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A comparative metagenomic and spectroscopic analysis of soils from an international point of entry between the US and Mexico



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ABSTRACT

The Paso del Norte region is characterized by its dynamic industries and active agriculture. Throughout the years, urban and agricultural soils from this region have been exposed to xenobiotics, heavy metals, and excess of hydrocarbons. In this study, samples of urban [domestic workshops (DW)] and agricultural-intended (AI) soils from different sites of Ciudad Juárez, Mexico were evaluated for their fertility, element content, and microbial diversity. Chemical analyses showed that nitrites, nitrates, P, K, Mg, and Mn were predominantly higher in AI soils, compared to DW soils ($p \leq 0.05$). The composition of soil microbial communities showed that Proteobacteria phylum was the most abundant in both soils (67%, $p \leq 0.05$). In AI soils, *Paracoccus denitrificans* was reduced ($p \leq 0.05$), concurring with an increment in nitrates, while the content of nitrogen was negatively correlated with the rhizobium group ($r^2 = -0.65$, $p \leq 0.05$). In DW soils, the Firmicutes phylum represented up to ~25%, and the relative abundance of Proteobacteria strongly correlated with a higher Cu content ($r^2 = 0.99$, $p \leq 0.0001$). The monotypic genus *Sulfuricurvum* was found only in oil-contaminated soil samples. Finally, all samples showed the presence of the recently created phylum *Candidatus saccharibacteria*. These results describe the productivity parameters of AI soils and its correlation to the microbial diversity, which are very important to understand and potentiate the productivity of soils. The data also suggest that soils impacted with hydrocarbons and metal(oid)s allow the reproduction of microorganisms with the potential to alleviate contaminated sites.

1. Introduction

Ciudad Juarez (Chihuahua, Mexico), located within the Paso del Norte Region and characterized by intense agricultural activities, has been impacted by a strong industrial growth. For decades, smelters have released contaminants to air, water, and soil (Díaz-Barriga et al., 1997; Pingitore et al., 2005), affecting human and environmental health (Rios-Arana et al., 2004). Metal(oid)s including cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), zinc (Zn), and copper (Cu) have been released to the environment, becoming of great concern because of their accumulation and toxicity (Del Toro et al., 2010). Although the smelters are no longer operating, there are still concerns regarding heavy metal pollution and accumulation in soil and water reservoirs

(Darby, 2012). Pingitore et al. (2005) performed a comprehensive study aimed at evaluating heavy metal(oid)s in soils from the city of El Paso. They found a positive correlation between the soil concentration of As, Ba, Cd, Cr, Cu, Pb, Ni, and Zn and the proximity to the smelters, which indicates a gradual increase.

The use of hydrocarbons and its derivatives have become the foremost source of energy for the industry. Their environmental by-products, as well as their release/spill commonly end up in bodies of water, air, and soil, threatening human health (Das and Chandran, 2011). Meanwhile, the application of higher doses of fertilizers can escalate a loss of soil fertility, as has been previously documented (Guo et al., 2010). Additionally, the excess of nitrogen or phosphorus leached from agricultural soils causes eutrophication of bodies of water (Conley

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et al., 2009).

The microbial diversity in soil is extremely high and much larger than any other group of eukaryotic organisms (Torsvik and Øvreås, 2002). This diversity has been achieved by the great heterogeneity, multiphase nature, and the chemical and biological properties of the soils (Daniel, 2005). Microorganisms represent a key biota on earth because they play central and unique roles in the nutrient cycle and in the health and performance of plants (Chowdhury et al., 2011). Since many of them have the capability to grow in contaminated environments and due to their ability to degrade hydrocarbons (Röling et al., 2002), to hyper-accumulate heavy metals (Nies, 1999; Gardea-Torresdey et al., 1998), and to transform xenobiotic compounds (Singh and Walker, 2006), they are persistently used and studied in bioremediation techniques.

Conventional methods to culture microorganisms are very limited, thus, a very small portion (< 1%) of them can be grown at laboratory conditions (Mocali and Benedetti, 2010). The recent usage of the Next Generation Sequencing (NGS) techniques has overcome these difficulties by allowing researchers to explore the diversity and function of the microbial communities, without the need of performing massive cultures. Indeed, the metagenomic information obtained by NGS approaches provide more genetic information than any other culturable methods (Rondon and Al, 2000).

To the best of our knowledge, there are no ecotoxicological studies about the interaction between the physicochemical properties, the chemical element content and heavy metal composition, and the microbial communities' structure from urban and agricultural soils of Ciudad Juárez, Chihuahua, México. Thus, the objective of this study was to characterize the chemical composition of urban and agricultural soils and to uncover the diversity of the resilient microbial communities by using spectroscopic and metagenomic approaches. This research set the basis to further develop effective approaches to improve soil fertility and to reduce the heavy metal and hydrocarbon compounds pollution.

2. Materials and methods

2.1. Soil sampling

Composite soil samples containing at least 20 sub-samples (depth, 0–20 cm) were taken from eight soils belonging to urban and agricultural sectors of Ciudad Juárez, México (Fig. 1). The agricultural-intended (AI) soils coordinates were: soil 1 (S1), 31°35.458' N 106°17.780'W; soil 2 (S2), 31°32.298' N 106°15.586'W; soil 3 (S3),

31°32.421' N 106°15.586'W; soil 4 (S4), 31°32.279'N 106°16.160'W; and soil 8 (S8), 31°40.0'N 106°22.36'W. The corresponding urban [domestic workshops (DW)] soils coordinates were: soil 5 (S5), 31°37.16'N 106°27.52'W; soil 6 S(6), 31°36.25'N 106°28.14'W; and soil 7 (S7), 31°38.43'N 106°26.52'W. The soil samples were saved in plastic bags and kept on ice during transportation. Once at the laboratory, the samples were stored at -20°C (for further physicochemical and spectroscopic measurements) and at -80°C (for further metagenomics analysis).

