JJEES

Jordan Journal of Earth and Environmental Sciences

JJEES (2024) 15 (2): 115-122
ISSN 1995-6681

Chemometric Evaluation of Residual Soil Contents: Application to Heavy Metals at Public Parks in Amman - Jordan

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Received September 19, 2023; Accepted November 15, 2023

Abstract

In this study, selected heavy metals, present in park soil samples from different areas in Amman-Jordan, were evaluated. Soil samples were collected from 30 different parks in 3 different areas during the summer season. The physiochemical properties that play a main role in metal mobility were determined, such as pH that showed neutral to alkaline values. The moisture content revealed low value due to the high evaporation level during sample collections, and finally, the organic matter content was full in high contents. Park soils' metal contents were extracted and then examined by flame atomic absorption spectroscopy. The data were exposed to principle component analysis and hierarchical cluster analysis to reveal the variations of park metal levels as a result of geographical park areas, to show the metals that were responsible for clustering parks, and finally, to facilitate the future metal's location prediction and parks clustering of unknown soil samples based on their metal contents. The results showed that thirty parks of different soil samples were grouped into two clusters. Among the parks, two areas of high traffic jams and construction processes were clustered together due to the distinct content of Pb, Cu, and Ni where the third area shows distinct content of Zn that was responsible for clustering parks of this area alone. Classification of park soils according to the similarities of their chemical contents based on Flame Atomic Absorption Spectrometry outcomes will help define the sources of the studied heavy metals and provide clear information about the common health symptoms / issues caused by exposure to these heavy metals in the areas that have been clustered in one group.

Keywords: *Park soils, Principal Component Analysis, Cluster analysis, Geographical prediction, Heavy metals.*

1. Introduction

Heavy metals are natural constituents of the earth-crust and are emitted to the environment in different ways: combustion of fossil fuel and road traffic, tears of tires, corrosion of building materials, automobile bodies, brake lining, industry, and human activities. Then, they are settled in soil, water, air, dust and sediments, fertilizer, and sewage. Consequently, they accumulate in the top layer (Mashal et al., 2017; Li et al., 2014; Aaseth et al., 2016; Kumar et al., 2015). Increasing industrialization has been accompanied throughout the world by the extraction and distribution of mineral substances from their natural deposits. Many of these substances have undergone chemical changes through technical processes, and are finally dispersed in solutions by different ways of effluent, sewage, dumps, and dust, into the water, the earth, and the air and thus into the food chain (Al Bakain et al., 2012a). Thus, the threat posed by heavy metals to human health and the environment has risen sharply; hence, there is rising public health interest in the possible effects of heavy metals on human health (appendix 1). Many investigations were carried out to determine the levels of heavy metals in different areas and materials worldwide, including sediment (Bany Yaseen et al., 2019; Irvine et al., 2009), street dust and surface soils (Tarawneh et al., 2021; Lee et al., 2005), and in different parks and gardens (Lorraine et al., 2015; Gu et al., 2018; Wang et al., 2019). Heavy metals can enter human bodies in many ways; the common modes of intake of external materials are inhalation of air into the lungs, ingestion of food, water, and at times non– food items into the gastrointestinal system and transfer through the skin intravenous, intramuscular and vaginal routes (Al Bakain et al., 2012a). Identifying the level of the heavy metals and their toxicity degree in the living areas is required, especially in the areas where children gather since they ingest quantities of soil dust when they play in parks or gardens more likely than adults with their habit of placing dirty fingers and objects into their mouths (Al Bakain et al., 2012a). Therefore, they are more susceptible to the intake of toxins than adults are which is risky since maximal brain growth and differentiation are found in the first few years of life.

Open parks are one of the favorite places for children to spend time. The distribution of heavy metals in the soil in parks is related to natural and anthropogenic sources (Lorraine et al., 2015; Gu et al., 2018; Wang et al., 2019) such as the food residues, application of irrigation water (surface or groundwater), and construction activities nearby the park's areas. Many studies focused on the concentration of heavy metals and their sources in different parks worldwide (Lorraine et al., 2015; Gu et al., 2018; Wang et al., 2019; Paukszto et al., 2018; El Hamiania et al., 2010). In the USA, Lorraine et al., 2015 evaluated Pb, As, and Cd levels in 12 parks in Los Angeles to find high levels of Pb (> 80 mg/Kg) in 11 gardens, where the As level was > 5 mg/Kg, then

