A Study on Soil Bacterial Population in Steel Company and Some Related Area in Erbil City in Relation to Heavy Metal Pollution

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ABSTRACT

Present study was conducted to investigate the impact of heavy metal pollution from Erbil steel company on soil physicochemical properties and bacterial population of surrounding soils. The highest bulk density, pH and total organic carbon were 0.774±0.001, 9.31±0.032, 36.447±0.005 g.Kg⁻¹ respectively were observed inside Erbil steel company. The concentrations of all the metals were higher within the steel company in comparison with the other sites and the maximum values were 292±0.577, 8.4±0.208, 259±0.577 and 7480±0.866 ppm for Pb, Cd, Cu and Zn respectively. The concentrations of Cd, Cu and Zn in Erbil steel company soil are exceeded the permissible limits for soil. The isolated bacterial genera in Erbil steel company soil were Acinetobacter, Bacillus, Micrococcus, Neisseria, Pseudomonas and Staphylococcus. These isolates are considered tolerant to alkaline pH and elevated levels of heavy metals from the steel company. Minimum Arthrobacter spp. (0.32×10⁵), proteolytic bacteria (0.57×10⁵) and asymbiotic nitrogen-fixers (0.12×10⁵) cfu.g⁻¹ dry soil were in the soil of steel company and minimum bacterial population was observed in Nawroz soil (0.05×10⁷) cfu.g⁻¹ dry soil followed by Erbil steel company soil (0.06×10⁷) cfu.g⁻¹ dry soil. The relationships between the heavy metals and the microbial populations were negative. The correlations among: pH, total organic C, Cu, Pb and Zn were significantly positive.

1. INTRODUCTION

Pollution of the environment with toxic heavy metals is spreading throughout the world with industrial progress. Various industrial, agricultural and military operations have released huge amounts of toxic heavy metals into the environment with deleterious effects on soils, water and air (Sobolev and Begonia, 2008; Zhang, et al., 2014 and Ahmad, 2014). Soil contamination by heavy metals like chromium (Cr), nickel (Ni), mercury (Hg), cadmium (Cd), lead (Pb) and copper (Cu) are consequently the most critical environmental problems as it poses significant impacts to the human health as well as the ecosystems (Octavia, 2013; Tamiru, et al., 2014 and Owolabi and Hekeu, 2014) because they cannot be naturally degraded like organic pollutants and they accumulate in different parts of the food chain (Šmejkalová, et al., 2014). Therefore; several studies have demonstrated that microbial parameters may be useful as indicators of changing soil condition caused by chemical pollution (Oliveira and Pampulha, 2006). Microorganisms are the first
biota that undergoes direct and indirect impacts of heavy metals. In polluted environments, the microbe’s response to heavy metals toxicity depends on the concentration and the availability of metals and on the action of factors such as the type of metal, the nature of medium and microbial species (Hassen, et al., 1998). Adverse effects of metals on soil microbes result in decreased decomposition of organic matter, reduced soil respiration, decreased diversity and declined activity of several soil enzymes (Lenart and Boroń, 2014). However, microorganisms have developed several mechanisms to tolerate such high concentrations of heavy metals and remediation by different processes including metals uptake (bioaccumulation) and often they are specific to one or a few metals. Therefore, from environmental point of view, isolation should focus on multiple heavy-metal resistance bacteria (Zhang, et al., 2014; Ahmad and Malik, 2012). Bacteria resistant to heavy metals can be used for detoxification and prevent further deterioration of contaminated sites (Owolabi and Hekeu, 2014). In a study done by Hookoom and Puchooa in (2013), showed that bacterial isolates were capable of growing in the presences of mercury, lead, silver, zinc and copper at varying concentrations and were identified to belong to the Bacillales, while in another study it was demonstrated that isolates were categorized under Pseudomonas species, Staphylococcus, Clostridium, Klebsiella species, Bacillus species, Listeria species, Streptococcus species and Proteus spp. after morphological and biochemical tests (Nath, et al., 2012 and Tamiru, et al., 2014). In this study, we selected soil samples from some areas with known pollution problems where heavy metals and other pollutants have emitted by Erbil steel company for several years. Our objective is to determine the effect of heavy metals on soil physicochemical properties and bacterial population, as well as isolation of soil bacteria that resist such heavy metals in the soil samples to be used to reduce the heavy metals caused by the long-term effects of industrial heavy metal pollution that can be used in bioremediation of heavy metals in future studies.

2. MATERIALS AND METHODS

2.1. Study area

Erbil Steel Company (ESC) situates on the west of Erbil city, that works to gathering iron scrap and bootless afterward, by some scientific steps, they will change to iron compounds. For the present study fourteen sites were selected on the base of their distance to the factory. The sites were (1) Erbil Steel Company, (2) Binberzy Gawra, (3) Khazna, (4) Nogharan, (5) Binberzy Bichuk, (6) Lajan, (7) Qaryatagh, (8) Shamamal, (9) Tarjan, (10) Sardasht, (11) Sahdawa, (12) Qalatasoran, (13) Nawroz and (14) Garden Soil in College of Science as control soil (Figure 1).

