Environmental contamination of potentially toxic elements (PTE) and its effect on soil microbial communities in former industrial cities of Northern Hungary (Salgótarján and Ózd)

by

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I. Introduction and objectives

Potentially toxic elements (PTE) are natural constituents of soil environment, however their concentration dramatically increased after industrialization that resulted in alteration of the soil quality (Liao et al., 2018; Tapia et al., 2018). Over centuries, anthropogenic activities (mining, manufacturing, industrial activities, etc.) have resulted in widespread metal(loid) contamination in urban areas that threatens the well-being of residents and the environment (Hiller et al. 2017). In 2019, Agency for Toxic Substances and Disease Registry ranked As, Pb, Cd, and Hg as the most dangerous chemicals and ranked them 1st, 2nd, 3rd, and 7th among the chemicals with great public health concern (CDC-ATSDR, 2019). Exposure of people to PTEs results in thousands of deaths and millions of mental disabilities every year. These elements and their compounds pose several health problems - such as cancer, neurological and kidney disease, birth defects, etc. (Tchounwou et al., 2013).

The most difficult problem associated with the contamination of soils with heavy metal(loid)s is that they cannot be naturally degraded like organic pollutants, and they can be accumulated in different parts of the food chain. Heavy metal(loid)s can slow down the speed of growth and reproduction of microorganisms, through functional disturbance, protein denaturation or the destruction of the integrity of cell membranes (Ghorbani et al. 2002). Studies have shown that the toxicity exerted by metals may suppress or can kill sensitive members of the microbial community and lead to a shift in community structure. However, to cope metal(loid) contaminants, soil microorganisms developed various resistance mechanisms. The main strategies through which they resist high concentrations of heavy metal(loid)s include efflux mechanisms, extracellular sequestration, biosorption, precipitation, alteration in cell morphology, enhanced siderophore production, intracellular bioaccumulation, etc. (Yu et al., 2017).

Previous studies reported that the use of microorganisms in bioremediation is one of the most economical and environmentally friendly methods by which soil microorganisms can neutralize or transfer contaminants to less harmful state through adsorption, precipitation, or transformation processes. The method is defined as a process to treat contaminated environments to regain healthy state (Abbas et al., 2018).

The study aims to explore the spatial distribution and enrichment of As, Pb, Hg, and Cd in two former industrial cities of Hungary, Salgótarján and Ózd, where the contaminants would form metal(loid) resistant species in the community and the ability of these specific microorganisms can be used in bioremediation processes. Therefore, a detailed characterization of microbial community/structure was performed to reveal the influence of soil physical and chemical parameters, as well as selected toxic compounds on the composition of prokaryotic communities. Additionally, health risk assessment of PTE was carried out to reveal exposure pathways, risky areas, and possible impact of PTE on residents in both cities.

II. Study area and sampling

Sampling was performed in two, former highly industrialized cities, Salgótarján and Ózd, located in a hilly area approx. 40 km distance from each other, where the region was significant brown coal, iron, and steel supplier. The foundation and development of the cities started in mid-18th century when brown coal deposits were discovered nearby both cities. Thereafter, iron and steel manufacturing, glassworks, mining machine factory, and later ferroalloy factory were established and operated for more than a century. In the 1980s the industrial production started to decrease, and after the collapse of the communist system the coal mines and industries were closed. However, the footprint of the significant and long-term anthropogenic industrial activities (coal mining, heavy industry, and later road and train transportation) can be recognized even today in both cities due to the presence of potential contamination sources (e.g., ruins of former smelters, coal mines, coal-fired power plant, slag hill, fly ash cone, etc.).

III. Material and Methods

The soil samples were digested by modified aqua regia digestion (1:1:1 HNO_3: HCl: H_2O) method at 95 °C on 15 g of < 0.075 mm milled aliquots for low to an ultra-low determination of elements in soils and were analysed by quadrupole-based ICP-MS (detection limit is 0.01 mg kg-1) at the Bureau Veritas Global Company in Canada. The organic content (total organic carbon, total nitrogen, ammonia, nitrate) of the soil samples was estimated at the Institute for Soil Sciences and Agricultural Chemistry by the loss-on-ignition method. Soil pH and conductivity (Eh) were tested in deionized water in a 1:10 soil-water ratio with a digital "Eijkelkamp 18.52.01 Multimeter" instrument. Soil particle analysis was performed with around 0.5 g of soil and 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.