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finally, Cd concentration was found to be < 2 mg/kg in all samples. In China (Gu et al., 2018), 28 urban soil parks in Guangzhou city were studied to find that Cd, Pb, Ni, Cu, Zn, and Mn were higher than their background value due to anthropogenic sources. Another study was carried out in China, where 34 soil samples from 6 parks in Jiaozuo-Henan Province were collected (Wang et al., 2019), and the results showed that the concentration of As, Zn, Co, and Ni was higher than the background value. In Tatra National Park-Poland, a high content of soluble compounds of the tested metals was obtained due to the long-term flow from the south and west of Poland to the studied area (Paukszto et al., 2018). In Morocco, 16 garden soil samples were collected from the area adjacent to a mining area (El Hamiania et al., 2010) to find that Cr concentration was above the acceptable value (50 mg Kg^{-1}), and Mn concentration was very high (i.e. exceeded 900 $mg.kg^{-1}$). The mobility of heavy metals in soil is affected by many factors: organic matter content, structure, moisture, and pH (Murray et al., 2004; Imperato et al. 2003). When pH decreases, the ions begin to break free of their compounds and hence, the solubility of the heavy metals in the form of ions will increase and that leads to the increase of the mobility in soils. High carbonate content enhances the pH, which promotes precipitation of metals in the soil. The change in pH during the extraction depends on the composition of the sample and affects the amount of the metal leaching considerably (Al Bakain et al., 2012a; Murray et al., 2004; Imperato et al. 2003).

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are useful tools in analytical chemistry that are used for classifications (Al Bakain et al., 2011; Al Bakain et al., 2021a; Al Bakain et al., 2020; Al Bakain et al., 2021b; Al-Hyasat et al. 2021; Liu et al., 2023). These tools were performed in many studies for many purposes to identify the content most important in distinguishing the sample varieties, to find the variation in chemical profiles as a result of different batches, to confirm whether the areas in the cluster analysis would also be grouped, and to reveal the compounds that were responsible for grouping the areas between clusters.

There is currently no available systematic clustering for the park soils' heavy metals content in Jordan. Hence, this study was implemented to examine the metal contents of 30 park soils. For the first time, park soils were obtained from different areas in Amman, extracted, and analyzed using Flame Atomic Absorption Spectroscopy (FAAS). The Zn, Pb, Cu, and Ni metals were evaluated to reveal all possible metals necessary for park clustering. Grouping the soil samples from different parks was accomplished, using unsupervised clustering methods (i.e. PCA and HCA). To the best of our knowledge, this study is the first to classify the park soil samples in Amman-Jordan and to use the PCA and HCA to predict the location for future park soil samples, considered for blind analysis.

2. Experimental

2.1. Sampling areas

Thirty public parks, distributed in different neighborhoods in Amman, have been selected as sampling areas (Table 1). The studied parks represent different locations, types of soil, ages of buildings around, street types (i.e. major or minor), and human activities. Sampling locations are shown in Figure 1.

2.2. Sampling

From each park, representative soil samples were collected during the summer season in Amman (i.e. August-October 2019), and then transferred to a polyethylene bag. Areas of the parks were varied from 675 m^2 to 8747 m^2. The number of the collected samples was varied according to the area of each park to be from 25 to 300 samples.

2.3. Chemicals and Instrumentations

All glassware, polypropylene test tubes, and funnels were initially cleaned with soap, washed thoroughly with tap and distilled water, and soaked overnight in 10 %HNO_3 (v/v) to remove any contamination by heavy metals. Centrifuge (Model, PK 130), oven (Binder, IP 20, Germany), muffle furnace (Carbolite, S33 6RB, England), water bath shaker (Jeio tech, BS-11, Korea), sieve 125 µm (Model Retsch, G.M, B.H, Germany), pH meter (HANNA, HI-2002 Edge, USA), and ultrasonic bath (Model, 50T, New York) were used in this work. A standard solution of Pb, Zn, Cu, and Ni of 1000 ppm was implemented to prepare the solutions based on the following concentrations: Zn: 0.05, 0.5, 1 and 2 ppm, Ni: 0.5, 1, 3, and 5 ppm, Cu: 0.05, 0.5, 1, 3 and 5 ppm, and Pb: 1, 2, 5, and 15 ppm. The HNO_3 analytical grade 65% (Scharleau, Spain) solvent was applied to extract the heavy metals from the soil samples. The standard Reference Material (SRM) NIST 1646 was purchased from Sigma-Aldrich. Determination of heavy metals was carried out on FAAS (Varian 55B model) according to the following conditions: Air acetylene was the fuel of 1.5 L/min fuel flow, slit width was 0.2 nm, lamp current was 5, 6, 5, 7 mA, and finally, the wavelengths were 217.0, 224.7, 213.9 and 232.0 nm for Pd, Cu, Zn, and Ni respectively.

