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THE EFFECT OF HISTORICAL POLLUTION ON MICROBIAL FUNCTIONAL KINETICS IN BIOREMEDIATED SOILS FROM BAIA MARE

Bianca POP, Roxana VIDICAN, Larisa CORCOZ, Vlad STOIAN

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

Corresponding author email: roxana.vidican@usamvcluj.ro

Abstract

This industry is a key contributor to environmental disturbances, with residual impacts such as contaminated water sources and heavy metal presence affecting local ecosystems. Microorganisms exhibit a high susceptibility to contamination by heavy metals and play a crucial role in the recycling of materials and the energy dynamics within the ecosystem. The revised Biolog-Ecoplate approach involves employing soil contaminated with heavy metals from Baia Mare at five polluted locations - Craica, Romplumb, Colonia Topitorilor, Ferneziu, and Urbis. The kinetics has been noted, referring to the percentage growth rate from 24 hours to the next 24 hours. The overall microbial functional profile exhibits multiple fast changes within a time frame of 24 hours, leading to a specific site-microbial activity. The results offer insights into significant characteristics of soil microbial functional communities affected by the presence of heavy metal pollution in all analyzed locations.

Key words: *functional microbial communities, biolog ecoplate, heavy metal toxicity, microbial community structure.*

INTRODUCTION

The progress in mining, metallurgy, industrialization, and urbanization has caused the introduction of heavy metals like lead (Pb), mercury (Hg), chromium (Cr), and cadmium (Cd), as well as metalloids such as arsenic (As) into the environment (Mishra et al., 2023). Many industrial byproducts that contain harmful heavy metals and metalloids can dissolve in water and combine with soil and water. As a result, the ecological environment is severely affected (Ahmed et al., 2022).
With rapid urbanization and industrialization in developing country, relatively and intensive short-term human activities will bring many organic pollutants (such as polycyclic aromatic hydrocarbons and phenols) and inorganic pollutants (such as heavy metals, arsenic compounds) into the urban environment (Li et al., 2018). Some heavy metals and metalloids (like As, Pb, Cd, and Hg) are not necessary for biological functions and can be harmful (Gall et al., 2015). These substances have been classified as dangerous by the United States Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2007; Khalid et al., 2017; Rai, 2018a).
On the other hand, certain heavy metals like Cu, Fe, Zn, and even Cr (III) are essential for metabolic processes in living organisms (Marschner, 2012). The relationship between soil, food crops, and the environment illustrates the interaction between abiotic and biotic factors (Rai et al., 2019).
Heavy metals are recognized for their ability to decrease or inhibit the functioning of soil enzymes, disrupt the transformation of carbon, nitrogen, and organic matter, and diminish both the biodiversity and biomass of microorganisms in soil (Giller et al., 2009). Consequently, this can result in the presence of particular microorganisms that are tolerant to heavy metals in soil environments (Giller et al., 2009).
Over the past few years, global concern has risen regarding soil contamination by heavy metals (HMs) because of their elevated toxicity, resistance to biodegradation, and prolonged accumulation (Fajardo et al., 2019). These heavy metals not only impact soil fertility but also interfere with the bacterial community, resulting in a reduction in biodiversity (Pan et al., 2020). Heavy metal pollution-induced stress can alter the attributes of bacterial communities (Lin et al., 2016). Sensitive soil bacteria may experience a decline

in both diversity and population, whereas resilient bacteria can easily adapt and proliferate, leading to the establishment of a distinct bacterial community structure. The presence of heavy metals continually influences both bacterial biomass and activity (Liu et al., 2020). In contrast to plants and animals, soil microorganisms are more sensitive to fluctuations in heavy metal levels due to their ability to react and adapt quickly to such stressors (Giller et al., 1998).

Heavy metals, soil, and bacteria have complex interactions. The presence of heavy metals significantly influences the microbial community structure, especially in soils with moderate to severe contamination (Li et al., 2017a). However, acidic wastewater from mining adds extra stress for microorganisms, as they have to cope with both heavy metals and acidity, further disrupting soil nutrient cycling (Pereira et al., 2014).

Hence, it is essential to comprehend how soil microbial diversity and composition changes under different degrees of heavy metal contamination (Azarbad et al., 2015).

Removing heavy metals from the environment is a significant challenge, as their decomposition, much like other pollutants, cannot be achieved through biological or chemical means (Sharma et al., 2023).

The purpose of this article was to investigate the kinetics and dynamics of microorganisms in soils polluted with heavy metals in Baia Mare, focusing on five pilot sites, utilizing the EcoPlate method to provide comprehensive insights into microbial activity and community structure.

MATERIALS AND METHODS

Soil samples were collected in 2023 from an ongoing experiment initiated in 2019 across five sites 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 soil heavy metal 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.

The EcoPlates functional guilds and groups are analyzed according to Stoian et al., 2022:

- CH - Carbohydrates; P - Polymers; CX - Carboxylic & acetic acids; AA - Amino acids; AM -Amines/amides;
- Water - W; Pyruvic acid methyl ester - CH1; Tween 40 - P1; Tween 80 - P2; α-Cyclodextrin - P3; Glycogen - P4; d-Cellobiose - CH2; α-d-Lactose - CH3; β-Methyl-d-glucoside - CH4; d-Xylose - CH5; i-Erythritol - CH6; d-Mannitol - CH7; N-Acetyl-d-glucosamine - CH8; d-Glucosaminic acid - CX1; Glucose-1-phosphate - CH9; d,l-α-Glycerol phosphate - CH10; d-Galactonic acid γ-lactone - CX2; d-Galacturonic acid - CX3; 2-Hydroxy benzoic acid - CX4; 4-Hydroxy benzoic acid - CX5; γ-Hydroxy butyric acid - CX6; Itaconic acid - CX7; α-Keto butyric acid - CX8; d-Malic acid - CX9; l-Arginine - AA1; l-Asparagine - AA2; l-Phenylalanine - AA3; l-Serine - AA4; l-Threonine - AA5; Glycyl-l-glutamic acid - AA6; Phenylethylamine - AM1; Putrescine - AM2.

The data analysis primarily adhered to traditional methods (Garland, 1997), calculating the Least Significant Difference (LSD), a statistical test used to determine if there are significant differences between the means±standard errors between in terms of various microbial parameters or characteristics affected by heavy metal pollution. Data analysis was performed in RStudio, version 2022.02.3 (RStudio Team. RStudio, 2019.), with packages “psych” (Revelle, 2019; Corcoz et al., 2022a) and “agricolae” (de Mendiburu, 2020; Corcoz et al., 2022b).