Bacterial strains were isolated based on the spreading and enrichment methods (for 2 weeks) on the nutrient agar medium supplemented with 200 µg/ml of respective metal salt compounds ($CdSO_4$, $HgCl_2$, $Pb(NO_3)_2$). In order to obtain metal resistant isolates, a minimum inhibitory concentration (MIC) analysis was performed. Soil community analysis were performed at selected eleven sampling sites (including control samples), either with high or low PTE content (sometimes at least one of the PTEs), to be able to compare the effect of the contamination. The bioinformatic analysis of raw sequencing reads was executed with MOTHUR v.1.40.5 using the MiSeq SOP protocol (accessed in 2019). The whole-genome shotgun and paired-end sequencing were performed by the Genomics Research Technology Support Facility (RTSF), Michigan State University (USA), on an Illumina MiSeq platform using the MiSeq standard v2 chemistry.

Non-carcinogenic and carcinogenic human health risk assessment was calculated by a health risk model suggested by the US Environmental Protection Agency. Univariate and multivariate statistical analysis, and the display of results fulfilled by R v2.5.1, and Past4 software. Dissimilarity of the bacterial communities was visually interpreted by non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarity method.

IV. Results and Discussion

Due to its multi-industrial background, Salgótarján soils depicted heterogenous distribution of PTEs. Whereas, at certain parts of the city, there was a similar pattern of elevated As and Hg, as well as Pb and Cd concentration. Despite that, the detected high PTE contents in Ózd soils illustrated clear patterns, particularly at around former and new industrial sites, slag contaminated soils, and coal mining areas. Analysis showed that in some sampling sites the concentration of PTEs exceeded the maximum permissible soil values for PTEs which represents significant PTE contamination in the cities. Moreover, Salgótarján soils were lesser contaminated with PTEs than Ózd soils, whereas in general, the enrichment of Pb and Cd was relatively higher than As and Hg, suggesting that with some exceptional sampling sites Hg and As might have originated from a natural source. Hooda, (2010) stated that PTEs in soils might have lithogenic or anthropogenic origins, however, in most situations, high PTE concentrations are linked to human-induced activities. The mean enrichment factors (EF) values decreased in the order of Pb>Cd>Hg>As in Salgótarján and Cd>Pb>Hg>As in Ózd. The average Salgótarján soil enrichment values showed a minimal enrichment of PTEs, however, all PTEs in Ózd samples depicted moderate to significant enrichment.

A significant ($p<0.05$) positive correlation was found among PTE content and physicochemical parameters, which show their strong association in both cities. Notably, Spearman’s correlation coefficient indicated a significant correlation between organic matter and PTEs in Salgótarján and between FE/Mn and PTEs in Ózd urban soils. Also, previous studies report that the abiotic redox reactions are the main factors controlling the mobility and transformation of PTEs on the surfaces of Fe(III)- and Mn-oxides, as well as ferrous species and humic substances mainly due to their high affinity and absorption (Alloway, 2013; Caporale & Violante, 2016).

Soil microbial community analysis revealed that Acidobacteria (9.8-14.4%), Actinobacteria (9.1-12.6%), Bacteroidetes (8.3-12.3%), Planctomycetes (8.9-12%), Patescibacteria (7.1-10.2%), Verrucomicrobia (4.1-9%), and Chloroflexi (4-7%) in Salgótarján, however, 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%) dominated Ózd soils. The taxonomic differences between contaminated and non-contaminated (control) sampling sites are especially visible at class and genus level. In contrast to control sample, a significant increase of Gammaproteobacteria, Deltaproteobacteria, Planctomycetacia, and Actinobacteria was observed in

Salgótarján urban soils (contaminated), whereas in Ózd urban soils the number of Deltaproteobacteria, Planctomycetacia, and Actinobacteria considerably decreased.

The archaeal community in all sampling sites of Salgótarján and Ózd was dominated by Thaumarchaeota (19.6-62.5%), Nanoarchaeota (6.7-60.8%), and Euryarchaeota (6.25-28%), and they cover the 95% of the archaeal communities.

