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Effect of an Equal Dose of Polymetallic Pollution on the Microbiological Characteristics of Two Soils with Different Organic Carbon Contents

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Abstract Reliable stable indicators are very important for assessing soil quality. This paper compares the dynamics of microbial parameters in two different soils (rich/poor in organic carbon), contaminated in laboratory experiments with an equal dose of heavy metals (HMs) after 30 and 90 days. For this purpose, the changes in the number, biomass, and taxonomic structure of bacterial and fungal communities were assessed in microcosm experiments using an organic carbon–rich ordinary chernozem (Ch-humus-rich soil) and a depleted carbon agrozem (Ag-humus-poor soil), spiked with high concentrations of a zinc, lead, and copper solution (1100 Zn + 660 Cu + 650 Pb mg per kg soil). At the 30th and 90th day from HM contamination, soil samples were collected for analyzing microbial community lipid markers by gas chromatography-mass spectrometry. The results show how both biomass and taxonomic structure of the bacterial and fungal communities analyzed were differently sensitive to HM contamination, depending on the type of soil. The Ag-humus-poor soil microbial community was significantly affected by the HM pollution with an increase in both fungi and bacteria and inside the latter several species changed their percentages. Differently, the Ch-humus-rich soil microbial community was not influenced by the HM addition. However, the negative impact of HM can manifest itself over time, so the microbial structure and its functioning cannot represent accurate indicators of the quality of all soil types. The results show that microbial characteristics should be taken into account only in a comprehensive assessment of soil quality in accordance with ISO 19,204:2017—soil quality TRIAD approach. This approach to environmental risk assessment combines biological data (from bioassay and ecological observations) with chemical analysis.

Keywords Soil quality indicators · Microbial lipid markers · Heavy metal pollution · Microbial community · Biomass · Biodiversity · Humus content

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1 Introduction

Soil contamination and degradation are critical issues worldwide having serious implications for the stability of the ecosystems and its functioning and ultimately human health (Moreira et al., 2019;

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Van der Perk, 2013). The development of new biotic indices to support chemical analysis for assessing soil degradation and chemical pollution is a priority area in environmental protection. Among various soil pollutants, heavy metals (HMs) are among the most diffuse and highly abundant group of toxic compounds (Torres-Cruz et al., 2018). HMs are elements of particular concern, because they do not undergo microbial degradation, remaining in the soils for long periods of time (Ali et al., 2013). High concentrations of HMs can exert selective pressure on soil microbiota affecting their growth, activity, and composition (Joshi et al., 2011; Mandal et al., 2014; Torres-Cruz et al., 2018; Madigan et al., 2019; Terekhova et al., 2018, 2021). Microorganisms have a key role in soil functioning, providing several ecosystem services that are essential to the sustainable functioning of natural and managed ecosystems. Microbial communities are vital for soil ecosystem functioning because they exist in enormous numbers and have an immense cumulative biomass and activity. Microbial communities are the most abundant form of life in the soil and can rapidly adjust to the environmental changes, thanks to their sensitivity and resilience (Fierer & Jackson, 2006; Fierer et al., 2010; Green et al., 2008; He et al., 2011; Ho & Chambers, 2019). Microorganisms are able to quickly respond to stress by changing their activity, biomass, and structure of their communities (Joshi et al., 2011; Torres-Cruz et al., 2018). Most of phenomena observed in the visible aboveground world are steered directly or indirectly by species, interactions, or processes in the belowground soil. In particular, being microbial communities involved in nutrient cycling and organic matter degradation, they can affect biodiversity and productivity of aboveground ecosystems. Microbial communities are the main responsible of soil homeostatic capabilities removing contaminants and providing key ecosystem regulating and supporting services such as soil fertility, resilience, and resistance to different stresses, including HMs (Bååth, 1989; Barra Caracciolo et al., 2020; Rajapaksha et al., 2004; Terekhova et al., 2018).

Mandal et al. (2018) report 18 pollution indices as useful tools for a comprehensive evaluation of contamination degree, especially in farmlands. The indices can give information not only on soil quality, but also a prediction of future ecosystem sustainability (Mandal et al., 2018). The choice of one of 18 pollution indices can depend on the type of soil to be considered. Nonetheless, the limitations of the "concentration criteria" for the assessment of pollutant content are obvious when it comes to the quality of soil or sediments serving as being an environment for living organisms (Chapman, 2007; Gąsiorek et al., 2017; Mahmoudabadi et al., 2015; McDonald et al., 2007; Ololade, 2014; Wu et al., 2014). Chemical indices are not useful for assessing soil ecological functions and mechanisms of destruction of organic matter. On the contrary, microbial parameters are recognized as sensitive and promising indicators of soil "health" (Barra Caracciolo et al., 2015; Giacometti et al., 2013; Kohler et al., 2016; Nunes et al., 2020; Perez-de-Mora et al., 2006; Terekhova et al., 2021; Veum et al., 2014; Winding et al., 2005). Only recently the attention has been focused on the maintenance of the structural and functional diversity of soil bacterial communities and the ways in which they might respond to various natural or anthropogenic disturbances (Barra Caracciolo et al., 2015; Bouasria et al., 2012). The beneficial relationships between soil microbial communities, plant quality, and soil ecosystem sustainability have been recognized; the large number and diversity of microorganisms in soils determine their high metabolic potential and capacity to respond to environmental stress (Zhang et al., 2014, 2019). Considering soil microbial populations essential for nutrient cycling and plant nutrient availability, preservation and maintenance of the composition and activity of microbial communities is fundamental for the sustainability of soil ecosystems (Kohler et al., 2016). In this context, the changes of soil microbial community structure and activity can reflect the anthropogenic impact on soils. For example, several bacterial taxa are known to resist HM toxic effects (Barra Caracciolo et al., 2020; Fajardo et al., 2019) or increase for responding to their toxic effects (Barra Caracciolo et al., 2015). Although several publications address this issue, experiments, which have tested microbial parameters as soil quality indicators of HM pollution, are quite scarce so far. Microbiological parameters for assessing the structure of microbial communities are various and among them, biochemical (e.g., phospholipid-derived fatty acid and the total ester-linked fatty acid methods) and molecular ones are the most commonly used (Rosencvet et al., 2019).