2.2. Soil samples collection

The soil samples were collected, during December 2014, using core sampler (10 cm height and 5 cm diameter) at the depth of 0-15 cm for the physicochemical and heavy metal study and using sterilized spatula for the bacteriological study. From each site, three replicated samples were taken and stored in sterile sealed plastic bags before being processed, and transported to laboratory for analysis (Pansu and Gautheyrou, 2006). Soils were air-dried at room temperature (25 C°), crushed and sieved through 2-mm stainless sieve to remove debris (Jaiswal, 2003). The samples then were processed in an isolated process for bacteria using the standard plate method following serial dilution for all bacterial groups.
2.3. Soil physicochemical analysis

Soil moisture content was determined by gravimetric method as described by (Jaiswal, 2003). Particle size distribution and soil texture was determined by hydrometer method according to (Ryan, et al., 2003). The pH and EC of the soils were determined using a calibrated pH-meter (JENWAY 3505) and an electrical conductivity meter (JENWAY 4510) in 1:1 (soil: water suspension) according to the method proposed by (Ryan, et al., 2003). Walkly-Black procedure (1934) was followed for determination of soil total organic carbon (Pansu and Gautheyrou, 2006). Mixed-acid digestion of 1g of each soil sample was done by using a mixture (HNO₃, HCl and H₂SO₄) at the ratio 15:5:2 (v:v) as given by (Hseu, et al., 2002), then the solutions were filtered through Whatman No.1 filter papers and used as test samples for analyses of Cd, Cu, Pb and Zn using Flame Atomic Absorption spectrophotometer (Pg instrument AA500 Atomic Absorption).

2.4. Media used for counting of bacteria

For counting of soil total: bacteria, *Arthrobacter* spp., proteolytic bacteria and asymbiotic nitrogen-fixers, the nutrient agar, soil extract agar (Atlas, 2005), nutrient gelatine agar (Atlas, 2004) and Ashbey’s nitrogen-free agar (Atlas, 2005) were used respectively. Serial dilution was prepared by adding one gram of each soil sample to 9 ml of sterile distilled water in sterilized test tubes, shaken well, a serial dilution of (10⁻⁷) were made in the same method of (Aneja, 2003).

2.5. Isolation and identification of bacteria

For the isolation of bacteria from soil samples, 1g of soil sample was serially diluted in sterile distilled water; 1 ml of soil suspension from 10⁻¹ to 10⁻⁷ was spread on the nutrient agar plate. The growth of the bacterial colonies was observed after 24 hrs of

![Figure 1: Map of Erbil city showing the studied area.](image-url)
incubation at 35°C in inverted position. The bacterial isolates were identified by using cultural, morphological and standard biochemical characteristics including: Gram’s staining and cell morphology, colony characteristics, motility, Catalase, oxidase, urease, H2S test and citrate utilization and stored at 4°C on slants of nutrient agar as described in (Holt, et al., 1994; Atlas, et al., 1995; Harley and Prescott, 2002 and Goldman and Green, 2009).

2.6. Statistical analysis
The experiment was designed in completely randomized (CRD) with three replication and the multiple comparison among the studied sites was done by using Duncan’s test at the level of significant of 0.05 by SPSS version 18 and Microsoft excel 2010 was used for creating the graphs. Person’s correlation was done to test the relationship among soil physicochemical characteristics, metal contents and bacteria groups from the studied sites at the level of (p<0.01) which considered as strong correlation.

Table 1: Physical properties of the studied soils, expressed by (mean ± S.E.).

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Site name</th>
<th>Moisture %</th>
<th>Bulk density</th>
<th>Total pore space%</th>
<th>Clay %</th>
<th>Silt %</th>
<th>Sand %</th>
<th>Texture class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Erbil Steel Company</td>
<td>14.51±0.002</td>
<td>0.774±0.001</td>
<td>22.59±0.024</td>
<td>23.39</td>
<td>32.17</td>
<td>44.44</td>
<td>Loam</td>
</tr>
<tr>
<td>2</td>
<td>Binberzy Gawra</td>
<td>19.99±0.016</td>
<td>0.588±0.008b</td>
<td>55.92±26.55</td>
<td>21.87</td>
<td>40.62</td>
<td>37.51</td>
<td>Loam</td>
</tr>
<tr>
<td>3</td>
<td>Khazna</td>
<td>16.51±0.003d</td>
<td>0.590±0.006d</td>
<td>55.72±0.002d</td>
<td>5.99</td>
<td>22.46</td>
<td>71.55</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>4</td>
<td>Nogharan</td>
<td>16.78±0.001f</td>
<td>0.627±0.001f</td>
<td>31.79±0.003f</td>
<td>6.01</td>
<td>27.04</td>
<td>66.95</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>5</td>
<td>Binberzy Bichuk</td>
<td>20.15±0.000a</td>
<td>0.639±0.002a</td>
<td>42.46±0.000a</td>
<td>29.74</td>
<td>32.88</td>
<td>37.38</td>
<td>Clay Loam</td>
</tr>
<tr>
<td>6</td>
<td>Lajan</td>
<td>20.30±0.001d</td>
<td>0.687±0.002d</td>
<td>35.60±0.004d</td>
<td>6.27</td>
<td>25.09</td>
<td>68.63</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>7</td>
<td>Qaryatagh</td>
<td>14.91±0.000c</td>
<td>0.677±0.001c</td>
<td>57.68±0.009c</td>
<td>17.63</td>
<td>35.26</td>
<td>47.11</td>
<td>Loam</td>
</tr>
<tr>
<td>8</td>
<td>Shammal</td>
<td>14.52±0.000f</td>
<td>0.607±0.001f</td>
<td>46.89±0.006f</td>
<td>14.62</td>
<td>35.10</td>
<td>50.28</td>
<td>Loam</td>
</tr>
<tr>
<td>9</td>
<td>Tarjan</td>
<td>20.59±0.120</td>
<td>0.564±0.002a</td>
<td>61.20±0.231a</td>
<td>28.33</td>
<td>22.04</td>
<td>49.63</td>
<td>Sandy Clay Loam</td>
</tr>
<tr>
<td>10</td>
<td>Sardasht</td>
<td>22.96±0.278</td>
<td>0.616±0.005d</td>
<td>57.65±0.015d</td>
<td>3.25</td>
<td>22.72</td>
<td>74.04</td>
<td>Loamy Sand</td>
</tr>
<tr>
<td>11</td>
<td>Sahdawa</td>
<td>22.77±0.021b</td>
<td>0.594±0.004d</td>
<td>55.42±0.023b</td>
<td>19.42</td>
<td>38.85</td>
<td>41.73</td>
<td>Loam</td>
</tr>
<tr>
<td>12</td>
<td>Qalatasoran</td>
<td>20.61±0.033</td>
<td>0.694±0.004d</td>
<td>47.98±0.006d</td>
<td>23.62</td>
<td>33.07</td>
<td>43.31</td>
<td>Loam</td>
</tr>
<tr>
<td>13</td>
<td>Nawroz</td>
<td>15.89±0.004b</td>
<td>0.677±0.001c</td>
<td>45.03±0.019b</td>
<td>5.94</td>
<td>20.81</td>
<td>73.25</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>14</td>
<td>Control (garden soil)</td>
<td>2.09±0.003b</td>
<td>0.721±0.003b</td>
<td>45.93±0.008b</td>
<td>17.87</td>
<td>33.19</td>
<td>48.93</td>
<td>Loam</td>
</tr>
</tbody>
</table>