2.2. Determination of fertility and salinity parameters

The fertility and salinity parameters, which include nitrates, nitrites, available P, organic matter, total nitrogen, pH, electrical conductivity (EC), and sulfates, were determined at the certified Laboratorio de Aguas-Suelos-Plantas y Alimentos from the Sonora Institute of Technology, Obregon City, Sonora, Mexico, following the Official Mexican Standard NOM-021-RECNAT-2000.

2.3. Quantification of macro and micronutrient in soils

Samples were oven dried for 72 h at 72°C . Then, 0.2 g of each soil were digested with 4 mL of *aqua-regia* and digested for 45 min at 115°C . The tubes were adjusted to 50 mL using deionized (DI) water (18 m Ω) and analyzed for K, S, P, Mg, Ca, Fe, Zn, Mn and for the heavy metal (oid)s Cu, Al, Pb, Se, As, Cr, Ni, and Cd, using inductively coupled plasma-optical emission spectroscopy (ICP-OES); Perkin-Elmer Optima 4300 DV. For quality control purposes, NIST reference material 2709a was used to validate the digestion and analytical method obtaining recoveries above 95%. For quality assurance of the elemental analysis blanks, spikes, and standards were read as previously described (Cota-Ruiz et al., 2018; Ochoa et al., 2017).

2.4. DNA preparation

DNA was extracted and purified following previous protocols (Sagar et al., 2014; Zhou et al., 1996) with modifications. Frozen soil samples (at -80°C) were slowly thawed to 4°C and 1 g was re-suspended in 1 mL of PBS (pH 8.0), vortexed and centrifuged at $3000 \times g$ for 10 min at 4°C . Supernatants were transferred into new microtubes and incubated for 60 min at 65°C with an equal amount of lysis buffer (1.5 M NaCl, 0.1 M EDTA, 4% SDS). Then, samples were centrifuged at $13,000 \times g$ for 5 min at 4°C and the supernatant was put into a new tube. The DNA was purified by phenol:chloroform (1:1) extraction,

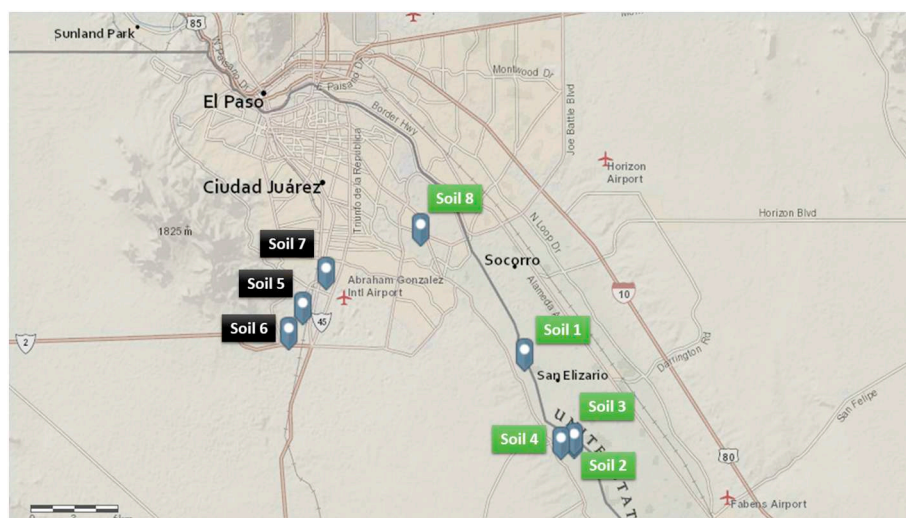


Fig. 1. Description of the sampled sites located in the urban and in the agricultural area of Ciudad Juárez, México.

followed by isopropanol precipitation. The pellets were washed with 70% ethanol and re-suspended in TE buffer. DNA quality was assayed by 260/280 absorbance ratio and by gel electrophoresis.

2.5. Metagenomic sequencing and analysis

The metagenomic sequencing and analysis were performed by IDIX SA de CV (Querétaro, México). The 16S ribosomal RNA gene was amplified targeting the hypervariable V3-V4 region. The fragments were sequenced on an Illumina MiSeq instrument (paired end, 2 × 250 mode). Sequencing data was evaluated for quality with FastQC v0.11.8 and preprocessed with Trimmomatic v0.36 (Bolger et al., 2014). Filtered reads were arranged into operational taxonomic units (OTUs) using Kraken v1.2.3 via The Galaxy Project (URL: usegalaxy.org) (Afgan et al., 2018; Wood and Salzberg, 2014).

2.6. Statistics

An ANOVA was performed to determine the experimental variance, while the Tukey's HSD test, followed by Bonferroni correction method, was used to distinguish significant differences between treatment means. Comparison were made with an error $\alpha = 0.05$, unless otherwise is stated. A Pearson coefficient correlation test was performed to evaluate the relationships between the microorganisms' relative abundance, soil physicochemical parameters, and nutrient element concentrations. The analysis was done with the program OriginPro.

3. Results and discussion

3.1. Fertility parameters

The fertility and salinity parameters of the sampled soils are shown in Table 1. As expected, the agricultural-intended (AI) soils had better fertility parameters, compared to soils collected from domestic workshops (DW) places.

