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Study of the effect of lead and cadmium pollution on the Soil and Wild Mushrooms Truffles (*Terfezia Tirmania*) From Al- Nukhib/Rutba District / Anbar Governorate / Iraq using spectroscopic analysis techniques

Nour Abbas Sadkhan (1)
Department of Biology Science, Mustansiriyah University, POX 46079, Iraq-Baghdad
Kcm4125@uomustansiriyah.edu.iq

Dr. Zied O. Ahmed (2)
Department of Computer Science, Mustansiriyah University, Iraq-Baghdad
zied_othman@uomustansiriyah.edu.iq

Dr. Reyam Naji Ajmi (3)
Department of Biology Science, Mustansiriyah University, POX 46079, Iraq-Baghdad
reyam80a@uomustansiriyah.edu.iq

Abstract:
Three sampling areas of soil and *Terfezia Tirmania* were conducted near the mushroom, far from it, and directly around the mushroom. For each site, representative soil samples were collected in sterile tools that were prepared, especially for this purpose, then placed in sealed polyethylene bags. Flame atomic absorption spectrometry was done to analyze samples of soil and mushroom for their concentrations of some heavy metals: lead and cadmium. The analyses showed that the highest concentrations of cadmium were in the soil far from the mushroom, 5.13 ppm, while the lowest concentrations were in it, 1.44 ppm. Results of lead also showed the highest concentrations in the soil far from the fungus, 1.05 ppm, while they were lower in the fungus, 0.68 ppm. The results showed that the fungus has a low absorption capacity for cadmium, and the existence of different concentrations depending on various distances is due to various pollution sources and the capability of lead to move in the soil. Therefore, *Terfezia Tirmania* can be used as a good bioindicator for heavy metal soil pollution with elements such as lead and cadmium, which are of environmental concern.

Keywords: Heavy metal pollution, *Terfezia Tirmania*, bioindicator, environmental uptake, Flame atomic absorption spectrometry

Note: This research is extracted from an M.Sc. thesis.

Introduction:

Environmental pollution by heavy metals represents one of the most crucial environmental issues related to agricultural and desert regions in general, with specific relevance for Anbar Governorate, Iraq. Examples of these metals include lead and cadmium, which modify the chemical and physical characteristics of soil, these modifications impede proper plant growth and adversely affect all the living organisms living in such soil. Among these plants, wild truffles *Terfezia Tirmania* have great nutritional and economic importance in the region but may be affected by the accumulation of these metals in the surrounding environment [1]. Soil pollution with lead and cadmium causes serious changes in its chemical and physical structure, leading to a decrease in the quality of agricultural crops. These toxic metals can be transferred from the soil to plants via their roots, accumulating in plant tissues. It is revealed that this pollution interferes with the absorption of essential nutrients by plants, resulting in reduced productivity and increased vulnerability to environmental stress [2]. Truffles are desert-growing wild fungi. Nonetheless, they grow in one of the harshest of such environments: the Anbar region. These fungi prove to be sensitive to heavy metal pollution since they accumulate metals from the soil around it , the fungi also form an environmental indicator that acts to assess the degree of pollution in the environment. According to [3] Terfezia tirmania, [Kingdom: Fungi/ Division:Ascomycota/ Subphylum: Pezizomycotina / Class: Pezizomycetes / Order: Pezizales/ Family: Terfeziaceae/ Genera: *Terfezia Tirmania*

Spectroscopy techniques like atomic absorption and ICP-MS are accurate methods for the measurement of heavy metals like lead and cadmium in soil and plants. These techniques help in understanding the extent of pollution and its distribution in the environment [4]. Heavy metals affect the nutrient balance in the soil, reducing its fertility other serious impacts of pollution are that it increases soil salinity, reducing its water-holding capacity and hence hindering plant growth and affecting the ecosystem as a whole [5]. The current study, therefore, aims at establishing how soil pollution with lead and cadmium has affected the physical and chemical properties of the soils and the growth of the wild truffles in Nukhayb areas, Anbar Governorate. The spectroscopy techniques allow studying the accumulation of heavy metals both in soil and truffles, determining the level of pollution impact on the sustainability of the local ecosystem. This research should develop effective environmental solutions for soil improvement and biodiversity enhancement in contaminated areas.