3.2. Soil chemical properties
Chemical properties of the studied soils are presented in table (2). The highest pH (9.31±0.032) was recorded in the soil of Erbil steel company, while the lowest pH (7.36±0.012) was recorded in Khazna soil. Significant differences (p<0.05) were observed between the studied sites. The highest EC was

3. RESULTS
3.1. Soil physical properties
Results of soil physical properties are presented in (table 1). The highest moisture content was (22.96±0.278) % observed in Sardasht soil, while the lowest moisture content was (2.09±0.003) % observed in control soil and significant differences (p<0.05) was observed among the studied sites. Bulk density of the studied soils was ranged between 0.564±0.002 and 0.774±0.001 in both Tarjan and Erbil steel company respectively. Between the studied sites, significant statistical differences were observed. The highest total pore space was 61.20±0.231 % in Tarjan soil and the lowest total pore space was 22.59±0.024 % in the soil of Erbil steel company. Significant differences were detected between the studied sites. Soil texture classes were varied from: loam, sandy loam, clay loam, sandy clay loam to loamy sand as shown in table (1).
458±0.289 µS.cm⁻¹ in the soil of Nogharan, while the lowest EC was 38±0.289 µS.cm⁻¹ in the soil of Qalatasoran. Significant differences (p<0.05) were observed between the studied sites. The highest total organic C was 36.447±0.005 g.Kg⁻¹ in the soil of Erbil steel company, while the lowest total organic C was 1.608±0.000 g.Kg⁻¹ in both of Binberzy Gawra and Binberzy Bichuk. Statistical analysis showed significant differences (p<0.05) between the studied sites.

### Table 2: Chemical properties of the studied soils, expressed by (mean ± S.E.).

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Site name</th>
<th>pH</th>
<th>EC µS.cm⁻¹</th>
<th>Total organic C g.Kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Erbil Steel Company</td>
<td>9.31±0.032</td>
<td>144±0.289</td>
<td>36.447±0.005</td>
</tr>
<tr>
<td>2</td>
<td>Binberzy Gawra</td>
<td>7.63±0.009</td>
<td>120±0.289</td>
<td>1.608±0.000</td>
</tr>
<tr>
<td>3</td>
<td>Khazna</td>
<td>7.36±0.012</td>
<td>270±1.732</td>
<td>6.074±0.013</td>
</tr>
<tr>
<td>4</td>
<td>Nogharan</td>
<td>8.3±0.006b</td>
<td>458±0.289</td>
<td>18.13±0.001</td>
</tr>
<tr>
<td>5</td>
<td>Binberzy Bichuk</td>
<td>8.1±0.029b</td>
<td>111±0.289</td>
<td>1.608±0.000</td>
</tr>
<tr>
<td>6</td>
<td>Lajan</td>
<td>7.83±0.003b</td>
<td>140±0.577</td>
<td>22.15±0.001</td>
</tr>
<tr>
<td>7</td>
<td>Qaryatagh</td>
<td>8.2±0.003b</td>
<td>112±0.866</td>
<td>4.28±0.000</td>
</tr>
<tr>
<td>8</td>
<td>Shamamal</td>
<td>8.25±0.003g</td>
<td>171±0.577</td>
<td>8.754±0.000</td>
</tr>
<tr>
<td>9</td>
<td>Tarjan</td>
<td>7.75±0.009b</td>
<td>172.5±1.44d</td>
<td>11.43±0.163</td>
</tr>
<tr>
<td>10</td>
<td>Sardasht</td>
<td>8.2±0.006c</td>
<td>290±0.289</td>
<td>21.26±0.722</td>
</tr>
<tr>
<td>11</td>
<td>Sahdawa</td>
<td>8.26±0.005d</td>
<td>163±0.289</td>
<td>19.47±0.001</td>
</tr>
<tr>
<td>12</td>
<td>Qalatasoran</td>
<td>8.01±0.003b</td>
<td>38±0.289</td>
<td>15.00±0.002</td>
</tr>
<tr>
<td>13</td>
<td>Nawroz</td>
<td>7.93±0.009e</td>
<td>111±0.289</td>
<td>21.26±0.018</td>
</tr>
<tr>
<td>14</td>
<td>Control (garden soil)</td>
<td>8.31±0.003b</td>
<td>75±0.289</td>
<td>9.64±0.014</td>
</tr>
</tbody>
</table>