2.4. Sample Pretreatment

For all representative soil samples, large debris and stones were removed, then the filtered soil was grounded, using a ceramic mortar and pestle to assure homogeneity, sieved through a 125 µm sieve, and finally, stored in polyethylene bags in a freezer at -20°C prior to analysis.

2.5. Moisture and Organic-Matter Content

The moisture content was determined by weighing around 1.0 g of each park soil sample, then was heated in an oven at 105ºC for 12 hrs. until reaching constant weight. The weight loss was calculated based on the difference between the final and the initial weight. The same dried sample was then transferred into a muffle furnace at 550º C for 4 hrs. in order to determine the organic matter content, which was then calculated gravimetrically based on the weight difference.

2.6. pH Measurement

The pH meter has been calibrated at pH 4 and 7 buffer solutions at room temperature. Then, the pH value of the water extract of each park soil sample was determined by dissolving the soil in milli-Q water (1: 2.5 wt/v) in 15 polypropylene test tubes.

Table 1. Description of the sampling areas in Amman- Jordan.

Park Group	Park No.	Park Name	Site
A	1	Queen Noor	Almadina
A	2	Ammar bin Yasir	Basman
B	3	Alabdallat	Marka
B	4	Iskan alamana	Marka
B	5	Alouda	Al Nasr
A	6	Khawla bent Alazwar	Yarmouk
A	7	Um almuminin	Ras Al Ain
A	8	Kafr Ra'i	Badr
A	9	Al Mutanabi	Zahran
A	10	Aldiyar	Zahran
A	11	Teacher Samir Al Rifai	Abdali
A	12	Bilal bin Rabah	Abdali
B	13	Alhilal	Tariq
C	14	The princess Badiea	Al Qwiesma
C	15	Juwaida	Al Qwiesma
C	16	Mutah	Khuraibet Al-Souq
C	17	AlMuqabilin Almuktabia	Al-Muqabilin
C	18	The Petra	Al-Muqabilin
B	19	Judge Moussa Al Saket	Sweileh
B	20	Um Omar	Sweileh
A	21	Almururia	Tlaa Al-Ali
A	22	The Hashemite Flag	Jubaiha
B	23	Al Manhal	Jubaiha
B	24	Independent Park	Shafa Badran
C	25	Hussain Al-Khawaja	Wadi Seer
B	26	Alshaeb	Wadi Seer
A	27	Alshilal	Wadi Seer
B	28	Abu Naseer 1	Abu Naseer
C	29	Albunaiat	Al-Muqabilin
C	30	The Honorable Rock	Ras Al Ain