Although the toxic effects of heavy metals on soil microbial populations have been extensively studied (Bååth, 1989; Rajapaksha et al., 2004), little is known about the effects on the overall microbial community (fungi and bacteria) in different soils, about the dynamics of microbial indicators over time in conditions of pollution.

In this context, the current work aims at analyzing the changes in the microbial structure (fungi and bacteria) of two different soils (one humus-rich chernozem and one humus-poor agrozem) spiked with HMs (1100 Zn + 660 Cu + 650 Pb mg per kg soil) in laboratory microcosms. For this purpose, soil samples were sampled at different times from adding HMs (0, 30, and 90 days) and molecules (i.e., higher fatty acids, aldehydes, hydroxyacids, and sterols) that compose microbial cell membranes were analyzed by mass spectrometry and used as phylogenetic biomarkers to describe the structure and size of the microbial communities. All results were compared with untreated soils (control microcosms). The different structure of the soil microbiota (fungi and bacteria) of the two soils with different humus status and contaminated with heavy metals made it possible to verify the significance of the phylogenetic biomarkers as useful biotic indices of soil quality.

2 Methods

2.1 Soil Sampling and Soil Characteristics

Soil samples from two different locations were used for experiments with the microcosm. The samples were collected in early summer (June 2016) from fallow soil plots to which traditional management applied during the previous year.

Ch-humus-rich soil—an organic carbon–rich ordinary chernozem (Corg—5.4%) (N: 51.105025 E: 40.308930, Voronezh region, Russian Federation) on which wheat had been grown the previous summer and Ag-humus-poor soil—a depleted carbon agrozem (Corg—1.5%) (Haplic Phaeozems (Hyposodic, Oxyaquic, FAO, 2014) (N: 47.912044 E: 45.375451, Kalmykia) from rice paddy to grow rice during the previous year.

At each location, four plots (at least 20 m × 20 m) were created with a 20-m space between each. At each plot, 10 random soil cores were taken at a depth of 10–15 cm using a soil core sampler. The soil cores from each plot were combined to obtain approximately 10,000 g of soil. Each sample was placed into a tagged plastic bag, sealed, and delivered to the laboratory at a temperature of about 10 °C.

In the laboratory, the soil samples were air-dried at room temperature (20 + 2 °C) for ~ 1 week (5–7 days)—for that, the soil samples were spread out on a tray. Before placing into the mesocosms, the soil samples were mixed using a scoop and passed through a 5-mm sieve to produce a homogeneous soil sample.

The basic soil edaphic parameters necessary for describing the selected samples were determined by standard methods: moisture (GOST 26,713–85), salt pH (GOST 26,484–85), aqueous pH (GOST 26,423–85), and organic matter content (GOST 26,213–91) (Table 1).

2.2 Setup of the Microcosm Experiments

The soil samples were freed from coarse plant fragments, sieved through a 5-mm sieve, and moistened to 55–60% of their total WHC. After a pre-incubation of 5 days (22 °C), each soil was divided into two parts. One part was used as control and the other one spiked with a high concentration of a heavy metal solution (1100 mg/kg Zn + 660 mg/kg Cu + 650 Pb) in the form of solid salts of copper sulfate ($CuSO_4$), lead nitrate ($Pb(NO_3)_2$), and zinc sulfate ($ZnSO_4$ $7H_2O$), which amounted to five times the tentative permissible concentrations (TPC) of each metal according to the regulatory document (hygienic normative)-GN

Table 1 Main characteristics of the soils used in the study

Soil	pH	Corg %	NH_4 mg/100 g	P_2O_5 mg/100 g	N %	WHC (water holding capacity) %	Soil use during the previous year
Agrozem (Ag)	7.3	1.5	0.72	16.8	0.1	95.9	Rice cultivation
Chernozem (Ch)	6.7	5.4	3.90	12	0.2	83.3	Wheat cultivation

2.1.7.2511–09. Then, aliquots (400 g each) of Ag or Ch soil were distributed in pots loosely closed, providing free air exchange. The experimental set was maintained at 22–24 °C for 90 days.

The choice of these pollutants was based on their widespread occurrence in soil and for their toxicity. In particular, Zn and Pb are classified as hazard class I, and Cu (less toxic) as hazard class II (Motuzova et al., 2014). In accordance with our earlier works, these concentrations can affect soil biological parameters (Pukalchik et al., 2015; Terekhova et al., 2018) and are similar to the amounts considered in other studies (Oladipo et al., 2018; Rose & Devi, 2018). However, this work was in particular focused on the effects of Zn, Cu, and Pb on natural microbiome of two different soils.

Each microcosm was performed in triplicate.

The four experimental conditions are summarized as follows:

- Ch microcosms—control of common chernozem (without adding salts of heavy metals);
- HMCh microcosms—common chernozem with the addition of heavy metals;
- Ag microcosms—control of agrozem (without the addition of salts of heavy metals);
- HMAg microcosms—agrozem with the addition of heavy metals.