### Table 3: Metal contents of the studied soils, expressed by (mean ± S.E.).

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Site name</th>
<th>Pb (ppm)</th>
<th>Cd (ppm)</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Erbil Steel Company</td>
<td>292±0.577</td>
<td>8.4±0.208</td>
<td>259±0.577</td>
<td>7480±0.866</td>
</tr>
<tr>
<td>2</td>
<td>Binberzy Gawra</td>
<td>10±0.289</td>
<td>N.D.</td>
<td>27±0.289</td>
<td>65±0.289</td>
</tr>
<tr>
<td>3</td>
<td>Khazna</td>
<td>13±0.289</td>
<td>N.D.</td>
<td>28±0.289</td>
<td>71±0.289</td>
</tr>
<tr>
<td>4</td>
<td>Nogharan</td>
<td>15±0.289</td>
<td>N.D.</td>
<td>31±0.289</td>
<td>92±0.289</td>
</tr>
<tr>
<td>5</td>
<td>Binberzy Bichuk</td>
<td>9±0.289</td>
<td>N.D.</td>
<td>25±0.289</td>
<td>56±0.289</td>
</tr>
<tr>
<td>6</td>
<td>Lajan</td>
<td>28±0.289</td>
<td>N.D.</td>
<td>35±0.289</td>
<td>151±0.289</td>
</tr>
<tr>
<td>7</td>
<td>Qaryatagh</td>
<td>12±0.289</td>
<td>N.D.</td>
<td>25±0.289</td>
<td>66±0.289</td>
</tr>
<tr>
<td>8</td>
<td>Shamamal</td>
<td>14±0.289</td>
<td>N.D.</td>
<td>45±0.289</td>
<td>67±0.289</td>
</tr>
<tr>
<td>9</td>
<td>Tarjan</td>
<td>15±0.289</td>
<td>N.D.</td>
<td>30±0.289</td>
<td>73±0.289</td>
</tr>
<tr>
<td>10</td>
<td>Sardasht</td>
<td>9±0.289</td>
<td>N.D.</td>
<td>26±0.289</td>
<td>70±0.289</td>
</tr>
<tr>
<td>11</td>
<td>Sahdawa</td>
<td>40±0.289</td>
<td>N.D.</td>
<td>36±0.289</td>
<td>93±0.289</td>
</tr>
<tr>
<td>12</td>
<td>Qalatasoran</td>
<td>14±0.289</td>
<td>N.D.</td>
<td>27±0.289</td>
<td>156±0.289</td>
</tr>
<tr>
<td>13</td>
<td>Nawroz</td>
<td>25±0.289</td>
<td>N.D.</td>
<td>32±0.289</td>
<td>106±0.289</td>
</tr>
<tr>
<td>14</td>
<td>Control (garden soil)</td>
<td>11±0.289</td>
<td>N.D.</td>
<td>27±0.289</td>
<td>50±0.289</td>
</tr>
</tbody>
</table>

The highest Pb was 292±0.577 ppm in the soil of Erbil steel company, while the lowest Pb was 9±0.289 ppm in the soil of both Binberzy Bichuk and Sardasht soils. Statistical analysis showed significant differences between the studied sites. The greatest value of Cd was in Erbil steel company soil, while in the other sites under the study, Cd was not detected. The highest Cu was 259±0.577 ppm in the soil the soil of Erbil steel company, while the lowest Cu was 25±0.289 ppm in the soil of Binberzy Bichuk. Significant differences were observed between the studied sites. Zinc values were ranged between 50±0.289 and 7480±0.866 ppm in control and Erbil steel company soils respectively. Statistical analysis showed significant differences between the studied sites. The concentrations of Cd, Cu and Zn analyzed in the soil of Erbil steel company are exceeded...
the permissible limits for soil according to EPA (2011).

### 3.4. Soil microbial population

By the present investigation, the isolated bacterial genera in the soil of Erbil steel company were: *Acinetobacter* spp., *Bacillus* spp., *Micrococcus* spp., *Neisseria* spp., *Pseudomonas* spp. and *Staphylococcus* spp. (Table 4). The negative relationships between the studied metals including Cu, Pb and Zn with the microbial populations are expressed in figures (2-5).

