24a	2.20± 0.20a	0.92± 0.25b	1.75± 0.34a	2.97± 0.02a	3.06± 0.15a	0.78± 0.10b	1.70± 0.04a
5_ROMP	0.41± 0.04c	0.37± 0.03b	0.70± 0.30b	0.37± 0.03c	0.85± 0.40b	0.81± 0.40c	0.37± 0.03c	0.32± 0.04c	0.37± 0.01b
5_URB	0.97± 0.15b	0.59± 0.06b	0.72± 0.04b	0.69± 0.07bc	0.73± 0.10b	0.86± 0.14c	0.61± 0.08bc	0.62± 0.12b	0.94± 0.42ab

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p < 0.05$ LSD test. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb. α -Keto butyric acid - CX8; d-Galactonic 2-Hydroxy benzoic acid - CX4; acid γ -lactone - CX2; γ -Hydroxy butyric acid - CX6; d-Galacturonic acid - CX3; 4-Hydroxy benzoic acid - CX5; d-Glucosaminic acid - CX1; Itaconic acid - CX7; d-Malic acid - CX9.

The analysis of Polymers and Amino acids groups shows significant differences between the activities recorded (Table 3). Colonia Topitorilor and Ferneziu frequently show the high values, while Romplumb and Urbis show the lowest ones. P1, P2, P3 and P4 groups, have the highest values in the Colonia Topitorilor site while Romplumb has the lowest values, indicating significant differences.

The AA1 functional microbiome reaches the highest value of 2.37 in the Ferneziu site, while in Urbis decrease significantly to 0.66. A similar pattern also occurs for AA2 and AA4, where the highest values are in Ferneziu site,

followed by Craica (AA2) and Colonia Topitorilor (AA4). For AA3 microbiome, the most suitable conditions were recorded in Colonia Topitorilor, in contrast with the lowest values recorded in Romplumb site. For the functional microbiome of AA5 and AA6, Colonia Topitorilor and Romplumb remains in opposite places, emphasizing the consistent differences between these sites and the ability of microbiome to proliferate.

In the amine category, AM1, Ferneziu has the highest average value, while Urbis has the lowest average one (Table 4). Therefore, there was observed a significant difference between

these two sites in term of specialized microbiomes. As for the AM2 group, the maximum value recorded is 1.75 (Colonia Topitorilor), while the lowest value of 0.64 is identified for Romplumb. The assessment of the basal community (WAT) revealed the

highest functional activity in Colonia Topitorilor, while Romplumb site presented a significant lower one. The other three sites recorded significant intermediate activities compared to the extremes.

Table 3. Site-specific differences in metabolic activity of polymers and amino acids functional groups

	P1	P2	P3	P4	AA1	AA2	AA3	AA4	AA5	AA6
5_CR	1.67± 0.02a	1.40± 0.25ab	0.58± 0.01bc	0.77± 0.17cd	2.33± 0.48a	2.74± 0.25a	1.23± 0.24bc	1.36± 0.35bc	0.70± 0.14bc	0.66± 0.07c
5_CT	1.85± 0.09a	1.81± 0.05a	1.7± 0.14a	1.73± 0.07a	1.84± 0.46a	2.02± 0.33a	2.21± 0.11a	2.11± 0.16ab	1.51± 0.23a	1.48± 0.20a
5_FR	1.63± 0.06a	1.44± 0.08a	1.50± 0.06a	1.56± 0.19ab	2.37± 0.15a	2.77± 0.07a	1.57± 0.41ab	2.15± 0.31a	0.93± 0.06b	1.04± 0.08b
5_ROMP	0.72± 0.27b	0.90± 0.16b	0.38± 0.03c	0.49± 0.12d	0.75± 0.21b	0.88± 0.41b	0.49± 0.10c	0.76± 0.22c	0.36± 0.01c	0.36± 0.01c
5_URB	0.81± 0.03b	0.90± 0.16b	0.81± 0.13b	1.11± 0.13bc	0.66± 0.07b	0.67± 0.09b	0.88± 0.28bc	0.67± 0.09c	0.59± 0.04bc	0.66± 0.06c

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p < 0.05$ LSD test. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb; Glycogen - P4; Tween 80 - P2; Glycyl-L-glutamic acid - AA6; α -Cyclodextrin - P3; L-Arginine - AA1; L-Phenylalanine - AA3; L-Serine - AA4; L-Asparagine - AA2; L-Threonine - AA5; Tween 40 - P1.