2.3 Microbial Community Characterization

To describe the structure of microbial communities and identify its changes under the influence of HM, aliquots of soil samples were collected 30 and 90 days from the start of the microcosm experiment.

The microbial community characterization was performed using the mass spectrometry of microbial markers (MSMM) (Osipov & Turova, 1997). This method is based on the high-precision determination of molecular markers of microorganisms such as higher fatty acids, aldehydes, hydroxyacids, and sterols that compose their cell membranes. The cell envelope of different organisms is known to have up to 200 fatty acids in its lipid structure, depending on the type of the cell (bacteria, fungus, plant, or animal). Some molecules are associated with only one taxon (Shekhovtsova et al., 2013) and can be used as phylogenetic biomarkers to describe the structure and size of natural microbial communities (Di Lenola et al., 2018; Rosencvet et al., 2019).

The cell number of such microorganisms was calculated by marker compound concentration using known data on fatty acid content in microbial cells and making allowances for preparation and device calibration conditions (White & Ringelberg, 1998). The program designed for the analysis and the algorithm for identification and computation of effective microbial cell number were based on the data pertaining to chemical composition of individual and collective markers, which characterized over 500 of microorganisms assumed to have formed the community. These data were taken from Sherlock MIDI Inc., USA, and https://ccug.se/collections/search?collection=entire, Sweden, and also private database of 750 strains as well as original publications from all the world (Bobbie & White, 1980; Wilkinson et al., 1989; Osipov & Turova, 1997; Spring et al., 2000; Rosencvet et al., 2019; Radnaeva et al., 2020).

The microbial lipid markers from soil samples were extracted and analyzed according to Osipov and Turova (1997). The fatty acids were extracted from the soil by an acid methanolysis (1 M HCl in methanol) followed by a silylation in BSTFA (N.O. bis-trimethylsilyl trifetaracetamide). The analysis of methyl trimethylsilylated esters was performed by the MSMM method, using an Agilent Technologies AT-5880/5975 gas chromatography mass spectrometer. This method has an advantage over PLFA (phospholipid fatty acids) due to the expansion of the spectrum of analyzed fatty acids, aldehydes, and sterols, as well as an increased sensitivity due to a mass spectrometer being used as a detector in mass fragmentography mode. The MSMM method is free from some of the limitations of traditional microbiological methods.

2.4 Statistical Analysis

For soil edaphic parameters, average values (n = 3) and standard errors were provided. For soil biotic parameters, average values (n = 3) of bacterial and yeast numbers of fungal biomass with their standard errors were provided. Bacterial community was divided into aerobes and anaerobes and bacterial phyla. The Shannon's, Simpson's, and Macintosh's indices were calculated for assessing the diversity of the bacterial community. The Gaussian mixture

model (GMM) was used to cluster multidimensional data on bacterial diversity according to their distribution. GMM allows data to be modeled by a set of Gaussian distributions. Usually, this model is used in a clustering framework and each Gaussian is supposed to correspond to one group. All calculations were performed with ExcelStat.

3 Results

3.1 Soil Microbial Community Characterization

A comparison of the dynamics of microbial communities in two soils was carried out for several groups of taxa and biomass of microorganisms.

The main taxa considered were bacteria, fungi, *Aspergillus*, and yeasts. Moreover, inside bacteria, several anaerobic (*Acetobacterium*, *Arthrobacter*, *Bacillus*, *Bacteroides*, *Bifidobacterium*, *Butyrivibrio*, *Clostridium*, *Desulfovibrio Enterobacteriaceae*, *Eubacterium*, *Propionibacterium*, *Ruminococcus*) and aerobic (*Acetobacter*, *Actinomadura*, *Aeromonas*, *Caulobacter*, *Agrobacterium*, *Corynebacterium*, *Cytophaga*, iron reducers, *Mycobacterium*, *Nitrobacter*, *Nocardia*, *Pseudomonas*, *Pseudonocardia*, *Rhodococcus*, *Riemirella*, *Sphingobacterium*, *Sphingomonas*, *Streptomyces-Nocardiopsis*, *Xanthomonas*) genera were identified.

The initial analysis of the lipid components showed that agrozem and chernozem soils were characterized by a similar number of bacteria (average value 10^6 cells/g) and yeast (average value 10^6 cells/g) number. Moreover, in the agrozem, a higher fungal biomass than in chernozem soil was found. Adding HMs influenced differently the microbial communities of the two soils. In the chernozem soil, the effect of HMs was not recorded in terms of variations of the number of total bacteria and yeast or fungi and *Aspergillus* biomass (Fig. 1).

Inside bacteria, four main taxa, such as *Firmicutes*, *Actinobacteria*, and *Proteobacteria*, were found in both Ag and Ch soils (Fig. 2). In the agrozem soil, *Proteobacteria* and *Firmicutes* were in lower and *Actinobacteria* in higher percentages in HM-spiked soil at 90 days. *Bacteroidetes* decreased over time in both Ag and HMAg conditions.

Very different results were obtained in the chernozem soil where adding HMs promoted the *Firmicutes* presence and a decrease in *Proteobacteria* and *Actinobacteria*, as found in both 30- and 90-day samplings (Fig. 2).

3.2 Aerobic and Anaerobic Bacteria

The structure of bacterial groups, conventionally divided into anaerobes and aerobes, changed in different ways in the two contaminated soils. At the 30th day of exposure, the ratio of aerobes and anaerobes in agrozem was similar (anaerobes—1.04×10^8 cells/g vs aerobes—1.00×10^8 cells/g). On the contrary, in chernozem soil, the aerobic bacteria prevailed (1.03 cells/g $\times 10^8$ higher than 0.83 cells/g $\times 10^8$). However, at the 90th day, aerobes increased in agrozem, while the ratio of aerobes to anaerobes in the chernozem soil remained stable (Table 2).