#### Table 4: The isolated bacterial genera in the soil of Erbil steel company.

<table>
<thead>
<tr>
<th>Main characteristics</th>
<th>Bacterial genera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell morphology</strong></td>
<td>Cocco-bacilli</td>
</tr>
<tr>
<td>Gram’s staining</td>
<td>-ve</td>
</tr>
<tr>
<td>Colony Color on</td>
<td>White</td>
</tr>
<tr>
<td>nutrient agar</td>
<td></td>
</tr>
<tr>
<td>Spores</td>
<td>No</td>
</tr>
<tr>
<td>Cell motility</td>
<td>-ve</td>
</tr>
<tr>
<td>Catalase</td>
<td>+ve</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+ve</td>
</tr>
<tr>
<td>Urease test</td>
<td>-ve</td>
</tr>
<tr>
<td>H₂S production</td>
<td>-ve</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>+ve</td>
</tr>
</tbody>
</table>

#### Figure 2: Correlation between heavy metals Cu (a), Pb (b) and Zn (c) and total bacterial population cfu. ×10⁷ g⁻¹ dry soil in the studied sites.

#### Figure 3: Correlation between heavy metals Cu (a), Pb (b) and Zn (c) and total *Arthrobacter* spp. ×10⁵ g⁻¹ dry soil in the studied sites.
The total number of counted bacteria from the studied sites is presented in Figure (6). Maximum bacterial population (19.71×10^7) cfu.g^{-1} dry soil was counted in control soil, while the minimum bacterial population (0.05×10^7) cfu.g^{-1} dry soil was counted in Nawroz soil.
Maximum *Arthrobacter* spp. was \( (1.23 \times 10^5) \) cfu.g\(^{-1}\) dry soil in Sardasht and the minimum *Arthrobacter* spp. was \( (0.32 \times 10^5) \) cfu.g\(^{-1}\) dry soil in the soil of Erbil steel company (Figure 7).

![Figure 7: Total number of *Arthrobacter* spp. \( \times 10^5 \) g\(^{-1}\) dry soil counted in different sites.](image)

The total population of proteolytic bacteria was ranged between \( 0.57 \times 10^5 \) and \( 3.16 \times 10^5 \) cfu.g\(^{-1}\) dry soil in both Lajan and Erbil steel company respectively (Figure 8).

![Figure 8: Total number of proteolytic bacteria \( \times 10^5 \) g\(^{-1}\) dry soil counted in different sites.](image)

Maximum number of total asymbiotic nitrogen-fixers \( (1.25 \times 10^5) \) cfu.g\(^{-1}\) dry soil was counted in Sardasht and the minimum number of total asymbiotic nitrogen-fixer was \( (0.12 \times 10^5) \) cfu.g\(^{-1}\) dry soil in the soil of Erbil steel company (Figure 9).

![Maximum number of total asymbiotic nitrogen-fixers.](image)
Figure 9: Total number of asymbiotic nitrogen-fixers×10^5 g^-1 dry soil counted in different sites.

3.5. Results of statistical correlation

Person’s correlation analysis (Table 5) showed high significant positive correlations (p<0.01) among: pH and Cu (r = 0.781), pH and Pb (r = 0.775) and pH and Zn (r = 0.773); total organic C and Cu (r = 0.676), total organic C and Pb (r = 0.708) and total organic C and Zn (r = 0.666); Cu and Pb (r = 0.994) and Cu and Zn (r = 0.996) as well as Pb and Zn (r = 0.994). On the other hand, a significant negative correlation between bulk density and total pore space (r = -0.682) was observed.

Table 5: Person’s correlation among physicochemical parameters, total number of microorganisms counted in the studied soils.

<table>
<thead>
<tr>
<th></th>
<th>Moisture</th>
<th>Bulk density</th>
<th>Total pore space%</th>
<th>pH</th>
<th>EC</th>
<th>Total organic C</th>
<th>Total Cu</th>
<th>Total Pb</th>
<th>Total Zn</th>
<th>Total no. of bacteria×10^7</th>
<th>Total Arthrobacter spp.×10^5</th>
<th>Total proteolytic bacteria×10^5</th>
<th>Total asymbiotic nitrogen-fixers×10^5</th>
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</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bulk density</td>
<td>0.503</td>
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<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Total pore space%</td>
<td>0.254</td>
<td>0.682**</td>
<td>1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.278</td>
<td>0.057</td>
<td>0.636</td>
<td>1</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>0.181</td>
<td>-0.395</td>
<td>-0.127</td>
<td>-0.016</td>
<td>1</td>
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<td></td>
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<tr>
<td>Total organic C</td>
<td>0.085</td>
<td>0.517</td>
<td>0.598</td>
<td>0.631</td>
<td>0.159</td>
<td>1</td>
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<tr>
<td>Total – Cu</td>
<td>-0.151</td>
<td>0.593</td>
<td>0.647</td>
<td>0.781**</td>
<td>-0.061</td>
<td>0.076**</td>
<td>1</td>
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<tr>
<td>Total – Pb</td>
<td>0.012</td>
<td>0.606</td>
<td>0.640</td>
<td>0.775**</td>
<td>-0.077</td>
<td>0.708**</td>
<td>0.994**</td>
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<tr>
<td>Total – Zn</td>
<td>-0.147</td>
<td>0.016</td>
<td>0.638</td>
<td>0.773**</td>
<td>-0.072</td>
<td>0.646**</td>
<td>0.996**</td>
<td>0.994**</td>
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</tr>
<tr>
<td>Total no. of bacteria×10^7</td>
<td>-0.661</td>
<td>0.014</td>
<td>0.189</td>
<td>-0.268</td>
<td>-0.066</td>
<td>-0.401</td>
<td>-0.220</td>
<td>-0.243</td>
<td>-0.216</td>
<td>1</td>
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<tr>
<td>Total Arthrobacter</td>
<td>0.321</td>
<td>-0.247</td>
<td>0.173</td>
<td>-0.046</td>
<td>0.428</td>
<td>0.144</td>
<td>-0.384</td>
<td>-0.375</td>
<td>-0.380</td>
<td>-0.221</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spp.×10^5</td>
<td>-0.119</td>
<td>0.098</td>
<td>0.140</td>
<td>-0.555</td>
<td>-0.490</td>
<td>-0.208</td>
<td>-0.300</td>
<td>-0.276</td>
<td>-0.285</td>
<td>0.489</td>
<td>-0.225</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total proteolytic</td>
<td>0.410</td>
<td>-0.099</td>
<td>0.308</td>
<td>-0.076</td>
<td>-0.091</td>
<td>0.278</td>
<td>-0.210</td>
<td>-0.180</td>
<td>-0.207</td>
<td>-0.075</td>
<td>0.395</td>
<td>0.249</td>
<td>1</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).
4. DISCUSSION