RESULTS AND DISCUSSIONS

Among the water group (WAT), it is observed that the highest level recorded is 107.51 in 3_CR. On the other hand, the lowest level is recorded in 3_FR, being 94.84, indicating a considerable difference between the two extremes. As for reading 4, there is a maximum level of 103.98 and a minimum of 87.91. These findings highlight significant differences between 4_ROMP and 4_CT. In relation to reading 5, the levels range between 101.99 and 99.14, with no significant difference between them (Table 1).

Table 1. Dynamics of basal and amines/amides functional groups in long-term contaminated sites

	WAT	AM1	AM2
3_CR	107.51±0.76a	102.87±3.36c	149.53±36.87ab
3_CT	103.01±3.25ab	144.76±18.14bc	179.93±14.29a
3_FR	94.84±2.71c	140.86±11.48bc	134.49±12.76abc
3_ROMP	104.28±1.5ab	108.88±9.69c	150.88±46.24ab
3_URB	99.56±0.9bc	104.96±0.34c	103.06±1.52bc
4_CR	100±0.41bc	102.53±1.92c	113.04±12.82bc
4_CT	87.91±1.57d	146.23±13.8bc	132.42±2.9abc
4_FR	100.91±3.54b	219.91±12.35a	183.36±23a
4_ROMP	103.98±1.79ab	164.34±61.98b	121.44±13.04bc
4_URB	101.21±0.18b	102.39±0.75c	108.55±3.37bc
5_CR	100.1±0.33bc	111.08±5.57c	107.85±4bc
5_CT	101.74±1.98b	104.12±7.29c	93.62±3.11c
5_FR	99.22±1.14bc	110.46±2.72c	95.88±4.48c
5_ROMP	99.14±2.97bc	116.36±6.13bc	105.11±3.1bc
5_URB	101.99±2.16ab	105.66±1.07c	111.53±2.32bc

Note: Means±s.e. followed by different letters present significant differences at $p<0.05$ based on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

In the amine group, phenyltinamine or AM1, it is highlighted that 3_CT records the highest value, i.e. 144.76. In contrast, the lowest value, i.e. 102.87, is observed for 3_CR, thus illustrating that there is no significant difference between 3_CT and 3_CR. Regarding reading 4, the values for 4_FR and 4_URB are significantly different. In the context of reading 5, the maximum value is 116.36 for 5_ROMP, while the minimum is recorded at 5_CT, where it is observed that there is no significant difference between them (Table 1). Among the putrescine or AM2 group, Within the putrescine or AM2 group, we observe that the highest value is recorded at 3_CT. On the other hand, the lowest value is identified at 3_URB, showing significant differences from the maximum value, but not from the other groups. Therefore, significant differences are found between 3_CT and 3_URB after 48 hours of incubation. For reading 4, the highest value is represented by 4_FR, while the lowest value is identified at 4_URB. In this context, it can be concluded that there are significant differences between these values after 72 hours of incubation. Concerning reading 5, the highest value is observed at 5_URB, while the lowest is recorded at 5_CT. Comparing these two values, it can be seen that there are no relevant differences (Table 1).

Within the pyruvic acid methyl ester family, CH1, 3_FR highlights with the highest value, marking a significant difference compared to the other groups. On the other hand, 3_URB has the lowest value, being clearly different from the groups with high values, but not from those with medium or low values. Therefore, 3_URB and 3_FR are significantly different, suggesting possible environmental variations or pollution influences. For 4_CR and 4_CT, there is a significant difference between them, with the values for 4_CT being considerably lower than those for 4_CR. In contrast, 4_FR, 4_ROMP and 4_URB do not show significant variations between them or from the other groups (4_CR and 4_CT). 5_ROMP stands out with the highest mean value, marking a significant difference from all other groups. On the other hand, 5_CT has the lowest mean value, differing from the high value groups, but similar to the medium value groups. In comparison, 5_ROMP and 5_CT are significantly different, with the values for 5_CT being much lower than those for 5_ROMP. Finally, 5_CR, 5_FR and 5_URB do not differ significantly from each other or from the other groups (Table 2).

Regarding the compound CH2 or D - cellobiose, it is noted that the maximum value is recorded at 3_CT, showing a significant difference compared to the other groups. In contrast, the lowest value is recorded at 3_URB, indicating significant differences from the maximum value, but not from the other groups. Thus, significant differences between 3_URB and 3_CT after 48 hours of incubation are evident. Regarding reading 4, the maximum value is represented by 4_CR, with significant differences from the other groups, while the lowest value is identified in 4_CT. Therefore, it can be concluded that there are significant differences between these values after 72 hours of incubation.

Table 2. Dynamics of carbohydrates functional guilds in long-term contaminated sites

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
3_CR	140.77±17.35abc	118.71±3.05de	103.67±1.07cd	136.41±30.23abcd	118.19±7.19abcd	111.28±3.38abcd	149.54±20.71bc	154.55±21.63bc	139.36±27.14a	110.96±2.79bc
3_CT	161.15±16.01a	212.49±33.17a	107.65±2.75cd	166.34±16.32a	127.15±6.46abc	111.18±4.41abcd	170.76±13.45b	186.82±16.83ab	126.92±8.3ab	112.2±3.46b
3_FR	161.46±14.53a	108.75±1.68e	95.86±1.74d	106.06±9.8cd	109.19±3.33abcd	101.79±0.58d	137.89±9.38bc	143.4±15.65bc	97.32±3.73c	134.92±5.43a
3_ROMP	124.37±21.27abc	106.02±1.6e	104.91±3.04cd	100.53±2.52d	102.88±1.14cd	107.46±1.76bcd	111.1±5.08c	107.6±1.13c	105.11±3.49bc	104.29±2.47bc
3_URB	103.7±0.96bc	102.28±1.33e	100.81±1.08cd	101.44±0.36cd	101.4±0.13d	104.91±0.54bcd	120.98±13.96c	105.52±1.87c	106.92±1.99bc	101.53±1bc
4_CR	155.15±4.48ab	201.5±55.43ab	103.18±0.24cd	112.32±7.44cd	133.97±26.12a	107.33±4.59bcd	225.09±16.24a	152.81±9.25bc	110.97±8.57bc	104.19±1.02bc
4_CT	96.48±2.13c	122.78±4.27de	176.08±19.8a	104.43±1.75cd	117.18±3.27abcd	146.33±8.94ab	113.32±2.91c	118.01±2.61c	126.82±9.69ab	107.59±4.87bc
4_FR	121.54±1.68abc	149.78±15.5bcde	114.78±15.57cd	154±32.22ab	128.86±7.05ab	139.54±1.29abcd	224.35±9.67a	222.37±50.81a	142.12±15.64a	107.65±10.72bc
4_ROMP	132.57±27abc	138.81±29.42cde	105.43±0.91cd	104.73±3.01cd	110.3±9.51abcd	144.21±40.58abc	150.37±31.03bc	108.27±1.67c	104.68±2.82bc	102.68±1.19bc
4_URB	115.95±10.84abc	134.75±9.8cde	105.7±2.54cd	115.23±10.53bcd	107.25±3.6bcd	104.31±1.62cd	130.03±25.39bc	113.4±7.26c	102.23±0.18bc	103.17±0.93bc
5_CR	132.3±23.68abc	177.82±14.46abcd	100.89±0.42cd	109.86±10.32cd	133.04±10.71a	125.76±22.17abcd	154.21±26.23bc	130.56±4.76c	101.12±0.75bc	103±1.41bc
5_CT	103.24±4.82bc	106.6±3.1e	149.55±14.35ab	97.47±0.93d	97.21±0.57d	127.56±3.99abcd	111.44±1.87c	104.74±2.45c	102.86±2.37bc	98.68±2.05c
5_FR	109.05±1.06abc	172.21±14.48abcd	181.38±17.32a	142.15±20.63abc	130.09±4.55ab	124.53±17.69abcd	133.34±2.8bc	121.58±13.17c	150.81±2a	107.04±8.56bc
5_ROMP	159.72±52.62a	185.87±26.49abc	133.34±30.89bc	99.55±3.1d	112.64±7.73abcd	152.68±23.29a	141.05±10.82bc	148.88±28.28bc	102.71±1.44bc	104.01±0.98bc
5_URB	119.93±2.76abc	140.64±10.39cde	127.05±6.46bcd	109.46±6.58cd	127.61±3.65abc	111.67±3.95abcd	121.2±7.5c	138.54±6.03bc	101.78±1.69bc	110.73±4.75bc