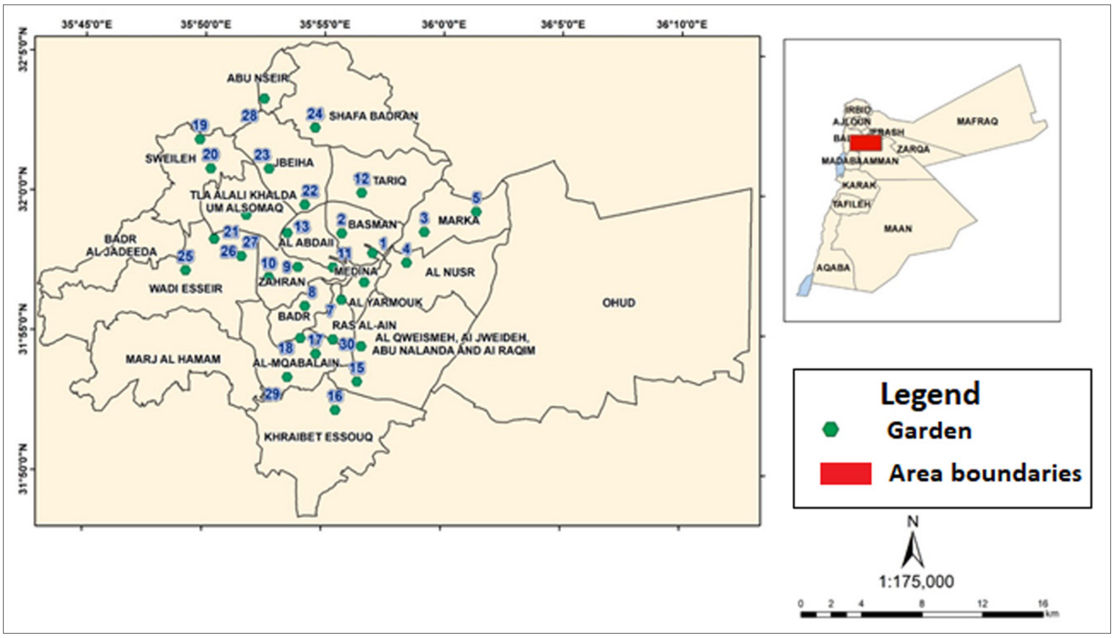


Figure 1. Map of the sampling sites in Amman (the numbers from 1 to 30 are the parks names as identified in Table 1).

2.7. Acid-Extractable Fraction of Heavy Metals

The extraction of heavy metals from park soil samples was carried out by weighing around 0.7 g of dry sample. Then, 15 mL of concentrated HNO_3 was added in 50 mL polypropylene test tubes, heated for 2 h at 95º C in a water bath shaker, sonicated for 30 mins in ultrasonic bath at 50º C, then centrifuged for 15 mins at 1000 rpm (Al Bakain et al., 2012a). The sample was then diluted with 1% HNO_3. Blank solutions and the SRM were prepared in the same way as employed for the real samples.

2.8. Validity and Quality Control

To evaluate the analytical precision and accuracy of the analytical procedure, blank reagents, replicate samples, and standard reference material were implemented. Blanks were prepared in a similar manner to that of the real samples and were analyzed before each measurement. All extractions and analyses were made in triplicate ($n = 3$) for quality assessment, and the average results were reported. The analysis of SRM was conducted and compared to the results reported in the certified values. The SRM was subjected to the extraction, and then the results were reported as the percent recovery.

2.9. Statistical data treatments

2.9.1 Multivariate analysis of park soil samples using unsupervised clustering methods

The main targets for applying multivariate analysis in chemistry are grouping, clustering, discriminating, and classifying objects (samples, compounds, or materials), besides modeling relationships among different analytical data (Al Bakain et al., 2020). In grouping or clustering, park soil samples would be grouped according to their chemical composition, elemental pattern, or technological properties. In classification, soil samples would be classified into known class membership based on their elemental pattern, chemical composition, or spectra (Otto, 2016; Sipos et al, 2005). Generally, there are two main methodologies adopted for grouping or clustering known as unsupervised and supervised methods. For unsupervised methods, a grouping of analytical data is carried out by projecting the high dimensional data into a lower dimensional space, and since there is no supervisor to relate the membership of samples to classes, then unsupervised methods are performed in a supervised manner (Al-Hyasat et al., 2021; Brereton, 2007). Both Principal Component Analysis PCA and Hierarchical Clustering HCA methods are typical examples of unsupervised methods (Otto, 2016; Brereton, 2007). In this study, HCA and PCA were performed to confirm whether the soil samples obtained from different parks would be grouped based on the analysis results of the heavy metal contents in each sample. PCA can reveal the metal/s that is/are responsible for grouping soil samples of origin. Such classification results may have value for environmental researchers in the discrimination and selection of park soil samples. These results may help to show the similarities and differences between soil profiles across Amman.

2.9.2 Arrangement of chromatographic data

The data were arranged as a data matrix X of samples and variables. For the current case, matrix X has the size of 30×30 (i.e. 30 parks) × 4 heavy metals. Matrix X was subjected to different clustering methods as will be discussed below. None of the collected samples were assigned to a class membership; hence, unsupervised clustering methods were applied (Al Bakain et al., 2020).

