

Research Article

Analysis of Some Heavy Metals and Organic Acids in *Ficus carica* Growing Adjacent in the Serpentine Soil in Sulaimani/Kurdistan, Iraq

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Serpentine soil refers to soil having magnesium-rich minerals such as chrysotile, lizardite, and antigorite. Fig (*Ficus carica*) is a plant of major importance in the world. It is a nourishing food and is used in industrial productions. This study analyzes the distribution of heavy metal contents such as nickel, cobalt, zinc, and manganese in different parts of this plant, such as its leaves, stems, and fruits. Furthermore, the organic acid content, including citric acid, fumaric acid, malic acid, and oxalic acid, was estimated. The studied area is known as Kunjirin which is a village located in the northwestern extension of the Zagros-fold-thrust belt in Mawat town, northeast of Sulaimani Province, Kurdistan Region of Iraq. The results show that there are significant differences in heavy metal contents among the plant organs except for zinc. The lowest level of heavy metals is in the fruits, while the highest amount of the heavy metal content is in the leaves. Moreover, the organic acids were unequally distributed in the plant organs. However, the lowest level of organic acids are found in the stem, while the highest concentration of the organic acids are found in the fruit part of the plant. The present study concludes that the level of heavy metals in the fruit part is within the legally admitted limits. This indicates that it is normal for human beings and animals to consume such fruits.

1. Introduction

The term “serpentine” refers to soil having magnesium-rich minerals such as chrysotile, lizardite, and antigorite. It has a sheet structure and a chemical composition close to $Mg_3Si_2O_5(OH)_4$ [1, 2]. Serpentine soils are the weathered product of serpentinites. These soils are often shallow and rocky. They consist of high concentrations of Fe and Mg, as well as heavy metal contents such as Ni, Cr, Cd, and Co. They also have low levels of nitrogen, phosphorus, and potassium.

The cation exchange capacities in this soil is often lower than in the agricultural soils.

Fruit plants are essential components of the universe, and they are one of the oldest forms of known food to humans [3, 4]. Microelements are important for the normal function and metabolism of organisms. They participate in numerous processes, such as enzymatic reactions (Zn, Co, Ni, Mn, Fe, Cr, and Al), glycolysis (Mn, Zn), nucleotide synthesis (Mg, Fe), organic acid transformation (Fe, Zn, Ni, and Mn), nitrogen exchange (Fe, Mo, Cu, Mn, and Co), and photosynthesis

(Fe, Ti, Mg, and Mn) [5]. Plant fruits, including the *Ficus carica* plant, are the source of either micronutrients or macronutrients. In a case study, different elements were assessed in a wild fig fruit. The fruit is rich with calcium ($10940 \mu\text{g g}^{-1}$), while it contains a low level of nitrogen ($90 \mu\text{g g}^{-1}$). The levels of various micronutrients are significantly different in this plant fruit. The concentration of some elements in *Ficus carica* are as follows: zinc ($60 \mu\text{g g}^{-1}$), iron ($310 \mu\text{g g}^{-1}$), nickel ($4 \mu\text{g g}^{-1}$), and cobalt ($80 \mu\text{g g}^{-1}$) [6].