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

Concerning reading 5, it is observed that the maximum value is recorded at 5_ROMP, while the lowest is recorded at 5_CT. Comparing these two values, significant differences are found, although no significant differences are identified between 5_ROMP and the other values (Table 2).

In relation to the substrate α - d - lactose (CH3), the highest value recorded is 107.65, while the lowest value, 95.86, is identified for 3_FR. The difference between the two extremes does not seem to be significant. For reading 4, a maximum value of 176.08 and a minimum of 103.18 is noted. Analysing these data, we can observe significant differences between 4_CT and 4_CR, as well as between 4_CT and the other values. As for reading 5, the maximum value is recorded at 142.15 and the minimum at 97.47, and the difference between them is considerable (Table 2).

For β-methyl-d-glucoside (CH4), the highest value recorded is found to be 166.34. In contrast, for 3_ROMP, we observe the lowest value, which is 100.53. This discrepancy is significant between the two extremes. Regarding the data from reading 4, we observe a maximum value of 154 and a minimum value of 104.43. Therefore, significant differences can be identified between 4_FR and 4_CT. Regarding reading 5, there is a maximum value of 142.15 and a minimum of 97.47 and the difference between them is significant (Table 2).

In substrate d, the maximum value recorded for Xylose or CH5 is 127.15, associated with 3_CT. In contrast, the lowest value, 101.4, is identified in 3_URB, with no significant difference between the two extremes. Concerning reading 4, a maximum value of 133.97 and a minimum of 107.25 is observed. In the light of these findings, no significant differences are shown between 4_CR and 4_URB, or between 4_CR and the other values. For reading 5, the maximum value is 133.04 and the minimum is 97.21, marking a significant difference between them (Table 2).

In substrate i - Erythriol (CH6), the highest value recorded is found to be 111.28 in 3_CR. In contrast, the lowest value is found in 3_FR, being 101.79, with an apparently insignificant difference between the two extremes. For reading 4, a maximum value of 146.33 and a minimum value of 104.31 are shown. Analysing these data, significant differences can be observed between 4_CT and 4_URB. For reading 5, the values recorded are 152.68 and 111.67 respectively, with a significant difference between them (Table 2). In group d - Mannitol (CH7), the highest value is observed in 3_CT, showing a considerable difference compared to the other groups. In contrast, the lowest value occurs in 3_ROMP, indicating significant differences from the maximum value, but not from the other groups. Hence, significant differences are found between 3_URB and 3_CT after 48 hours of incubation. As for reading 4, the maximum value is recorded at 4_CR, while the minimum value is at 4_CT, with significant differences from the other non-overlapping groups. These differences are evident at 72 hours of incubation. For reading 5, the maximum value is recorded at 5_CR and the lowest at 5_CT. Analysis of these values shows no significant differences after 96 hours of incubation (Table 2).

In the context of dataset N, it is observed that group 3_CT stands out with the highest value, indicating a significant discrepancy compared to the other groups. On the other hand, the 3_URB group records the lowest value, showing a significant difference between 3_CT and 3_URB, which may suggest variations in the environmental setting or the impact of pollution. Regarding reading 4, the values for 4_FR and 4_ROMP are significantly different, with the values for 4_ROMP being considerably lower than those for 4_FR. Regarding reading 5, although the maximum value is 148.88 and the minimum is observed at 5_CT, these differences are not significant (Table 2).

In the glucose-1-phosphate group (CH9), it is observed that 3_CR records the highest value of 139.36, while the lowest value of 97.32 is recorded in 3_FR, showing a significant difference between these two. As for reading 4, the values for 4_FR and 4_URB are notably different. In the context of reading 5, the maximum value is 150.81 for 5_FR, while the minimum value is recorded in 5_CR, showing a significant difference between them. Significant differences are also shown between 5_FR and the other groups (Table 2).

Analysing the presence of d,1-α-glycerol phosphate in comparison to the other substrates, it is observed that it shows low levels of uptake. Specifically, glycerol phosphate suggests that the microorganisms in the examined soil are less efficient in their metabolic process. The maximum value is recorded in 3_FR, while the minimum value appears in 3_URB, showing significant differences between them. For reading 4, no significant differences are found between 4_FR, which has the highest uptake, and 4_ROMP, which records the lowest value. This indicates that the substrate present, glycerol phosphate, shows low uptake compared to other carbohydrate types, suggesting that the community of microorganisms capable of metabolising it is relatively smaller than for other elements in the previous groups (Table 2).

In the case of the substrate d-glucosaminic acid (CX1), it is noted that 3_CT shows the highest value. In contrast, 3_URB has the lowest value, showing a significant difference between 3_CT and 3_URB, which may reflect differences in the environment or the impact of pollution. For reading 4, the values for 4_CT and 4_ROMP are significantly different, with the values for 4_ROMP considerably lower than those for 4_FR. For reading 5, the maximum value is 169.34 and the minimum is observed for 5_CT, but these differences are not significant (Table 3).

