Chemical Fractionation and Risk Assessment of Some Heavy Metals in Soils, Assiut Governorate, Egypt

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Abstract
Toxic elements behavior in Egyptian soil need more studies for the enhancement of soil quality and human health protection. The current study focuses on the mobility assessment of chemical species of As, Pb, Cr, Ni, Cu and Co in soils due to anthropogenic emission using sequential extraction (SE) and risk assessment code (RAC). Generally, the studied soils are alkaline calcareous loam soil. It has a low organic matter (OM) that ranges from 1 to 3.8% and is enriched with As, Pb, Cr, Ni, Cu and Co, which preferentially accumulate in soil clay and silt fraction. SE revealed that the non-residual (bioaccessible) fraction contains about (70.7%) As, (65.2%) Co, (58.8%) Cr, (28.4%) Cu, (6.8%) Ni and (64.5%) Pb, indicates the risk of pollution with As, Co, Cr, and Pb. RAC values indicated a high risk of soil pollution with As, medium risk of Co and Pb, low risk of Cu and Ni, and no risk of Cr. The results pointed out the geogenic source of Cr, mixed source of Co, Cu, and Ni and the anthropogenic source of As and Pb. The spatial distribution analyses of these metals indicated the role of urbanization, traffic, and industry on the releasing of toxic metals into the environment.

Keywords: Sequential extraction; Risk assessment code; Heavy metals; Geogenic; Anthropogenic; Bioavailability; Assiut.

1. Introduction
Chemical fractionation of soil's heavy metals content becomes necessary to reveal their potential risks affecting plants, animals, and human health [1]. These metals emanate either with low concentrations from natural soil-forming processes (Weathering of parent rock and pedogenic processes) or with alert concentrations from anthropogenic sources (such as industrial, urbanization and agricultural processes), domestic effluent, fossil fuels combustion and atmospheric depositions [2]. Heavy metals stability, toxicity and non-biodegradability in soil have increased their negative impact on humans, directly or indirectly [3]. Heavy metals bioavailability and mobility in soil are strongly based on their chemical form and soil physicochemical characteristics that varied with various environmental conditions [4, 5]. Furthermore, plant cover type affects heavy metals in the soil. The growth of plants provides organic acids into the rhizosphere, which can be chelated with heavy metals from carbonate and/or reducible fractions, leading to the release of these metals into the environment [6].

Determining heavy metals species distribution in soil has great importance in assessing their bioavailability and mobility [7]. Nowadays, evaluation of metal chemical forms in the soil is a necessary issue in metal risk assessment and remediation expediencies [1, 2, 5]. Consequently, this will help prevent plant toxicity by identifying the available and unavailable fraction of a metal [4]. The sequential extraction steps, since proposed by Tessier [8], have been applied widely to study chemical speciation of metals. Several modified procedures were proposed elsewhere to determine the extractable heavy metals load in soils and sediments [9-12]. The application of sequential extraction procedures led to the development of an important index for assessing metal mobility and bioavailability risks [11-13]. The Risk Assessment Code (RAC) is applied to evaluate the environmental risks induced by the unstable
species of toxic heavy metals. The water-soluble, exchangeable and carbonate fractions of the heavy metals represent the highest mobile and bioavailable fractions of the soils [13].

The assessment of the metal load and behavior in agricultural soils becomes an essential issue to prevent its bioaccumulation and the associated risks to humans. However, previous researches on soil, in the study area, mainly focused on the total metals content without any point on species, mobility and bioavailability of these metals, which can reduce their effects on the environment [2, 14, 15].

The alluvial soil of the Nile Valley is the most important resource for Egypt food security. It represents one of the oldest agricultural soils worldwide and the oldest civilization was flourished on this soil. Egypt is one of the agricultural countries that mainly depend on the Nile valley soil. Thus, the present work has been dealt with physicochemical properties of soil, the total metals content; their different species, sources, and bioavailability.

2. Materials and Methods

2.1. Study area

The northern part of Assiut Governorate was the target of this study (Fig. 1), owing to complex activities; including large industries (food, detergents, fertilizers, chemical and cement), urbanization and agriculture. Besides, there is the main sewage plant (El-Madabgh sewage plant) that is used to irrigate many crops [15]. Even though, the importance of soil and the intensive human activities in the study area, there is a lack in the data about the behavior of heavy metals pollution in the soil and the expected risks. Geologically, the main source of this alluvial soil is the mafic/ultramafic rocks in the Ethiopian hinterlands [16, 17]. Consequently, the weathering of these rocks leads to the release of ferromagnesian minerals that are characterized by the presence of considerable concentrations of Cr, Cu, Co and Ni [17] into the Egyptian soil.