Metal ions cannot freely move across the cellular membrane due to their charge. Therefore, ion transport into cells has to be mediated by membrane proteins known as transporters. Transmembrane transporters possess an extracellular binding domain to which the ions attach just before the transport. The binding domain is receptive only to specific ions and is responsible for transportation. The transmembrane structure facilitates the transfer of bound ions from extracellular space through the hydrophobic environment of the membrane into the cell [7]. Convection (or mass flow) and diffusion are two mechanisms responsible for metal transport from the bulk soil to the plant roots. Due to convection, soluble metal ions move from soil solids to root surfaces. From the rhizosphere, water is absorbed by roots to replace water, which is transpired by the leaves. Water uptake from the rhizosphere creates a hydraulic gradient directed from the bulk soil to the root surface. The concentration gradient drives the diffusion of ions toward the depleted layer surrounding the roots [8]. Some plants can regulate metal solubility in the rhizosphere by exuding a variety of organic compounds from the roots [9]. Metal uptake by plants can be affected by several factors, including metal concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants, and plant age. It is generally accepted that the metal concentration in soil is the main factor. In addition, soil pH, which is correlated negatively with metals in plants, plays an important role in governing metal uptake by plants [10, 11]. Tessier et al. (1979) reported that the metals in the soil are associated with several fractions such as follows: (a) free metal ions in soil solution; (b) absorption to inorganic soil constituents; (c) bounding to soil organic matter; (d) precipitation; and (e) embedding in the structure of the silicate minerals [12].

Oliveira et al. (2008) demonstrated that the organic acids are primary metabolites, which can be found in great amounts in all plants, especially in their fruits [13]. Production of organic acids are related to photosynthesis and carbohydrate metabolism [14]. Organic acids, such as citrate, fumarate, malate, succinate, and oxaloacetate, play an important role in several major metabolic pathways in plants. These can include fatty acid biosynthesis and oxidation, glyoxylate acid cycle, and carbohydrate biosynthesis. They also have a significant role in the citrate cycle of the respiratory process [15]. Citric acid induces the activities of all antioxidant enzymes and reduces the production of reactive oxygen species and electrolyte leakage in different parts of sunflower. For instance, citrate and malate are supposed to be responsible for the chelation and transportation of nickel into vacuoles [16].

The human body requires an equilibrium amount of nutrients including vitamins, minerals, sugars, pectins, and

fibers. These nutrients that are required in the human body can be obtained by the consumption of plant fruits. The monitoring of heavy metals in plants represents a possibility to evaluate the degree of pollution in different environmental mediums such as soil, water, and air [17]. Absorption of excessive amounts of elements by plants above their physiological need may have phytotoxic effects. In contrast, insufficient amounts of these may decrease their yields and alimentary value. Therefore, their determination in various mediums is very important [18]. Each metal in the plant body has a special biological function. The deficient, sufficient, and toxicity range of each metal in plant leaves is different. For example, in fenugreek plants, activities of antioxidant enzymes, electrolyte leakage, and reactive oxygen species all enhanced with increasing concentration of applied Cu [19].

Fig (*Ficus carica*) is rich in vitamins, mineral elements, water, and fat matter [20]. This plant can withstand the temperature between -4°C and 45°C [21]. Fig has been cultivated since the 4th century B.C. It was introduced into the New World by Spanish explorers during the 16th century. It can also be grown as an attractive ornamental tree or shrub [22]. Productive fig trees have relatively low leaf N, P, and K concentrations (2.1%, 0.1%, and 1.0% dry weight, respectively) in July, although its tissue contains Mn and Ca. The concentration of these metals often exceed those typically found in other deciduous species growing in the same soils. Seasonal variations of fig leaf nutrients are similar to other tree crops [23]. It has a characteristically milky juice, which has proteolytic activity [24].

The studied area is adjacent to serpentine soil. The soil is located with an altitude of 1090 m, but the studied area is located in the lower part of this type of soil, with approximately an altitude of 1000 m. The serpentine soil contains a high concentration of heavy metals. These heavy metals could be eroded into the studied area and accumulated in the body of the cultivated plants. Thus, one of the commonly cultivated plants, *Ficus carica*, has been chosen to study the concentration of the heavy metals in its different organs and to clarify the concentration of these metals to legally admitted limits to human consumption.