Table 4. Site-specific differences in metabolic activity of basal activity and amines functional groups

	AM1	AM2	WAT	AWCD
5_CR	0.72± 0.04b	1.08± 0.38ab	0.58± 0.04b	0.74± 0.02b
5_CT	1.90± 0.12a	1.75± 0.02a	0.80± 0.07a	0.94± 0.10ab
5_FR	2.11± 0.09a	1.29± 0.14ab	0.56± 0.02b	1.09± 0.01a
5_ROMP	0.75± 0.36b	0.64± 0.22b	0.33± 0.01c	0.23± 0.09c
5_URB	0.65± 0.05b	0.67± 0.08b	0.59± 0.07b	0.18± 0.04c

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p < 0.05$ LSD test. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb. Putrescine - AM2; WAT – basal respiration;; Phenylethylamine - AM1; AWCD - Average Well Color Development.

High levels of CH7 in Craica soils indicate increased microbial activity, associated with an efficient decomposition of organic substrates (Figure 1). In contrast, low CH4 values in Romplumb soils may reflect an inhibition of microbial activity, associated with heavy metal toxicity. A high activity of polymers decomposition, especially P1 in Ferneziu soils, suggests a significant number of microorganisms capable of degrading complex compounds. On the other hand, low values of P3 in Craica soils suggest a reduced microbial diversity and a limited capacity to degrade polymeric substances, most likely due to heavy metal contamination. High levels of carboxylic and acetic acids, such as CX7 in Ferneziu soils, indicate the activity of microbial communities

under stress conditions. However, low values of CX8 in Romplumb soils could be a sign of microbial inhibition caused by heavy metal toxicity. High amounts of amino acids such as AA4 in Ferneziu soils show active protein synthesis and favourable metabolic condition of microorganisms. In contrast, the lower AA6 values in Romplumb soils suggest a potential metabolic stress due to metal contaminants, which interfere with protein biosynthesis. High levels of amines and amides, such as AM1 in Ferneziu soils, indicate an increased microbial activity related to protein degradation, while low AM2 values in Urbis soils may suggest an inhibition of microbial functions due to the presence of heavy metals.

Regarding the functional guilds, as associated similar microbiomes, it was observed that the highest value is recorded for the CX guild (Table 5). In contrast, the lowest value can be

associated with the amine category guild or AM. This contrast between the two guilds reflects the significant differences in specific parameters (Table 5).

Groups	CR.	CT.	URB.	FR.	ROMP
Carbohydrates CH1, CH2, CH3, CH4, CH5, CH6, CH7, CH8, CH9, CH10	CH7↑				CH4↓
Polymers P1, P2, P3, P4	P3↓			P1↑	
Carboxylic and acetic acids CX1, CX2, CX3, CX4, CX5, CX6, CX7, CX8, CX9				CX7↑	CX8↓
Amino acids AA1, AA2, AA3, AA4, AA5, AA6				AA4↑	AA6↓
Amines/Amides AM1, AM2			AM2↓	AM1↑	

Figure 1. Variation trends in microbial functional groups as affected by historical pollution with heavy metals. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb

Table 5. Site-specific differences in metabolic activity of community and functional guilds

	SUM	P	CH	CX	AA	AM
5_CR	41.1± 2.20b	4.42± 0.30b	12.43± 1.99b	13.46± 0.80b	9.04± 0.25a	1.80± 0.37b
5_CT	54.3± 1.00a	7.1± 0.32a	16.47± 0.30a	15.9± 0.77ab	11.1± 0.64a	3.65± 0.14a
5_FR	51.3± 0.60a	6.15± 0.08a	13.90± 0.69ab	17.0± 0.33a	10.8± 0.79a	3.4± 0.22a
5_ROMP	17.7± 2.96c	2.49± 0.51c	5.57± 0.69c	4.62± 1.04c	3.62± 0.91b	1.41± 0.59b
5_URB	24.1± 3.31c	3.64± 0.32b	8.28± 1.31c	6.77± 0.94c	4.16± 0.64b	1.32± 0.13b

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p < 0.05$ LSD test. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb. Polymers (P); SUM - sum of all activity recorded; Amino acids (AA) and Amines/amides (AM); Carboxylic & acetic acids (CX); Carbohydrates (CH);

The NMDS ordination project the variations in microbial activities and community similarities in each of the different heavy metal polluted sites in Baia Mare. The analysis was performed based on the site locations, the distances within site-specific communities and between different sites, along with the guild vectors, which indicates the metabolic pathways of the microorganisms (Figure 2). The central point of the NMDS projection highlights the main carbon sources available, such as amino acids, amines, polymers, carbohydrates, carboxylic acids, which are situated along the axis 2. The Craica site is located in the center of the ordination, indicating a moderate microbial activity in soils with a relative low pollution. It is positive correlated with vectors AM and AA, suggesting high metabolic activity and a diverse microbial community capable of using a wide range of substrates. A higher concentration of amines and amino acids is