Regarding the -galactonic acid y-lactone group (CX2), the highest value observed is 167.58 in 3_C, while the lowest value of 100.32 occurs in 3_URB, indicating a significant difference between these two extreme values. For reading 4, the maximum value is 180.87 and the minimum is 103.65, showing significant differences between 4_CT and 4_URB. In reading 5, the maximum value recorded is 109.84 and the minimum is 95.57, but the difference between them is not considered significant (Table 3).

Table 3. Dynamics of carboxylic acids functional groups in long-term contaminated sites

	CX1	CX2	CX3	CX4	CX5	CX6	CX7	CX8	CX9
3_CR	115.27±6.48c	112.14±6.81c	122.99±14.21cd	112.21±1.64b	174.65±40.5ab	184.4±48.12abcd	112.21±3.67c	100.48±9.11cde	110.8±7.88c
3_CT	135.51±1.86bc	167.58±16.25ab	171.95±11.8abc	121.12±8.61b	186.47±19.81a	205.68±28.86abc	165.01±26.8bc	96.21±3.16de	169.42±24.12abc
3_FR	112.99±1.12c	147.04±17.08b	200.7±3.64a	106.94±0.93b	142.48±15.86ab	252.2±40.35a	122.1±2.42c	95.27±6.04e	112.95±7.43c
3_ROMP	104.96±1.97c	105.47±2.33c	109.6±4.07d	109.05±1.26b	124.67±15.5ab	140.52±31.34cde	108.04±1.49c	116.42±4abc	107.51±2.29c
3_URB	104.3±2.31c	100.32±1.09c	103.06±0.44d	107.54±1.11b	105.54±1.11b	102.46±0.3e	102.95±0.39c	100.27±1.69cde	102.89±1.33c
4_CR	218.78±60.23a	112.86±11.33c	176.25±25.41ab	103.14±0.32b	185.1±34.9a	222.4±11.51ab	195.28±87.34b	115.05±7.9abcd	200.36±53.12ab
4_CT	141.1±7.43bc	108.09±0.75c	106.49±6.81d	159.96±18.41a	121.99±4.53ab	123.46±3.5de	162.98±8.75bc	123.37±5.63ab	119.95±5.26bc
4_FR	195.61±12.05ab	180.87±18.96a	175.85±11.04ab	123.87±23.21b	186.39±22.9a	183.49±24.03abcd	338.41±13a	133.13±12.97a	151.1±45.8abc
4_ROMP	106.09±1.08c	103.26±0.92c	148.21±38.9bcd	101.28±0.59b	166.3±60.38ab	158.57±48.63bcde	104.08±1.89c	93.46±1.56e	106.17±4.59c
4_URB	117.53±8.49c	103.65±1.22c	107.92±2.34d	106.3±1.36b	106.06±3.21b	107.35±2.72e	103.97±0.5c	100.81±0.21cde	104±2.83c
5_CR	169.34±47.75abc	107.59±2.26c	172.67±37.5abc	112.46±4.45b	138.86±35.43ab	131.96±21.16de	122.69±4.34c	118.3±5.93abc	135.66±27.22bc
5_CT	112.03±1.39c	95.57±1.7c	102.08±6.51d	109.28±3.84b	103.93±7.46b	106.32±2.92e	105.69±1.38c	109.52±5.69bcde	97.02±2.09c
5_FR	147.5±8.4bc	109.84±2.03c	107.98±3.93d	115.43±13.78b	113.55±6.4ab	113.86±5.84de	122.58±0.86c	101.71±4.16cde	220.34±58.59a
5_ROMP	116.2±13.58c	104.19±2.41c	124.12±21.83cd	101.79±2.51b	123.41±16.03ab	105.46±1.01e	107.2±1.18c	96.87±8.41de	108.41±3.78c
5_URB	154.99±45.63abc	101.97±3.45c	118.94±10.27d	106.72±0.82b	117.89±6.91ab	140.08±10.5cde	101.09±2.69c	104.6±8.39bcde	154.84±47.14abc

Note: Means±s.e. followed by different letters present significant differences at $p<0.05$ based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

Within the d-galacturonic acid or CX3 group, the highest value was recorded at 3_FR, while the lowest value was observed at 3_URB. This shows significant differences between 3_FR and 3_URB after 48 hours of incubation, but not compared to the other groups. In reading 4, the maximum value was recorded at 4_CR and the minimum at 4_CT, indicating significant differences after 72 hours of incubation. For reading 5, the highest value was observed at 5_CR and the lowest at 5_CT, indicating significant differences between these two values, although no significant differences were found between 5_CT and the other values (Table 3).

In the 2-hydroxy benzoic acid substrate (CX4), it is notable that there is no difference between the maximum value, 3_CT, and the minimum value, 3_URB, and regardless of the value, there are no significant differences in the other groups. Regarding reading 4, the highest value is indentified at 4_CT, with 159.96, showing significant differences from the other groups, while the lowest value is at 4_ROMP. Hence, it can be concluded that there are relevant differences between these values after 72 hours of incubation. Regarding reading 5, the highest value is observed at 5_FR and the lowest at 5_CT. Comparing these two values, no significant differences are observed after 96 hours of incubation (Table 3).

For the substrate 4-hydroxy benzoic acid (CX5), the highest value recorded is 186.47 in 3_CT, while the lowest value of 107.54 is identified in 3_URB, showing a remarkable difference between these two extreme values. In reading 4, a maximum value of 186.39 and a minimum value of 106.06 are observed, marking notable differences between 4_FR and 4_URB. In reading 5, the maximum value is 138.86 and the minimum is 103.93, but the difference between them is not considered significant (Table 3).

In the y-hydroxy butyric acid or CX6 group, the highest value recorded is 252.2 and belongs to 3_FR. In contrast, the lowest value of 102.46 is observed in 3_URB, showing a significant difference between the two extremes. For reading 4, the values range from a maximum of 222.4 to a minimum of 107.35, indicating notable differences between 4_CR and 4_URB. For reading 5, the values range from a maximum of 131.96 to a minimum of 105.46, with no significant difference between them (Table 3).

Within the itaconic acid group (CX7), 3_CT has the highest value of 165.01. In contrast, 3_URB has the lowest value of 102.95, indicating that there is no relevant variation between 3_CT and 3_URB. As for reading 4, the values for 4_CR and 4_URB are considerably different. For reading 5, the maximum value of 122.69 is observed at 5_CR and the minimum value at 5_URB, with no significant difference between them. Also, no significant differences are observed between the other groups (Table 3).

