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Exploring the relationship between metal(loid) contamination rate, physicochemical conditions, and microbial community dynamics in industrially contaminated urban soils

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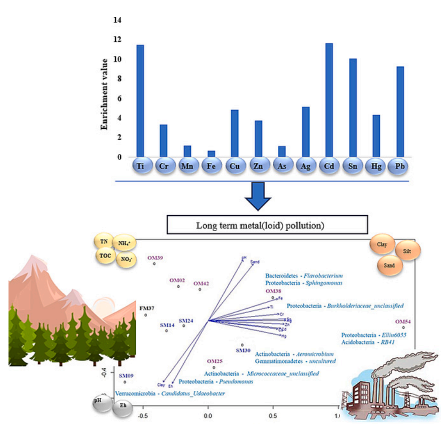
HIGHLIGHTS

- Contamination shifted the proportion of microbial communities.
- Number of rare taxa disappeared in metal(loid) polluted samples.
- Archaeal communities significantly reduced due to metal(loid) pollution.
- The amount of organic material substantially decreased in contaminated soils.
- All contaminated sites pose a severe and persistent health risk to children.

GRAPHICAL ABSTRACT



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ABSTRACT

Increasing metal(loid) contamination in urban soils and its impact on soil microbial community have attracted considerable attention. In the present study, the physicochemical parameters and the effects of twelve metal (loid) pollution on soil microbial diversity, their ecotoxic effects, and human health risk assessment in urban soils with different industrial background were studied in comparison with an unpolluted forest soil sample. Results showed that urban soils were highly contaminated, and metal(loid) contamination significantly influenced structure of the soil microbial communities. In all samples the bacterial community was dominated by Proteobacteria, and on the level of phyla characteristic differences were not possible to observe between polluted and control sampling sites. However, clear differences emerged at class and genus level, where several rare taxa disappeared from contaminated urban soils. Simper test results showed that there is 71.6 % bacterial OTU and 9.5 % bacterial diversity dissimilarity between polluted and control samples. Ratio of Patescibacteria,

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Armatimonadetes, Chlamydiae, Fibrobacteres, and Gemmatimonadetes indicated a significant ($p < 0.05$) positive correlation with soil Zn, Cr, Pb, Sn, Cu, Mn content, suggest that metal(loid)s strongly influence the structure of microbial community. In contrast, the presence of metal(loid) contamination in urban soils has been found to significantly reduce the population of Archaeal communities. This can be attributed to the depletion of organic matter caused by contamination that reached a minimum of 0.5 m/m% for nitrate and 0.9 m/m% for total organic carbon. The values of urban soil pH were close to neutral, ranging from 5.9 to 8.3.

The findings of ecotoxicology test are alarming, as all the studied urban soil sites were cytotoxic to soil microorganisms, and in one site metal(loid) contamination reached genotoxic level. Moreover, all the metal(loid) contaminated sites pose severe and persistent health risk to children, highlighting the urgent need for effective measures to mitigate metal(loid) pollution in urban areas.

1. Introduction

Over centuries anthropogenic activities (mining, manufacturing, etc.) have resulted in widespread metal(loid) contamination in urban areas (Alloway, 2013; Hooda, 2010). The principal increase of metal (loid)s occurred after their usage in various industrial products such as pipes, steel, glass, paint, battery, ammunition, etc. Continuous accumulation of metal(loid) contaminants can change the physical, chemical, and biological properties of the urban soils. Industrially contaminated urban soils have great environmental significance due to their potential impact on human health and the ecosystems. Understanding the characteristics, behaviour and fate of contaminants, as well as characteristic microbial communities in these soils can help identify potential sources of pollution, assess the extent of contamination, and guide appropriate remediation efforts (Khademi et al., 2019; Lenart-Boroń and Boroń, 2014; Luo et al., 2019; Radomirović et al., 2020). The behaviour and toxicity of metal(loid)s depend also on the soil inorganic ion content, the chemical forms of metal(loid)s and their solubility, soil fractions, pH, temperature, soil organic matter composition, etc. (Caporale and Violante, 2016; Friedlová, 2010). Long or high levels of toxic exposure can cause damage to a majority of the organs and organ systems, with particular emphasis on the central nervous system and kidneys, potentially resulting in death in case of humans (Jaishankar et al., 2014; Park and Zheng, 2012).

The long term exposure to elevated concentrations of metal(loid)s in soil can result alteration of the structure of the microbial community too as these compounds can be toxic also for microorganisms (Sheik et al., 2012; Yao et al., 2016). Even at low concentrations, metal(loid)s can accumulate in microorganisms that can damage cell structure, disrupt metabolism, denatures proteins, DNA, and other molecules.

Numerous studies have investigated the impact of metal(metalloid)s on soil microbial community structure, revealing a general adverse effect of their accumulation on microorganisms (Chu, 2018; Liu et al., 2022; Nwuche and Ugoji, 2008). However, other reports suggest no clear relationship between metal(loid) pollution and bacterial community structure (Zhang et al., 2016; Zhu et al., 2013). The shifts in microbial community structure are influenced not only by metal(loid) concentrations but also by other parameters: soil physicochemical properties, including organic matter content, pH, and soil texture, not only affect the toxicity of metal(loid)s but also contribute to the alterations in microbial community structure (Lu et al., 2022; Song et al., 2018; Zhang et al., 2016). Therefore, it is crucial to assess the effects of metal(loid)s on microbial community composition in soils with similar physicochemical properties. Although, some studies have examined the shifts in bacterial community composition caused by metal(loid) pollution over extended periods, the majority of research have focused on the effects of single metal(loid)s or metal(loid) mixtures on soil microbes, with limited consideration of interactions between different metal(loid)s. Addressing this research gap, our paper studies the interactions between various metal(loid)s and their impact on urban soil microbial communities. Thus, understanding changes in diversity and structure of soil microbial community is crucial for understanding their ecological impact as well. Soils with permanent metal(loid) contamination leads to the development of various resistance mechanisms in microbial communities to adapt and cope with pollution (Gomathy and Sabarinathan, 2015). Microorganisms can neutralize or transform contaminants to less harmful state through adsorption, precipitation, or transformation processes, which is known in bioremediation (Abbas et al., 2018). It is more efficient and environmentally friendly than current physicochemical remediation methods, which are rather

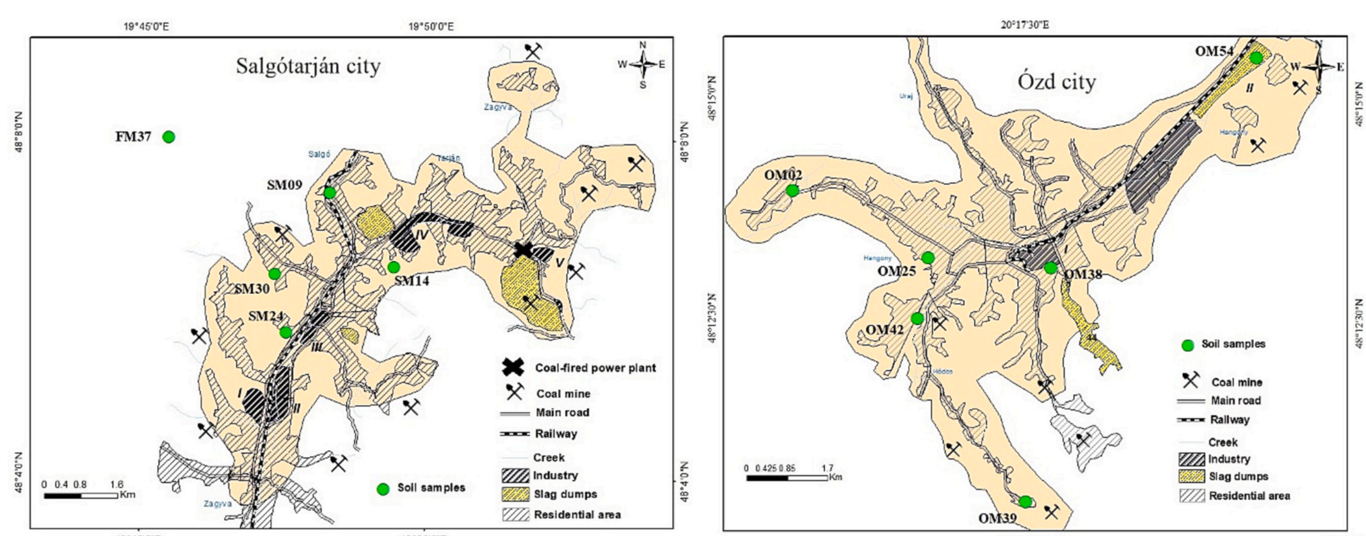

Fig. 1. The maps showing the location of urban soil sampling sites collected for microbiological study and positions of the local anthropogenic sources (industries, slag dumps, coal mines) in Salgótarján (left) and Ózd (right). Sample FM37- brown forest soil considered as control sample.

expensive and have various drawbacks (Gaur et al., 2014).

The present study aims 1) to measure the concentration of twelve metal(loid)s and physicochemical parameters from urban soils of ten differently contaminated sites from two cities in comparison with a control brown forest sample, 2) to analyse the effects of metal(loid) contamination on microbial community structure 3) to identify and isolate bacterial strains that could be potentially used in bioremediation processes and 4) to evaluate the ecotoxicological effect and human health risk assessment associated with the metal(loid) contamination. The hypothesis of this study proposes that metal(loid) pollution and physicochemical parameters significantly influence the structure of microbial communities in heavy industrially contaminated urban soils, though these factors even interact with one another, too. It is expected that higher concentrations of metal(loid)s and partially altered environmental conditions will lead to distinct shifts in the composition and diversity of microbial community structure, ultimately affecting the overall ecosystem functioning in these contaminated soil environments. Additionally, it is hypothesized that the elevated concentrations of metal(loid)s in contaminated soils may pose a risk to human health and soil microorganisms, particularly for residents exposed to these pollutants through ingestion, inhalation, and dermal contact. The study seeks to assess the potential non-carcinogenic health risks, especially for children, and determine the extent of the hazard posed by metal(loid) contamination in these urban environments.

The findings of this study contribute to the ongoing efforts to understand the impact of anthropogenic activities on urban environments and provide valuable insights into the development of effective bioremediation strategies for addressing metal(loid) contamination in polluted areas.

2. Material and methods

2.1. Sample collection and preparation

The study was conducted in metal(loid) polluted areas of Salgótarján (STN) (48°06′34.4"N 19°48′43.4″E) and Ózd (OZD) (48°13′17.8"N 20°16′56.6″E) cities in Hungary. Salgótarján, a multi-industrial city, is primarily contaminated by coal-fired power plant, whereas contamination in Ózd is dominated by iron-steel industry. Both sites have suffered from metal(loid) pollution for several decades (Abbaszade et al., 2022 and reference therein).