Analysis showed that in Salgótarján and Ózd soils, some of the rare taxa (at genus level) exist either only at the contaminated samples or uncontaminated soils. Among them *Sulfurifustis, and Candidatus Moranbacteria*, together with other rare genera exist only in the urban samples (contaminated) that can be due to their high tolerance to soil elemental contamination, including PTEs. On the other hand, genera, such as *Nitrobacter, Filomicrobium, Lactobacillus*, etc. in Salgótarján soils and *Azomonas, Methyloterrigena, Emticicia*, etc. in Ózd soils, were found only in control (uncontaminated) samples, which can be due to their sensitivity to metal contamination.

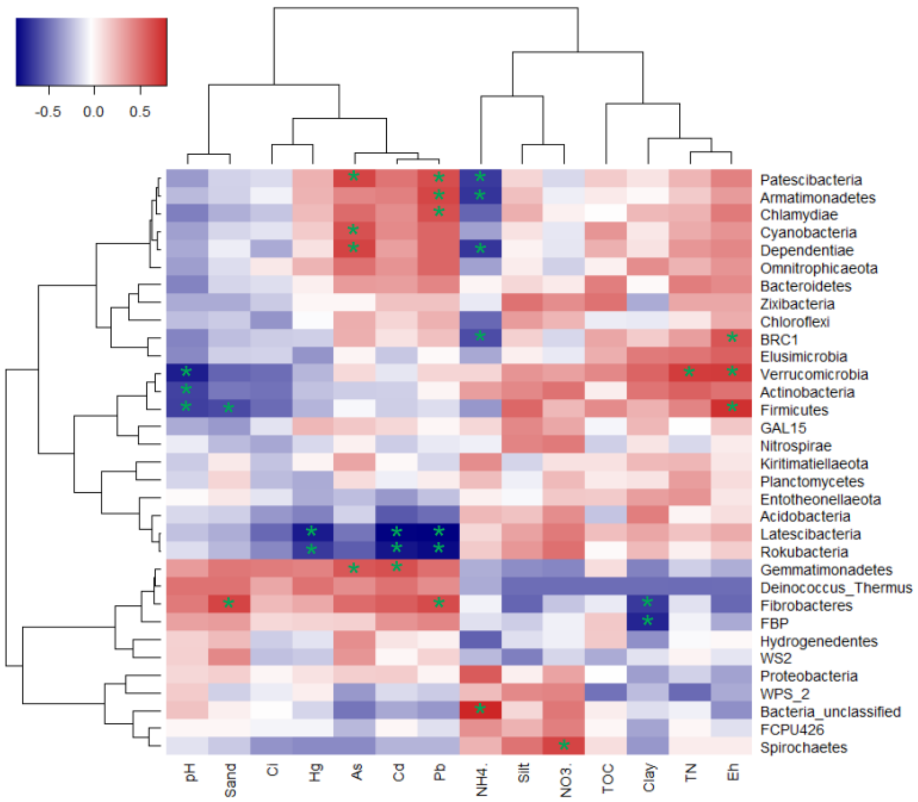

Figure 1. The Spearman correlation heatmap shows the connection between environmental factors and Bacteria phyla. Correlation significance values (p<0.05) are shown by stars.

It is noticed that the content of heavy metal(loid)s significantly correlated 15 bacterial phyla and 19 bacterial genera ($p < 0.05$). At the level of bacterial phyla, the relative abundance of bacteria in urban soil samples with levels of heavy metal(loid)s was significantly related to the physical and chemical properties of the soil. In particular, Patescibacteria, Armatimonadetes, Dependentiae, Verrucomicrobia, Firmicutes, Fibrobacteres, BRC1, FBP, Spirochaetes, Actinobacteria, and Gemmatimonadetes were significantly correlated with SOM, pH-Eh, and soil texture. Additionally, a strong metal resistance was observed in Rokubacteria and Latescibacteria to almost all analysed metal(loid)s (Figure 1). Our results suggested that these bacteria phyla significantly related to the content of the studied PTEs as similarly reported by Y. Liu et al., 2022. This suggests that the combinations of soil physicochemical factors (SOM, soil texture, pH-Eh) and heavy metal(loid)s were responsible for alterations in the bacterial community (Chodak et al., 2013).