2.9.3 Principal component analysis:

PCA is a data compression method based on the correlation among variables. It aims to group those correlated variables, while replacing the original ones by a new set, called the principal components (PCs), onto which the data are projected (Al Bakain et al., 2012b). These PCs are completely uncorrelated and are built as a simple linear combination of the original variables. It is important to point out here that the PCs contain most of the variability in the data set, albeit in a much lower dimensional space. The first principal component, PC1, is defined in the direction of maximum variance of the whole data set. PC2 is the direction that describes the maximum variance in the orthogonal subspace to PC1. The subsequent components are taken orthogonal to those previously chosen and describe the maximum of the remaining variance (Liu et al., 2023; Otto, 2016; Brereton, 2007). Once the redundancy is removed, only the first few principal components are required to describe most of the information contained in the original data set. In this study, two data matrices were built. The data matrix X (30×30) is decomposed into two matrices: T (score matrix) and L (loading matrix), using a suitable PCA algorithm. The first step in PCA is the computation of loadings. Mathematically, the loadings are the eigenvectors of the matrix (XXT). There are several methods to estimate the eigenvectors, such as singular value decomposition (SVD) and NIPALS (non-linear iterative partial least-squares) in the order of explained proportion of the variations in X until a certain pre-established number of components (Al Bakain et al., 2020). The loadings are grouped into a matrix L. The collected loadings are orthonormal, meaning they are both orthogonal and normalized. The relationship between the original matrix X, the loading matrix U, and the score matrix T is described (Otto, 2016; Brereton, 2007):

$$X = TLT \tag{1}$$

Mathematically, matrix X is decomposed in the product of two matrices, T and L, on the condition that L is formed by orthonormal columns. T is obtained as T = XTL. In this work, the size of X is 30×30, while size T is 30×h and L is h×30, where h is the number of factors needed to decompose matrix X. The optimum number of factors (h) is necessary to create the optimum number of loadings and scores and produce informative discrimination among soil samples (Al Bakain et al., 2020). As initial variables are assigned into four different groups (i.e. 4 heavy metals), then it is possible to classify soil samples based on heavy metals` levels.

2.9.4 Hierarchical clustering analysis

The main strategy of unsupervised methods is based on cluster analysis where the soil samples are aggregated stepwise according to the similarity of their features or variables (i.e., contents of heavy metals). As a result, hierarchically ordered clusters are created. In HCA, the collected data is displayed in a certain way to emphasize their natural clusters and patterns in a two-dimensional space. The results are often presented in the form of dendrograms, which allow quick visualization of clusters and correlations among tested samples. The similarity between samples can be evaluated following a suitable distance measure, which is commonly applied in pattern recognition. Euclidean distance d (which is a special case for Minkowski distance) between samples is estimated as (Brereton, 2007):

$$d_{i,k} = [\sum_{k=1}^{K} (x_{i,k} - x_{j,k})^2]^{1/2} \quad (2)$$

where K and i/j are the number of variables measured for samples and indices for samples respectively. Estimations would be made using the main principal components of the original data after decomposition by PCA. Initially, $d_{i,k}$ is estimated between all samples (i.e., every sample is to be compared with the remaining samples) to create the distance matrix. The similarity or aggregation between samples is then estimated using the weighted average linkage method (Brereton, 2007).

2.10. Statistical software

The statistical analysis, including principal component analysis PCA and hierarchical cluster analysis HCA, was performed using Chemoface 1.61 software which runs under MatlabVR (Mathworks, 8.6, USA).

3. Results and Discussion

3.1. Physico-chemical soil properties

Moisture (Mois) content, organic matter content (OM), and pH have been evaluated (Figure 2). The pH is a test for measuring the binding strength and potential mobility of heavy metals in soil (Al Bakain et al., 2012a). Metal solubility decreases at high pH values, which leads to metal precipitation. The results of pH measurements showed that all park soil samples in the 3 areas (A, B, and C) were neutral to alkaline (i.e. pH > 7). The pH values were varied between 7.86 and 12.27, with an average of 8.52. This result referred to the difference in waste composition that might affect the pH values, such as the food residues, application of irrigation water (surface or groundwater), construction activities near the parks area, and cleaning rate of the park areas. Moreover, the nature of the soil in Jordan is almost calcareous (i.e. high carbonate content), which raises the pH values (Al Bakain et al., 2012a). Carbonate provides a buffer against the mobilization of metals and facilitates metal's precipitation (Sipos et al., 2005).

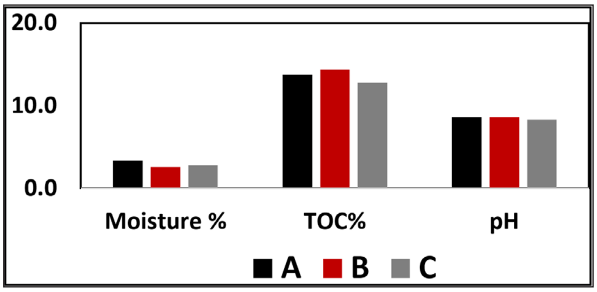


Figure 2. Average physico-chemical soil properties for the studied parks in Amman.