Comparing the keto-butyric acid substrate or CX8 with other groups shows that it has lower absorption levels. This indicates that the microorganisms in the soil analysed are less efficient in metabolising this type of substance. At reading 3, the highest value is at 3_ROMP and the lowest is 95.27, with no significant differences. For reading 4, the highest value is 133.13 and the lowest is 93.46, showing significant differences between 4_FR and 4_ROMP. For reading 5, the maximum value is 118.3 and the minimum 96.87, with a significant difference between them (Table 3).

Within the d-malic acid group (CX9), 3_CT has the highest mean value, while 3_URB has the lowest mean value. However, 3_URB is not significantly different from the groups with high, medium or low values, indicating that there are no notable differences in environmental or pollution impact between 3_CT and 3_URB. In contrast, 4_CR and 4_URB show significant differences, with the values for 4_URB being considerably lower than those for 4_CR. The groups 4_FR, 4_ROMP and 4_URB show no significant differences between them or from 4_CR and 4_CT. In group 5, 5_FR has the highest mean value, indicating a significant difference from all other groups. Group 5_CT has the lowest mean value and, although it differs from the groups with high values, it is similar to those with mean values. In comparison, 5_FR and 5_CT are significantly different, with the values for 5_CT being much lower than those for 5_FR. The groups 5_CR, 5_ROMP, 5_CT and 5_URB do not differ significantly from each other and from the other groups (Table 3).

In the Tween 40 group (P1), 3_CR shows the highest value of 138.37. In contrast, the lowest value of 108.18 is recorded for 3_URB, indicating that the differences between 3_CR and 3_URB are not significant. On reading 4, significant differences are observed between 4_FR and 4_URB, suggesting possible environmental changes or the impact of pollution on soil microbiota. For reading 5, the maximum value is 146.97 for 5_FR and the minimum value is 110.35 for 5_CT, with no significant differences between them, suggesting greater homogeneity of environmental conditions or impact on soil microbiota (Table 4).

In the Tween 80 or P2 group, values for 3_URB are observed to be much lower than those for 3_CT, reflecting variations in environmental conditions or the effects of pollution on the soil microbiota at those sites. In term of reading 4, there is a maximum value of 149.37 and a minimum of 113.66. In the light of these findings, no significant differences between 4_FR and 4_URB can be shown. For reading 5, there is a maximum value of 185.44 and a minimum of 125.33 and the discrepancy between them is significant (Table 4).

As for the α-cyclodextrin or P3 group, 3_CT is observed to have the highest value, while 3_ROMP has the lowest value, suggesting that there are no significant differences between these two variables. Regarding reading 4, the values for 4_CT and 4_CR are significantly different. In the context of reading 5, 5_FR records the highest value, while 5_CR records the lowest value, with significant differences (Table 4).

Among the glycogen group (P4), 3_CT is shown to have the highest value, while 3_URB has the lowest value. This significant discrepancy between 3_CT and 3_URB may reflect variations in the environment or the effects of pollution. As for the readings for variant 4, the values for 4_CR and 4_FR are considerably different, with 4_CR having considerably lower values than 4_FR. In reading 5, the highest value recorded is 152.1 and the lowest is observed at 5_CR, confirming the significant differences between these values (Table 4).

It is observed that in the 1-Arginine or AA1 group, the highest value is recorded at 3_CT, while the lowest value is identified at 3_URB, showing significant differences between the two, but not with respect to the other groups. Significant differences between 3_URB and 3_CT after 48 hours of incubation are thus observed. As for reading 4, the highest value is recorded at 4_CR, with significant differences from the other groups whose letters do not coincide, while the lowest value is observed at 4_URB. In this situation, it can be concluded that there are significant differences between these values after 72 hours of incubation. Concerning reading 5, the highest value is recorded at 5_CR, while the lowest is observed at 5_CT. Comparing the two values, it is found that there are no significant differences between them (Table 4).

Within the 1-Asparagine group (AA2), the highest value recorded is 364.34, while in 3_URB the lowest value is found, namely 103.45, indicating a significant difference between the two extremes. As for reading 4, there is a maximum value of 192.03 and a minimum of 102.95. Following these observations, no significant differences can be shown between 4_CR and 4_URB, as well as between 4_CR and the other values. In relation to reading 5, the maximum value recorded is 142.82, while the minimum is 88.46, but the difference between them is not significant (Table 4).

Table 4. Dynamics of Polymers and Amino acids functional groups in long-term contaminated sites

	P1	P2	P3	P4	AA1	AA2	AA3	AA4	AA5	AA6
3_CR	138.37±17.53ab	132.67±18.98abc	109.83±2.6bcd	115.38±3.89de	134.14±13.95cd	221.9±64.88b	108.02±3.84cde	131.08±22.93bc	103.62±2.85d	108.26±3.02cd
3_CT	128.21±2.04ab	136.84±11.03abc	125.29±6.18bc	135.6±4.12bcd	194.14±25.06abc	224.91±31.06b	139.06±6.9abcd	173.8±4.71ab	113.31±8.61bcd	107.71±5.33cd
3_FR	112.23±3.38b	121.74±5.74c	105.05±9.31cd	123.17±5.51de	146±7.69cd	364.34±69.34a	106.39±2.35de	123.87±9.04bc	101.41±2.24d	102.78±0.66d
3_ROMP	118.53±4.46ab	119.82±23.33c	105.82±4.09cd	110.41±5.99de	125.81±18.49cd	158.21±50.54bcd	89±18.11e	113.93±5.49bc	107.41±1.96d	107.66±2.07cd
3_URB	108.18±1.14b	102.91±2.77c	106.2±1.55cd	103.68±1.55e	101.41±1.41d	103.45±1.17cd	105.09±2.62de	107.42±2.55bc	104.89±2.67d	104.46±1.8d
4_CR	146.98±25.52ab	127.88±6.52bc	103.06±1.65d	114.48±11.23de	269.29±77.85a	192.03±15.82bc	128.86±12.17abcde	146.56±22.49bc	101.25±0.31d	105.33±1.72cd
4_CT	140.34±8.4ab	129.53±7.46bc	162±7.28a	128.59±3.09bcde	129.4±4.36cd	121.4±2.21cd	166.08±16.78a	156.96±11.62bc	124.98±2.92bc	117.42±3.11cd
4_FR	170.45±22.09a	149.37±12.11abc	156.86±16.53a	167.89±11.57a	237.91±23.15ab	145.04±14.61bcd	151.4±17.46abc	238.79±62.68a	153.97±2.92a	144.09±3.55a
4_ROMP	115.98±23.36b	136.57±14.03abc	111.26±6.18bcd	115.02±2.09de	164.34±51.36bcd	147.89±44.91bcd	103.64±3.46de	123.72±10.72bc	104.01±1.54d	105.4±4.38cd
4_URB	100.87±11.06b	113.66±4.81c	108.03±2.68cd	124.68±6.42de	106.3±3.75d	102.95±1.82cd	107.86±4.21cde	107.43±2.06bc	103.56±1.72d	102.13±0.75d
5_CR	144.96±35.35ab	181.35±55.92ab	106.14±3.5cd	111.45±7.13de	128.87±21.27cd	142.82±38.37bcd	158.3±11.89ab	130.09±17.08bc	127.86±9.42b	106.25±1.37cd
5_CT	120.98±3.64ab	125.33±3.29c	106.34±2.76cd	119.99±1.94de	86.53±20.15d	88.46±14.37d	115.56±2.51bcde	101.82±6.22c	127.57±13.53b	136.77±19.01ab
5_FR	146.97±10.28ab	142.94±11.44abc	168.97±11.4a	151.2±3.36abc	123.57±5.93cd	97.27±3.33cd	160.87±18.6a	144.23±29.15bc	112.28±1.94cd	121.28±3.59bc
5_ROMP	130.82±36.24ab	185.44±19.66a	112.12±8.86bcd	125.12±22.75cde	116.03±16.06cd	120.76±14.62cd	140.77±36.78abcd	165.49±37.9bc	106.88±2.23d	101.63±2.85d
5_URB	142.87±12.3ab	144.05±13.86abc	129.3±5.29b	152.1±16.05ab	109.39±3.93d	115.02±1.25cd	131.04±21.03abcde	108.62±3.63bc	107.72±3.23d	114.15±5.23cd