Among AI soils (S1, S2, S3, S4, and S8), S4 showed the greatest content of nitrates ($68.86 \pm 3.81 \text{ mg kg}^{-1}$) and sulfates ($27.21 \pm 0.08 \text{ meq L}^{-1}$, respectively ($p < 0.0001$). The high content of nitrates in S4 represents an advantage for agriculture, since nitrates can be used by plants to satisfy their nitrogen requirements. On the other hand, nitrates accumulation in soil conveys an environmental concern, since it can be easily leached out to water reservoirs (Zhou et al., 2016), threatening the human health as it interferes with the hemoglobin-oxygen binding process. In this study, we found that S4, S1, and S2 exhibited the highest nitrates concentrations (68.86 mg kg^{-1} , 41.99 mg kg^{-1} , and 24.69 mg kg^{-1} , respectively, Table 1). These soils had the lower relative abundance of the denitrifying bacteria *Paracoccus denitrificans* (0.22%, 4.22%, and 2.33% respectively, Fig. 2A). Conversely, S7, S5, and S6 showed lower nitrate concentration (0.4, 1.08, and 9.3 mg kg^{-1} , respectively) but the higher relative abundance of *P. denitrificans* (13.11%, 15.89%, and 62.33%, respectively). This negative correlation could be the result of the denitrifying participation of this bacterial species in the conversion of NO_3

to N_2 , with the concomitant depletion of nitrates (Abaye et al., 2005). The only exception was S8, which showed 4.24 mg kg^{-1} of nitrates and a relative abundance of 1.2% of *P. denitrificans* (Table 1, and Fig. 2A).

S3 showed the highest concentration of nitrogen ($0.21 \pm 0.01\%$ ($p < 0.005$) and nitrites ($5.19 \pm 0.13 \text{ mg kg}^{-1}$ ($p < 0.0005$), although the latter was not statistically different from S4 ($4.20 \pm 0.5 \text{ mg kg}^{-1}$) ($p \leq 0.05$) (Table 1). The total amount of nitrogen can be augmented by anthropogenic activities (such as fertilization). Also, the plants and microorganisms inhabiting the ecosystem could also increase the nitrogen derivatives as nitrite and nitrates (Fornara and Tilman, 2008). Since a large amount of nitrogen in soils (up to 80% of the total nitrogen used by plants) can be a consequence of the mycorrhizal fungi and the nitrogen-fixing bacteria activity (Van Der Heijden et al., 2008), the latter mainly represented by the rhizobiales, we evaluated the relative abundance of the rhizobium group across soils. Results showed a significantly negative correlation between their relative abundances and the nitrogen content ($r^2 = -0.65$, $p < 0.05$) (Supplementary Table 2). These results suggest that the human fertilization process is somewhat interfering with the nitrogen fixation process; however, functional experiments regarding enzyme or bacterial activity would be needed to confirm it.

The larger percentage of organic matter was observed in S2 and S3 ($3.97 \pm 0.06\%$, and $3.63 \pm 0.21\%$, respectively) ($p < 0.005$), but not statistically different from S8 (Table 1) ($p < 0.05$). The content of organic matter positively correlated with the relative abundance of *Actinobacteria* group, ($r^2 = 0.67$, $p < 0.05$, Supplementary Table 2). The AI soils with the higher pH were S2 (8.16 ± 0.02) and S8 (8.08 ± 0.03) ($p < 0.0001$), which are considered “moderately alkaline,” while the rest of the AI soils (S1, $\text{pH } 7.87 \pm 0.02$; S3, $\text{pH } 7.69 \pm 0.03$; S4, 7.64 ± 0.02) can be defined as “mildly” alkaline (Table 1) (Brady and Weil, 2013; Meena et al., 2006). In our study, the pH value did not correlate ($p < 0.05$) with the relative abundance of the most representative groups of bacteria (Fig. 3), probably because of the pH values did not strongly differ in the analyzed soils (the mean values ranged from 7.64–8.16). However, the pH value had a significant positive correlation with the Shannon diversity index ($r^2 = 0.63$, $p \leq 0.05$) (Supplementary Table 1 and Supplementary Table 2), which is in line with previous studies reporting that the pH resulted in a good predictor of the bacterial diversity through the soils (Lauber et al., 2009; Nicol et al., 2008).

Because the apparent soil electrical conductivity (EC) is affected by physicochemical properties, it has been settled as an excellent tool to analyze the spatial variations of several edaphic conditions of soil samples (Corwin and Lesch, 2005). The EC values were obtained in order to characterize the 8 locations mentioned in this study. We found that the higher EC was detected in S4 ($2.83 \pm 0.03 \text{ mS cm}^{-1}$) and 1 ($2.62 \pm 0.02 \text{ mS cm}^{-1}$) ($p < 0.0001$). The EC negatively correlated ($p < 0.05$) with the organic matter, total N, K, and P, and with the relative abundance of *Bacteroidetes*, *Candidatus Saccharibacteria*, as well as with the Shannon diversity-index (Supplementary Table 2). Contrarily, the relative abundance of *Proteobacteria* group positively correlated with the EC of the AI soils ($r^2 = 0.79$, $p < 0.01$) (Supplementary Table 2). The EC measures the content of salt in soils, thus, it directly

Table 1

Physicochemical parameters of sampled soils. Different letters mean significant differences at $p \leq 0.05$.