The soil samples from area A showed high pH values since this area consists of new hotels, shopping centers, and office buildings in Amman (Abdali). Moreover, areas A and B had big construction phases during our sample collection.

Regarding the moisture content of the 30-park soil, the values were low, and there was no variation among the three areas as shown in Figure 2. The highest moisture content was 3.33% at area A followed by 2.78% and 2.57% for areas C and B respectively. These results are related to the high temperature recorded in Amman during the sample collection (August-October 2019) with an average of 38°C.

The average organic matter (OM%) content was 13.77%, 14.38%, and 13.78% for areas A, B, and C respectively. The OM% in the studied areas ranged between 7.97% and 21.07%. The differences in OM % values between the studied areas can be attributed to the organic contaminants caused by the residues of food, animal, cartons, papers, and car emissions such as fuel and oils, smelting, chemical fertilizers, mining emissions, in addition to grass and vegetation that cover the soil (Al-Khashman et al., 2006) at the studied park soil areas.

3.2. Total heavy metals

In order to assess the accuracy of the results obtained by the analytical method implemented in this study, two methods were provided: blank solutions and SRM. The recovery of the heavy metals in NIST (1646 a) was (95.60%, 105.04 %, 97.21 %, and 85.52%), and the detection limit was 0.01, 0.10, 0.02, and 0.07 mg/L for Zn, Pb, Cu, and Ni respectively. The averages of heavy metals concentrations (mg/Kg) in the 30 investigated park soil samples are illustrated in Figures 3 and 4.

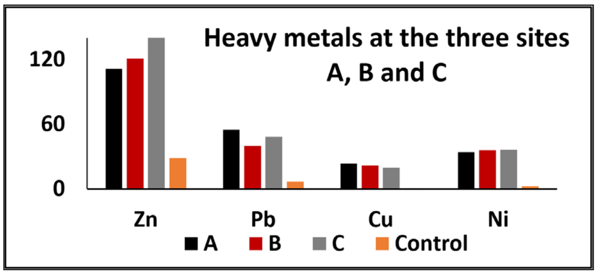


Figure 3. The average total concentrations (mg/Kg) of heavy metals in the soils of the three park areas.

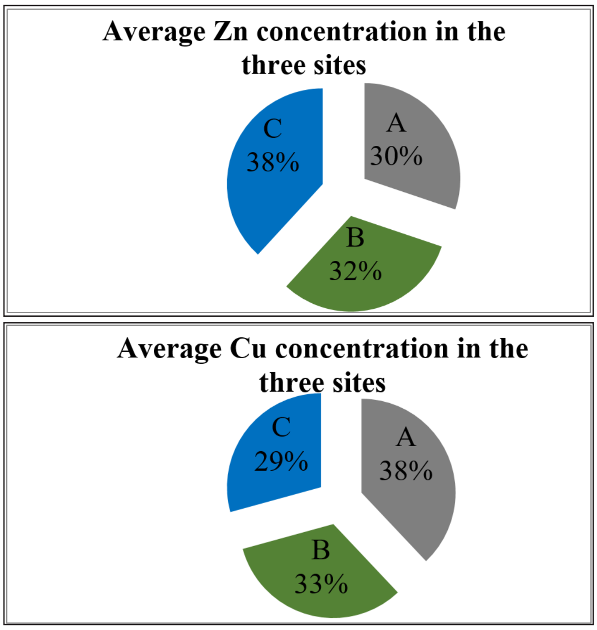
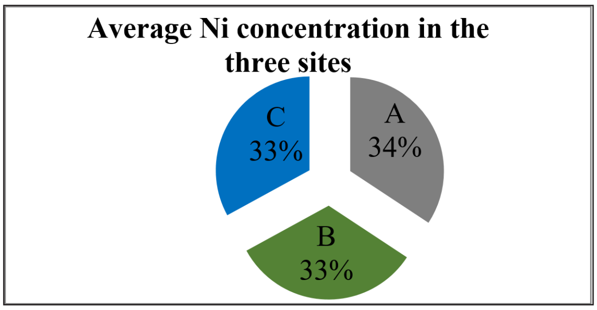


Figure 4. Average concentrations of each metal in the three studied areas.