2.2. Sampling and analyses

Twenty soil samples (0–30 cm) were collected using a stainless-steel shovel from different sites (Fig. 1) and kept in polyethylene bags. Soil samples were air-dried, mildly crushed and sieved through a 2mm nylon sieve for further analysis. The pH values of the soil samples were measured at a soil/water ratio of 1:1 (g/mL) using a pH meter (Hanna HI 991301). Particle-size distribution was determined using hydrometer (ASTM 152H) method [18]. Calcium carbonate percentage (CaCO3%) was estimated by the titrimetric methods. Modified Walkley and Black method [19] was applied to determine soil organic matter percentage (SOM %) by chromic acid digestion. A small part of each sample was oven-dried at 110° C for 3 h, ground to pass through a 63µm sieve and homogenized for analysis.

For the determination of total metal concentration (TC), exactly 1 g of pulverized (<63 µm) soil sample was attacked with aqua regia (HNO3: HCl = 1: 3). After digesting for 3 h at 240 °C, the solution was transferred to a volumetric flask, which was then filled to 50 ml with bi-distilled water. The elements were determined in the extract by the atomic absorption spectroscopy (Buck Scientific 205 AA). ArcGIS (10.4.1) [20] was used to produce the spatial distribution maps of elements using Inverse distance weighting to create independent raster layers show the link between the element concentrations at the different points within the study area. This technique has been used to conduct environmental monitoring, understand and to predict pollutant spread [21, 22].

The five-step sequential extraction of Phuong [23] was used (Fig. 2) to separate heavy metals into: exchangeable fraction (F1), carbonate fraction (F2), oxidizable fraction (F3) bound to organic matter, reducible fraction (F4) bound to Fe/Mn oxides, and residual fraction (F5). Analysis of standard reference material, blank samples, and analytical duplicates was run for quality control and quality assurance. The validation of sequential extraction results was assured by the calculation of the recovery percent (R %) (Equation 1) [5].

\[
R\% = \frac{100\times (F1+F2+F3+F4+F5)}{TC} \quad (1)
\]

The risk assessment code (RAC) developed by Perin et al. [24] and applied by [4, 5, 25] for assessment of heavy metals mobility. It is the proportion of metals concentration in the exchangeable and carbonates fractions to the total concentration (Equation 2: There are five classes based on RAC value; no risk (RAC < 1), low risk (1 ≤ RAC <10), medium risk (10 ≤ RAC <30), high risk (30 ≤ RAC <50) and very high risk (RAC ≥ 50).

\[
RAC = \frac{100\times (F1+F2)}{(F1+F2+F3+F4+F5)} \quad (2)
\]
Fig. 1. Study area and sampling sites location map.

Fig. 2. Flowchart of applied SE method.
3. Results and Discussion

3.1. Physicochemical Characteristics

The soil physicochemical parameters; pH, texture, mineral composition, organic matter, redox potential, metal source, and loading rate controlled the fate of toxic elements in the soil environment [26]. In the northern part, on the western bank of River Nile, in Assiut Governorate, the main soil characteristics averages; pH, OM, CaCO$_3$, sand, silt and clay were 7.67, 2.4%, 9.1%, 33.1%, 36.9% and 28.9%, respectively (Table 1). The alkaline nature of the soils can enhance the precipitation of heavy metals because their solubility inversely proportional to the pH of the soil (Fig. 3). Thus, the alkaline environment will lead to the accumulation of heavy metals [27]. In addition to the pH of the soil, OM is the primary contributor to retain heavy metals in the soil through the formation of complexes or as exchangeable forms [9]. Based on the USDA [28] classification of soil texture, the soil was mostly loam (Fig. 4). This sandy nature of soil may be due to the depletion of humus from the soil [29]. The content of heavy metals in soil is inversely proportional with the soil particle sizes and accumulates in the clay and silt fraction of soil [9]. This can be supported by the positive correlation between metals and both silt and clay (Fig. 5). The bioavailability and toxicity of metals decrease with increasing particle size [30].