At day 30, the species *Rhodococcus equi* and *Propionibacterium jensenii* were dominant in both the agrozem (29.26 cells/g 10^6 and 15.59 cells/g 10^6, respectively) and chernozem (34.5 cells/g 10^6 and 21.34 cells/g 10^6, respectively) soils. However, their abundances increased markedly only in the Ag soil where heavy metals were present (*Rhodococcus equi*: 44.5 cells/g 10^6 at 30 days and 166.16 cells/g 10^6 at 90 days, respectively; *Propionibacterium jensenii*: 36.71 cells/g 10^6 at 30 days and 502.9 cells/g 10^6 at 90 days, respectively).

In addition, *Clostridium* OPA*, *Clostridium pasteurianum*, and *Acetobacter* sp. were dominant in the agrozem, while *Arthrobacter* sp. and *Streptomyces-Nocardiopsis* in the chernozem soil. At the 90th day, the patterns of bacterial dominance in the agrozem samples did not change and the abundance of *Clostridium* OPA*, *Clostridium pasteurianum*, and *Acetobacter* sp. further increased.

Several genera of agrozem (*Clostridium* OPA*, *Clostridium pasteurianum* and *Acetobacter* sp., *Propionibacterium jensenii*) exhibited higher abundance than the initial ones and compared to those found in the chernozem soil (Table 2).

In chernozem, the diversity of the bacterial communities was stable between HM-spiked and control soil (Table 3). The Shannon, Simpson, and Macintosh indices remained virtually unchanged during polymetallic contamination in the first observation period (30 days) and only a slight decrease at 90 days was found in the Shannon index.

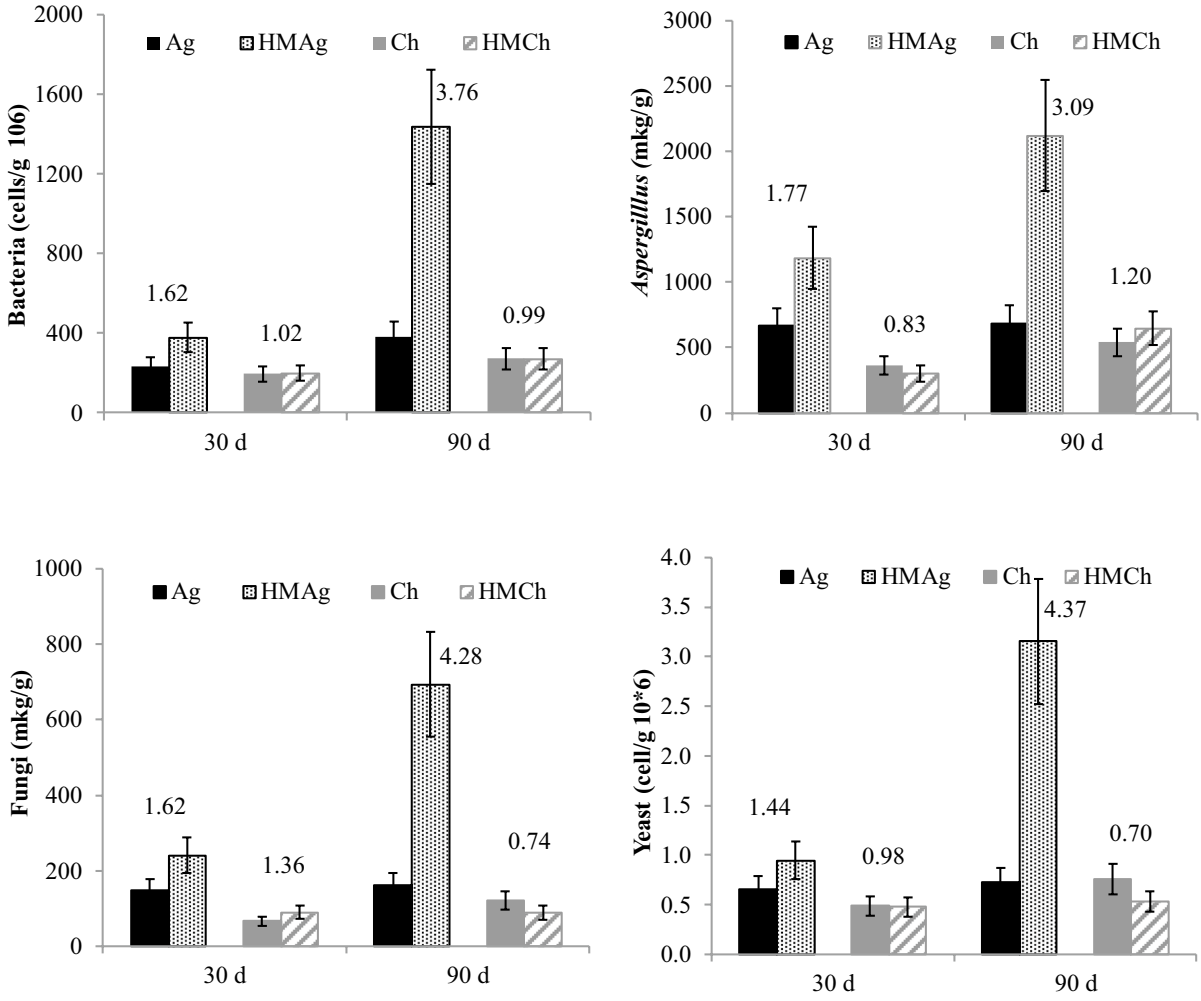

Fig. 1 Microbial community characterization in terms of number of bacteria and yeasts (cells/g soil), fungi, and *Aspergillus* biomass (mg/kg soil) in agrozem (Ag), HM-spiked agrozem (HMAg), chernozem (Ch), and HM-spiked chernozem (HMCh) at days 30 and 90

HM addition in the humus-depleted agrozem stimulated a slight increase in bacterial diversity (Table 3, 30 days). The values of the Shannon diversity index increased (the Simpson and Macintosh indices remained unchanged), possibly due to the additional introduction of the NO_3^- anion together with heavy metals. However, 90 days from HM pollution, all indices decreased (Shannon, Simpson, and Macintosh).