Sample	N- NO_2 (mg kg^{-1})	N- NO_3 (mg kg^{-1})	Available P (mg kg^{-1})	Organic matter (%)	Total nitrogen (%)	pH	EC (mS cm^{-1})	SO_4^{-2} (meq L^{-1})
Soil 1	1.79 ± 0.1^d	41.99 ± 1.62^b	15.13 ± 0.01^c	1.72 ± 0.01^{de}	0.09 ± 0^d	7.87 ± 0.02^b	2.62 ± 0.02^d	14.13 ± 0.06^c
Soil 2	3.30 ± 0.55^{bc}	24.69 ± 1.29^c	11.40 ± 0.01^e	3.97 ± 0.06^c	0.14 ± 0^c	8.16 ± 0.02^a	1.89 ± 0.02^e	8.45 ± 0.02^g
Soil 3	5.19 ± 0.13^a	12.03 ± 0.28^d	21.39 ± 0.02^a	3.63 ± 0.21^c	0.21 ± 0.01^a	7.69 ± 0.03^c	1.83 ± 0.05^e	9.37 ± 0.06^f
Soil 4	4.20 ± 0.5^{ab}	68.86 ± 3.81^a	12.07 ± 0.03^d	1.52 ± 0.08^e	0.08 ± 0^d	7.64 ± 0.02^{cd}	2.83 ± 0.03^d	27.21 ± 0.08^d
Soil 5	0.48 ± 0.03^{ef}	1.08 ± 0.00^g	20.11 ± 0.1^b	22.48 ± 0.02^a	0.18 ± 0.01^b	7.47 ± 0.04^e	5.98 ± 0.11^b	48.88 ± 0^a
Soil 6	0.01 ± 0.01^f	9.30 ± 0.43^e	2.48 ± 0.02^h	4.85 ± 0.32^{bc}	0.09 ± 0^d	7.54 ± 0.03^{de}	7.91 ± 0.01^a	39.49 ± 0.02^b
Soil 7	1.46 ± 0.02^{de}	0.40 ± 0.02^h	2.77 ± 0.01^g	15.21 ± 0.07^{ab}	0.17 ± 0.01^b	7.87 ± 0.03^b	3.41 ± 0.08^c	30.11 ± 0.04^c
Soil 8	2.19 ± 0.05^{cd}	4.24 ± 0.09^f	3.91 ± 0.01^f	2.56 ± 0.00^{cd}	0.13 ± 0.01^c	8.08 ± 0.03^a	1.52 ± 0.05^f	8.46 ± 0.01^g

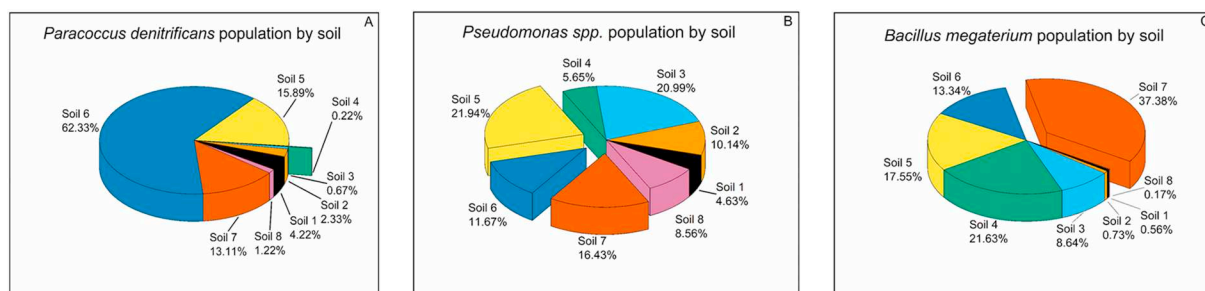


Fig. 2. Comparison of the relative abundance of different microorganisms among the analyzed soils. Each panel only separately compares the relative presence of A) *Paracoccus denitrificans*, B) *Pseudomonas* spp., and C) *Bacillus megaterium*, across the agricultural intended and domestic workshop soils.

expresses the salinity of the soil. Contrasting results regarding the organic matter or nitrogen content have been previously documented as a function of the salinity increment in soils (Sardinha et al., 2003). Additionally, it has been documented that the salinity of soils directly affects the structure and abundance of microorganisms by interfering with their osmotic homeostasis (Chowdhury et al., 2011; Wichern et al., 2006). As previously mentioned, in our study we found a negative correlation ($r = -0.66$, $p < 0.05$) between the salinity and the relative abundance of the *Bacteroides* group. In agreement with these results, Ibekwe et al. (2010) found that the Bacteroidetes abundance group tended to decrease when the soils were exposed to higher salinities, however, another study demonstrated that this group was one of the most dominant in the hypersaline ecosystem called “La Sal del Rey” (southern Texas) (Hollister et al., 2010). On the other hand, we found a strong correlation between salinity and the relative abundance of the Proteobacteria group ($r^2 = 0.8$, $p < 0.01$), which points that these microorganisms are well adapted to these environments. Similar results were reported by Canfora et al. (2014), demonstrating that the Proteobacteria was the most relative abundant group (95.95%) across the salinity gradient of the tested soils. The microorganisms within Proteobacteria group belong to one of the biggest clusters that occupy almost every ecosystem.

Among DW soils (S5, S6, and S7), the higher content of nitrates, sulfates, the pH value, and the EC were found in S6 ($9.30 \pm 0.43 \text{ mg kg}^{-1}$), S5 ($48.88 \pm 0.00 \text{ meq L}^{-1}$), S7 (7.87 ± 0.03), and S6 ($7.91 \pm 0.01 \text{ mS cm}^{-1}$), respectively (Table 1) ($p < 0.05$). As shown in Supplementary Table 2, the content of nitrates positively correlated with the relative abundance of Actinobacteria spp.