![Fig. 3 Relationship between soil pH and its content of elements.](image)

![Fig. 4. USDA diagram of soil classification including the studied soil samples](image)

Table 1. Physicochemical characteristics of the studied Assiut soil.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>OM</th>
<th>CaCO$_3$</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>As</th>
<th>Co</th>
<th>Cr</th>
<th>Cu</th>
<th>Ni</th>
<th>Pb</th>
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<tr>
<td>Unit</td>
<td></td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>µg/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Mean</td>
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<td>2.4</td>
<td>9.1</td>
<td>33.1</td>
<td>36.9</td>
<td>28.9</td>
<td>15.6</td>
<td>37.7</td>
<td>121.7</td>
<td>33.9</td>
<td>97.9</td>
<td>39.2</td>
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<tr>
<td>Median</td>
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<td>7.5</td>
<td>27.3</td>
<td>39.6</td>
<td>30.3</td>
<td>15.4</td>
<td>40.8</td>
<td>128.9</td>
<td>35.2</td>
<td>88.3</td>
<td>36.2</td>
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<td>0.7</td>
<td>7.9</td>
<td>24.0</td>
<td>18.7</td>
<td>8.1</td>
<td>3.6</td>
<td>11.8</td>
<td>30.5</td>
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<td>8.9</td>
</tr>
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<td>1.0</td>
<td>0.5</td>
<td>5.1</td>
<td>3.5</td>
<td>15.0</td>
<td>4.8</td>
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<td>45.3</td>
<td>13.3</td>
<td>43.0</td>
<td>28.9</td>
</tr>
<tr>
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<td>3.8</td>
<td>34.0</td>
<td>72.5</td>
<td>64.3</td>
<td>47.5</td>
<td>20.5</td>
<td>50.5</td>
<td>154.6</td>
<td>53.2</td>
<td>324.8</td>
<td>59.2</td>
</tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30.8</td>
<td>21.8</td>
<td>27.6</td>
<td>10.9</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>7.9</td>
<td>54</td>
<td>20</td>
<td>22</td>
<td>25</td>
</tr>
</tbody>
</table>

(1) After Goher [31] (2) after Kabata-Pendias and Mukherjee [32] (-) No available data

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The studied soil showed severe contamination with heavy metals for human activities, As (15.6 µg/g), Co (37.7 µg/g), Cr (121.7 µg/g), Cu (33.9 µg/g), Ni (97.7 µg/g) and Pb (39.2 µg/g) (Table 1). These concentrations were 1.5-5 times higher than the background concentrations in the world agricultural soil and Egyptian sediments (Table 1). Unfortunately, the elevated total metal content in the soil will increase its mobility. The enrichment of these toxic metals in the upper soil layers and bottom sediments is referred to the intensive human activities in recent decades [15, 33]. The spatial distribution of the studied metals (Fig. 6) illustrated that the highest concentration of metals was almost concentrated around the Assiut City and Fertilizers factory indicating the role of urbanization, crowded traffic and industry in releasing toxic metals into the environment. In the XRD analysis, the primary mineral phases in the studied area are quartz, albite, calcite, montmorillonite and vermiculite [34]. The inter-relationship between the studied metals indicated the association of Co, Cr, Cu and Ni (Fig. 7). This may be attributed to the presence of these metals in the mafic-ultramafic source rocks of the studied soil. On the other hand, the positive correlation between Pb and both of As, Co, Cr, Cu, Ni and Pb (Fig. 8) indicated the contribution of the anthropogenic activities in the study area in the pollution of soil with these metals.

3.2. Sequential Extraction

The obtained results (Table 2 and Fig. 9) revealed that, the non-residual fraction (F1+F2+F3+F4) contained about As (70.7%), Co (65.2%), Cr (58.8%), Cu (28.4%), Ni (6.8%) and Pb (64.5%) which represent the proportion of metal that is mobile under environmental conditions change [10]. The non-residual fraction contains the metal load from anthropogenic inputs [35]; unfortunately, metals in this fraction are more available to plants than the residual fraction [4]. In this study, the recovery values of all metals ranging from 94.4% to 99.1% showing good accordance between total concentrations and the sum of fractions [5].

The exchangeable fraction (F1) contained about 35.6%, 7.9%, 0%, 1.3%, 0.6% and 9.2% of As, Co, Cr, Cu, Ni and Pb, respectively, of the total metal extract. This fraction supposedly is the most potentially mobile fraction, because it is includes the weakly sorbed metals in soil matrix; particularly those in weak electrostatic interactions and ion-exchange process [36]. So, it is influenced by the cation exchange capacity (CEC), clays and organic matter present in soils [37]. Changes in the conditions of environment such as pH decrease, ionic composition, adsorption-desorption reactions [1, 5] can be released this fraction into the environment. It is the most available fraction to biota and most easily leached to groundwater [23]. Unfortunately, arsenic (As) mainly concentrated in this fraction, with value (5.4 µg/g) greater than the world soil (Table 2). The contribution of carbonate fraction (F2) was 16% for As, 18.7% for Co, 0% for Cr, 7.1% for Cu, 4.6% for Ni, and 14.8% for Pb (Table 2), which could be released when environmental conditions change [38]. Fractions (F1 and F2) contain the weakly bound element on soil matrix, and thus considered the most labile and bioavailable fractions [7].
Fig. 6. Spatial distribution maps of the studied heavy metals contents in the study area soil.