Moisture and nutrients are essential for the growth of microorganisms in the soil (McKinney, 2004). Soil water affects the moisture available to organisms as well as soil aeration status, the nature and amount of soluble materials, the osmotic pressure, and the pH of the soil solution (Paul, 2007). By the present study the moisture ranged between 2.09±0.003 and 22.96±0.278 % and this observation comes in agreement with those of (Stanley, et al. 2014) who observed a range of soil moisture content from 3.35±0.02 to 20.10±0.20 % in Lafarge cement factory in Nigeria. Bulk density, pore space and soil particle size distribution and texture are considered among the physical soil quality indicators which reflect effects on root growth, speed of plant emergence and water infiltration (Martinez, et al., 2010). Bulk density of the studied soils by this study was ranged between 0.564±0.002 and 0.774±0.001 in both Tarjan and Erbil steel company respectively and the same values were observed by (Vanita, et al., 2014) from four different zones of Amritsar, Punjab (India). Grain size and pore volume are the most important factors controlling the percolation of water and ventilation within the soil which affect living microorganisms (Mirsal, 2008). In the present study the highest total pore space was 61.20±0.231 % in Tarjan soil and this possibly refer to its texture class which is sandy clay loam and the lowest total pore space was 22.59±0.024 % in the soil of Erbil steel company which has a loam soil texture (Table 1) and this come in agreement with the statement of (Paul, 2007), who state sandy-textured soils having a higher mean pore size.

Soil pH, salinity, organic matter content, phosphorus availability, cation exchange capacity, nutrient cycling, and the presence of contaminants such as heavy metals, organic compounds, radioactive substances, etc are among the chemical quality indicators of soil (Martinez, et al., 2010). Soil pH influences a number of factors affecting microbial activity, like solubility and ionization of inorganic and organic soil solution constituents, and these will in turn affect soil enzyme activity (Paul, 2007). Our finding revealed that contamination in Erbil steel company increased soil pH to 9.31±0.032 in comparing with other studied soils and regarding to this finding, the observation of (Anjaneyulu, et al., 2011) who observed that the soil polluted with iron slag in pig iron plant located in India increased soil pH, electrical conductivity and organic carbon as well as the observation of (Stanley, et al., 2014) who observed a pH range of 8.20 - 9.50 in Lafarge cement factory in Nigeria, seem to confirm our findings. The highest total organic C was 36.447±0.005 g.Kg\(^{-1}\) in the soil of Erbil steel company and (Hiroki, 1992 and Jiang, et al., 2014) observed as the same as the present finding in Ohta City in Japan and from commercial apple orchards in Fujisaki Town in Japan, respectively.

The most significant anthropogenic sources of heavy metals in soils depend upon human activities such as mining, smelting, electroplating, use of pesticides and fertilizer, municipal sewage sludge, atmospheric deposition, etc, and above the defined levels, heavy metals are enough to cause risk to human health, plants, animals and aquatic biota. The heavy metals essentially become contaminants in the soil and water environment because of their excess generation by natural and man-made activities, transfer from mines to other locations where higher exposure to humans occurs, discharge of high concentration of metal waste through industries, and greater bioavailability (Dixit, et al., 2015). Lead is a
common environmental contaminant found in soils. Unlike other metals, Pb has no biological role, and is potentially toxic to microorganisms. Studies have shown that long-term heavy metal contamination of soils has harmful effects on soil microbial activity, especially microbial respiration and enzyme activities. Moreover, habitats that have high levels of metal contamination show lower numbers of microbes than uncontaminated habitats (Sobolev and Begonia, 2008). By the present study the highest Pb was 292±0.577 ppm in the soil of Erbil steel company and similar observation was found by (8) in a contaminated soil of an arable field in the center Portugal. Moreover, the greatest value of total Cd, Cu and Zn was in Erbil steel company soil. However (Stanley, et al., 2014) observed higher levels of metals including Pb, Cd, Cu, Cr, Fe and Ni in Lafarge cement factory than the other comparing sites in their study. Moreover, significant differences were observed between the studied sites regarding to the determined metals in this study and this may refer to the emissions of metallurgical dust which are spread according to the wind direction and particle size while soil is the main receiver of heavy metals in dry land (Barcan, 2002). In the vicinities of steel plants the concentration of copper exceeds several thousand ppm and the pollution remains for a long time, even after the operation of mines or steel plants had been stopped (Kabata-Pendias and Pendias, 1999). The fine fractions of dust are enriched with lead, arsenic, and zinc. The concentrations of Cu, Cd and Zn analyzed in the studied soils are exceeded the permissible limits for soil according to (EPA, 2011 and Warmate, et al. 2011) and their accumulation over time can adversely affect the type and number of soil microorganisms (Stanley, et al. 2014).