2. Materials and Methods

2.1. Studied Area Description. The studied area is known as Kunjirin village, which is located in the northwestern extension of the Zagros-fold-thrust belt in Mawat Town, Sulaimani Province, Kurdistan Region of Iraq. Serpentine occurrences within the Zagros Suture Zone have received a special emphasis on their importance in thrusting mechanisms. This area lies at $35^{\circ} 49' 210''$ N, $45^{\circ} 29' 271''$ E and is located 20 km southeast of Mawat town, 25 km northeast of Sulaimani city. Some ecological information of the studied area are presented in the Meteorology Center of Agriculture in Sulaimani city. For instance, the temperature is ranging from -3°C in January to 44°C in July, the annual mean temperature is 22°C , the annual mean precipitation is approximately 820 mm, the rainfall has been between October and May, and there is no rainfall during the summer. The studied area is surrounded by mountains and natural pasture land in

addition to the presence of scattered small agricultural land, where rural farmers cultivate orchards with fruits such as figs, vines, apricot, pomegranates, pears, and peaches, and also annual summer vegetables such as tomato, okra, eggplant, green pepper, and onion.

2.2. Sample Collection. The studied area was determined according to the presence of flora, such as the fig plant, adjacent to the serpentine geological formation. Samples of plant specimens were selected all over the studied area. The leaves, stems, and fruits of *Ficus carica* were collected from one-year growth and during the fruit maturation (August to September). The corresponded soil samples were also collected from the studied area to determine the concentration of the heavy metals. All of the plant and soil samples were randomly taken in 6 replicates by using a stainless knife for the plant samples and auger for the soil samples.

2.3. Drying, Grinding, and Storage of Samples. To stop the enzymatic reaction and stabilize the specimens, the samples were dried in a dust-free oven at a temperature of 80°C for 24 hours after brushing the plant samples. The samples were then stored in a moisture-free atmosphere. The plant tissues were reduced by using a grinder to make a 0.5–1.0 mm particle size to ensure homogeneity and to easily destroy organic matter [25]. The samples were finally stored in clean plastic containers.

2.4. Dry Ashing and Analysis of Heavy Metals. A high-temperature combustion technique was followed to make dry ash from the plant tissues. An amount (0.5 g) of the dried sample was weighed on a sensitive balance in a borosilicate crucible. The samples were then heated at 500°C in a muffle furnace for a long time (8 hours). Then, 25 ml of 2 M HCL was used to dissolve the remainder ash. The prepared solution was then filtered through a filter paper to remove excess particles. The heavy metals were lastly determined in the solution using an automatic absorption flame spectrophotometer (Phoenix -986 Biotech) with a detection limit of 95%. Analytical grade nitric acid (HNO₃) and HCl were supplied from Fischer Scientific. Standards and samples were diluted using deionized water. The standard operating parameter was applied as follows: Zn (213.9 nm), Ni (232 nm), Mn (279.4 nm), and Co (395 nm). The glassware was washed and rinsed before use. Standards of these heavy metals were prepared in nitric acid. Six different concentrations (0 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm) were prepared from the standard solutions to obtain a calibration curve after diluting the stock standard solution of concentration 1000 ppm [25].

2.5. Organic Acid Analysis. Common organic acids in the plant tissues were separated and identified by using high-performance liquid chromatography (HPLC, Gilson). Standard substances of organic acids were purchased from Sigma-Aldrich. Water was purified (18MΩcm⁻¹ quality) from New Human Power I (Korea). One gram of the plant powder was extracted with methanol:water 50:50 (v/v) (50 ml), for 20 minutes following the hot extraction method. The extracted solution was filtered through a filter (Milli-

pore). The mobile phase was vacuum-filtered through a 0.45 μm nylon filter. The samples (20 μl) were analyzed by using the Supelcogel C-60 1 H column, following a mobile phase of 0.15% phosphoric acid and detecting at 210 nm UV. The chromatographic separation of these compounds was performed at room temperature. The analysis was run at a flow rate of 1 ml min⁻¹ with 10 min run time. Stock standard solutions of organic acids were dissolved in ultrapure water to a concentration of 1000 ppm. The solutions were stored in a dark place at 4°C. The calibration curve was made by diluting the stock solution in the mobile phase to obtain final concentrations between 0 ppm and 100 ppm [26, 27].