Table 2. Sequential extraction average concentrations of metals in the Assiut soil.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Exchangeable (F1)</th>
<th>Carbonate (F2)</th>
<th>Organic (F3)</th>
<th>Reducible (F4)</th>
<th>Sum F1+F2+F3+F4</th>
<th>Residual (F5)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/g %</td>
<td>µg/g %</td>
<td>µg/g %</td>
<td>µg/g %</td>
<td>µg/g %</td>
<td>µg/g %</td>
<td>%</td>
</tr>
<tr>
<td>As</td>
<td>5.4 35.6</td>
<td>2.4 16.0</td>
<td>0.8 5.6</td>
<td>2.0 13.5</td>
<td>10.6 70.7</td>
<td>4.4 29.3</td>
<td>97.3</td>
</tr>
<tr>
<td>Co</td>
<td>2.9 7.9</td>
<td>6.9 18.7</td>
<td>6.5 17.7</td>
<td>7.7 20.9</td>
<td>24 65.2</td>
<td>12.9 34.8</td>
<td>97.6</td>
</tr>
<tr>
<td>Cr</td>
<td>0.00 0.0</td>
<td>0.0 0.0</td>
<td>0.2 0.1</td>
<td>70.9 58.7</td>
<td>71.1 58.8</td>
<td>49.8 41.2</td>
<td>99.1</td>
</tr>
<tr>
<td>Cu</td>
<td>0.4 1.3</td>
<td>2.3 7.1</td>
<td>3.2 9.8</td>
<td>3.4 10.2</td>
<td>9.3 28.4</td>
<td>23.7 71.6</td>
<td>97.6</td>
</tr>
<tr>
<td>Ni</td>
<td>0.6 0.6</td>
<td>4.4 14.6</td>
<td>1.5 1.6</td>
<td>0.0 0.0</td>
<td>6.5 6.8</td>
<td>89.9 93.2</td>
<td>98.5</td>
</tr>
<tr>
<td>Pb</td>
<td>3.4 9.2</td>
<td>5.4 14.8</td>
<td>8.5 23.1</td>
<td>6.4 17.4</td>
<td>23.7 64.5</td>
<td>13.0 35.4</td>
<td>94.4</td>
</tr>
</tbody>
</table>

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Fig. 7. Relationship between Cu and both of Co, Cr and Ni (µg/g) in Assiut soil.

Fig. 8. Relationship between Pb and both of As, Co, Cu and Ni (µg/g) in Assiut soil.

Fig. 9. Relative distribution of the studied toxic metals among the various soil fractions in Assiut soil.
The mean concentration of As, Co, Cr, Cu, Ni and Pb in the oxidizable (organic) fraction (F3) was 0.8, 6.5, 0.2, 3.2, 1.5 and 8.5 µg/g, respectively corresponding to 5.6%, 17.7%, 0.1%, 9.8%, 1.6% and 23.1 of the total concentrations, respectively (Tables 2). The reducible fraction (F4) contains about 13.5%, 20.9%, 58.7%, 10.2%, 0% and 17.4% of the total concentrations of As, Co, Cr, Cu, Ni and Pb, respectively (Table 2). It has appeared that reducible fraction is an important pool for Co, Cr and Cu in the studied soil. Heavy metals in this fraction are bound to Fe/Mn oxides/hydroxides and will be released into the environment along with the decomposition of these compounds [39] and changing of the environmental conditions, such as pH and redox potential [1]. Generally, fractions (F3 and F4) are sensitive to environmental changes and hence can be mobilized under suitable conditions such as redox reactions [40].