Soil organisms are sensitive indicators of quality and health because the diversity and abundance may be related to many functions such as decomposition of organic matter, plant and root development (competition), sequestration and detoxification of heavy metals, pesticides and other pollutants and presence of pathogens in soil and plant (Martinez, et al., 2010). Microorganisms play vital roles in soil fertility and primary production through organic matter decomposition and nutrient cycling. When some stress factors such as temperature, extreme pH or chemical pollution such as heavy metals are imposed on a natural environment, soil biota can be affected as well as the ecological processes these microorganisms regulate (Oliveira and Pampulha, 2006). Microorganisms are the first biota that undergoes direct and indirect impacts of heavy metals. Some metals (e.g. Fe, Zn, Cu, Ni, Co) are of vital importance for many microbial activities when occur at low concentrations. These metals are often involved in the metabolism and redox processes. Metals facilitate secondary metabolism in bacteria, actinomycetes and fungi (Weinberg, 1990). However, high concentrations of heavy metals may have inhibitory or even toxic effects on living organisms. Adverse effects of metals on soil microbes result in decreased decomposition of organic matter, reduced soil respiration, and even if they do not reduce their number, they depress their biodiversity and declined activity of several soil enzymes (Hafeburg and Kothe, 2007). Some of the general changes in morphology, the disruption of the life cycle and the increase or decrease of pigmentation are easy to observe and evaluate (Hafeburg and Kothe, 2007). Soil microbial population responses to heavy metal contamination provide a relevant model for ecological studies to assess the influence of environmental characteristics. Several studies have demonstrated that metals influence microorganisms by affecting their growth,
morphology and biochemical activity (Wyszkowska, et al., 2008). Various metals may affect different microbial populations and the resulting impact may vary depending on the metal whose limit concentrations in soils were exceeded. For instance, the pollution of soils with copper affects microorganisms that take part in nitrification and mineralization of protein compounds (Kabata-Pendias and Pendias, 1999). Pollution of the soil with heavy metals negatively influence the soil microbial properties depending upon the soil pH, organic matter content and other soil chemical properties (Anjaneyulu, et al., 2011). Bacteria are the most important group of microorganisms in the environment. Their basic mission is to convert dead biological matter to stable materials that can be recycled back into new biological matter, keeping the biological cycle functioning without disruption (McKinney, 2004). Studies on the effects of metals on soil bacteria have been conducted showing that short term contact causes the selection of resistant bacteria within weeks. A more prolonged exposure to metals slowly selects resistant bacteria. On the other hand long term exposure to metals leads to the selection/adaptation of the microbial community which then thrives in polluted. The presence of different metals together may also have greater adverse effects on the soil microbial biomass/activity and diversity than those caused by single metals at high concentrations (Wyszkowska, et al., 2008). By this investigation, the isolated bacterial genera in the soil of Erbil steel company were: Acinetobacter spp., Bacillus spp., Micrococcus spp., Neisseria spp., Pseudomonas spp. and Staphylococcus spp.; regarding to this finding many workers have isolated different bacterial genera in polluted soils. According to the observation of (Hookoom and Puchooa, 2013), the bacterial isolate capable of growing in the presence of Pb, Zn and Cu isolated in Mauritius were belonged to Bacillus spp.; as well as (Tang, et al., 2014) isolated Bacillus spp. in contaminated soils of Shanjia which can tolerate Pb, Zn and Cr; however, (Banerjee, et al., 2015) isolated four varieties of Bacillus spp. from the ash dyke sample of four thermal power plants of Chhattisgarh, i.e., Bharat Aluminium Company (BALCO), Chhattisgarh State Electricity Board (CSEB), Korba, Thermal Power Cooperation (NTPC), Bilaspur and KSK Akaltara, Chhattisgarh; while (Sevgi, et al. 2010) isolated Pseudomonas spp. and Bacillus spp. in Mersin industrial area of Turkey. Moreover, (Owolabi and Hekeu, 2014) isolated Aeromonas spp., Arthrobacter spp., Corynebacterium spp., Pseudomonas spp. and Streptococcus spp. from six contaminated sites in Lagos and Ota, Nigeria; and (Stanley, et al., 2014) isolated 4 bacterial genera belonging to Alcaligenes, Bacillus, Pseudomonas and Micrococcus in Lafarge cement factory in Nigeria. However, one of the reasons of decreasing biodiversity of microorganisms in heavy metal polluted soils is the selection for tolerant species or strains. Metal exposure may lead to the establishment of tolerant microbial populations, that are often represented by several Gram-positive genera such as Bacillus, Arthrobacter and Corynebacterium or Gram-negatives, e.g. Pseudomonas, Alcaligenes,Ralstonia or Burkholderia (Piotrowska-Seget, et al., 2005). It was shown that the impact of heavy metals on the bacterial metabolism depends on the growth form. The resistance towards metals seems higher in consortia than in pure cultures (Sprocati, et al., 2006).