The urban soil samples were obtained from various locations within Salgótarján, including park (SM09), playgrounds (SM14, SM24), and roadside (SM30). In Ózd, urban soil samples were collected from four different playgrounds (OM02, OM25, OM39, OM42) and two industrial sites (OM38, OM54) (Fig. 1). Industrial residues (coal ash or smelter slag) were observed in all sampling sites, except sample SM14 and OM02 (SM14 old playground from the former industrialized region, OM02: playground from the agricultural area of Ózd, ~ 6 km from the former industry). In addition, a brown forest soil sample, as control sample, was obtained from a forest located approximately 7 km away from the Salgótarján urban areas (FM37; 48°08′44.0"N 19°45′56.0″E). The sampling locations were chosen based on previous environmental geochemical study of Pb (Abbaszade et al., 2022) and among them, SM09, SM14, OM02, OM25 and OM39 were classified as low polluted, SM24, SM30, OM38, OM42, and OM54 were classified as highly polluted, whereas FM37 (from nearby forest) was considered as unpolluted control sample.

Samples were collected in May 2018 from 0 to 10 cm soil depth: plant residues (e.g., roots) and other eukaryotes residues (e.g., small larvae) were removed, then with a sterile spatula the excavated soil was sampled into sterile falcon tubes following standard microbiological rules. The samples were taken to the laboratory in cooling bag (4–6 °C) and were kept cool till further processing. For elemental and physicochemical analysis, urban soil samples were collected (Demetriades and Birke, 2015) and delivered to the laboratory in plastic bags.

2.2. Element analysis

In bulk samples, the total concentration of 12 metal(loid)s, including Ti, Cr, Mn, Fe, Cu, Zn, As, Ag, Cd, Sn, Hg, Pb were measured. The soil samples were air-dried in the laboratory and passed through 2-mm sieves to obtain a representative portion. For homogenization, the coning and quartering procedures were applied (Hooda, 2010). Visible organic materials (e.g., worms, grasses, roots) and urban debris (e.g., bricks, concrete, waste) were removed before grinding, mortaring, and pulverization. The modified aqua regia digestion (1:1:1 HNO_3: HCl: H_2O) method was used at 95 °C on 15 g of <0.075 mm milled aliquots for low to an ultra-low determination of metal(loid)s in soils (Reimann et al., 2009) and were analysed by PlasmaQuant MS Elite (Analytik Jena, Germany) inductively coupled plasma mass spectrometer (ICP-MS) (detection limit is 0.01 mg kg^{-1}). The analytical quality was controlled using certified reference materials (DS11, NIST981, and NIST-983; Table S1). In all cases, the relative standard deviation (RSD) for elemental concentration measurements was <3 % RSD.

2.3. Enrichment factor analysis

The following equation was used to calculate the enrichment factor (EF) of each metal(loid) to estimate the enrichment levels of the selected hazardous metal(loid)s in the soil samples (Dragović et al., 2008; Z. Wang et al., 2018):

$$EF = \frac{(Ci/Cref)sample}{(Ci/Cref)control} \quad (1)$$

where C_i is the hazardous metal(loid) concentration in mg kg^{-1} and C_{ref} is the content of the reference element for normalization in mg kg^{-1}. Because of their low variability in soil samples, K, Al, Fe, Mn, Ti, and Sn are usually applied as reference elements in EF calculations (Cui et al., 2014; Gąsiorek et al., 2017). In the current study, as the Al was not a potential contaminant, it was used for normalization. The enrichment levels of toxic metal(loid)s in the samples were classified as follows: minimal enrichment ($EF \leq 2$), moderate enrichment ($2 < EF \leq 5$), significant enrichment ($5 < EF \leq 20$), very high enrichment ($20 < EF \leq 40$), and extremely high enrichment ($EF > 40$) based on the calculated EF values (Khademi et al., 2019), which can reflect the origin of the toxic metal(loid)s as well (Gąsiorek et al., 2017; Zacháry et al., 2015).

2.4. Grain size analysis

Prior to the particle analysis, around 0.5 g of urban soil was mixed with an optimal amount of Na-pyrophosphate ($Na_4P_2O_7$) to disaggregate particles (Abdulkarim et al., 2021), which was further performed by applying to FRITSCH ultrasonic cleaner. The mixture was kept overnight so that the aggregates were detached and then was analysed by Laser Scattering Particle size distribution analyser PARTICA 950-V2 LA instrument at the Research and Instrument Core Facility of Sciences, Eötvös Loránd University, to identify soil texture. Based on measurements, urban soil particle sizes were assessed in three size categories: <8 μm (clay), 8–63 μm (silt), and > 63 μm (sand) (Konert and Vandenberghe, 1997; Thomas et al., 2021).

2.5. Eh-pH analysis

The air-dried urban soil samples (1 g) were mixed with 10 mL of distilled water for Eh-pH analysis. The mixture was kept rotating for 30 min. Soil pH and conductivity (Eh) were tested in a prepared solution in a 1:10 soil-water ratio with a digital Eijkelkamp 18.52.01 Multimeter instrument (Yu and Rinklebe, 2015) at the Department of Microbiology, Eötvös Loránd University.

Table 1
Concentration of metal(loid)s and physical parameters s in the studied urban soil samples TN - total nitrogen, TOC – total organic carbon, C_i – inorganic carbon ($CaCO_3$). *Al concentration in soil samples were used only for the enrichment analysis.

		Control sample	Salgótarján (STN) urban soil samples				Ózd (OZD) urban soil samples					
		FM37	SM09	SM14	SM24	SM30	OM02	OM25	OM39	OM42	OM38	OM54
Chemical/physicochemical parameters	Unit	Brown forest	Park	Playground		Roadside	Playground				Industrial area	
Ti	mg kg^{-1}	5.00	130	200	150	90.0	90.0	120	90.0	250	310	350
Cr	mg kg^{-1}	6.09	19.8	21.6	29.4	20.9	20.1	21.7	18.9	148.8	49.0	444.4
Mn	mg kg^{-1}	357	355	509	602	354	452	390	409	3612	3590	2946
Fe	m/m %	2.1	2.7	2.5	2.4	2.0	2.0	2.3	2.2	4.4	9.9	11.6
Cu	mg kg^{-1}	7.10	22.7	24.9	245	135	12.8	18.1	23.2	74.5	140	384
Zn	mg kg^{-1}	54.2	74.7	117	188	402	54.5	91.4	94.8	526	778	4655
As	mg kg^{-1}	6.80	8.50	73.6	26.1	15.7	4.00	7.70	10.3	23.8	27.1	35.9
Ag	mg kg^{-1}	0.05	0.07	0.08	0.72	0.19	0.05	0.49	0.10	0.85	1.30	4.40
cCd	mg kg^{-1}	0.20	0.70	0.60	1.40	1.60	0.10	0.40	0.40	3.00	5.20	62.9
Sn	mg kg^{-1}	0.50	4.40	3.00	13.8	7.30	0.80	1.60	1.70	11.7	34.3	90.8
Hg	mg kg^{-1}	0.10	0.40	0.10	0.10	0.30	0.04	0.50	0.10	0.90	0.70	4.90
Pb	mg kg^{-1}	16.0	80.0	31.5	1692	433	14.8	36.3	23.5	251	596	1674
Al*	m/m %	0.46	1.56	1.57	1.32	1.12	1.08	1.28	1.04	1.68	1.35	1.75
$NH4^+$	mg kg^{-1}	1.6	3.3	2.1	1.6	2.8	7.8	5.2	10.4	5.9	3.6	2.0
$NO3^-$	mg kg^{-1}	2.1	4.4	5.3	4.9	10.5	20.7	7.2	7.7	4.3	2.1	0.5
TN	m/m %	0.2	0.2	0.2	0.4	0.3	0.2	0.1	0.3	0.2	0.1	0.0
TOC	m/m %	2.8	1.7	1.7	1.9	4.0	1.3	1.2	2.9	1.4	1.9	0.9
C_i	m/m %	4.5	1.8	0.1	0.6	0.6	0.5	4.2	0.4	9.2	16.4	8.1
pH		7.7	5.9	6.6	7.6	7.4	7.6	7.6	7.5	7.7	7.9	8.3
Eh		−35.5	59.0	20.1	−29.4	−22.6	−41.2	−36.4	−32.8	−44.7	−55.2	−77.5
Soil texture	Clay V %	10.9	20.0	13.4	12.3	7.1	9.8	14.0	11.2	11.0	6.6	4.7
	Silt V %	39.9	48.7	45.9	42.5	59.2	46.2	52.5	45.8	39.7	33.6	23.0
	Sand V %	49.2	31.3	40.8	45.2	33.8	44.0	33.5	43.0	49.3	59.8	72.2

2.6. Soil organic content (SOC) analysis

The organic content analysis was performed by the method of Hargitai/Tyurin (Molnár et al., 2019) and the method of Kjeldahl (Conkinlin, 2014) for organic carbon and nitrogen species, respectively, at the Centre for Agricultural Research, Hungarian Academy of Sciences.

2.7. Isolation and identification of bacteria

Bacterial strains were isolated and later maintained on nutrient agar medium (5 g/L peptone, 3 g/L meat extract, 15 g/L agar; DSM medium 1) supplemented with 200 μg/mL of respective metal salt compounds ($CdSO_4$, $HgCl_2$, $Pb(NO_3)_2$). The number of cultivable metal(loid) tolerant bacteria was determined (CFU values) after the dilution series method. Bacterial strains were randomly isolated from all plates.

After purification, bacterial strains were identified based on 16S rRNA gene sequencing: the genomic DNA of the bacterial strains was extracted using a DNA extraction kit (DNeasy Power Lyzer Microbial Kit, Qiagen, Germany). The 16S rRNA gene of the bacterial strains was amplified (PCR) from the extracted genomic DNA using the universal primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGCTACCTTGTT ACGACTT-3′) at the department of Microbiology, ELTE, and sequenced at LGC Genomics, Berlin, Germany. The 16S rRNA gene sequences of the strains were compared to the reference sequences in the EzTaxon database (Yoon et al., 2017) to identify closely related bacteria/taxa. The obtained 16S rDNA gene sequences were deposited in the GenBank under accession number MT765153 - MT765173.

2.8. Minimum inhibitory concentration (MIC) analysis

The minimum inhibitory concentration (MIC) analysis was performed to obtain high metal(loid) resistant strains (Maity et al., 2019; Neethu et al., 2015). Bacterial strains were transferred to elevated concentrations of metal(loid) salts ($CdSO_4$, $HgCl_2$, $Pb(NO_3)_2$ and As_2O_3) on nutrient agar medium (DSM medium 1) that contains 200 μg/mL, 500 μg/mL, 800 μg/mL, 1000 μg/mL, 1500 μg/mL, 2000 μg/mL, 2500 μg/mL, 3000 μg/mL, 5000 μg/mL, 10000 μg/mL, 15000 μg/mL of Pb, Cd, Hg and As, respectively. Besides the individual effect, isolates were exposed to the complex effect of metal(loid) salts (900 μg/mL Pb, 600 μg/mL Cd, and 600 μg/mL Hg) to check their multi-elemental tolerance, as well. Additionally, MIC values were checked by low phosphate Tris-salt mineral medium supplemented with metal(loid) salt additives ($CdSO_4$, $HgCl_2$, $Pb(NO_3)_2$ and As_2O_3,) in various concentrations (500–800 μg/mL for Pb, 500–1500 μg/mL for Cd, 300–500 μg/mL for Hg, 100–220 μg/mL for As).