2.6. Soil Analysis. Soil samples were randomly collected in different sites of the studied area. This area was approximately 700 m far from the lower part of the serpentine soil. The composite soil sample from each site was taken at different depths, from the upper part to the lower part of the soil profile, 0–30 cm, 30–60 cm, and 60–90 cm (6 subsamples were collected by using a stainless auger). The soil samples were then air-dried at room temperature for 7 days to minimize microbial activity [28]. Following drying, the soil samples were ground by a grinder and sieved (2 mm). The AB-DTPA extraction method was followed to identify the heavy metals in the soil. This method is a multielement soil test for alkaline soils and is an approach that is used to extract metals from soils. For the preparation of the AB-DTPA reagent, 9.85 g of DTPA (diethyltriaminepentaacetic acid) was added to 4500 ml of deionized water. This solution was shaken for five hours constantly on a hot plate to dissolve the DTPA. The molarity of DTPA solution was 0.005 M. After that, 79.06 g of ammonium bicarbonate (NH₄ HCO₃) was added to 900 ml of the 0.005 M DTPA solution. The pH of the AB-DTPA extraction reagent was adjusted to 7.6 by adding some drops of 2 M hydrochloric acid (HCl). To extract the heavy metals in the soil samples, 10 g of the soil was added into a 125 ml conical flask, and then 20 ml of the extraction reagent (AB-DTPA) was added to the conical flask and shaken on a shaker for 15 minutes. The extracted solution was filtered. The heavy metals were lastly analyzed using a similar atomic absorption flame spectrophotometer as used for the heavy metal analysis in the fig plant [25].

2.7. Statistical Analysis. The different group means were compared by using a one-way analysis of variance ANOVA, with the SPSS, version 22 software. The data was shown as mean ± standard error. To clarify significant differences among mean values at the probability level of ($p < 0.05$), the Duncan test was used.

3. Results

3.1. Heavy Metal Contents in Fig. Figure 1 shows the distribution of the heavy metals in the leaf, stem, and fruit of the 6 samples in the fig. The cobalt level varied statistically significant among the different organs. The highest concentration of cobalt was found in the leaf (8.2 μg g⁻¹), but it was below the detectable limits amount of cobalt in the fruit of the fig. The highest concentration of nickel was in the leaf

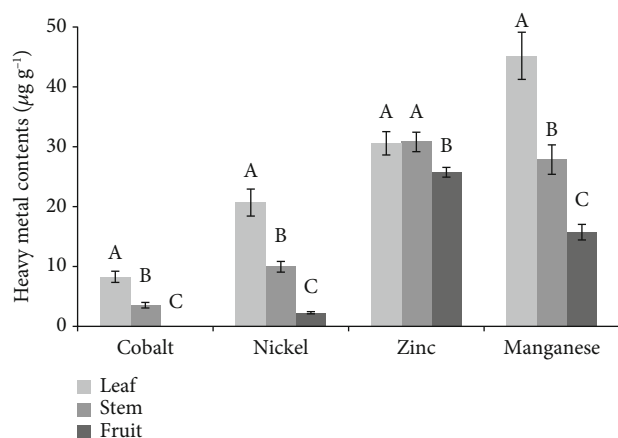


FIGURE 1: Heavy metal content ($\mu\text{g g}^{-1}$ of dry matter) in fig. Different letters indicate significant differences among organs at the ($p < 0.05$).

($20.7 \mu\text{g g}^{-1}$), and the lowest concentration of nickel was in the fruit ($2.3 \mu\text{g g}^{-1}$). In addition, the highest concentration of manganese was found in the leaf ($45.2 \mu\text{g g}^{-1}$) with the lowest concentration of this metal in the fruit ($15.8 \mu\text{g g}^{-1}$). Zinc contents show no significant differences between the leaf and stem of the plant ($30.5 \mu\text{g g}^{-1}$, $30.8 \mu\text{g g}^{-1}$), respectively. However, the lowest concentration of zinc was found in the fruit ($25.7 \mu\text{g g}^{-1}$). The total amount of heavy metals in the leaf, stem, and fruit of the fig were $104.7 \mu\text{g g}^{-1}$, $72.3 \mu\text{g g}^{-1}$, and $43.8 \mu\text{g g}^{-1}$ of dry matter, respectively.