According to GMM, the largest changes in the structure of the bacterial community in agrozem with the introduction of HM on the 90th day compared to the control on the 30th day occurred in groups 3 and 4 (Table 4, Fig. 3). The main of the species is reported to be able to hydrolyze difficult oxidized organic matter under aerobic (*Rhodococcus equi*) and anaerobic (*Propionibacterium jensenii*) conditions, as well as associative nitrogen fixers under aerobic (*Acetobacter* sp.) and anaerobic (*Propionibacterium jensenii*) conditions. It is the dominance of these types of bacteria that significantly distinguishes the structure of the bacterial community in the samples of chernozem and agrozem with HM on the 90th day of the experiment (Table 4, Fig. 3).

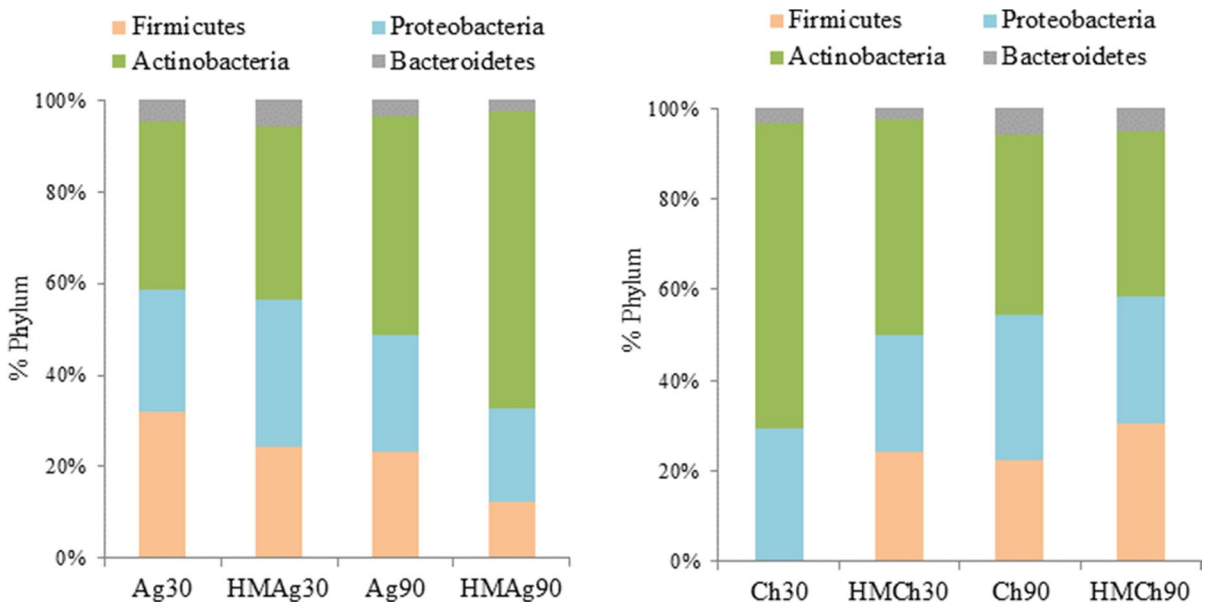

Fig. 2 Relative abundance (%) of the main bacteria phyla in agrozem (Ag and HMAg) and chernozem (Ch and HMCh) soils at 30 and 90 days

4 Discussion

The HM pollution affected significantly the humus-poor soil (agrozem) microbial community structure and an overall increase in bacteria, yeasts, fungi, and *Aspergillus* was observed, especially after 90 days from the HM spike. Differently, the humus-rich soil (chernozem) microbial community remained almost unchanged (Table 2). The increase in fungi can be associated to positive modifications of the bacterial community (Di Lenola et al., 2018). In fact, development of filamentous fungus in contaminated soils has been reported for a moderate soil contamination in a previous work (Terekhova, 2007). Biochemical variations in the composition of phospholipids and other membrane lipids can vary, depending on the degree of exposure, the initial lipid composition, genetic resistance of fungi, and their ability to adapt to extreme conditions (Fedoseeva et al., 2021).

The analytical method used made it possible not only the identification but also the quantification of microorganisms at different taxonomic levels (Bobbie & White, 1980; Stead et al., 1992; Osipov & Turova, 1997; Verkhovtseva et al., 2002). It has also the ability to dissociate the superposition of the entire pool of microbial markers enabling to assess the contribution of any out of hundreds of microorganism species present in a sample (White, 1988; Spring et al., 2000; Pagès et al., 2015; Rosencvet et al., 2019). Finally, this method has also the advantage, comparing to traditional microbiological methods, to be culture independent.