2.9. Next-Generation Sequencing (NGS)

Soil community analysis was performed on the selected eleven samples (Fig. 1) to be able to compare the effect of the metal(loid) contamination on the microbial community structure. The isolation of the community DNA from the selected eleven soil samples was conducted according to the manufacturer instructions via Ultraclean® PowerSoil DNA Kit (MoBio, Carlsbad, CA, USA). The cell walls were destroyed mechanically by Retsch Mixer Mill MM400 (Retsch, Germany), shaking at 30 Hz for 2 min. The Bacteria and Archaea communities were identified based on the partial 16S rDNA gene sequence after the PCR amplification of V3-V4 regions by universal primers (S-D-Bact-0341-b-S-17 forward (5′-CCT ACG GGN GGC WGC AG-3′) and S-D-Bact-0805-a-A-21 reverse (5′-GAC TAC HVG GGT ATC TAA TCC-3′) for Bacteria, and S-D-Arch-0519-a-A-19 (5′ – CAG CMG CCG CGG TAA – 3′) and S-D-Arch-0855-a-A-20 (5′ – TCC CCC GCC AAT TCC TTT AA – 3′) for Archaea in triplicate and the mixture was used for sequencing (Toumi et al., 2021). The DNA sequencing was fulfilled at the Michigan State University (USA) by Genomics Research Technology Support Facility (RTSF). Identification of genomic bases and FastQ format conversion were performed by Illumina Real-Time Analysis (RTA) v1.18.54 and Illumina Bcl2fastq v2.19.1, respectively.

The bioinformatic analysis of raw sequencing reads was executed with MOTHUR v.1.40.5 software (Kozich et al., 2013) using the MiSeq SOP protocol (accessed in 2019: https://mothur.org/wiki/miseq_sop/). After a quality trimming (deltaq = 10) the raw reads were dereplicated, and the sequences were aligned against SILVA template alignment; then, the desired aligned region was screened. Following filtering gaps, the potentially chimeric sequences were detected via chimera.vsearch tool and removed. Additional reduction of sequence noise with pre-

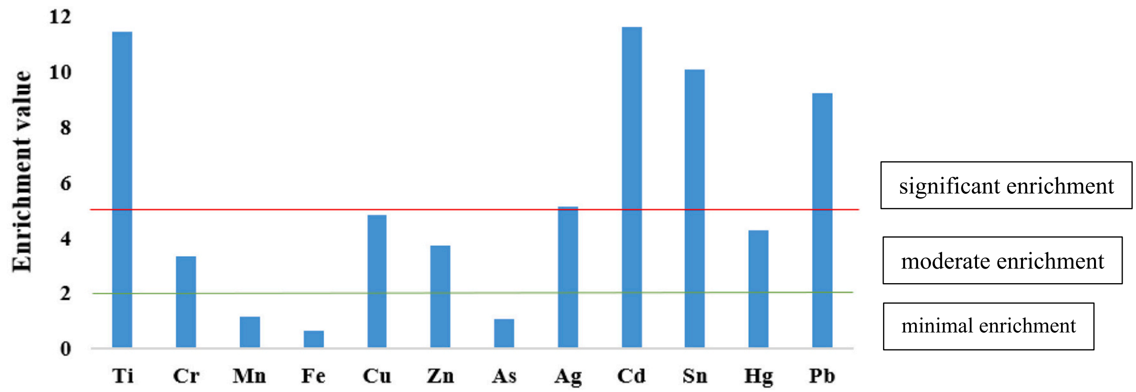

Fig. 2. The average enrichment factor (EF) of the selected metal(loid)s in the studied Salgótarján and Ózd urban soil samples. Minimal enrichment (EF ≤ 2), moderate enrichment (2 < EF ≤ 5), significant enrichment (5 < EF ≤ 20), very high enrichment (20 < EF ≤ 40), and extremely high enrichment (EF > 40).

clustering followed the taxonomic classification using the SILVA taxonomy database (https://mothur.org/wiki/silva_reference_files/) with a >80 % bootstrap support value considered for the successful classification.

Species richness and diversity indices were assessed with inverse Simpson (1/D) (Simpson, 1949), Shannon-Weaver (Shannon, 1948), Chao1 (Anne, 1984), and ACE (Hughes et al., 2016) indices, as well as depicted with rarefaction curves based on the subsampled reads from MOTHUR. The sequence raw reads were deposited under the BioProject PRJNA643801 accession number in the NCBI SRA database.

2.10. Ecotoxicology test

The ecotoxicology test was performed with the water-soluble fraction of the metal(loid)s in soil by mixing 1 g soil with 40 mL 0.11 mol acetic acid solution (Sigma) at the Department of Microbiology, Eötvös Loránd University. The mixture was rotated for 16 h and after centrifugation the supernatant was used for further analysis. The cytotoxicity and genotoxicity of the soil samples were analysed by SOS Chromotest V6.5 (Environmental Bio-Detection Products Inc.), a quantitative bacterial assay, as suggested by (Legault et al., 1996; Malachova, 1999). The enzymatic activity of the bacterial indicator strain *E. coli* PQ37 determines the genotoxic activity of the soil samples via the β-galactosidase and alkaline phosphatase, which were determined by optical densities at 620 nm and 405 nm, respectively by Tecan Sunrise microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

The SOS induction factor (SOSIF) was calculated by the following equation using β-gal - β-galactosidase and G- alkaline phosphatase activity (determined by OD):

$$\mathrm{SOSIF} = \frac{\beta - \mathrm{gal}}{\mathrm{G}} \tag{2}$$

2.11. Human health risk assessment

Non-carcinogenic human health risk assessment based on elemental analysis (Table 1=) was calculated by a health risk model suggested by the US Environmental Protection Agency (RAIS, 2017; US EPA, 1989b, 1989a). The study covers the ingestion, inhalation, and dermal pathway exposure for metal(loid)s, which can result from hand-to-mouth action, dropped food, direct soil or dust consumption and inhalation, dermal contact, etc.

The non-carcinogenic risk assessment was done by the following equations (RAIS, 2017):

$$\mathrm{ADI}_{\mathrm{children/adults}} = (\mathrm{C}^{*}\mathrm{EF}^{*}\mathrm{ED}^{*}\mathrm{IR}^{*}\mathrm{CF})/(\mathrm{AT}^{*}\mathrm{BW}) \tag{3}$$

$$\mathrm{HQ}^{i} = ADI^{i}/RfD^{i} \tag{4}$$

ADI represents the average daily intake of metal(loid)s; C - concentration of metal(loid)s at sampling site (mg kg^{-1}); EF - exposure frequency, 350 days/year; ED - exposure duration that is considered 6 years for children and 30 years for adults; IR - ingestion rate that is 200 mg kg^{-1} for children and 10 mg kg^{-1} for adults; CF – conversion factor (10^{-6}), AT - average time = 365*ED; BW - average body weight, which is 15 kg for children and 70 kg for adults. Hazard quotient (HQ) > 1 indicates possible adverse health effects, whereas HQ < 1 means no adverse health effect. The oral reference dose (RfD) of metal(loid)s is shown in (Table S5) (Kamunda et al., 2016; C. Liu et al., 2016; RAIS, 2017; US EPA, 1989b, 1989a).

The non-carcinogenic effect on the population for n number of metal (loid)s is the sum of all the HQs owing to individual metal(loid)s. According to the USEPA, this is referred as the Hazard Index (HI) and is represented by the following equation:

$$\mathrm{HI} = \sum\nolimits_{k=1}^{n} HQk \tag{5}$$

where HQ_k is a value of metal(loid) k. The exposed population is unlikely to suffer negative health impacts if the HI value is less than one. If the HI value is more than one, there may be cause for concern about non-carcinogenic effects (US EPA, 1989a).

2.12. Statistical analysis

The normality of the data was assessed using the Shapiro-Wilk test due to the limited number of samples. To account for the non-normal distribution of specific features, the data underwent a Box-Cox transformation prior to the statistical analysis which is a more effective method for data normalization (Daimon, 2014; McGrath et al., 2004). To extract a reduced number of independent metal(loid) components in urban soils, principal component analysis (PCA) was performed. The relationship between soil microbial community and metal(loid)/physicochemical parameters was examined and illustrated by R 2.5.1 (R Core Team, 2020) via vegan (Oksanen et al., 2019) and ggplot2 packages (Wickham, 2010). The dissimilarity of the bacterial communities (using OTU data) was visually interpreted by non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarity method. The relationship between operational taxonomic units (OTUs) and environmental factors was analysed by the Envfit function using the vegan package (Oksanen et al., 2019). Differences in the environmental factors (soil organic content, soil texture, Eh-pH) and metal(loid)s in sampling sites were checked by one-way ANOVA ($p < 0.05$ considered significant). The OTU dissimilarities between sampling sites/cities were analysed by the Simper test on PAST4 software (https://www.nhm.uio.no/english/research/resources/past/). Community structure differences were checked by the PERMANOVA test, using Adonis with 9999

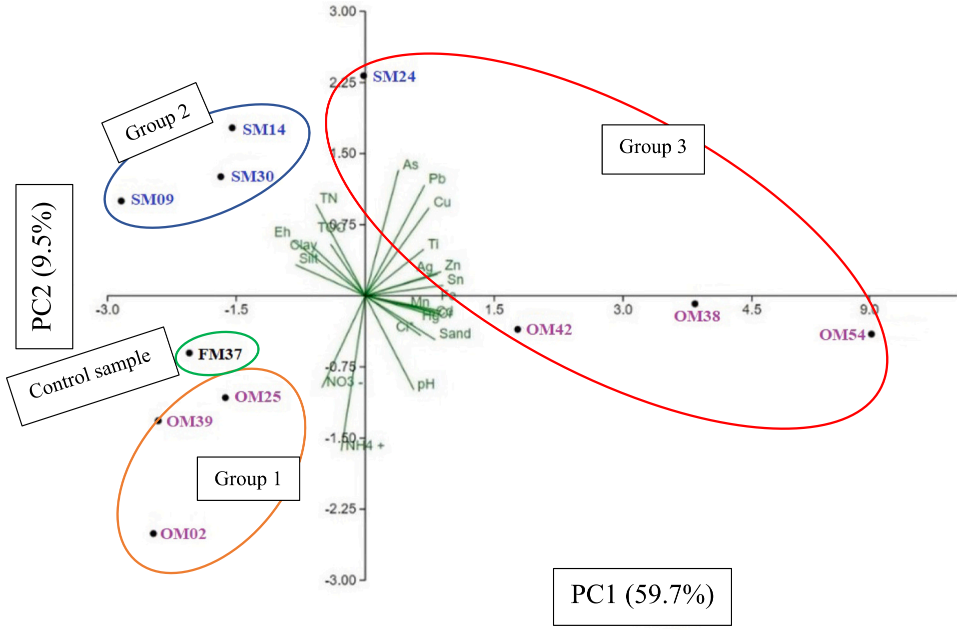

Fig. 3. Principal component analysis (PCA) of the urban soil physicochemical parameters and metal(loid) content. The figure illustrates the three studied sample groups and the possible effects of the environmental physicochemical factors (Table 1) on samples.

permutations on the vegan package (Anderson, 2001).