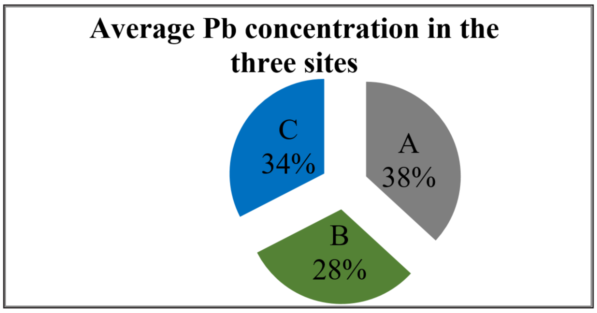


Figure 4 (contin.) Average concentrations of each metal in the three studied areas.

Generally, zinc occurs naturally in soil with about 70 mg/kg in crustal rocks and also from the high density of vehicular traffic (Al-Khashaman et al., 2006). The three park areas showed high levels of Zn (115.60, 121.17, 146.21 mg/kg at A, B, and C respectively) since these areas have intense traffic jams with busy vehicles movements all over the day. Since Zn exists in the carburetors, pumps, and automobile door panels, hence, high levels of Zn are expected. Moreover, the majority of the three park areas are located on major or minor busy streets, nearby bus stations, have busy cafes with outdoor Hookah and high traffic jam neighborhoods. The average concentration of Zn of the control sample was 28.68 mg/Kg; this indicates that the soil samples in all parks have higher levels than the control area as seen previously in Figure 3.

Regarding Ni, the average concentrations at all park samples in all areas were close to each other (37.72, 36.07, and 36.27 mg/Kg, in A, B, and C respectively). This result refers to the usage of Ni in many specific and recognizable consumer products, including stainless steel, alnico magnets, coinage, rechargeable batteries, and special alloys and green tint in glass (Davis, 2000).

For lead, the average concentration was 54.86, 45.58, and 48.48 mg/kg respectively. The average concentration of Pb in the control sample was 6.83 mg/Kg. In fact, the main source of Pb in the completely studied areas is attributed to the automobile exhausts that use Pb as an anti-knock agent in the combustion of gasoline. Since the majority of public park areas have very intense traffic jams, hence a high percentage of Pb is expected.

Finally, the average concentration of Cu was found to be 25.38, 21.90, and 19.56 mg/kg at A, B, and C areas respectively. The sources of Cu are wires, electric device tailing, and plating materials that use copper to protect the surface of the brass alloy, bars, and winding in the motor and generator of the buses (Kartal et al., 2006).

3.3. Statistical data treatment

As indicated in Figure 5-A, two main clusters collect the 30 parks at the three areas. Cluster of group I collects 6 parks, where cluster II collects 24 parks. HCA analysis revealed the same and/or comparable constituents of park soil contents of heavy metals. It is clearly appeared that cluster II (22 parks from locations A and B, and only 2 parks from location C) are overlapped which means that these parks' soils have similar or very comparable metal contents. Regarding group I, it has only parks from area C, which means that the park soils of this area have the same metal contents. To clarify these results, the average concentrations have been taken, and HCA has been re-carried out to reveal Figure 5-B. The order of the average Zn and Pb contents in all the areas was C > B > A and A > C > B, respectively. Cu and Ni metals showed the same order of A > B ≈ C. Location C shows distinct content of Zn that clusters the parks from area C together, but far from locations A and B, which means that Zn is responsible for clustering C alone. This result clearly appeared on PCA, Figure 5-C which revealed that the data loading collected was 99.99% of the whole information from PC1 and PC2.