observed, while CX and CH are present with lower activities. The proximity to the P vector indicates that the microbial diversity shows an affinity towards polymers. Colonia Topitorilor is located close to the Ferneziu site, which suggests a similar microbial composition in these two locations. It is related to vectors CX and P, but is not directly related to AA, indicating low utilization levels for these last substrates. This association reflects a moderate level of heavy metal pollution. The microbial community at this site appears less diverse in comparison to Craica, with an ability to adapt to pollution. In case of the lower heavy metal pollution, this allows the microorganisms to avoid metabolizing amino acids. Ferneziu shows similar microbial activity, but with some differences. It has a strong link with AM, which means that the microorganisms here utilize them at higher rates. However, it is

also associated with CX, which suggests that these substrates are strongly decomposed. This shows that the microorganisms in Ferneziu site are adapted to decompose both AM and CX substrate groups, suggesting that they have been exposed to pollution for a long time. Romplumb is significantly different from the other sites, because it is the most isolated on the ordination. This position shows that it has a unique microbial community affected by heavy heavy metal pollution. Because it has no specific links to the substrates (AA, CX, P, CH,

AM), it is interpreted that toxic pollution has decreased microbial diversity and forced them to adapt to these difficult conditions. This difference suggests that the microorganisms in Romplumb are either highly specialized or that their activity is limited by toxic stress. The community is composed of heavy metal-resistant species and showed lower values of metabolic activities, which indicates the difficulties encountered by microorganisms to persist in this environment.

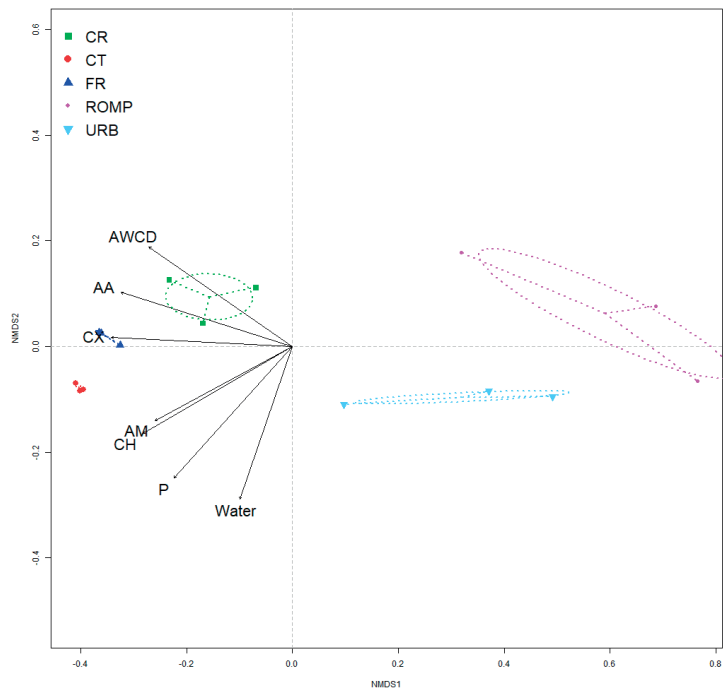


Figure 2. Variation trends in microbial functional groups as affected by historical pollution with heavy metals. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb; AA - Amino acids; AM - Amines/Amides; P - Polymers; CH - Carbohydrates; CX - Carboxylic & acetic acids; SUM - total metabolic activity; AWCD - Average well color development; WATER - basal activity

Urbis is also different from the other sites, but it is closer to the center of the ordination than Romplumb, indicating that pollution has a moderate impact. Regarding the fact that this site is not related to the analysed vectors, its location indicates a combined influence of heavy metal pollutants. Microorganisms at this site are affected by pollution stress, which reduces their diversity and activity. Heavy metal pollution supports the growth of

specialized microorganisms that can tolerate contaminants, but their metabolic activity is more affected than in sites with lower heavy metal pollution.

CONCLUSIONS

Microbial activity and diversity decreased with the degree of pollution, showing the impact of contamination on the entire soil microbiome.

Polymers guild showed the highest activity in Colonia Topitorilor site, with a similar trend of carbohydrates and the maximum form of activities.

Site Ferneziu induced the highest activity for CX and AM, while the AWCD recorded its maximum.

The most intense polymer and carbohydrate activity was observed in soils less contaminated with heavy metals.

There are significant differences between the microbial communities of polluted sites in Baia Mare. Craica, Colonia Topitorilor and Ferneziu sites show diverse metabolic activity, with microorganisms adapted to utilize different carbon and nitrogen sources. In contrast, Romplumb and Urbis are isolated and reflect specialized microbial communities adapted to extreme pollution conditions.

There is a visible ability of microorganisms to adapt to chemical stress in different contaminated environments, leading to site-specific functional microbiome patterns.

ACKNOWLEDGEMENTS

This paper is part of a PhD study in the thematic area of “*The traceability of heavy metals in historically polluted sites and the possibilities for their return to agriculture*”, conducted by the first author B.P., under the coordination of Prof. dr. Roxana Vidican (R.V.).

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