3. Results and discussions

3.1. Environmental parameters and metal(loid) content of the urban soil sites

The concentration of the analysed metal(loid)s and environmental parameters in the studied urban soil samples are presented in Table 1. The physicochemical/environmental parameters and metal(loid) concentrations in the soil samples distributed heterogeneously among the samples. Differences in the metal(loid) concentrations and environmental parameters of the sampling sites significantly ($p < 0.05$) differed (53 %). The results of enrichment study were calculated for soil samples in both cities relative to the control (brown forest soil) sample value of metal(loid)s in soil (Fig. 2 and Fig. S1). The mean EF values decreased in the order of Cd > Ti > Sn > Pb > Ag > Cu > Hg > Zn > Cr > Mn > As > Fe in Salgótartján (STN) and Ózd (OZD) samples. Except As, Fe and Mn, the other elements depicted moderate to significant enrichment of metal (loid)s. Especially in two STN samples (SM24-playground, and SM30-roadside), and three OZD samples (OM38-industry, OM42-playground, and OM54-industry) significant accumulative enrichment of all, the analysed metal(loid)s was observed (Fig. S1).

The Principal Component Analysis of the analysed metal(loid)s and environmental parameters (Table 1) formed three distinct groups with 59.7 % PC1 and 9.5 % PC2 (Fig. 3). Group 1 contains the control FM37 (brown forest soil) and urban soils from playgrounds: OM02, OM25 and OM39 which are considered as low or no metal(loid) polluted samples.

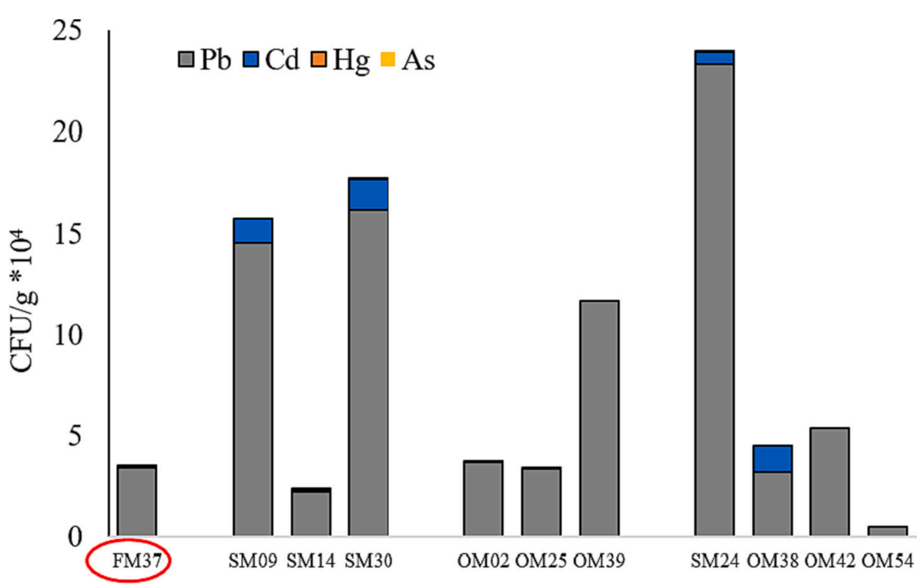

Fig. 4. Resistant colony forming unit (CFU) counts in Pb, Cd, Hg, and As salt amended nutrient media plates from STN and OZD urban soils. The number of Hg and As resistant bacteria are very low (or there is not), thus, it cannot be seen in the figure. Sample FM37 is the control sample. The samples were grouped based on the grouping obtained from PCA analysis.

Table 2
Isolated bacterial strains and their tolerance to the selected metal(loid) salts. All listed strains resisted the complex effect of metal(loid)s (Pb-900 μg/mL, Cd-600 μg/mL, Hg-600 μg/mL).

Strain ID	Isolated species/ closest relatives	NCBI accession number	Studied metal(loid)s			
			Pb (μg/mL)	Cd (μg/mL)	Hg (μg/mL)	As (μg/mL)
S9E8P	*Bacillus pseudomycoides* DSM 12442	MT765153	1600	600	600	800
S14E3P	*Bacillus pseudomycoides* DSM 12442	MT765154	1600	600	600	400
S14E4P	*Aminobacter aminovorans* DSM 7048	MT765155	1600	600	600	–
S14E6H	*Azospirillum brasilense* TSH58	MT765156	1300	600	800	–
S14E9P	*Ochrobactrum tritici* SCII24	MT765157	1600	800	600	1000
S3010P	*Arthrobacter gyeryongensis* DCY72	MT765158	1600	600	600	100
S9E3P	*Bacillus thuringiensis* ATCC 10792	MT765159	1600	600	600	600
S9E4H	*Bacillus megaterium* NBRC 15308	MT765160	1500	2000	1200	1000
S14E1C	*Cupriavidus campinensis* WS2	MT765161	3000	15,000	1400	200
S14E3C	*Cupriavidus campinensis* WS2	MT765162	3000	10,000	1400	200
S14E4C	*Cupriavidus campinensis* WS2	MT765163	3000	15,000	1500	400
S14E6C	*Cupriavidus campinensis* WS2	MT765164	3000	15,000	1500	200
S24E2P	*Bacillus thuringiensis* ATCC 10792	MT765165	1600	600	600	600
S24E3C	*Cupriavidus campinensis* WS2	MT765166	3000	4500	1400	100
S24E7C	*Delftia acidovorans* 2167	MT765167	3000	3000	1400	200
S24E7H	*Pseudomonas frederiksbergensis* JAJ28	MT765168	1500	2000	1200	–
S24E9P	*Brevibacterium frigoritolerans* DSM 8801	MT765169	1600	600	600	600
S30E2H	*Pseudomonas kunmingensis* HL22-2	MT765170	1500	2000	1200	–
S30E3H	*Pseudomonas kunmingensis* HL22-2	MT765171	1500	2000	1200	100
S303Pb	*Bacillus idriensis* SMC 4352-2	MT765172	1600	600	600	800
SK27P	*Bacillus simplex* NBRC 15720	MT765173	1600	600	600	800

- No bacterial growth.

Samples in this group were not influenced significantly by anthropogenic or industrial activities as much as the following two groups. Group 2 includes samples SM09-park SM14-playground, and SM30-roadside with moderate metal(loid) pollution, low pH, high Eh (oxidation reactions), and high clay and silt fractions (Fig. 3). These urban soils are rich in soil organic matter, and it is supposed that they still preserve certain natural condition (and feature) of soils. Group 3 is noted with the highest metal(loid) pollution and lowest organic content for urban soils SM24-playground, OM38- former industry, OM42-playground and OM54- new industry. These soil samples were significantly affected by anthropogenic activity and thus the soil organic content decreased, and grain size fractions dominated by sand fraction (Fig. 3). Study by Seddaiu et al. (2013) determined that disturbed soils contained lower levels of total organic matter. A negative correlation between Eh and pH, as well as soil silt and sand fraction (Fig. 3) indicate the wide-scale impact of metal(loid)s and organic content on sampling sites, was similarly reported also by Lee et al. (2002). On the other hand, in our study a positive correlation ($p < 0.05$) between clay fraction and soil organic content was observed and also stated by previous studies due to the capacity of clay to hold organic material (e.g., Zhang et al., 2016 and references therein).

3.2. Cultivation of metal(loid) tolerant/resistant Bacteria

Due to high metal(loid) contamination in the studied urban soil environment of both cities, the number of metal(loid) tolerant bacteria was relatively high (Fig. 4). Cultivation analyses revealed a high number of bacteria tolerant to 200 μg/mL Pb and Cd salts, ranging from $0.5*10^{4}$ to $23.3*10^{4}$ CFU for Pb and from $0.007*10^{4}$ to $1.5*10^{4}$ CFU for Cd. However, there is very less or no growth on Hg and As salts amended plates (Fig. 4). In total, 220 isolates were obtained from the plates and exposed to elevated concentrations of the selected metal(loid) salts to identify minimum inhibitory concentration (MIC) values. However, among them, only 21 strains were able to grow in elevated metal(loid) concentrations and they were identified by 16S rRNA gene sequence (Table 2). The MIC values are different for bacterial species and strains, ranging between 1300 and 3000 μg/mL for Pb, 600–15,000 μg/mL for Cd, 600–1500 μg/mL for Hg, and 100–1000 μg/mL for As. Remarkable differences among species in terms of the selected metal(loid) tolerance were observed, especially members of *Cupriavidus*, *Bacillus,* and *Pseudomonas* genera were noticeable (Table 2) as was also reported by other studies (Gupta et al., 2012; Mihdhir and Assaeedi, 2016; Monsieurs et al., 2011). A single *Delftia acidovorans* strain S24E7C and *Pseudomonas* spp. depicted considerable resistance to Pb, Cd, and Hg, whereas significant As tolerance was observed only in strains of *Bacillus* species. Due to their sensitivity, certain strains did not grow in the As salt-containing medium (Table 2).

The fact that the isolated bacterial strains are originated from the high metal(loid) contaminated sites may explain their great tolerance to metal(loid)s (Abou-Shanab et al., 2007). In natural conditions, soil bacteria are exposed to metal(loid)s in solution or adsorbed on soil colloids, which leads to the selection of tolerant bacteria in the population. On the other hand, due to adaptation, microorganisms isolated from metal(loid) contaminated habitats frequently show tolerance to multiple contaminants (Abou-Shanab et al., 2007). Among the bacterial strains, *Cupriavidus* sp. illustrated (Table 2) comparatively high resistance to the four selected metal(loid)s (particularly Cd) and was selected for further identification of resistance mechanisms through genomic analysis (Abbaszade et al., 2020). The candidate strain could be a potential candidate to bioremediate contaminated soils which offers eco-friendly and sustainable environment.

3.3. Results of the diversity index calculations of the samples

The alpha diversity of the 16S rRNA gene sequencing was used to calculate the taxonomic richness and diversity (Table S2) of the samples. The number of bacterial OTU ranged between 1844 and 2349 in all polluted urban soils, whereas in the control sample, the value is 1701. It is revealed that only 37–53 % of the total bacterial community is abundant >1 % in all studied urban soil samples. Among the diversity indices, the Chao1 and ACE are particularly effective in estimating the species richness and was relatively low in the control sample (1704 and 1722, respectively). Thus, higher species richness was observed in contaminated urban soil samples (Table S2). Other diversity indices (Shannon and Inverse Simpson) of the microbial communities in the control and metal(loid)-polluted samples were relatively similar, despite a high difference in metal(loid) and environmental factors (Table S2).