Thanks to the MSMM method, the composition of the agrozem and chernozem bacterial communities was investigated in detail and bacterial species identified inside the main phyla found (Fig. 2 and Table 2). The number of bacteria in agrozem increased by 63% (Table 2) 30 days after the HM addition and this increase can be due to the selection of bacterial species able to respond in different ways to the metal contamination. The proportion of *Actinobacteria* increased to 65%, with 1.5–2.2 times the number of anaerobic bacterial species (HMAg 90 days, Table 2). The dominant species of the microbial community were the *Firmicutes* and anaerobic nitrogen fixer *Clostridium pasteurianum* and the *Actinobacteria Propionibacterium jensenii*, which increased from 3 to 10 times (Table 2). The proliferation in *Firmicutes* species can be due to their known capability to resist and remove HMs (Fajardo et al., 2019; Johnson et al., 2019; Pan et al., 2017). Several species of *Firmicutes* are recognized as able to resist and remove Pb, Cu,

Table 2 Number of aerobic and anaerobic bacteria in untreated samples agrozem (ag) and chernozem (Ch) and soils spiked with heavy metals (HMAg and HMCh) at 30 and 90 days

Bacteria (N. cells/g 10^6)	Sampling time (days)							
	30d		90d		30d		90d	
Anaerobes	Ag	HMAg	Ag	HMAg	Ch	HMCh	Ch	HMCh
Acetobacterium sp.	1.63	0	0	0	2.74	0	0	4.73
Arthrobacter sp.	7.53	10.9	16.27	29	13.32	11.41	7.88	7.39
Bacillus sp.	0.58	0	1.84	1.84	0	1.16	3.38	3.05
Bacillus subtilis	4.4	7.46	0	0	4.6	3.27	8.86	8.13
Bacteroides fragilis	2.28	4.44	1.08	4.65	0.14	0.15	3.83	3.69
Bacteroides hypermegas	0.14	0.33	0.16	0.29	0.13	0.14	0.2	0.16
Bacteroides ruminicola	1.51	3.29	2.36	5.03	1.07	1.08	2.15	1.78
Bifidobacterium sp.	0.18	$<10^4$	$<10^4$	$<10^4$	0.33	0	$<10^4$	$<10^4$
Butyrivibrio 1–2-13	4.57	$<10^4$	$<10^4$	$<10^4$	$<10^4$	$<10^4$	$<10^4$	$<10^4$
*Clostridium OPA**	11.34	13.23	14.22	27.85	8.13	12.94	12.55	11.73
Clostridium pasteurianum	31.05	47.54	46.19	76.87	8.32	18.53	21.08	35.67
Clostridium perfringens	0.27	0.36	0.13	1.8	0.06	0.11	0.24	0.25
Desulfovibrio sp.	8.45	10.62	6.25	17.51	3.11	5.38	9.73	5.98
Enterobacteriaceae	1.42	0.86	2.55	6.43	4.45	0.98	1.12	$<10^4$
Eubacterium lentum	1.76	$<10^4$	$<10^4$	$<10^4$	$<10^4$	$<10^4$	$<10^4$	$<10^4$
Propionibacterium jensenii	15.59	36.71	38.13	502.9	21.34	17.55	28.77	22.47
Propionibacterium sp.	2.16	0	5.43	$<10^4$	7.89	5.98	$<10^4$	0.44
Ruminococcus sp.	8.88	11.78	10.46	42.68	7.99	7.47	8.2	4.94
Total anaerobes	103.74	147.52	145.07	716.85	83.62	86.15	107.99	110.41
*Clostridium OPA**—*C. omelianskii*, *C. pasteurianum*, *C. acetobutyricum*								
Aerobes								
Acetobacter sp.	10.35	24.33	19.82	66.44	9.32	8.96	19.75	14.04
Actinomadura roseola	0.9	1.57	1.84	1.84	0.71	0.65	2.35	1.7
Aeromonas hydrophila	6.51	15.87	0	0	0	3.94	6.92	7.32
Agrobacterium radiobacter	0.71	0	13.1	13.1	6.13	3.53	4.9	1.66
Caulobacter sp.	6.22	11.32	12.56	54.44	4.99	4.61	10.16	13.04
Corynebacterium sp.	1.96	5.11	8.08	32.51	4.15	3.58	4.96	4.3
Cytophaga sp.	2.38	4.38	2.91	6.89	1.42	1.26	3.24	2.18
Iron reducers	$<10^4$	$<10^4$	$<10^4$	0.6	$<10^4$	$<10^4$	0.24	0.21
Mycobacterium sp.	2.12	3.51	$<10^4$	5.12	0.84	0.89	$<10^4$	$<10^4$
Nitrobacter sp.	2.39	2.68	3.42	50.4	2.73	1.56	3.88	3.04
Nocardia carnea	1.74	2.9	1.39	$<10^4$	1.38	0	1.95	2.14
Pseudomonas fluorescens	5.52	11.38	3.17	7.9	5.58	5.63	2.73	3.83
Pseudomonas putida	1.34	4.98	2.29	3.87	1.7	1.68	3.68	2.56
Pseudomonas vesicularis	4.16	8.51	5.91	12.57	1.96	3.62	6.8	5.51
Pseudonocardia sp.	5.41	8	6.32	19.16	4.47	3.98	5.59	5.55
Rhodococcus equi	29.96	44.5	50.19	166.16	34.5	29.14	34.34	29.35
Rhodococcus terrae	0.52	$<10^4$	3.51	0	0	1.48	5.5	2. 57
Riemirella sp.	2.58	5.43	2.69	7.76	1.39	1.02	3.85	3.21
Sphingobacterium spiritovorum	0.73	1.42	1.44	4.27	1.12	0.97	0.71	0.68
Sphingomonas adgesiva	0.74	1.47	1.09	0	0.77	0.81	0.69	0.72
Sphingomonas capsulata	1.1	2.13	1.2	3.6	1.04	1.01	0.81	1

Table 2 (continued)

Bacteria (N. cells/g 10^6)	Sampling time (days)							
	30d		90d		30d		90d	
Streptomyces-Nocardiopsis	5.97	11.55	18.58	45.97	14.72	10.84	5.43	6.03
*WARB***	1.92	5.16	3.84	6.36	1.39	1.91	2.5	1.81
Xanthomonas sp.	2.72	6.54	4.262	9.66	2.35	3.13	3.84	3.07
Total aerobes	97.95	182.74	167.612	518.62	102.66	94.2	134.82	112.95

WARB** Wolinella-Acholeplasma-Roseomonas-Burkholderia

and Zn in different ways, such as through metallothionein production (Kushwaha et al., 2018), bioadsorption, and bioaccumulation inside the cell (Chen et al., 2015; Kushwaha et al., 2018).