($r^2 = 0.92$, $p < 0.05$) and *Candidatus saccharibacteria* ($r^2 = 0.97$, $p < 0.005$), and with the Fisher alpha diversity index ($r^2 = 0.89$, $p < 0.05$). The content of sulfates positively correlated with the presence of the Firmicutes group ($r^2 = 0.94$, $p < 0.01$) but negatively with the relative abundance of Proteobacteria ($r^2 = -0.85$, $p < 0.05$). The pH value positively correlated with the relative amount of Proteobacteria ($r^2 = 0.97$, $p < 0.01$), while the salinity negatively correlated with the relative abundance of the latter group ($r^2 = -0.91$, $p < 0.05$). Additionally, the higher percentage of nitrogen was presented in S5 (0.18 ± 0.01) and S7 (0.17 ± 0.01) ($p < 0.005$). The nitrogen content negatively correlated with the Fisher alpha diversity index ($r^2 = -0.92$, $p < 0.01$), and also negatively with the relative amounts of Actinobacteria ($r^2 = -0.95$, $p < 0.005$) and *Candidatus Saccharibacteria* ($r^2 = -0.97$, $p < 0.005$).

When comparing DW with AI soils, the higher percentage of organic matter was observed in DW soils, specifically in S5 (22.48 ± 0.02) and S7 (5.21 ± 0.07) (Table 1). Also, the higher content of sulfates (average of $39.49 \pm 9.39 \text{ meq L}^{-1}$), and the greater conductivity values (average of $5.77 \pm 2.25 \text{ meq L}^{-1}$) were obtained in DW soils, compared to their counterparts AI soils (Table 1) ($p < 0.05$). It is possible that hydrocarbons and their derivatives end up in this kind of urban places as a result of spills or as byproducts. Additionally, the lower content of nitrites was obtained in DW soils ($0.65 \pm 0.74 \text{ mg kg}^{-1}$ for DW soils in comparison to $3.33 \pm 1.4 \text{ mg kg}^{-1}$ for AI soils) ($p < 0.05$), which correlates with a higher relative amount of *P. denitrificans* in DW soils (Fig. 2A). Thus, possibly this bacteria is playing a significant role in the denitrifying process.

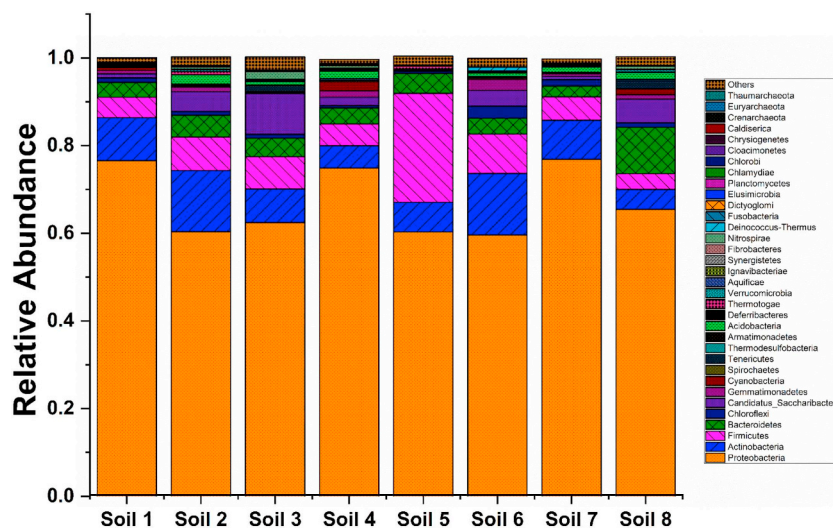


Fig. 3. The relative abundance of the most dominant bacteria phyla obtained from 16S rRNA genes in soil. The relative abundances were calculated from all sequences categorized lower than the domain level.

3.2. Element soil composition

3.2.1. Agricultural soils

The macro (K, S, P, Mg and Ca) and micronutrient (Fe, Zn, Cu, Mn, and Ni) contents for AI soils are shown in Table 2.

The content of K and S did not show difference in all tested soils ($p < 0.05$). The K values obtained in this study (average of 3681 mg kg^{-1}) are higher in comparison to the values reported for other agricultural soils (average of 69.25 mg kg^{-1}) (Darilek et al., 2009). The amount of P was higher in S2 and S3 (1872.80 ± 72.67 and $2048.43 \pm 112.44 \text{ mg kg}^{-1}$, respectively), compared to S4 ($1487.07 \pm 56.63 \text{ mg kg}^{-1}$) (Table 2) ($p < 0.05$). Interestingly, the highest availability of P was found in S3 ($21.39 \pm 0.017 \text{ mg kg}^{-1}$) (Table 1) ($p < 0.05$), which is similar to the values reported for farming soils from the northwest of Bosnia and Herzegovina (Grujic et al., 2018). The solubilization of P is a key process to make it available for plants uptake (Shen et al., 2011). The biological players in charge to solubilize inorganic phosphates are the phosphate-solubilizing microorganisms (PSMs). They largely solubilize P due to their capacity to decrease the pH in soils by releasing either organic acids and/or protons (Gyaneshwar et al., 2002). In our study, we found a negative correlation between the available P and the pH ($r^2 = -0.62$, $p < 0.05$) in AI soils (Supplementary Table 2), being the S3 the one with the lowest pH and the highest P availability (Table 1). The PSMs include the alpha-Proteobacteria, Rhizobium group, and strains belonging to *Bacillus*, *Pseudomonas*, and *Enterobacter* genera (Khan et al., 2009). All of them were, to some extent, well represented not only in S3 but in the rest of the AI soils. Due to the diverse number of PSMs found across the analyzed soils, probably their metabolic activities vary between soils. Also, it is possible that other factors such as the mineral content of soils can affect P availability (Hinsinger, 2001).