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

In the 1-phenylalanine (AA3) group, it is observed that the highest value is recorded at 3_CT, while the lowest value is identified at 3_ROMP. These differences are significant compared to the maximum value, but not compared to the other groups. Thus, significant differences are found between 3_CT and 3_ROMP after 48 hours of incubation. For reading 4, the highest value is represented by 4_CT, while the lowest value is identified at 4_ROMP. In this case, it can be concluded that there are significant differences between these values after 72 hours of incubation. As for reading 5, the highest value is observed at 5_FR, while the lowest is recorded at 5_CT. Although there are significant differences between these two values, no significant differences are identified between 5_FR and the other values (Table 4).

For the 1-Serine group (AA4), it is noted that 3_CT records the highest value, i.e. 173.8. In contrast, the lowest value of 107.42 is observed for 3_URB, indicating that there is no significant difference between 3_CT and 3_URB. Concerning reading 4, the values for 4_FR and 4_URB are significantly different. In the context of reading 5, the maximum value is 165.49 for 5_ROMP, while the minimum is recorded at 5_CT, where it is found that there is no significant difference between them (Table 4).

Group 1-Threonine or AA5 shows no discrepancy between the highest value, 3_CT, and the lowest, 3_FR, while no significant differences are observed between groups at other value levels. Regarding reading 4, the highest value is recorded at 4_FR, totalling 153.97, indicating significant differences from the other groups, while the lowest value is evident at 4_CR. Therefore, it can be concluded that there are significant differences between these values at 72 hours of incubation. Regarding reading 5, the maximum value is observed at 5_CR, while the minimum is recorded at 5_ROMP. Analysing these two values, significant differences can be observed after 96 hours of incubation (Table 4).

In relation to the glycyl-l-glutamic acid group or AA6, the highest value recorded is 108.26, while the lowest value of 102.78 is identified in 3_FR, but the difference between them does not seem to be significant. Regarding reading 4, a maximum value of 144.09 and a minimum of 102.13 is observed. Analysing these data, significant differences can be identified between 4_FR and 4_URB, as well as between 4_FR and the other values. For reading 5, the maximum value recorded is 136.77, while the minimum is 101.63, and the difference between them is significant (Table 4).

Within the methyl ester group of pyruvic acid, or CH1, it is evident that CR has the highest value at 275.79. Conversely, the lowest value, 144.17, is seen in URB, highlighting a significant difference between CR and URB.

Regarding the substrate CH2, or D-cellobiose, it is notable that there is no difference between the highest value, CT, and the lowest value, URB. Additionally, regardless of the value, no significant differences are observed in other groups (Table 5).

In the context of the α-d-lactose substrate (CH3), the highest recorded value is 277.83. In contrast, the lowest value, 135.36, is identified in URB, highlighting a significant difference between these two extremes (Table 5).

In the context of β-methyl-D-glucoside (CH4), the greatest value is recorded for FR. On the other hand, the lowest value is recorded for ROMP, showing significant differences from the maximum value, but not from the other groups. Therefore, significant variations are observed between FR and ROMP (Table 5).

In the context of the substrate D-xylose, or CH5, considering the second reading, with CR having the highest value and ROMP the lowest, we can observe significant differences between these values.

Within the context of the substrate i-erythritol (CH6), it is notable that ROMP shows the highest value. In contrast, URB has the lowest value, highlighting a significant difference between ROMP and URB. This may reflect environmental differences or the impact of pollution (Table 5).

In the d-mannitol group (CH7), the higher value recorded is 494.31 in CR. In contrast, the lower value is 206.43 in URB, indicating a significant difference between these two extremes.

Within the context of the N-acetyl-D-glucosamine group (CH8), it is observed that the greatest value is recorded in FR. Conversely, the lowest value is identified in URB, indicating significant differences compared to the maximum value. Therefore, significant differences are noted between FR and URB after 24 hours of incubation (Table 5).

Among the glucose-1-phosphate (CH9) group, FR has the maximum value, indicating a significant difference from the other groups. URB has the minimum value, being substantially different from the groups with high values, but not from those with medium or low values. Thus, URB and FR are significantly different (Table 5).

For the substrate d,1-α-glycerol phosphate (CH10), it can be observed that the highest recorded value is 154.31 in FR. In contrast, the lowest value is identified in ROMP, reaching 111.34, with a significant difference between these extremes being evident (Table 5).

In the case of the substrate Acid d-glucosamine (CX1), it can be observed that the maximum value recorded is 368.48 in CR. In contrast, the lowest value is found in ROMP, namely 129.19, showing a significant difference between these two extremes (Table 6).