While, the residual fraction (F5) content of As, Co, Cr, Cu, Ni and Pb were 29.3%, 34.8%, 41.2%, 71.6%, 93.2% and 35.4%, respectively of the total concentrations, equivalent to 4.4, 13, 49.8, 89.9, 23.7 and 13 µg/g, respectively. The residual fraction is governing the distribution of Ni and Cu, indicating the incorporation of them within the crystalline structures of the minerals and hence their immobility under natural conditions. Consequently, Ni and Cu source in the studied soil was natural; resulted from the weathering of mafic/ultramafic rocks in Ethiopian highlands. Although (Ni and Cu) are stable in the environment along with the decomposition of OM represents a good binding site for heavy metals adsorption. Tillage and harrowing processes increase air (oxygen) circulation and reduce vertical water movement and thus lead to organic matter oxidization and releasing organic-linked metals [43]. Heavy metals that attach to these organic constituents may become available because most of them are water-soluble. Also, seasonal agricultural processes will encourage the emission of metals associated with reducible fraction in the soil owing to photo-redox reactions of sunlight.

### 3.3. Risk assessment code (RAC)

The RAC showed different values between soil samples and individual metals [42]. Arsenic was highly mobile in the studied samples with RAC>50 (Table 3). Co and Pb were of medium risk. Cu and Ni were of low risk. Cr was of no risk. The trend of RAC of these metals is Cr<Ni<Cu<Co<As. The detected high levels of available heavy metals forms (high RAC) in the studied soils may be because of more frequently mechanized arable farms compared to forest and tree plantation. The seasonal agricultural cycle in the study area and associated tillage and harrowing bring up the soil from the rhizosphere to top surface and bury organic matter. The surface of heavy metals is Cr<Ni<Cu<Co<As. The detected high levels of available heavy metals forms (high RAC) in the studied soils may be because of more frequently mechanized arable farms compared to forest and tree plantation. The seasonal agricultural cycle in the study area and associated tillage and harrowing bring up the soil from the rhizosphere to top surface and bury organic matter. The surface of OM represents a good binding site for heavy metals adsorption. Tillage and harrowing processes increase air (oxygen) circulation and reduce vertical water movement and thus lead to organic matter oxidization and releasing organic-linked metals [43]. Heavy metals that attach to these organic constituents may become available because most of them are water-soluble. Also, seasonal agricultural processes will encourage the emission of metals associated with reducible fraction in the soil owing to photo-redox reactions of sunlight.

Table 3. RAC values of selected Heavy Metals.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>As</th>
<th>Co</th>
<th>Cr</th>
<th>Cu</th>
<th>Ni</th>
<th>Pb</th>
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<tr>
<td>1</td>
<td>70.6</td>
<td>24.5</td>
<td>0</td>
<td>5.9</td>
<td>5.6</td>
<td>22.5</td>
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<tr>
<td>2</td>
<td>64.2</td>
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<td>7.5</td>
<td>27.8</td>
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<tr>
<td>3</td>
<td>48.4</td>
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<td>0</td>
<td>11.0</td>
<td>20.7</td>
<td>20.2</td>
</tr>
<tr>
<td>4</td>
<td>57.2</td>
<td>27.3</td>
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<td>4.3</td>
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</tr>
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<td>5.7</td>
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<td>4.0</td>
<td>22.8</td>
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<tr>
<td>7</td>
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<td>8.2</td>
<td>5.7</td>
<td>23.7</td>
</tr>
</tbody>
</table>

Low RAC of Cr, Cu, and Ni can be explained as evidence of incorporation of these metals within the crystal lattice of minerals (geogenic source of elements) and hence relatively low biological availability, reactivity, and liability of these metals in the environment [44, 45]. The low RAC values of...
these metals can be attributed to the presence of the higher levels of the extracted metals in F4 and F5 (readily non mobile) and low levels were within F1 and F2 (mobile fractions). The absorption and loss of metal labile fractions from agricultural soil during crops harvesting, can lead to the release and redistribution of these metals from the non-labile fractions to retain equilibrium in the soil.

4. Conclusions

The geochemical studies presented an evaluation of soil physicochemical properties characteristics and sources, spatial distribution, accumulation and bioavailability of As, Co, Cr, Cu, Ni, and Pb in alluvial soils of Assiut, Egypt. The findings indicated that the critical contamination in the studied area is linked to human activity spots as proved by the established spatial distribution maps for the studied heavy metals. The average concentrations of the studied metals were higher than Egypt's source sediments and world soil. Sequential extraction results showed that the bioaccessible fractions of As, Co, Cr, Cu, Ni and Pb were about 70.7%, 65.2%, 58.8%, 28.4%, 6.8% and 64.5%, respectively of the total extract. Arsenic showed the highest bioavailability in surface soils. The RAC values showed very high potential risk of the mobility of As, medium risk from Co and Pb, low risk from Cu and Ni, and no risk from Cr in the investigated soils.

5. Conflicts of interest

There are no conflicts to declare

6. Formatting of funding sources

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8. References


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