Due to the high level of metal contamination in the environment, from both cities, the number of metal tolerant colonies were significantly high. However, isolated 22 strains were able to grow in elevated metal(loid) concentrations and especially *Cupriavidus*, *Bacillus* and *Pseudomonas* spp. were noticeable as was reported by other studies (Gupta et al., 2012; Mihdhir & Assaeedi, 2016). Among the isolated strains, *Cupriavidus campinensis* S14E4C illustrated comparatively high resistance to PTEs, particularly to Cd and was selected for further identification of resistance mechanisms through genomic analysis. The genome sequence analysis of the strain revealed wide number of metal(loid) resistance genes and was considered a potential strain which can be used in bioremediation of metal(loid) contaminated soils.

Analysis showed that the average non-carcinogenic risk assessment values are similar in Salgótarján and Ózd indicating the hazard quotient (HQ) of PTEs for adults and children is below the safe value (HQ<1) in ingestion, inhalation, and dermal exposure. High values suggested that PTE pollution could represent a significant non-carcinogenic health risk at the contaminated sites and these urban areas pose a high non-carcinogenic health risk for children compared to adults as was similarly reported by previous studies (Gržetić et al. 2008; Tepanosyan et al. 2017). The lifetime carcinogenic risk for adults and children was estimated separately from the average influence of each PTE for all the pathways. Based on the calculated carcinogenic risk ADI values, the excess lifetime cancer risks values were computed, and the results showed that in both cities, there is low carcinogenic risk for adults and children.

V. Thesis points

1. Industrial activities caused significant potentially toxic elements (PTE) contamination in Salgótarján and Ózd urban soils. In both cities, there is a heterogenous distribution of As, Pb, Hg, and Cd, and the concentrations were all higher than their correspondent geochemical background values. Except for Hg in Salgótarján, all PTE in both cities exceeded the maximum permissible soil values. It was concluded that in Salgótarján urban soils, organic content plays a significant role on Pb, Hg, Cd, and Fe on As mobility, whereas, in Ózd urban soils, Fe and Mn most likely constraints PTE mobility, thus bioavailability on the surface environment (*Abbaszade et al. 2022*).

2. Compared to local geochemical background (control) samples, there is a minimal enrichment of studied PTE (< 2) in Salgótarján soils, whereas, in Ózd soils, the enrichment is significantly high (2< x <16) and primarily found around industrial sites. Analysis of local contamination sources indicated that in both cities, PTE concentration results from various contamination sources, whereas local coal ash in Salgótarján and smelter slag in Ózd are the predominant pollution sources. This suggests that anthropogenic input from industrial operations was substantial enough to cause significant alterations in soils (*Abbaszade et al. 2022*).

3. Proteobacteria, Acidobacteria, Planctomycetes, Actinobacteria, and Bacteroidetes are dominant phyla in selected Salgótarján and Ózd urban soils, however, concentration differences of contaminants and other soil parameters (organic matter, soil fractions, pH) affected soil community and structure in various ways. It was found that the low soil PTE and high organic content enhanced the diversity, on the other hand, high PTE contamination depleted soil organic matter and resulted in a diversity decrease. A shift in community composition was observed particularly in rare taxa, where high PTE contamination killed the sensitive members of the communities and increased the number of PTE-resistant genera.

4. A high number of metal(loid) resistant isolates indicate selective pressure of contaminants formed resistant bacterial communities, and these communities developed various resistance mechanisms. The comprehensive examination of the most metal(loid) resistant bacterial strain, *Cupriavidus campinensis* S14E4C, revealed that the strain can grow in high metal(loid) exposed environments and carry a significant number of metal(loid) resistant genes encoding various resistance mechanisms. Thus, the strain is effective and suitable for bioremediation, which can transfer toxic compounds to less or non-toxic compounds (*Abbaszade et al. 2020*).

5. Arsenic, lead, mercury, and cadmium contaminants can cause significant health issues and endanger local resident's life. The non-carcinogenic health risk assessment, performed especially on kindergarten, playgrounds, and parks, suggested both cities as safe areas for adults, but it can be hazardous to children's health. Thus, high PTE contaminated sites are not suggested for cultivation and playground purposes; however, further investigations are required (*Abbaszade et al. 2022*).

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