Methodology:

Description and collection of samples from the study area :

The samples were collected from the study sites in March in 2024. Samples were collected from three areas: Terfeziaceae samples , Soil samples close to Terfeziaceae, and Soil samples far from Terfeziaceae . Soil Samples After the identification of the sites, which are near the growth of wild mushrooms, the collection and transportation of soil using clean tools made of materials that do not contain polyethylene or stainless steel, like shovels, should be used. They must be sterilized or washed well with nitric acid or distilled water to avoid sample contaminations. Soil samples are collected from a depth of 15-30 cm using a shovel or a special tube to collect soil from several points on the site, then mixed together to obtain a representative sample according [4]. The sample amount is about 500 grams to kilogram of soil at each point ،the soil samples are placed in sealed polyethylene bags at a suitable temperature between (5-10 C), and are usually stored in the cold to avoid decomposition or unwanted chemical reactions before analysis and sending the samples to the laboratory for analysis that allows measuring the concentration of heavy elements. For the purpose of quality assurance and pollution control, strict steps must be implemented to ensure that the samples are not contaminated during the collection and transportation process.

Fungi Samples were collected from locations known to have using a scalpel or sharp knife to cut the mushroom from the base. The mushroom samples were placed in clean, sealed plastic bags and stored at low temperature (ice pack) between (0-4 C) to avoid any decomposition before analysis, taking into account documenting all information related to sample collection, such as location, date, time and proximity to sources of contamination . According [6], to take all necessary measures to avoid contamination during collection and transportation, the World Health Organization (WHO) was relied upon. (2015). Guidelines for sampling and analysis of soil, water and biological materials. Heavy metal analysis using spectroscopic techniques: Spectroscopic techniques flame atomic absorption spectroscopy (FAAS) were used to measure lead and cadmium concentrations in the samples. The samples were prepared appropriately by grinding, heating and dilution before analysis to obtain accuracy in the mea To analyze the concentrations of cadmium and lead in soil and mushroom samples using Flame Atomic Absorption Spectroscopy (FAAS), the following procedures are followed according [7]:

1. Sample preparation: Soil: Soil samples are collected from different locations near and far from the mushroom. The samples are then dried in an oven at a low temperature (about 40-60 °C), and then ground into a fine powder. Mushrooms (*Terfezia Tirmania*): The mushrooms are collected, washed to remove dirt, then dried and ground.
2. Sample preparation for analysis: An aqueous solution is prepared from the samples by dissolving the sample in concentrated nitric acid (HNO3) or hydrochloric chloride (HCl) to break down the organic and mineral compounds in the sample. After the reaction, the solution is filtered to obtain the mineral solution to be analyzed.
3. Using the FAAS device: Calibration of the device: The flame atomic absorption device is calibrated using standard solutions containing known concentrations of cadmium and lead. These solutions are used to create a calibration curve that helps in determining the concentration of metals in the samples. Sample preparation: After calibration, the solution extracted from the sample is introduced into the FAAS device. In this device, the sample is heated using a flame that emits radiation, and the light absorption is measured at the wavelengths specific to cadmium (228.8 nm) and lead (283.3 nm) and measurement: When the metal atoms pass through the flame, they absorb a certain amount of light at the wavelengths specific to each metal, and this information is used to determine the concentration of the metal in the sample based on the absorption equation compared to the standard curve according [8].
4. Analysis and interpretation: After obtaining the absorbance values, the concentrations of cadmium and lead in the samples are calculated based on the standard curve equation. The results of the concentrations in soil and mushrooms are compared to determine the levels of metal contamination in the environment [9].

5. Environmental and health interpretation: The concentrations of metals were analyzed to deduce the level at which soil and fungal contamination is affecting the local ecosystem. If they are above the limits, they could indicate an environmental and health hazard [10].

The statistical data analysis was done by calculating the arithmetic mean and standard deviation (Standard Deviation) for both cadmium and lead in the samples of Terfeziaceae fungi and soil (near and far) using ANOVA statistical tests to determine the presence of statistically significant differences between the different concentrations in the samples [11].