The content of Mg was greater in S1 and S8 (7688.67 ± 562.09 and $7355.70 \pm 377.64 \text{ mg kg}^{-1}$, respectively), compared to S4 ($4993.27 \pm 474.07 \text{ mg kg}^{-1}$) (Table 2) ($p < 0.01$). These results are higher in comparison to the values (125 mg kg^{-1}) reported for organic farming soils (Mäder et al., 2008). The S4 presented the lowest concentration of Ca ($17.57 \pm 1.39 \text{ mg kg}^{-1}$) ($p < 0.001$). Since the stability of soils has been linked to the Ca:Mg proportion, in this study we compared these values. The results showed that the AI soils had similar Ca:Mg ratio with an average of 4.12, which indicate that these soils are stable, as ratios lower than 1.0 lead to an increment in particle dispersion, negatively affecting the stability (Moore et al., 2004).

The content of Fe and Al did not differ in all analyzed soils ($p < 0.05$). Among the AI soils, the Zn was only detected in S8 ($31.26 \pm 7.19 \text{ mg kg}^{-1}$), reflecting the nutrient deficiency regarding this element in the rest of the AI soils. The content of Cu was higher in S8 ($30.64 \pm 1.43 \text{ mg kg}^{-1}$) compared to S4 ($14.23 \pm 3.87 \text{ mg kg}^{-1}$) ($p < 0.01$). The amount of copper positively correlated with the pH in AI soils ($r^2 = 0.55$, $p < 0.05$). It has been claimed that the amount of adsorbed Cu by soil increases with pH (Bradl, 2004). However, since the retention mechanism lowers Cu solubility (Kumpiene et al., 2008), lesser Cu availability for plants that grow on these soils should be expected. The higher concentration values of Mn were obtained in S1 and S8 (360.14 ± 17.64 and $353.81 \pm 1.09 \text{ mg kg}^{-1}$, respectively) in comparison to soil 4 ($202.04 \pm 13.98 \text{ mg kg}^{-1}$) ($p < 0.01$). Metal (oids) Se, As, Cr, Pb, Ni, and Cd were also analyzed but they were not detected in AI soils.

3.2.2. Domestic workshop soils

Three different soils (S5, S6, and S7) from oil-contaminated domestic workshop areas were analyzed for macro and micronutrients, and for heavy metals (Table 2). There were no statistical differences in all macronutrients (K, S, P, Mg, and Ca) nor in the micronutrients Fe and Mn analyzed ($p < 0.05$).

The concentration of Cu was higher in S7 ($676.18 \pm 74.61 \text{ mg kg}^{-1}$) compared to soil S5 ($116.30 \pm 8.14 \text{ mg kg}^{-1}$) and S6

Table 2
Elemental composition of the analyzed soils. Different letters denote significant differences at $p \leq 0.05$.

Sample	K (mg kg^{-1})	S (mg kg^{-1})	P (mg kg^{-1})	Mg (mg kg^{-1})	Ca (g kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Al (g kg^{-1})	Pb (mg kg^{-1})
Soil 1	3551.9- 7 ± 27- 5.86 ^{ab}	868.9- 4 ± 33- 7.89 ^b	1712.2- 4 ± 45- 22 ^{ab}	7688.6- 7 ± 56- 2.09 ^a	34.5- 7 ± 1.6- 1 ^b	15.8- 6 ± 0.7- 0 ^a	N.D.	21.6- 5 ± 2.4- 9 ^{de}	360.1- 4 ± 17- 64 ^a	13.6- 2 ± 2.6- 9 ^{ab}	N.D.
Soil 2	3868.1- 6 ± 26- 0.88 ^a	898.0- 6 ± 36- 9.65 ^b	1872.8- 0 ± 72- 67 ^a	6520.9- 8 ± 62- 6.30 ^{ab}	27.3- 3 ± 2.0- 4 ^b	13.3- 7 ± 0.8- 7 ^{ab}	N.D.	21.6- 6 ± 2.6- 9 ^{de}	257.4- 0 ± 18- 44 ^{ab}	12.4- 2 ± 2.9- 0 ^{ab}	N.D.
Soil 3	3632.7- 3 ± 27- 6.33 ^a	983.8- 4 ± 36- 8.11 ^b	2048.4- 3 ± 11- 2.44 ^a	6604.3- 5 ± 42- 5.34 ^{ab}	26.0- 8 ± 1.2- 5 ^b	13.7- 9 ± 0.5- 5 ^{ab}	N.D.	22.7- 0 ± 2.2- 6 ^{de}	261.5- 4 ± 8.1- 5 ^{ab}	12.3- 6 ± 2.5- 7 ^{ab}	N.D.
Soil 4	2786.8- 3 ± 49- 4.34 ^{abc}	687.8- 2 ± 34- 6.82 ^b	1487.0- 7 ± 56- 63 ^b	4993.2- 7 ± 47- 4.07 ^{bc}	17.5- 7 ± 1.3- 9 ^c	11.2- 8 ± 0.8- 0 ^{ab}	N.D.	14.2- 3 ± 3.8- 7 ^c	202.0- 4 ± 13- 98 ^{bc}	9.84 ± - 2.68 ^{ab}	N.D.
Soil 5	1885.3- 3 ± 40- 4.81 ^{bc}	1443.5- 4 ± 42- 3.84 ^{ab}	869.2- 8 ± 58- 54 ^c	3129.4- 9 ± 19- 0.52 ^d	77.6- 4 ± 1.4- 9 ^a	7.73 ± - 0.09 ^b	296.5- 3 ± 32- 58 ^a	116.3- 0 ± 8.1- 4 ^b	51.9- 8 ± 7.3- 6 ^d	5.15 ± - 1.39 ^b	145.3- 7 ± 1- 0.30 ^b