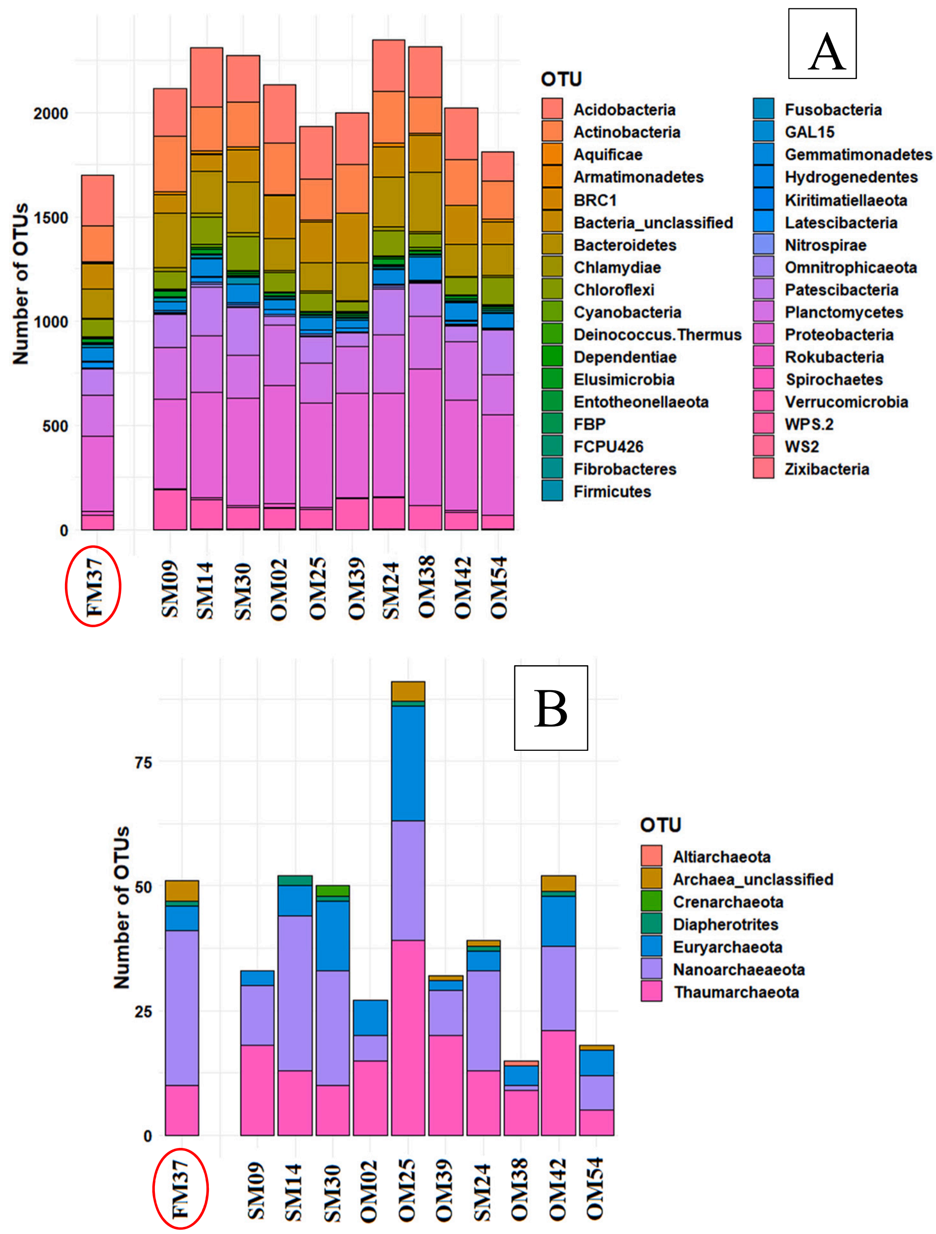

Fig. 5. Abundance of bacterial (A) and archaeal (B) phyla in STN and OZD urban soil samples, obtained by NGS sequencing. FM37 is the control soil sample.

The Simper test showed 9.5 % dissimilarity in diversity between control and polluted soils. Earlier studies (Luo et al., 2019; Zou et al., 2021) reported that the grade of metal(loid) pollution does not mean a simple positive or negative linear relationship with microbial composition and community structure. For instance, the low and medium concentrations of metal(loid)s can promote, however, long-term metal(loid) exposure can hinder microbial diversity (Luo et al., 2019; Sobolev and Begonia, 2008).

Archaea OTU ranged from 15 to 91 (Table S2). The highest archaeal OTU number was found in urban soil OM25-playground (91 OTU), one of the lowest metal(loid) containing samples. Whereas the lowest value was defined in highest metal(loid) contaminated urban soils: OM38-industry and OM54-industry (15 and 18, respectively). Generally, archaea are known for their strong resistance to environmental stress factors, however early studies reported that soil physiochemical parameters, particularly soil metal(loid) content and organic matter, were strong determinants of archaeal community variation (R. Sandaa et al., 1999; Zhao et al., 2020).

3.4. Structure of the soil microbial community based on amplicon sequencing

Analysis indicated that in all sample on the level of phyla, the bacterial community was dominated by Proteobacteria, which constituted 20–22.8 % of the total soil bacterial community in STN and 25–28 % in OZD urban soils. In STN samples, their abundance followed by Acidobacteria (9.8–12.5 %), Actinobacteria (9.1–12.6 %), Bacteroidetes (8.6–12.3 %), Planctomycetes (8.9–12 %), Patescibacteria (7.5–10.2 %), Verrucomicrobia (4.5–9 %), and Chloroflexi (4–7.1 %) (Fig. 5A). In OZD samples, however, the abundance of Proteobacteria was followed by Planctomycetes (10–13.8 %), Acidobacteria (7.7–13.1 %), Bacteroidetes (7–12.3 %), Actinobacteria (7.6–11.7 %), Patescibacteria (2–11.7 %), Verrucomicrobia (3.6–7.3 %), and Chloroflexi (2.5–7.1 %) (Fig. 5A). The abundance of unclassified bacteria ranged between 3.6 and 6.6 % in STN and 3.6–12.0 % in OZD samples. The above-mentioned phyla cover 91 % of the soil community (Fig. 5A). The relative abundance of each phylum varied among the sampling sites, and the overall average dissimilarity of phyla was only 16.22 % between polluted and control samples. Thus, on

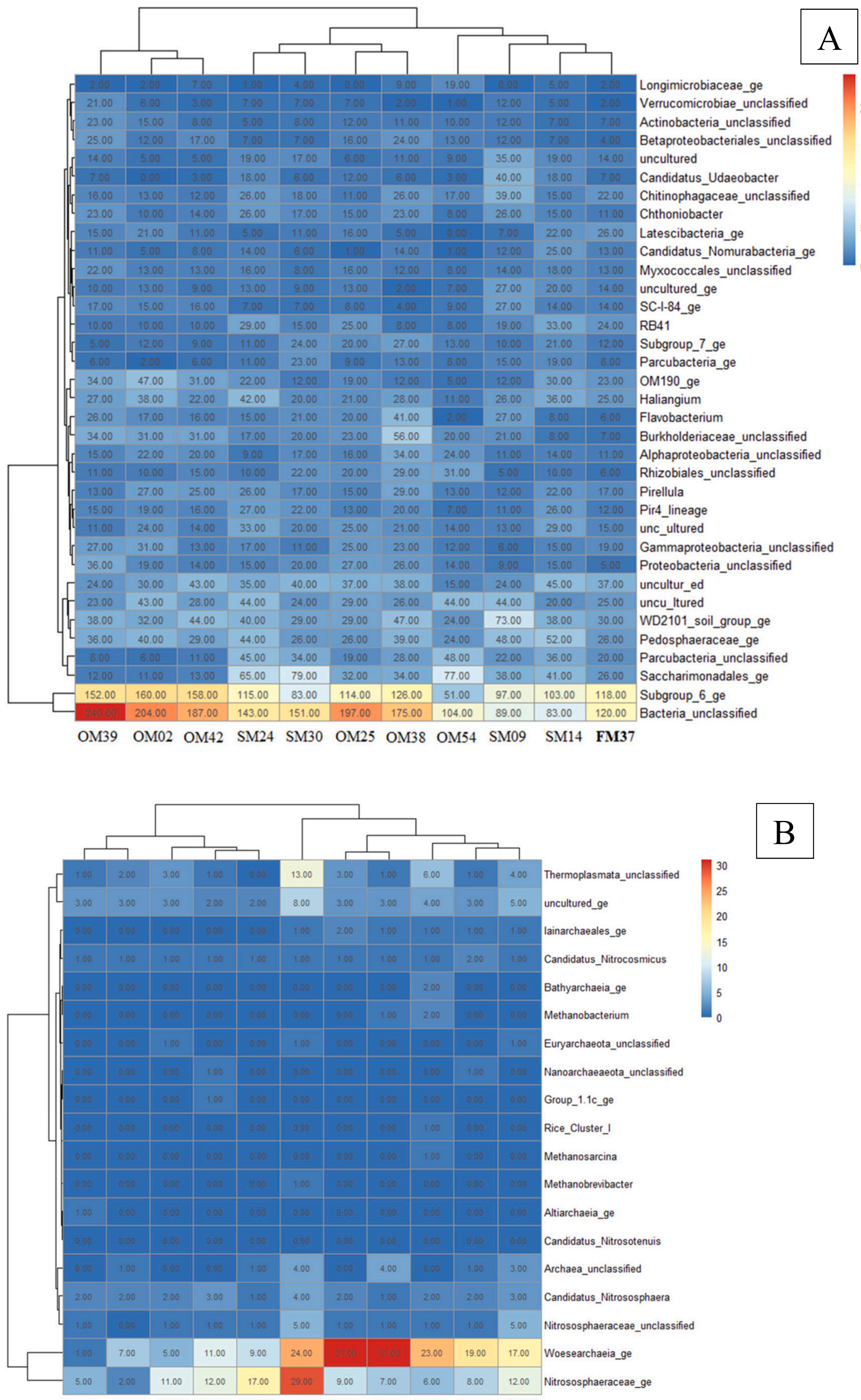

Fig. 6. The most abundant (>1 %) bacterial (A) and archaeal (B) OTUs (genus level) in STN and OZD urban soil samples. Plot illustrates the number of OTUs. FM37 is control sample.