In a similar way, some soil propionibacteria species not only fix atmospheric nitrogen, enriching the soil with it, but also form exopolysaccharides which protect bacteria from toxic metals because it can have anionic functional groups which sequestrate positive metals (Gupta & Diwan, 2017). Moreover, the twofold increase in the number of *Desulfovibrio* sp. was presumably due to the introduction with HMs of the anion SO_4^{2-}, necessary for the metabolism of this sulfate reducer. The number of associative nitrogen fixers pertaining to *Acetobacter* sp. and to *Rhodococcus equi*, which have a high hydrolytic ability (Alvarez, 2010), increased significantly among aerobic species. In addition, the proportion of aerobes such as *Caulobacter* sp., *Corynebacterium* sp., *Nitrobacter* sp., and the generalized group *Streptomyces-Nocardiopsis* increased as well.

In the chernozem soil, no significant change in the overall number of bacteria in the soil with HM occurred. However, an increase in the proportion of some *Firmicutes* species such as *Clostridium pasteurianum* and *Desulfovibrio* sp. at day 30 was observed; both species are presumably capable to resist HMs (Barreiro et al., 2015; Cabrera et al., 2006; Fajardo et al., 2019).

Abiotic factors such as soil use, pH, prevalence of anaerobic vs aerobic conditions, and above all the organic carbon, and its content in humic substances, are key factors for structuring microbial communities and make them more stable to environmental stress. The microbial community developed in the Ch-humus-rich soil was more complex and stable and with bacterial populations able to resist and respond to metal stress. Moreover, the soil structure, thanks to the humus substance, was able to act as metal chelants. These results are consistent with reports by other authors which the combination of heavy metals and soil physicochemical properties can have different impacts on microbial community composition (Lin et al., 2019; Zhao et al., 2019).

Differently, the Ag-humus-poor soil, owing to lack of humic structure, was not able to form complexes with HMs and consequently the microbial community was more exposed to their toxic effects. However, 30 and 90 days from contamination, several bacterial species able to respond to HMs increased. At the end of the experiment, in fact *Clostridium pasteurianum* and *Propionibacterium jensenii* became the dominant species in the bacterial community.

Table 3 Shannon (H), Simpson (U), and Macintosh (D) indices in agrozem (Ag) and chernozem (Ch) and soils spiked with heavy metals (HMAg and HMCh) at 30 and 90 days

Soil type	Agrozem				Chernozem			
Time	30 d		90 d		30 d		90 d	
Soil samples	Ag	HMAg	Ag	HMAg	Ch	HMCh	Ch	HMCh
H	4.10	4.31	4.20	3.74	4.37	4.35	4.61	4.44
U	0.79	0.78	0.76	0.57	0.78	0.79	0.80	0.79
D	0.93	0.93	0.92	0.80	0.93	0.94	0.94	0.93

Really, the analysis of lipid markers made it possible to evaluate the changes in the agrozem microbial community due to HM addition, showing how microbiological parameters give additional information on the contamination occurrence and above all on its effects on soil ecosystems.

5 Conclusions

The analysis of the microbial community structure is very useful for evaluating the effects of contamination on soil ecosystems. In particular, the detailed information obtained from the microbial marker mass spectrometry (MSMM) method made it possible to know in detail which species were affected by the HM addition within the microbial community. The overall results show how microorganisms living in a soil depleted in organic carbon (agrozem) were very sensitive to heavy metal presence and a significant shift in the microbial structure was observed. The content of organic matter seems to be a strong factor regulating the stability of microbial communities, and making them resistant to HM effects. The overall results suggest how both abiotic (e.g., organic carbon, contamination) and biotic (microbial community structure) factors need to be taken into consideration for evaluating the quality state of a soil.

Concluding, our work provides evidence against using alone the total microbial biomass indicators as they are not reliable bio-indicators of the state of soil microbiota in case of polymetallic pollution of soils that differ in humus status. Structural changes in bacterial communities are more pronounced under the influence of this type of chemical pollution in a soil with a low organic carbon content. The organic matter seems to be a stronger deterrent regulating the state of microbial cenosis than the presence in the soil samples of zinc, copper, and lead 5 times the estimated permissible concentration. Overall, the data obtained do not contradict the known facts about a higher resistance to negative effects of soil ecosystems rich in humus.

The quantitative differences found in this work demonstrate that the use of only these microbiological indicators (total number of bacteria and fungi, including yeast forms) is not exhaustive for evaluating soil quality. In practical recommendations, a set of potentially effective microbiological parameters must be clarified based on the humus state of soils and the history of its anthropogenic use.

The microbial community of the rich humus soil was not affected by heavy metals during the study period; however, the negative effect may appear later.