3.2. Heavy Metal Contents in Soil of the Studied Area. There was a different distribution of heavy metals in the soil depths (Figure 2). The highest concentration of cobalt was in the soil surface ($3.35 \mu\text{g g}^{-1}$), while the lowest concentration of this metal was found in the depth 60-90 cm ($1.7 \mu\text{g g}^{-1}$). Nickel contents show significant differences between 0-30 cm and 60-90 cm of the soil depth ($25.6 \mu\text{g g}^{-1}$ and $13.2 \mu\text{g g}^{-1}$), respectively, while nickel contents show no significant differences between 0-30 cm and 60-90 cm with 30-60 cm of the soil depth ($21 \mu\text{g g}^{-1}$). The amount of zinc shows a statistical difference in the soil depth. The highest concentration of zinc was found on the soil surface ($16.7 \mu\text{g g}^{-1}$). The amount of manganese shows no significant differences between 0-30 cm and 30-60 cm (25.4 , $24.7 \mu\text{g g}^{-1}$). However, the lowest concentration of the manganese was found in the depth of 60-90 cm ($15.275 \mu\text{g g}^{-1}$). The total amounts of heavy metals in 0-30 cm, 30-60 cm, and 60-90 cm soil depths were $71.1 \mu\text{g g}^{-1}$, $60.9 \mu\text{g g}^{-1}$, and $37.4 \mu\text{g g}^{-1}$, respectively.

3.3. Organic Acid Contents in Fig. Organic acids in the fig organs were differently distributed (Figure 3). Citric acid contents show significant differences between the fruit and the leaf ($28.7 \mu\text{g g}^{-1}$ and $14.3 \mu\text{g g}^{-1}$), respectively. Fumaric acid contents show no significant differences between leaves and stems ($22.4 \mu\text{g g}^{-1}$ and $20 \mu\text{g g}^{-1}$), respectively, while the highest concentration of fumaric acid was found in the fruit ($36.9 \mu\text{g g}^{-1}$). Malic acid contents show no significant differences between leaves and stems ($12.7 \mu\text{g g}^{-1}$ and $5.5 \mu\text{g g}^{-1}$), respectively. However, the highest concentration of fumaric acid was found in the fruit ($61.4 \mu\text{g g}^{-1}$). The amount of oxalic

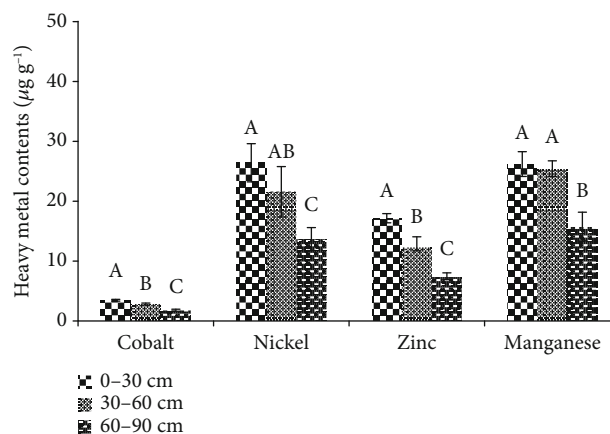


FIGURE 2: Heavy metal content ($\mu\text{g g}^{-1}$ of dry weight) in the soil depths. Different letters indicate significant differences in the soil depths at the ($p < 0.05$).