It is an environmental and biological analysis of the effect of high concentration of lead and cadmium on the chemical and physical properties of the soil, such (pH), mineral composition, and its water-holding capacity with the examination of the accumulation of heavy metals in Terfeziaceae fungi through the comparison of cadmium and lead accumulation levels in fungi near and far from the contaminated soil.

Following [12], the following procedures are used in analyzing acidity or pH, mineral composition, and water-holding capacity of the soil.

1. Measurement pH: Equipment needed: pH meter or pH test strips. A soil sample representative of the studied area is collected, and to the soil sample, distilled water is added in specific proportions (like 1:1) to obtain an aqueous soil solution, record the value and analyze it to determine the degree of acidity or alkalinity in the soil a low pH indicates acidity, while a high pH indicates alkalinity.

2. Soil mineral composition analysis: This requires the use of Flame Atomic Absorption Spectroscopy (FAAS) after collecting a soil sample and drying it, then grinding it to obtain a fine powder, then preparing the aqueous solution by adding nitric acid (HNO_3) or hydrochloric acid (HCl) to break down organic matter and prepare standard solutions for mineral elementssuch as iron, magnesium, calcium, potassium, phosphorus to measure the concentration of mineral elements in the soil sample.

3. Measuring the soil's water retention capacity: Using a soil water retention capacity determination device or a field capacity measurement device, after collecting uncontaminated soil samples

(depth ranging from 10-30 cm), the sample is moistened with water until the soil is saturated, then the sample is weighed after adding water and left for a period to allow the excess water to drain. After draining, measure the amount of water absorbed and stored in the soil by measuring the weight before and after water retention, then calculate the water retention capacity using the following equation the water retention capacity of soil, also known as Field Capacity, is:

Field Capacity=Initial Weight Final Weight−Initial Weight×100 ………. [13]

- **Final Weight** is the weight of the soil after excess water has drained out (at field capacity).
- **Initial Weight** is the weight of the soil before the water was added or after it has been fully saturated.

Statistical analysis of the study:

Descriptive statistics: The arithmetic mean and standard deviation are calculated to measure the variation in lead and cadmium concentrations in different samples and the test is for the presence of statistically significant differences between groups (soil near and far Terphysiae and fungi). If the p-value is less than 0.05, the differences are significant. The correlation analysis gave a study of the relationship between minerals in soil and fungi using Pearson's correlation. These analyses are used to assess the risks of pollution to the environment and health[14].

Results and Discussion:

Table 1 show the average concentrations of lead (Pb) and cadmium (Cd) in samples of Terfeziaceae fungi and soil taken from different areas (near and far from the fungi). These data provide insights into the levels of contamination in these samples as follows:

1. Cadmium (Cd) Concentrations: Terfeziaceae Fungi , the average cadmium concentration in the fungi was 1.4367 parts per million (ppm) with a standard deviation of 0.8121. Soil (near Terfeziaceae), the average cadmium concentration in the soil near the fungi was 3.5319 ppm, with a standard deviation of 0.6975, soil (far from Terfeziaceae): The average cadmium concentration in the soil far from the fungi was 5.1333 ppm, with a standard deviation of 1.5038. Cadmium concentrations increase with distance from the fungus, with soils further away appearing to have the highest concentrations of cadmium. The standard deviation indicates that cadmium levels in soils close to the fungus are less variable than in soils farther away, which may indicate that contamination in these areas is relatively homogeneous or that the fungus absorbs cadmium less efficiently than other metals [14].

2. Lead (Pb) concentrations: Terfeziaceae fungus, the mean lead concentration in the fungus was 0.6811 ppm, with a standard deviation of 0.9785, soil near Terfeziaceae: The mean lead concentration in soils close to the fungus was 0.8967 ppm, with a standard deviation of 0.2416, soil far from Terfeziaceae: The mean lead concentration in soils far from the fungus was 1.0546 ppm, with a standard deviation of 0.4135. Lead concentrations in the samples ranged from relatively low levels. However, the highest lead concentrations were in the soil farthest from the fungus. The low standard deviation in the soil near the fungus suggests that contamination in that area is less variable and more homogeneous than in the distant soil [15].