Table 5. Dynamics of carbohydrates functional groups from 24 to 96 hours in long-term contaminated sites

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
CR	275.79±4.64a	412.74±89.3a	107.90±0.56c	168.51±40.7ab	206.75±35.2a	150.67±29.1ab	494.31±33.9b	312.11±55.7ab	161.73±46.3ab	119.14±4.55b
CT	160.63±18.8bc	277.64±44.1ab	277.83±16.0a	169.36±16.9ab	145.25±11.3b	206.84±11.3ab	216.94±25.0b	230.78±21.0ab	168.03±27.3ab	118.71±0.58b
FR	214.09±20.0abc	283.77±49.7ab	204.54±43.3ab	219.58±29.2a	183.18±13.8ab	176.90±25.2ab	409.80±8.88ab	377.82±84.1a	209.85±30.5a	154.31±15.0a
ROMP	237.05±52.1ab	277.23±72.8ab	148.05±35.7bc	104.61±2.67b	127.78±13.2b	238.26±71.4a	246.06±72.0ab	174.44±36.0b	112.83±2.29b	111.34±2.23b
URB	144.17±13.6c	192.19±8.81b	135.36±7.40bc	127.55±11.4b	138.72±5.34b	122.28±5.71b	206.43±78.6ab	166.94±19.7b	111.17±0.90b	115.93±4.51b

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

Within the group of galactonic acid γ-lactone (CX2), it is observed that the highest value is recorded in FR. Conversely, the lowest value is identified in URB, indicating significant differences compared to the maximum value. Therefore, significant differences are noted between FR and URB (Table 6).

In the d-galacturonic acid or CX3 group, according to the second reading, we observe significant differences between the maximum and minimum values, represented by FR and URB, respectively (Table 6).

In the substrate 2-hydroxy benzoic acid (CX4), it is observed that CT has the highest value. In contrast, ROMP has the lowest value, highlighting a notable difference between ROMP and CT. This may indicate environmental differences or pollution effects.

In the case of 4-hydroxy benzoic acid (CX5) substrate, CR presents the highest level, while URB shows the lowest level. This shows substantial distinctions between CR and URB, with no discernible variation between the other groups (Table 6).

Within the y-hydroxy butyric acid or CX6 group, there is a notable contrast in values. FR stands out with a value of 503.11, while URB lags behind with a value of only 153.75, showing a substantial disparity between the two.

In the CX7 itaconic acid category, FR is the one with the highest average, while URB is far behind FR in value. Consequently, FR and URB show notable differences (Table 6).

Examining the substrate keto-butyric acid or CX8, relative to the other groups, shows that it has lower levels of uptake. Specifically, this substrate indicates that the microorganisms in the soil analysed are less efficient in metabolising this substance. Hence, at reading 2, the highest value is observed at CR and the lowest is 104.78, with no significant differences.

In the d-malic acid group (CX9), it is observed that the highest value is recorded at FR. In contrast, the lowest value occurs at ROMP, showing significant differences from the maximum value, but not from the other groups that have overlapping letters. Therefore, significant differences are found between FR and ROMP (Table 6).

Table 6. Dynamics of carboxylic acids functional groups from 24 to 96 hours in long-term contaminated sites

	CX1	CX2	CX3	CX4	CX5	CX6	CX7	CX8	CX9
CR	368.48±41.3a	138.65±25.7bc	346.78±41.8ab	130.28±6.86ab	390.31±33.6a	502.10±72.6a	263.91±112.85bc	135.34±9.84a	273.87±45.8ab
CT	214.44±13.6b	173.47±18.7b	190.05±31.6bc	212.11±31.2a	241.82±44.9ab	273.13±48.5ab	280.20±32.9b	129.77±8.48a	200.55±40.6abc
FR	323.72±3.97a	289.01±31.4a	380.27±22.3a	159.91±50.6ab	309.53±69.3ab	503.11±19.1a	507.17±28.5a	129.22±17.5a	313.77±17.7a
ROMP	129.19±14.4b	113.35±1.35bc	227.10±108.95abc	112.39±2.46b	271.69±126.14ab	265.51±138.19b	120.55±3.50bc	104.78±5.78a	123.92±8.72c
URB	190.80±58.8b	105.93±2.38c	132.85±14.9c	122.00±2.06b	131.64±6.05b	153.75±10.1b	108.19±2.80c	105.98±10.1a	169.73±58.9bc

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

In the second reading of the Tween 40 group (P1), it is observed that FR records the highest value. On the other hand, the lowest value appears at URB, showing notable differences compared to the maximum value. Therefore, after 24 hours of incubation, significant differences are observed between FR and URB.

In the Tween 80 or P2 group, ROMP shows the highest numbers while URB shows the lowest numbers, indicating minimal contrast between ROMP and URB (Table 7).

In group α - Cyclodextrin or P3, FR is found to have the highest figure of 274.79, while CR has the lowest value of 120.16. This highlights a significant discrepancy between FR and CR.

Regarding the glycogen group (P4), it is observed that FR has the highest value while CR has the lowest value, indicating a significant difference between the two. This difference can be attributed to variations in the environment or the impact of pollution.

In the Arginine or AA1 group (Group 1), there's a notable contrast in levels. The highest measurement, 437.79, is observed in the CR condition, whereas the lowest, 117.64, is found in URB. This variation between the highest and lowest values is considerable (Table 7).

In the analysis of group 1-Aspargine (AA2), it is noted that the highest concentration is recorded in CR. In contrast, the lowest level is recorded in URB, indicating significant differences compared to the maximum value, but not compared to the other groups having the same associated letter. Therefore, significant differences are evident between URB and CR.

In the 1-phenylalanine group (AA3) category, the highest value recorded reached 267.04, while the lowest value, 116.10, was observed in ROMP. This notable difference between the highest and lowest value is relevant.

In category 1-Serine (AA4), FR has the highest value of 382.11, while URB has the lowest value of 125.71. This highlights the significant differences between FR and URB (Table 7).

In the first category, threonine or AA5, in reading 2, CT shows the highest value, 182.92, which differs markedly from the values of the other groups. In contrast, the lowest value is observed in URB. Consequently, it suggests notable distinctions between these values.

We can identify significant differences between FR and ROMP within the glycyl-l-glutamic acid or AA6 group, with a maximum value of 179.39 and a minimum of 115.00 (Table 7).