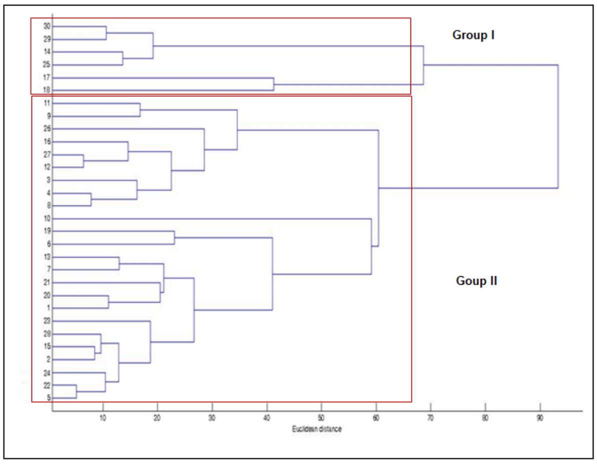


Figure 5A. Dendrogram of the 30 park soil-origin samples of metal contents

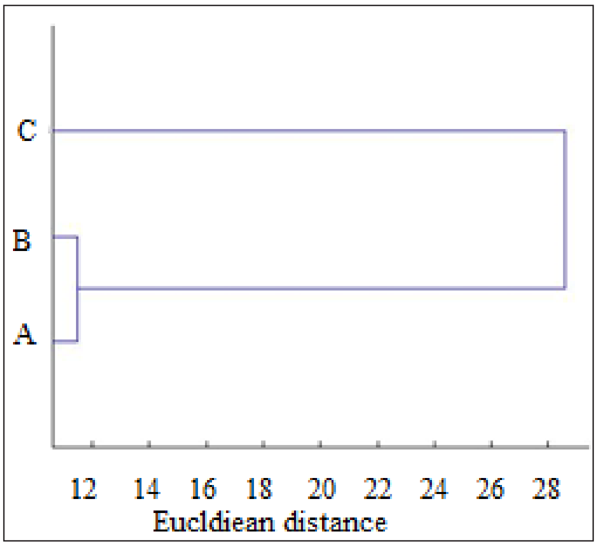


Figure 5B. Dendrogram of the 30-park soil origin samples of metal contents in average.

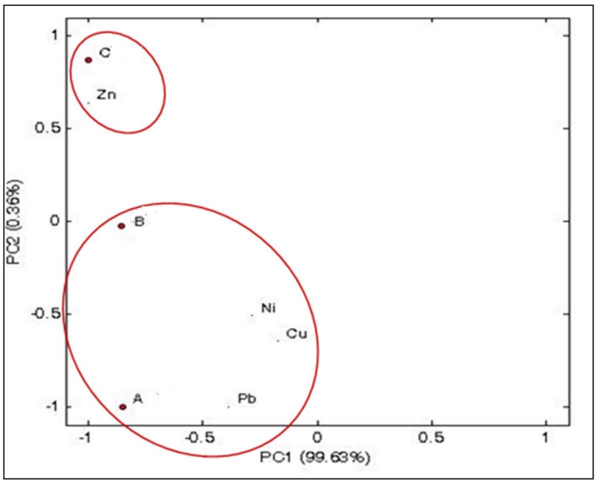


Figure 5C. PCA output: bi-plot obtained from the 30 origin parks soil contents.

4. Conclusion

This study has been carried out in order to achieve SDG#3, good health and well-being, since it detects sensitive metals that affect human health, hence, this will raise the awareness of the major sources of heavy metals that may lead to health issues. Based on the results of the current study, the average pH values at all park soil samples in Amman area were neutral to alkaline, where the moisture contents were low due to the evaporation rate based on the high temperature in the summer that decreases the moisture content. Regarding the organic matter, it was found at a high level due to the high level of organic contamination in all areas. FAAS was used to evaluate the Zn, Pb, Cu, and Ni contents of 30 park soil samples in 3 areas. The metal concentrations showed higher levels than the control areas at the whole park. The metal contents were used to group soil samples with the help of PCA and HCA. The grouping outputs uncovered the geographical origin of soil samples. By HCA and PCA, the 30-park soil origins were classified into two clusters: 24 parks from areas A and B due to the close contents of Pb, Cu, and Ni, and 6 parks in area C due to the distinct constituents of Zn.

Acknowledgment

This project was implemented, thanks to the financial support provided by (1) The University of Jordan, (2) L’Agence Universitaire de la Francophonie-Moyen Orient (AUF - Moyen Orient), and (3) Elsevier Foundation.

Conflict of Interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Appendix 1. (Aaseth et al., 2016; Li et al., 2014; **Lorraine** et al., 2015).

Element	Toxicity
Lead	Inhibits different biological processes in nervous system, heart, and kidneys, causing headaches, anemia, and death.
Cadmium	Hazardous for the nervous system, kidneys, liver, lungs and could also affect bone. Causing fever, chills, body aches, and joint pain.
Iron	Duo to increasing the concentration level of Fe above the threshold value that causes abdominal pain, vomiting, lethargy, and diarrhea.
Copper	Accumulating of copper leads to several problems like hypotension, abdominal pain, liver damage, kidneys and brain.
Zinc	Loss of taste, infertility, birth defects, chronic diarrhea, kidney disease, white spots on fingernails.
Nickel	Hazardous to the respiratory system, immune system, nasal cavities and sinuses, and the skin.