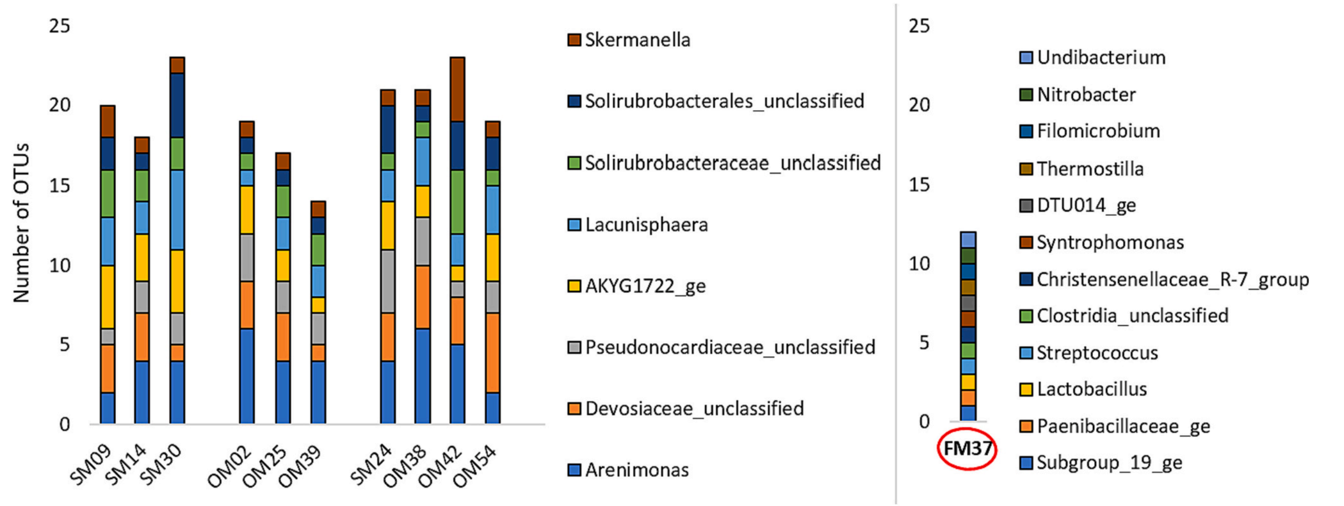

Fig. 7. Identified rare taxa (abundance <1 %) (at genus level) in STN and OZD urban soil samples. Sample FM37 represent control sample. Some of the taxa exist in polluted soils and in control, too.

the level of phyla, characteristic differences were not visible between polluted urban soil sampling sites and the control sample. However, the observed differences in relative abundance indicated that certain bacterial phyla responded differently to the analysed metal(loid)s. Compared to the control sample, the metal(loid)-polluted urban samples were characterized by a low abundance of Acidobacteria and a higher abundance of Proteobacteria and Patescibacteria (Fig. 5A). Though, it can often be relative: as some members of the prokaryotic community, which is sensitive to metal(loid)s die, on the other hand, can multiply more in the lack of competitors. Similar patterns for Proteobacteria were reported by Sandaa et al. (2001) and Li et al. (2017a). On the other hand, higher Deltaproteobacteria OTU was observed in metal(loid) polluted STN samples but lower OTU in metal(loid) polluted OZD samples. Studies stated that in metal(loid) polluted soils, members of Proteobacteria (e.g. genera *Pseudomonas, Sphingomonas*, etc.) showed high relative abundance and are typically resistant to metals (Yang et al., 2020). Wang et al. (2019a, 2019b) reported that low concentration of Cd appears to increase, whereas higher Cd levels inhibit microbial proliferation. Similar results were observed in the case of the members of Planctomycetes and Actinobacteria phyla in our study. These varying responses to metal(loid)s may be attributed to varied environmental circumstances and ability to use numerous forms of organic materials such as carbon, nitrogen and energy sources (Bouskill et al., 2010).

The archaeal community in all STN and OZD urban soils was dominated by Thaumarchaeota (20–62.5 %), Nanoarchaeota (6.7–59.6 %) and Euryarchaeota (6.25–28 %), these phyla covered the 95 % of the archaeal communities (Fig. 5B). Crenarchaeote appeared only in the urban soil SM30-roadside, and can be associated with metal contamination as stated by Li et al. (2017a) for archaeal metal resistance. The highest number of OTU (at the level of phyla) was observed in urban soil OM25-playground, however, the lowest number of archaeal phyla was observed in urban soil OM38- industry and OM54-industry (Fig. 5B), the highest metal(loid) polluted samples. The Simper test revealed an average dissimilarity of 36.12 % among archaea phyla between samples from the control samples and metal(loid) polluted sampling sites. Observations shows in high metal(loid) contaminated sites (OM38- industry and OM54-industry) the number of archaea OTUs (at the level of phyla) was substantially less than in control sample and less polluted urban soils (Fig. 5B).

The taxonomic differences between the metal(loid) polluted and controlsamples were visible at class and genus level. In contrast to the control sample, ratio of significantly higher Gammaproteobacteria, Deltaproteobacteria, Planctomycetacia and Actinobacteria was observed in STN urban soils, whereas the number of Deltaproteobacteria, Planctomycetacia, and Actinobacteria OTUs in OZD urban soils were considerably lower. A characteristic abundance of Latescibacteria_cl in control sample, on the other hand, Actinobacteria, Planctomycetacia, and Gammaproteobacteria in metal(loid) polluted STN and OZD samples were observed (Table S3).

At the genus level, >1 % of genera represented 37–53 % of the total community. Among them, *Subgroup_6_ge* 9 (Acidobacteria) is the most abundant taxonomic unit in all STN and OZD urban soils, followed by *Saccharimonadales_ge* (Fig. 6). Analysis indicated clear differences emerged at the genus level. It was noticed that the relative abundance of *Latescibacteria_ge* in control samples and unclassified Betaproteobacteriales, Burkholderiaceae and Proteobacteria in the metal(loid) polluted urban soils were substantially high, which can be associated with their sensitivity and resistance to different metal(loid) contaminants (Fig. 6), especially to As and Cd (Ayala-Muñoz et al., 2020; Yu et al., 2022). These results indicate that the content of metal(loid)s can be responsible for the variations in the bacterial community structure.

Performed Simper test proved the significant OTU dissimilarity with 71.56 % among polluted and control samples. Main contribution originated from the genus *Pseudomonas* (Proteobacteria), *MB-A2-108_ge* (Actinobacteria), *RB41* (Acidobacteria). The biggest variation among the urban soils (83.83 %) was observed in sample OM54-industry, which is strongly enriched in metal(loid) concentration. It was observed that the number of Alphaproteobacteria (*Ellin6055* genus) and Blastocatellia (*RB41* genus) in sample OM54-industry, whereas Gammaproteobacteria (*Burkholderiaceae_unclassified* genus) in urban soils OM38-industry and OM54-industry (two high metal(loid) polluted samples) are characteristic.

Archaea often make only a small percentage of the microbial community in soils, in this study, the archaeal community obviously responded to metal(loid)s contamination. In the case of Archaea, the most abundant classes in our samples belong to Thermoplasmata, Woesearchaeia, and Nitrososphaeria. Among the samples, most abundant archaeal OTU was the most dominant at sample OM25-playground, which is characterized by low metal(loid) pollution and high soil organic content, with the highest number of Thermoplasmata and Nitrososphaeria OTUs (Fig. 5). The lowest number of the mentioned archaeal classes were observed in the highest metal(loid)-polluted samples (Table S4).

At the genus level, the abundance of *Woesearchaeia_ge, Nitrososphaeraceae_ge* and *unclassified Thermoplasmata* was particularly noticeable in STN and OZD urban soils and were representing 6.7–60.8 %, 11.1–53.1 %, and 3–14.3 % of the communities, respectively (Fig. 6). Due to their membrane structure, ability to use diverse energy sources,

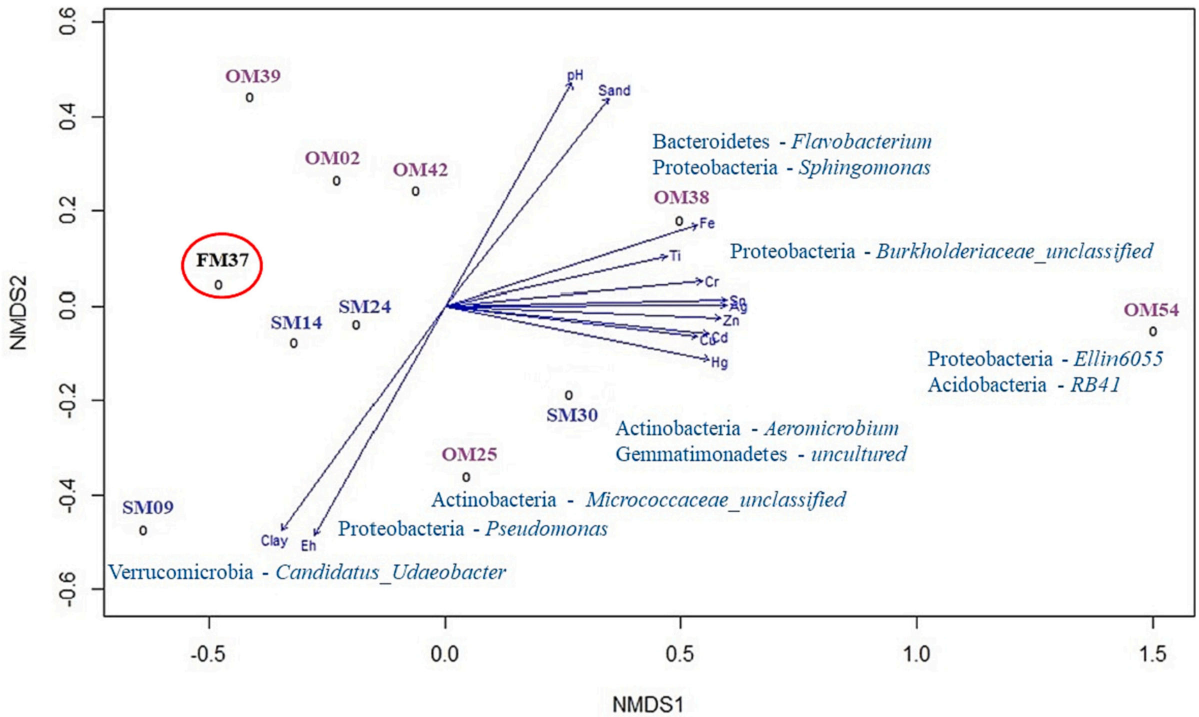

Fig. 8. Non-metric dimensional scaling (NMDS) analysis of bacterial OTUs with the fitted metal(loid)s and environmental physicochemical factors ($p < 0.05$) in STN and OZD urban soil samples. Sample FM37 indicates the control sample. The stress level is 0.08. The characteristic phyla and genera in sampling sites are shown next to the sampling sites (identified by Simper test).

and reduce metal(loid)s by ammonia, sulphide or sulphur, archaea play an important role in regulating the toxicity of metal(loid)s in the soils (Li et al., 2017a). In this study, the number of abundant archaeal genera did not follow any patterns, however, there was a significant decrease of *Nitrososphaeraceae_ge* and *Woesearchaeia_ge* in the high metal(loid)-polluted sites (OM38-industry and OM54-industry) (Fig. 6). According to previous studies, low concentrations of metal(loid)s can promote the growth of communities, whereas higher quantities have an inhibitory effect that frequently results in negative correlated outcomes (Yu et al., 2021). Thus, organic-rich and low metal(loid)-bearing urban soil (OM25-playground) and the control sample might promote the archaeal community (Fig. 6).

As the abundance of the rare genera (abundance <1 %) was responsible for 47–63 % of the total community, their potential impact on soil processes cannot be ignored. Analysis showed that in STN and OZD urban soils, some of the rare taxa (at genus level) existed either only at the polluted or control samples (Fig. 7). Among them *Skermanella, Lacunisphaera, Arenimonas,* together with other unclassified rare genera (Fig. 7), exist only in metal(loid)polluted samples. This might be due to their gained resistance to the metal(loid)s in the soils (Sun et al., 2022; G. Wang et al., 2021). These taxa might also be present in the control sample, but their quantity is under the detection limits. On the other hand, genera, such as *Nitrobacter, Filomicrobium, Lactobacillus, Streptococcus, Subgroup-19-ge*, etc. were found only in control sample (FM37) and could be associated with low metal and high total organic carbon content (Table 1; Fig. 7). It was reported that rare and abundant taxa do not necessarily react to environmental changes in the same way (Liang et al., 2020), and, in general, the higher relative abundance of a given taxon is not necessarily connected to increased activity. Compared to rare taxa, the observed abundant genera typically occupy a wider range of niches and have access to a wider resource, making them better adaptive to environmental changes. Though the members of "rare taxa" are often relatively slow-growing, they may become active in more advantageous conditions (Shade et al., 2014; Xu et al., 2021).