From the present study, it is apparent that soil bacterial population are affected by heavy metal levels in the studied soils ranging from 9 to 292 ppm for Pb, 25 to 259 ppm for Cu and 50 to 7480 ppm for Zn. Although it is known that the number of bacteria decreased in soil contaminated with Pb of a concentration of 3,500 ppm soil (Bisessar, 1982), the present
report provides the first evidence for the inhibitory effects of heavy metals on soil microorganisms at lower concentrations in the soil of Erbil steel company. During the present investigation, the minimum bacterial population \((0.05 \times 10^7)\) cfu.g\(^{-1}\) dry soil was counted in Nawroz soil, in this regard, (Oliveira and Pampulha, 2006) observed higher number of culturable aerobic bacteria in a contaminated soil in Portugal. Moreover, (Anjaneyulu, et al., 2011) observed that bacterial population was decreased two to three folds in polluted soils in pig iron plant in India. Minimum \textit{Arthrobacter} population was observed in the soil of Erbil steel company during this study. In a laboratory study conducted by (Wyszkowska, et al., 2008) showed that lead and zinc have reduced \textit{Arthrobacter} population in contaminated soils with cadmium, copper, lead and zinc. Nitrogen fixing bacteria are one of the more interesting groups of bacteria found in soil and are very important microorganisms in nitrogen deficient soils. These bacteria have the ability to take nitrogen gas from the atmosphere and fix it into ammonia nitrogen in their cell proteins. Unfortunately, nitrogen fixation requires considerable energy and occurs only when ammonia, nitrite, or nitrate nitrogen is not available in the soil (McKinney, 2004). Minimum number of total asymbiotic nitrogen-fixers was observed in the soil of Erbil steel company. However, (Oliveira and Pampulha, 2006) observed higher number of asymbiotic nitrogen-fixers in a contaminated soil in Portugal in comparing with the soil of Erbil steel company \((0.12 \times 10^5)\) and this may refer to the type and source of pollutants in this area so he observe lower metal contents in such soils.

The correlations among physicochemical, metal contents and microbial population in the studied soils were determined by Person’s correlation. Strong positive correlations were found among Cu, Pb and Zn and this may indicate that they are from the same source according to the statement of (Stanley, et al., 2014). This situation come in agreement with the observations of (Hiroki, 1992) who observed a positive correlation \((r = 0.964-0.984**)\) among the content of Cu, Zn and Cd and the observation of (Stanley, et al., 2014) who observed a strong positive correlation between Fe, Pb and Cd. Moreover, there were negative relationships among the contents of Cu, Pb and Zn and the population of microorganisms in the present study. Many authors have reported that the soil microbial population is strongly affected by heavy metals. For example the population of bacteria and actinomycetes decreased in soil contaminated with Pb at 21,320 mg.kg\(^{-1}\) soil and Zn at 1,273 mg.kg\(^{-1}\) soils (Hiroki, 1992). Furthermore, negative correlations were found by (Huang, et al., 2009) between Pb concentrations and microbial amounts and biological activities. In a similar study in the steel alloy factory in China, (Jiang, et al., 2014) observed that the elevated chromium loadings resulted in changes in the activity of the soil microbe by the indication of a negative correlations between soil microbial population and chromium contents. Moreover, in other similar study in iron and still factory in Libya, (Mlitan, 2013) found that the soil samples which exposed to the factory effluent were the poorest in fungal population and diversity compared to control. It is difficult to determine whether Cu Pb or Zn affected the soil microorganisms, because there were highly positive correlations \((0.994-0.996)\) between the concentration of the three metals and the changes in the concentration of the heavy metals in the field were similar to each other. It is thus suggested that the metals affected microorganisms in combination with each other.

The relation among pH and Cu, Pb and Zn were positive, as pH increased, the levels of
these metals were all increased; and this probably because soil pH influenced the dissolution of heavy metals, low pH may result in increased solubility and high availability of heavy metals for plant roots, while high pH result in accumulation of heavy metals in soil as stated by (Zhao, et al., 2015). Furthermore, a positive correlation was found among Cu, Pb and Zn levels in the studied soils and similar observation was found by (Hu, et al., 2013) in surface soils from lands of six different areas in Guangdong Province, China and this suggests that they probably originated from the same common sources.

5. CONCLUSIONS

This study showed that the levels of soil bulk density, pH, total organic carbon and heavy metals in the soil of Erbil steel company were higher than the other studied sites. The adverse effect of this is noticeable by the population of the soil microorganisms, which were generally low in this site. It is observed that the concentrations of the analyzed metals except for Pb in the soil of Erbil steel company were exceeded the permissible limits for soil. Moreover, it is noticeable that the pH, total organic carbon and the metal contents are in parallel increasing with each other. The isolated bacterial genera in the soil of Erbil steel company are considered tolerant to alkaline pH and heavy metals from the steel company. Further studies on the effect of steel company on rhizosphere microorganisms, diversity and community structure analysis need to be conducted.

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