(continued on next page)

Table 2 (continued)

Sample	K (mg kg ⁻¹)	S (mg kg ⁻¹)	P (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Ca (g kg ⁻¹)	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Al (g kg ⁻¹)	Pb (mg kg ⁻¹)
Soil 6	1867.8- 4 ± 53- 9.99 ^{bc}	3029.2- 0 ± 81- 2.33 ^a	560.7- 4 ± 82- 16 ^c	3497.0- 4 ± 13- 1.37 ^{cd}	90.1- 7 ± 9.2- 4 ^b	11.8- 1 ± 1.5- 3 ^{ab}	79.4- 4 ± 10- 12 ^b	31.5- 2 ± 1.7- 1 ^{cd}	51.4- 3 ± 19- 33 ^d	5.72 ± - 1.51 ^{ab}	N.D.
	1668.8- 1 ± 48- 4.97 ^c	1664.1- 1 ± 45- 1.29 ^{ab}	680.4- 1 ± 11- 7.73 ^c	3744.9- 0 ± 19- 7.42 ^{cd}	109.0- 5 ± 31- 78 ^a	13.4- 7 ± 4.0- 2 ^{ab}	386.3- 7 ± 18- 2.61 ^a	676.1- 8 ± 74- 61 ^a	101.1- 3 ± 60- 08 ^{cd}	7.26 ± - 1.33 ^{ab}	36.0- 7 ± 2- 2.60 ^b
	4566.0- 6 ± 25- 7.41 ^a	936.0- 1 ± 48- 5.21 ^b	1854.4- 6 ± 78- 29 ^{ab}	7355.7- 0 ± 37- 7.64 ^{ab}	32.7- 4 ± 0.4- 0 ^b	14.7- 8 ± 0.2- 0 ^{ab}	31.2- 6 ± 7.1- 9 ^b	30.6- 4 ± 1.4- 3 ^{cd}	353.8- 1 ± 1.0- 9 ^a	15.9- 0 ± 1.0- 4 ^a	N.D.

(31.52 ± 1.71 mg kg⁻¹) ($p < 0.05$). The content of Cu strongly correlated with the relative abundance of the class Proteobacteria ($r^2 = 0.99$, $p < 0.0001$) in DW soils, which indicate that the microorganisms within this group are well adapted to high amounts of this metal. A previous study performed on field soils treated with Cu²⁺ showed that the Proteobacteria group was one of the major bacteria phyla represented (Wakelin et al., 2014). Another study also demonstrated that increasing concentrations of Cu in soil (up to 500 mg kg⁻¹) stimulated the presence of alpha and beta-Proteobacteria in the rhizosphere of *Elsholtzia splendens*, a copper accumulator plant (Wang et al., 2008), which indicates the importance of these microorganisms in the symbiosis process. Since members of the genus *Pseudomonas* tolerates high concentrations of heavy metals (Abaye et al., 2005; Wakelin et al., 2009), we compared their relative abundance in the samples from DW and AI locations, and we found that the highest relative abundances of *Pseudomonas* spp. were mostly observed in the oil-contaminated DW areas (up to 21.94% at the S5), except by the S3 that belongs to the group of AI locations (20.99%, Fig. 2B).

For the rest of the elements (Se, As, Pb, Cr, Ni, and Cd), only Pb was detected in S7 and S5. The greater content of Pb was presented in S5 (145.37 ± 10.30 mg kg⁻¹) in comparison to the S7 (36.07 ± 22.60 mg kg⁻¹) ($p < 0.05$) (Table 2).

3.3. Main representative groups of bacteria and its environmental significance

The Proteobacteria phylum was the most relative abundant group between the all analyzed soils, with an average value of 67% (Fig. 3) ($p < 0.05$), which is in line with previous soil reports (Janssen, 2006; Spain et al., 2009). This phylum comprises a great variety of microorganisms involved in important processes such as the nitrogen, the carbon and the sulfur cycles (Campbell et al., 2006; Dixon and Kahn, 2004). In this study, the alpha, beta, and gamma Proteobacteria represented > 80% of Proteobacteria. Within AI soils, the highest relative abundance for the alpha, beta, and gamma Proteobacteria was found in S4, S1, and S3, respectively. For the DW soils, the highest relative abundance for alpha, beta, and gamma Proteobacteria was found in S7, S5, and S5, respectively. Additionally, the epsilon-Proteobacteria subclass was also found in all explored soils, although in lower relative amounts (Supplementary Table 3). The epsilon subclass of Proteobacteria have gained attention since they have been identified in a wide variety of environments such as anoxic marine waters and hydrothermals (Hügler et al., 2005), from oil-contaminated places (Hubert et al., 2012). Additionally, their ability to degrade hydrocarbons has also been reported (Keller et al., 2015). Particularly, only the oil-contaminated S7 showed the presence of the genus *Sulfuricurvum*, a microorganism that can grow in crude oil and can oxidize sulfur, sulfide, and thiosulfate (Supplementary Table 3) (Kodama and Watanabe, 2004).

The Actinobacteria phylum was the second most represented group reaching up to ~14% of the relative abundance in S2 and S6 (Fig. 3) ($p < 0.05$). These microorganisms have been identified to be present in a higher proportion in soils and many of them have the potential to degrade organic compounds such as insecticides (Briceño et al., 2012), and also can accumulate heavy metals (Polti et al., 2014).