3.5. The relative impact of environmental parameters and metal(loid)s on community composition

Based on the nonmetric-multidimensional scaling (NMDS) ordination analysis, it was revealed that the enrichment of the metal(loid)s considerably affected the diversity of bacterial communities (Fig. 8). Metal(loid)s significantly correlated with NMDS1 and NMDS2, indicating that these factors were important for explaining the variations in the bacterial community structure (Table S5). Especially, the impact of the metal(loid)s on the samples SM30-roadside, OM38-industry, and OM54-industry is very strong, whereas the community in the SM09-park sample is mainly affected by low soil pH value and clay fraction. The taxa (based on Simper test) with high load for each sampling site were shown on Fig. 8, which revealed minor clusters with the OTU similarity between STN and OZD urban soils. However, among them, samples SM09-park, OM25-playground, SM30-roadside, OM38-industry, and OM54-industry represented higher dissimilarities than the other samples. One of the highest variations (OTU difference) was observed in sample OM54-industry, where the anthropogenic metal(loid) input (from industry) altered the soil texture and became rather sand fraction dominated compared to other samples. A significant shift in Proteobacteria (*Ellin6055_ge, unclassified Burkholderiaceae*) and Acidobacteria (*RB41_ge*) were observed at this site (Fig. 8). The high relative abundance of genera in two highest metal(loid) contaminated urban soils: sample OM54 - industry, OM38 – industry, and in moderate metal(loid)-enriched samples (SM09-park, OM25-playground, SM30-roadside) indicated patterns (Fig. 8), confirms that the enrichment of metal(loid)s increases the metal(loid) resistant members in the polluted sites as was similarly shown by Duan et al. (2021); Luo et al. (2018) and Yu et al. (2021). This finding correlates well with the earlier research, which stated that among the community, the members of the class Gemmatimonadetes and genus *Aeromicrobium* correlate positively with Cd (Sun et al., 2022), as well as *Pseudomonas* species can resist multiple metal (loid)s, including Zn, Hg, Cu, Cr and Cd species in high concentrations (Malik and Jaiswal, 2000). This reveals that the microbial communities

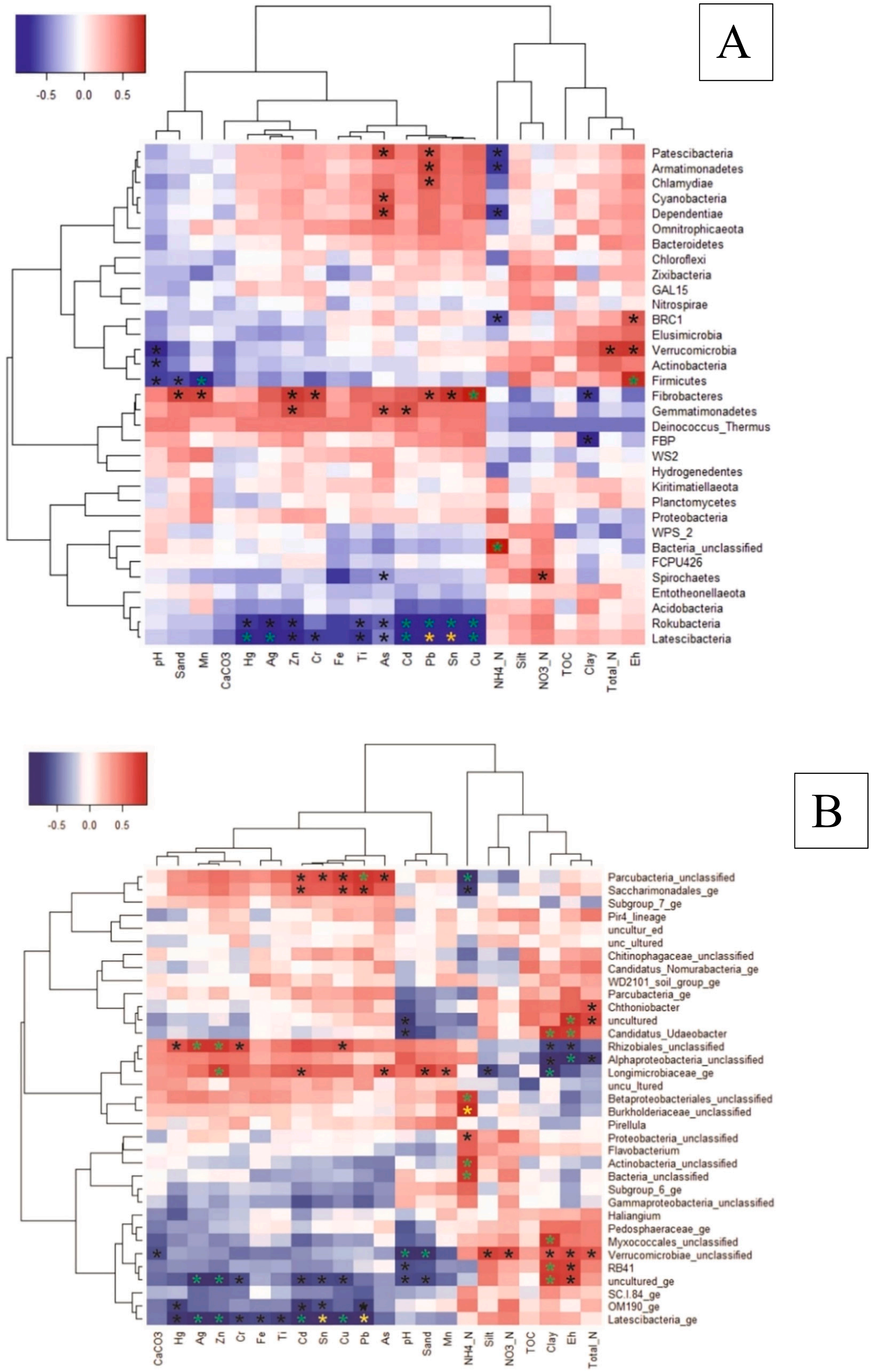

Fig. 9. Spearman correlation heatmap shows the connection between environmental factors, metal(loid)s, and Bacteria phyla (A) or genera (B) in STN and OZD urban soil. Correlation significance values ($p < 0.05$) are shown by starts (<0.05 by black, <0.01 by green, and < 0.001 by yellow). Background colours refer to the Spearman's rank correlation coefficient (from −1 to +1).

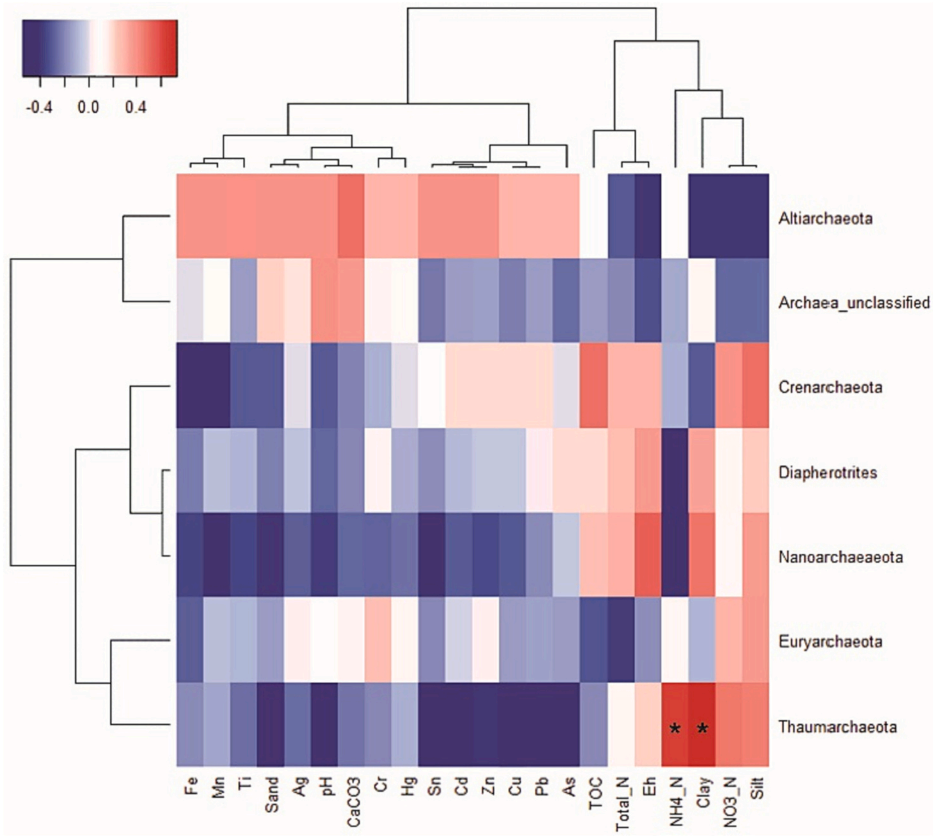

Fig. 10. Spearman correlation heatmap showing the connection between environmental factors, metal(loid)s, and archaeal phyla in STN and OZD urban soil. Correlation significance values (p < 0.05) are shown by stars.

of the metal(loid)-polluted samples differed significantly from the control sample. However, though the SM14-playground, SM24-playground, OM02-playground, OM39-playground, and OM42-playground are polluted in various degrees (less or more depending on site) with metal (loid)s, they show an OTU similarity to FM37 control sample (Fig. 8). No correlation with metal(loid)s was observed in these sampling sites (Fig. 8), however, the similarity of the communities can be explained by the low anthropogenic impact (Yao et al., 2017).

Among the communities, taxa at phylum and genus level illustrated a strong correlation with the studied metal(loid)s and according to Spearman correlation analysis, the members of the soil microbial communities respond differently to environmental factors and metal(loid)s (Fig. 9A and B). It is noticed that the content of metal(loid)s significantly correlated with 10 bacterial phyla (Fig. 9A) and 7 abundant bacterial genera ($p < 0.05$) (Fig. 9B). In particular, a positive correlation between Patescibacteria and As, Pb; Armatimonadetes, Chlamydiae and Pb; Cyanobacteria, Dependentiae and As; Fibrobacteres and Zn, Cr, Pb, Sn, Cu, Mn; and Gemmatimonadetes and Zn, As, Cd was observed, respectively. However, Rokubacteria and Latescibacteria illustrated a strong negative correlation with most of the analysed metal(loid)s (Fig. 9A). At the level of bacterial genera, *Saccharimonadales_ge* and *Longimicrobiacea_ge*, as well as unclassified Rhizobiales, and Parcubacteria were significantly related to Hg, Cd, Cu, Sn, Pb, and As, respectively. It is observed that among the genera, all studied metal(loid)s, particularly Hg, Cd, Sn, and Pb are negatively influencing OM190_ge and Latescibacteria_ge (Fig. 9B). Our results suggest that these bacterial phyla and genera are significantly related to the content of metal(loid) that was also demonstrated by Liu et al. (2022) for relation between community and Zn, Cu, Cd and Pb. Li et al. (2017a, 2017b) found that soil microorganisms respond to long-term metal(loid) pollution by changing the composition and structure of their microbial communities rather than changing their diversity and evenness.