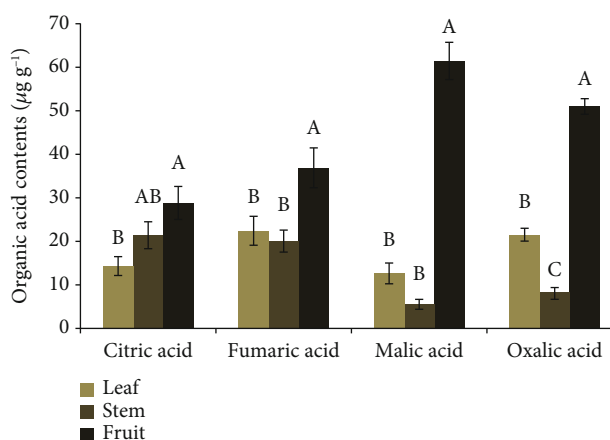


FIGURE 3: Organic acid content ($\mu\text{g g}^{-1}$ of dry matter) in the fig organs. Different letters indicate significant differences among organs at the ($p < 0.05$).

acid shows statistical differences between the organs. The highest concentration of oxalic acid was found in the fruit ($51 \mu\text{g g}^{-1}$), while the lowest concentration of oxalic acid was found in the stem ($8.1 \mu\text{g g}^{-1}$). The total organic acids in leaves, stems, and fruits of the fig were $71.7 \mu\text{g g}^{-1}$, $55.1 \mu\text{g g}^{-1}$, and $178.9 \mu\text{g g}^{-1}$, respectively.

4. Discussion

This study demonstrates that the heavy metals were distributed nonuniformly among the different organs of the *Ficus carica* (Figure 1). These results were in agreement with published data from many other studies [29–31]. As an example, it is investigated that heavy metals are not equally distributed in the pomegranate plant body [32]. The highest content of heavy metals is in the leaves of plants because these tissues have the highest metabolic activities, such as photosynthesis and transpiration, and the mobility of metals from leaves to other parts of the plant is different, whereas plant fruits contain the lowest level of the metals. The tested heavy metals in the *Ficus carica* could be arranged in descending order

according to their amounts in the plant: $Mn \geq Zn > Ni > Co$. It might be related to a considerable difference in mobility and physiological function of the elements in the plant tissues. In the plant body, for instance, P, K, Mg, and Zn are more mobile than Ca, Fe, and Mn [33]. As shown in Figure 1, the amount of zinc and manganese in the dry fruit of the *Ficus carica* was 25.7 and 15.8 $\mu\text{g g}^{-1}$, respectively, while Sattar et al. have reported that the amounts of zinc and manganese in the dry fruit of fig were 4.9 and 4.9 $\mu\text{g g}^{-1}$, respectively [34]. This variation might be related to their habitat, soil chemical components (metal concentration, organic matter, clay, and soil pH), and adjacentness of this plant to the serpentine soil. Furthermore, the lowest level of the tested metals was in the fruit of the plant, without any detectable amount of cobalt. It is reported that metal requirements for the human body are 8000-11000 $\mu\text{g day}^{-1}$ for zinc [35-37], 2000 to 3000 $\mu\text{g day}^{-1}$ for manganese [35-38], 200 to 800 $\mu\text{g day}^{-1}$ for nickel [36, 37, 39], and 150-500 $\mu\text{g day}^{-1}$ for cobalt. Above this level, the metals might be toxic and not safe for the human body.

The high nickel contents in the analyzed plant leaves were in agreement with the report of Eboh and Thomas (2005), who clarified that nickel level in cannabis leaves was 10.40 $\mu\text{g g}^{-1}$ dry weights [40]. High nickel concentration in fig might be related to the soil being contaminated with significant amounts of nickel, 29 $\mu\text{g g}^{-1}$ dry weight, from the serpentine body, which is adjacent to the studied area [26]. Soil contamination with heavy metals can affect concentrations in plants [11]. Other reasons can be related to the high solubility of nickel in the soil solution [30], easy mobility of nickel in plants [33], serpentinite weathering, erosion of soil to the cultivated area under the effect of the topography, slope length, soil erodibility, raining, and runoff factors [41, 42]. Some other studies have shown that the nickel concentration in leaves of plants ranges from 0.05 to 5.0 $\mu\text{g g}^{-1}$ Ni dry weight [10]. This amount of nickel is essentially less compared to the amount of this element in the fig plant. Therefore, the studied area may be contaminated with heavy metals, especially nickel.