It can be concluded that microbial characteristics should only be taken into account in a comprehensive

Table 4 Results by microbial class according to GMM

Class	1	2	3	4
	Bacteroides fragilis	*Agrobacterium radiobacter*	*Acetobacterium* sp.	*Actinomadura roseola*
	Bacteroides hypermegas	*Bacillus* sp.	*Acetobacter* sp.	*Aeromonas hydrophila*
	Bacteroides ruminicola	*Butyrivibrio 1–2-13*	*Bacillus subtilis*	*Arthrobacter* sp.
	Bifidobacterium sp.	*Clostridium pasteurianum*	*Caulobacter* sp.	*Clostridium OPA**
	Clostridium perfringens	*Enterobacteriaceae*	*Pseudomonas putida*	*Corynebacterium* sp.
	Cytophaga sp.	*Mycobacterium* sp.	*Pseudonocardia* sp.	*Propionibacterium jensenii*
	Desulfovibrio sp.	*Rhodococcus terrae*	*Rhodococcus equi*	*Propionibacterium* sp.
	Eubacterium lentum		*WARB***	*Pseudomonas fluorescens*
	Iron reducers			*Pseudomonas vesicularis*
	Nitrobacter sp.			*Streptomyces-Nocardiopsis*
	Nocardia carnea			
	Riemirella sp.			
	Ruminococcus sp.			
	Sphingobacterium spiritovorum			
	Sphingomonas adgesiva			
	Sphingomonas capsulata			
	Xanthomonas sp.			

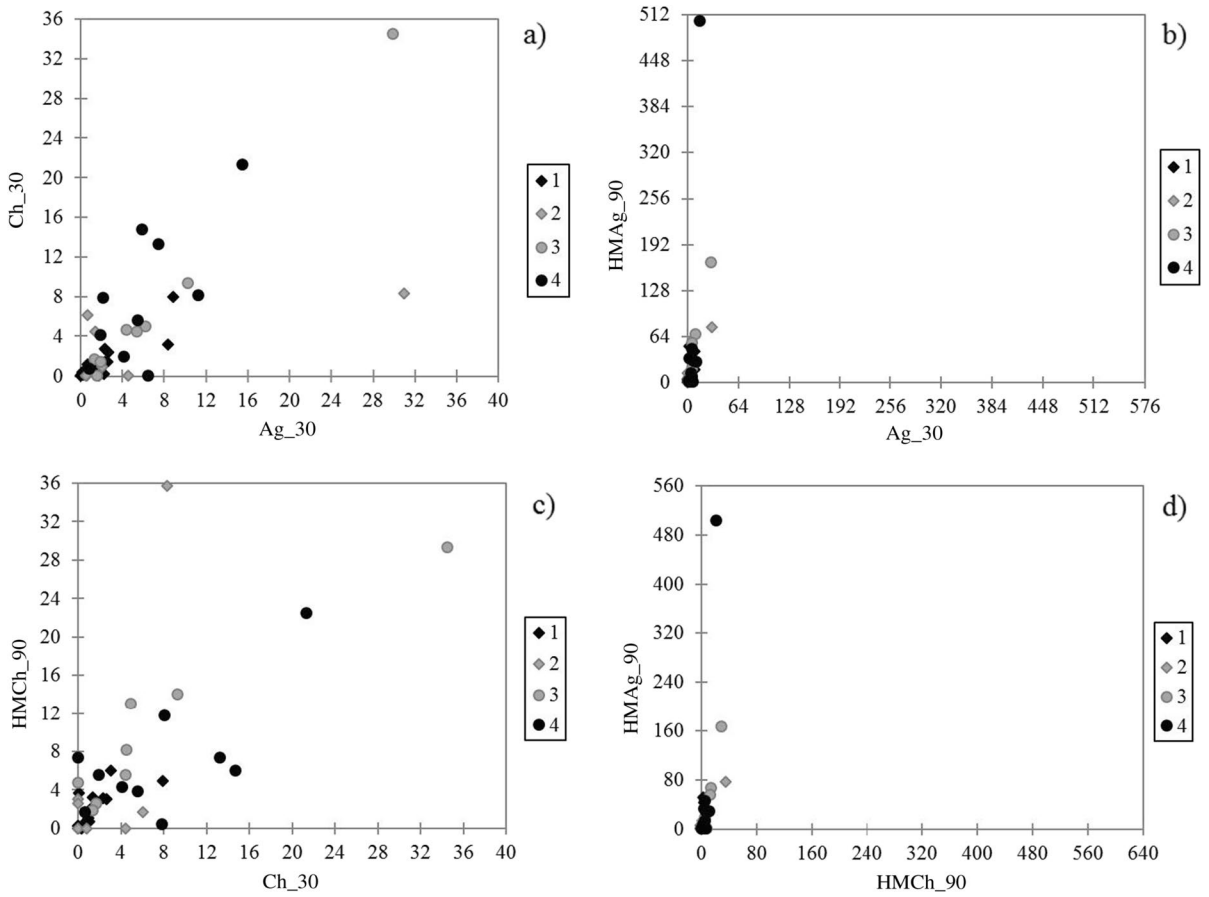

Fig. 3 **a–d** Map classification of control and treated soil samples according to GMM

assessment of soil quality in accordance with ISO 19,204: 2017—TRIAD approach to soil quality. This approach combines chemical data, toxicity testing, and ecological data of a site to determine the effect of contamination on the ecosystem (Chapman, 2007; McDonald et al., 2007; Pukalchik et al., 2015).

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Author Contribution Vera Terekhova: conceptualization, writing—original draft, supervision, funding acquisition, project administration; Elena Fedoseeva: validation, formal analysis, writing—original draft; Anna Barra Caracciolo: writing—review and editing; Anastasiya Kiryushina: investigation, visualization, writing—review and editing; Nadezda Verkhovtseva: methodology, investigation.

Declarations

Competing Interests The authors declare no competing interests.

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