On the other hand, changes in the bacterial community structure are not influenced only by urban soil metal(loid) content, but a variety of environmental factors, including soil organic matter content, moisture, pH, or soil type. The driving effect of soil organic matter (SOM), pH, and redox potential on metal bioavailability and thus on soil microorganisms were reported in a number of studies (Wang et al., 2019a, 2019b; Epp Schmidt et al., 2019; Mhete et al., 2020). Therefore, high soil SOM, silt, and clay fraction, as well as low pH in the studied sampling sites, can be a significant regulator for soil microbial community rather than metal (loid) concentration (Fig. 9) (Huang et al., 2021; Nakatsu et al., 2005; Yavitt et al., 2021) that might explain the relative impact of these parameters. However, in highly polluted samples, lower levels of total organic matter were observed and also stated by several studies (e.g., Seddaiu et al., 2013). The relative abundances of specific groups of bacteria in soils, such as the Acidobacteria and Proteobacteria (Gammaproteobacteria and Betaproteobacteria), have been demonstrated to be highly influenced by soil pH (Yun et al., 2016). Whereas, except direct effect on the community, changes in pH influence the solubility and bioavailability of metal(loid)s by converting active metal(loid)s into inactive chemical forms or vice versa (Petruzzelli et al., 2020). Lee et al. (2015) reported that a decrease in soil pH and redox potential could increase the mobility of soluble metal(loid)s and thus the toxicity. This suggests that the combinations of soil physicochemical parameters (SOM, soil texture, pH-Eh) and metal(loid)s content were responsible for alterations in the bacterial community.

A significant positive correlation was observed between ammonia oxidizing Thaumarchaeota vs soil ammonia content and clay fraction (Fig. 10), which was similarly reported by Brochier-Armanet et al. (2008). That explains the less abundance of Archaea OTU in metal(loid) contaminated samples, which were characterized by low organic content (Fig. 5). There was no sign of the relation between soil metal(loid) content and archaeal phyla.

Table 3
Ecotoxicology test results of STN and OZD urban soils and control sample - FM37. SOSIF-induction factor (average).

Soil samples	SOSIF	Cytotoxicity	Genotoxicity
FM37	0.1	+	–
SM09	0.56	+	–
SM14	0.34	+	–
SM24	0.37	+	–
SM30	0.16	+	–
OM02	0.32	+	–
OM25	0.46	+	–
OM38	1.2	+	+
OM39	0.38	+	–
OM42	0.97	+	–
OM54	0.38	+	–

+ genotoxic. IF≥1.2 is considered genotoxic.

3.6. Results of the ecotoxicological test of the soil samples

Previous studies have evaluated the toxicity of metal(loid) pollutants on soil enzyme activity, which is an important indicator of soil quality (Aponte et al., 2020; Maphuhla et al., 2022; Yeboah et al., 2021). It was found that metal(loid) pollution can significantly reduce soil enzymatic activity, which can lead to a decrease in soil fertility and a decrease in crop yields. Thus, ecotoxicology tests can provide important information about the potential impact of environmental pollutants on living organisms and the environment. In this study, the ecotoxicological effect of metal(loid)s in the soil samples were presented by SOS induction factor (SOSIF) and the values in the metal(loid) polluted and control samples are given in Table 3. The results of SOS Chromotest, obtained from this study showed a strong positive cytotoxicity and negative genotoxicity in all samples (including control one), except sample OM38-industry one of the highest metal(loid) polluted samples. The main reason for the cytotoxicity at low contaminated and even at FM37 control samples can be the cumulative impact of soil metal(loid) content and high nitrate content. Studies show that high levels of nitrate result in nitrite accumulation in soils which is poisonous or inhibitory to microorganisms (Albina et al., 2019; Yarbrough et al., 1980).

Results showed that the soil leachate (with water) activated a DNA repair process in *E. coli* PG37 bacteria and the initiation of the SOS response is a sign that the bacteria cells were exposed to mutagenic and genotoxic chemicals present in the analysed leachate, which resulted in DNA damage. Compared to the contaminated samples, a relatively low IF was observed in unpolluted control sample (FM37) (Table 3). A strong toxicity of analysed metal(loid)s is reported by various studies, however it strongly connected to their bioavailability (Leita et al., 1995; Olaniran et al., 2013; Rieuwerts et al., 1998). Maisto et al. (2011) reported a high soil toxicity only at high metal(loid) concentrations where soil metal (loid) bioavailability would be higher as well. To prove the statement, additional genotoxicity and chemical tests are needed to assess the toxicity profile of soil samples from these industrial sites.

3.7. Human health risk assessment

The health risk estimation based on the total concentration of what elements represent a long-term threat and can overrate the authentic health risk (Liu et al., 2016). In polluted areas, the total concentration of the studied metal(loid)s in the urban soils alerted the sampling sites which were considered as highly contaminated areas (Fig. 3). At these sites, the metal(loid) contents exceed the maximum threshold value for the acceptable concentration of metal(loid)s in soils assigned by the Hungarian government (HUGD, 2009). Therefore, an additional assessment of human health risk was performed according to US EPA models (RAIS, 2017; US EPA, 1989b, 1989a) on metal(loid) content to reveal the risk possibility of high metal(loid) contaminated sites for STN and OZD urban soil samples. The results were calculated with the reference dose (RfD) (Table S6) and average daily intake (ADI) values of the metal(loid)s for adults and children based on eqs. 3, 4, and 5 (material and methods). Chronic (non-carcinogenic) health risk assessment analysis indicating the hazard quotient (HQ) of metal(loid)s for adults is below the safe value (HQ < 1) in ingestion, inhalation, and dermal

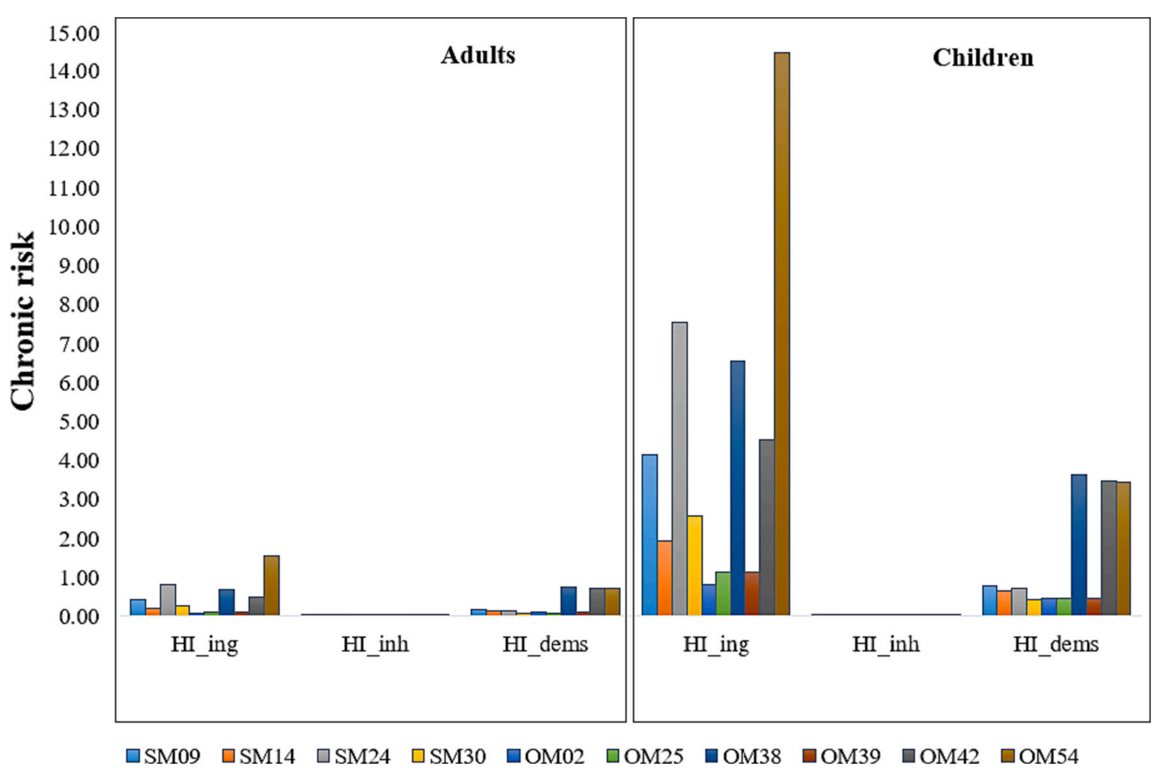

Fig. 11. Non-carcinogenic (chronic) health risk in STN and OZD urban soil samples though three pathways. Health risk was calculated based on HI (hazard index) values. Ing – ingestion, inh - inhalation, and dems - dermal pathways. Samples with value of chronic risk >1 considered potentially risky for human health.

exposure (Fig. S2). On the other hand, HQ values for As in sample SM09-park and SM14-playground; Pb in sample SM24-playground and SM30-playground; As, Pb, Fe, and Mn in sample OM38-industry; As and Mn in sample OM42-playground; As, Pb, Cd, Fe, Cr, and Mn in sample OM54-industry, were higher than the safe level (>1) for children (Fig. S2). Results showed that besides urban soils obtained from industrial areas, particularly park and playground samples are significantly contaminated and thus accumulation of metal(loid)s could pose health risk to residents. Additionally, the combined effect of metal(loid)s was higher than safe level and could carry possible health risk to adults and mainly children (HI > 1) (Fig. 11). High values suggested that metal(loid) pollution could represent a significant non-carcinogenic health risk at the contaminated sites, which pose a higher non-carcinogenic health risk for children compared to adults, as was similarly reported by previous studies (Gržetić and Ahmed Ghariani, 2008; Tepanosyan et al., 2017). The findings also show that the ingestion pathway mostly contributes to non-carcinogenic risk in both adults and children, followed by the dermal pathway. Inhalation is the least dangerous pathway for metal(loid) exposure (Fig. 11).

4. Conclusions

The study examined metal(loid) concentrations, soil physicochemical parameters, and their impact on the urban soil microbial community in two contaminated urban environments (Salgótarján and Ózd) compared to an uncontaminated forest sample. The data analysis revealed that metal(loid) contamination led to an increase in soil sand grain size fractions and a decrease in soil organic content. The research demonstrated that the impact of metal(loid) contamination shifted community composition, but soil organic content and pH also were significant regulators. It was revealed that high metal(loid) concentrations could be cytotoxic to soil microorganisms but not necessarily genotoxic. These findings offer valuable insights into the extent and consequences of metal(loid) pollution in urban soils, which offers valuable information for the development of effective environmental management strategies. Additionally, the study highlighted the potential use of metal(loid)-resistant/tolerant bacterial strains for bioremediation in terrestrial environments, providing a sustainable and eco-friendly solution to urban ecological issues. The research has implications for addressing human health concerns related to metal(loid) pollution, particularly in sampling sites, such as playgrounds and parks with elevated metal(loid) concentrations that pose a chronic health risk, especially for children. Thus, understanding the levels and sources of metal(loid) contamination in urban soil is crucial for assessing the associated health risks for individuals residing in affected areas.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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