The amount of heavy metals in the soil depths decreased from the surface soil, from 0-30 cm to lower portions of 30-60 cm and 60-90 cm (Figure 2). The amount of cobalt in the depth of 0-30 cm was 3.3 $\mu\text{g g}^{-1}$, but it decreased to 2.6 $\mu\text{g g}^{-1}$ and 1.7 $\mu\text{g g}^{-1}$ in the levels of 30-60 cm and 60-90 cm, respectively. The distribution of the metals in the soil depths might correlate to the organic matter content in the soil [41], and soil type can affect the solubility, mobility, and amount of metals in nature. This distribution of metals in the soil of the studied area might not be reflected in their concentrations in the fig plant. Other factors, such as solubility of the metals in soil solution and types of plants, can affect the bioaccumulation of the metals in the plant organs.

This data shows the unequal distribution of organic acids among the different parts of plant organs (Figure 3). These findings are consistent with Falade et al. (2003) data [43]. They indicate that orange juice contains a high level of ascorbic acid but a low level of citric acid. Lime juice is rich with citric acid, while pineapple juice contains a low level of organic acids. The dominant acids in the studied fig fruit

were malic acid and oxalic acids (61.42, 51 $\mu\text{g g}^{-1}$), respectively, while Karadeniz (2004) reported that the dominant acid in grapefruit, orange juices, and tangerine was malic acid with concentration 4.03 g l^{-1} , 7.79 g l^{-1} , and 5.29 g l^{-1} , respectively [44]. In addition, Byrne et al. (1991) reported that the dominant acid in peach is malic acid (50% to 60% of total) [45] and that malic and quinic acids were the principal organic acids in the plum fruits [46]. However, the present data show that malic and oxalic acids are the principal organic acids in the fig fruit with concentrations of 61.42 $\mu\text{g g}^{-1}$ and 51 $\mu\text{g g}^{-1}$, respectively. The variation of organic acid concentration in fruits may result in the period of fruit harvesting, ash contents and variation in the genetic matter of plant species, and environmental factors such as temperature. The high levels of some organic acids in the plant organs, especially in the fruit, may indicate that they have been involved in the translocation of metals or organometallic complexes and phytochelatins in the plant. An example would be a strong correlation present between ascorbic, citric, and total organic acid content in the plant fruits, and the enhancement of mineral availability [47].

5. Conclusions and Recommendations

To conclude, the present study indicates that the heavy metal and organic acid concentrations in the fig plant were varied in the different parts of the plant. Moreover, the results indicated that the soil of the studied area might have been contaminated with heavy metals, especially nickel, from its adjacent serpentine soil. The presence of the heavy metals in the soil reflected their contents in the plant. Particularly, the highest contents of heavy metals were found in the fig leaves, while the lowest contents of heavy metals were found in the plant fruit. The total amounts of the selected heavy metals in the fruit of the fig plant were within the legally admitted limits. Thus, the fig fruit of the studied area could be consumed by human beings and animals.

As future aspects of this study, similar data can be obtained from other cultivated plants and vegetables, the estimation of amino acids and phytochelatins in cultivated plants of this studied area (which may contribute to the heavy metal tolerance), and the analysis of heavy metals in serpentinophytes, such as oak, for comparing concentrations in the cultivated plants.

Data Availability

This paper used the collected samples in the countryside and record the data in the lab.

Conflicts of Interest

The authors confirm that they have no conflicts of interest regarding the publication of this paper.

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