Table 7. Dynamics of polymers and amino acids functional groups from 24 to 96 hours in long-term contaminated sites

	P1	P2	P3	P4	AA1	AA2	AA3	AA4	AA5	AA6
CR	271.88±38.4a	298.05±81.2a	120.16±5.69c	150.62±30.0b	437.79±115.31a	534.45±109.65a	224.75±39.3ab	239.62±41.2bc	134.55±12.8ab	121.13±3.96b
CT	217.21±11.2ab	225.21±33.3a	214.76±3.02b	209.65±12.9b	218.58±64.5ab	240.21±53.9ab	267.04±30.8a	278.36±31.1ab	182.92±31.4a	172.99±25.6a
FR	281.25±43.8a	257.48±18.7a	274.79±28.6a	313.39±31.2a	428.69±48.3a	493.98±38.9a	262.76±54.8a	382.11±30.3a	175.12±1.93a	179.39±3.29a
ROMP	165.47±38.7b	298.63±57.6a	132.62±16.0c	160.14±33.6b	246.49±94.8ab	313.74±171.69ab	116.10±3.74b	241.13±73.5bc	119.62±6.32b	115.00±1.58b
URB	156.46±25.0b	170.30±26.0a	148.34±7.39c	196.07±20.0b	117.64±2.32b	122.52±3.38b	149.89±29.3b	125.71±8.59c	116.97±4.58b	121.53±2.61b

Note: Means±s.e. followed by different letters present significant differences at *$p<0.05$* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

The results show that substrate water or WAT is highest in CR and lowest in CT. This discrepancy is significant compared to the maximum value recorded, but not so significant compared to the other groups with overlapping letters. Therefore, significant differences are observed between CR and CT (Table 8).

In the amine category, phenyltinamine or AM1 has the highest average value, while URB has the lowest average value. Therefore, there is a significant difference between URB and FR.

As for the putrescine or AM2 group, the maximum value recorded is 236.55, while the lowest value of 124.76 is identified for URB. The difference between the two extremes appears to be negligible (Table 8).

Regarding high carbohydrate levels, such as CH2, CH1 and CH1 in CR(2), CT(3), CR(4) and ROMP(5) soils show intense microbial activity, suggesting more efficient degradation of organic matter. Lower CH1, CH3, CH4, CH5 values in ROMP(2), FR(3), CT(4), CT(5) soils may signal an inhibition of microbial activity due to the presence of heavy metals that are toxic to microorganisms (Figure 1).

The high polymer values, namely P1, P2 AND P4 in FR(2), CR(3), FR(4) and ROMP(5) soils, indicate a significant presence of microorganisms capable of degrading complex materials. At the same time, lower P2, P3 and P4 in CR(2), URB(3), URB(4) and CR(5) soils indicate a decrease in microbial diversity and capacity to break down polymeric substances due to heavy metal contamination (Figure 1).

Table 8. Dynamics of basal and amines/amides functional groups from 24 to 96 hours in long-term contaminated sites

	WAT	AM1	AM2
CR	107.61±1.31a	117.20±7.73b	186.50±59.7a
CT	92.133±3.7925c	219.37±33.5ab	222.74±18.0a
FR	94.884±3.5595bc	343.34±42.0a	236.52±36.4a
ROMP	107.40±2.66a	229.54±115.98ab	201.18±78.7a
URB	102.77±2.61ab	113.57±2.02b	124.76±4.72a

Note: Means±s.e. followed by different letters present significant differences at *p<0.05 based* on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

Increased carboxylic and acetic acid concentrations, namely CX3, CX6, CX7 in FR(2), FR(3), FR(4) and FR(5) soils suggest active communities to stressful environments.

The decreased CX2 and CX8 levels in ROMP(2), FR(3), ROMP(4), CT(5) soils may be a warning sign of inhibition due to heavy metal toxicity (Figure 1).

Elevated values of amino acids, AA1, AA2 and AA4 in FR(2), FR(3), CR(4), ROMP(5) soils indicate active protein synthesis and good metabolic health of microbial communities. Lower values, AA1, AA3 and AA6 in ROMP(2), ROMP(3), URB(4) and CT(5) soils may show metabolic stress caused by metal contaminants, affecting protein synthesis (Figure 1).

The high values of amines and amides, AM1, AM2 in FR(2), CT(3), ROMP(4) and ROMP(5) soils indicate a microbial activity of protein degradation. Smaller concentrations, AM1 and AM2 in URB(2), URB(3), URB(4) and CT(5) soils reveal an inhibition of microbial function caused by the presence of heavy metals (Figure 1).

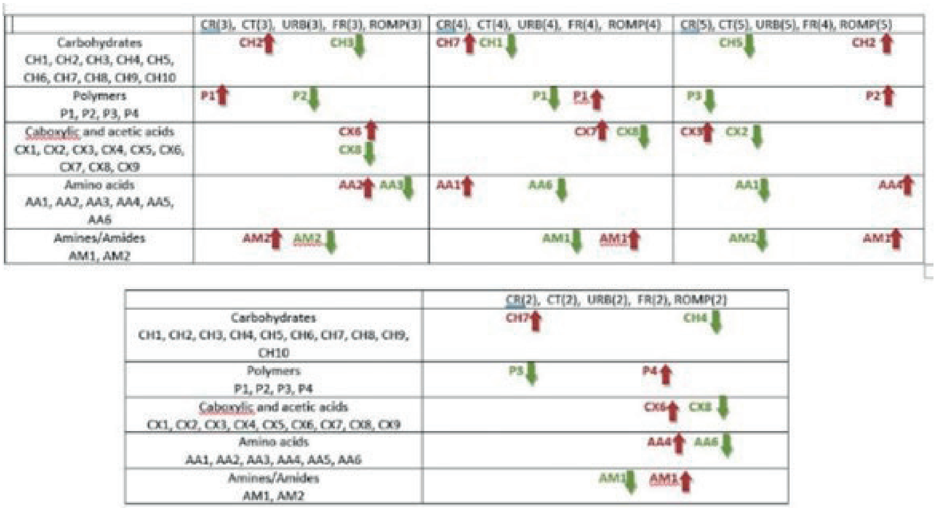

Figure 1. Trends of functional groups asociated with contaminated sites

CONCLUSIONS

The presence of heavy metals significantly alters the functional kinetics of microorganisms in Baia Mare soils. They reduce microbial diversity, moreover they disrupt nutrient cycling and decrease soil fertility. Microorganisms show different responses to contamination. Susceptible ones reduce their diversity and population, while resistant species adapt and proliferate.

This paper highlights significant differences in microbial activity between polluted sites in Baia Mare, such as Craica and Colonia Topitorilor, which show varying microbial functional profiles and growth rates.

The method used effectively measures the functional responses of the microbial community to contamination, while providing detailed insights into how different substrates metabolise in polluted environments.

The research highlights the need to understand soil microbial dynamics in the face of heavy metal pollution, which is essential for developing effective bioremediation strategies and ensuring soil and thus environmental health.

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