The Firmicutes raised up to ~25% in the S5 (Fig. 4A), while in the rest of the soils their relative abundance was significantly lower ~6% (Fig. 3) ($p < 0.001$). As presented above, in S5 there were larger amounts of Cu (116.30 ± 8.14 mg kg⁻¹) and Pb (145.37 ± 10.30 mg kg⁻¹). This positive correlation suggests that the microorganisms within the Firmicutes group are well adapted to heavy metals-contaminated environments and could be involved in their cell-accumulation. Indeed, it has been reported that *Firmicutes* spp. can inhabitant atmospheres with larger amounts of heavy metals (Ellis et al., 2003; Sun et al., 2010). Remarkably, in our study, the *Bacillus* spp. were the most relative abundant bacteria within Firmicutes group (up to

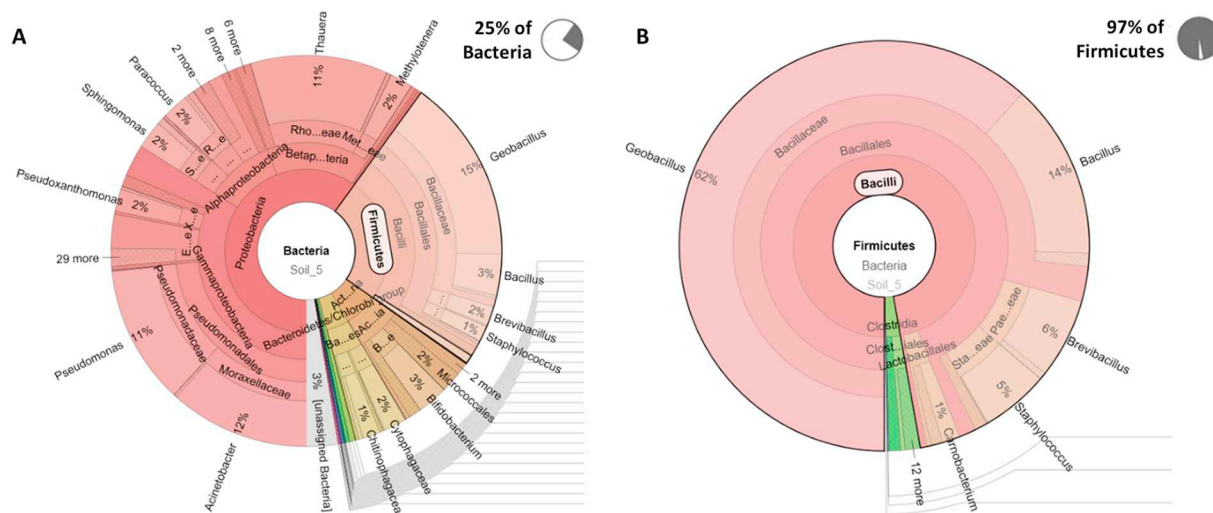


Fig. 4. Analysis of the bacteria relative abundance in the domestic workshop soil 5. Panel A) shows the relative abundance of the *Firmicutes* group and panel B) shows the relative abundance of *Bacilli* subclass.

97%) (Fig. 4B), which suggest that they could participate in the hyperaccumulation of the heavy metals, as has been formerly demonstrated (Çolak et al., 2011). A previous study on *Bacillus megaterium* reported that this species is resistant to lead-contaminated environments (Roane, 1999). Accordingly, in the present study, we found that the higher relative abundance of *B. megaterium* was presented in the S7 (37.38%, $p < 0.05$) which was contaminated with lead, in comparison to the rest of the AI soils (Fig. 2C) ($p < 0.05$).

The Bacteroidetes phylum did not show differences among the analyzed soils, with a relative abundance average of ~5% ($p < 0.05$). Interestingly, the recent coined *Candidatus Saccharibacteria* (Albertsen et al., 2013), formerly known as the TM7 phylum, was found in all environments tested (average of ~3%) being higher represented in S3 with ~9% in relative abundance, although not different from S8 (Supplementary Table 3) ($p < 0.05$). *Saccharibacteria* spp. has been now identified as a phylogenetic diverse group with important roles in the degradation of organic compounds (Kindaichi et al., 2016), which points these microorganisms as strong candidates to be used in bioremediation approaches.

4. Conclusions and future work

This study set the basis to explore the capability of some microorganisms to remove contaminants. To the best of the authors' knowledge, this is the first report that explores the interaction of the chemical composition with the microbial diversity in urban and agricultural soils from the Paso del Norte region. The AI soils showed better fertility parameters in comparison to the DW soils. For the AI soils, we found that parameters such as the nitrates content or the availability of P, somehow behave as a function of the microbial community dynamics. On the other hand, the greater percentage of organic matter, sulfates, and the higher conductivity values were found in DW soils. The latter results can be expected since hydrocarbons and their derivatives finish up in these urban places as a result of spills or as byproducts. Additionally, a positive correlation between the presence of heavy metals in oil-contaminated places and the microorganisms of the Firmicutes and Proteobacteria groups, the *Sulfuricurvum* spp. and *Pseudomonas* spp. genus, and *Candidatus saccharibacteria* were found, which suggests that these microorganisms tolerate heavy metals and hydrocarbon contaminated environments. Further studies such as omics approaches, which describe the functional role of the above-mentioned microorganisms, are fundamental to gaining a deeper understanding, to improve soil productivity, and to reduce the contamination of soils.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2018.12.055>.

Conflict of interest

The authors declare no conflict of interest.

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