Comparing the levels of cadmium and lead, it can be noticed that cadmium concentrations are higher in all samples when compared with lead concentrations. Probably this is because different contamination sources exist, or there is different mobility of these metals in the environment. Cadmium may be more stable in the soil or be better absorbed by the fungus. The higher concentration of cadmium in the soil near the Terfeziaceae than in the fungus may be an indication that cadmium is more abundant in the soil or the fungus does not absorb it as efficiently as other metals. The highest lead concentration in the soil that was furthest from the fungus may indicate that lead has a greater mobility in soil or contamination levels are quite variable over distances [16]. [17] showed that soils in industrially polluted areas typically have higher concentrations of lead and cadmium, which is consistent with the trend observed in soil samples away from fungi, [18] also showed that cadmium tends to accumulate more in soil than in plants, which is consistent with the higher concentrations of cadmium in soil. These results are supported by [19] who showed that cadmium and lead can significantly affect plant growth and accumulate in plant tissues, but at varying rates. [20].Noted that fungi can uptake heavy metals from the environment, making them effective bioindicators. In comparison, the lower cadmium concentrations here may indicate that the uptake of cadmium is limited or that fungi are more discriminating in the uptake of cadmium. Standard deviations (SDs) show the variation in contamination levels: the higher the standard deviation in the soil samples, the more variable the contamination will be in that area, probably because of different industrial, agricultural, or human sources of contamination. These findings agree with previous research that emphasized environmental contamination by these heavy metals and their effects on local ecosystems.

Table 1 Cadmium and Lead Concentrations in Soil and Terfeziaceae Fungi Samples in Al-Nukhaib/Al-Ratbah Area, Anbar Governorate

Sample	Cadmium Mean	Lead Mean	Cadmium Std	Lead Std
Terfeziaceae	1.4367	0.6811	0.8121	0.9785
Soil (Near Terfeziaceae)	3.5319	0.8967	0.6975	0.2416
Soil (Far from Terfeziaceae)	5.1333	1.0546	1.5038	0.4135

According to Table 2, the pH values in the samples ranged from 6.8 to 7.2, indicating a neutral or near-neutral soil. A pH value between 6 and 7 is ideal for most plants, Terfeziaceae close to soil: recorded pH = 7.2, indicating that the environment is close to neutrality, Terfeziaceae far from soil: recorded pH = 7.1, which is very close to the previous value, Soil (close to *Terfezia Tirmania*): recorded pH = 6.8, indicating that the soil is closer to mild acidity, Soil far from *Terfezia Tirmania* : recorded pH = 7.0, which is somewhat neutral. In this case, there are no significant differences between the samples, indicating that the acid composition of the soil is not significantly affected by proximity or distance from the fungus [15].

As for the mineral composition, magnesium (Mg) was recorded: the highest percentage was in the soil close to *Terfezia Tirmania* (1.8) and the lowest percentage was in the soil far from *Terfezia Tirmania* (1.6). This suggests that the soil near the fungus may contain more magnesium, which is an important element for plant growth. The soil near the fungus had the highest value (4.0), followed by the soil far away (3.7), indicating a positive effect of mineral elements on the soil structure near the fungus. The soil near Terfeziaceae had the highest value (1.2) compared to the soil far away (1.0), which may indicate a higher concentration of salts in the soil near the fungus, which may affect the water holding capacity. Potassium (K) had lower values in the soil near the fungus (0.5) compared to the soil far away (0.6). This value may indicate that the fungus consumes potassium in the soil near it. The water holding capacity was as follows: Terfeziaceae near the soil: recorded a water holding capacity of 35%, Terfeziaceae far from the soil : recorded a water holding capacity of 32%. Soil close to the *Terfezia Tirmania*: recorded a water retention capacity of 40%, Soil far from the *Terfezia Tirmania* : recorded a water retention capacity of 38%. It is observed that soils close to the Terfeziaceae whether close or far retain more water than the fungus. This could be due to the soil composition which contains mineral components that help in retaining water better.

According to previous studies on the effect of mineral elements and pH on the water retention capacity of soils, the results here are in agreement with studies that suggest that soils rich in magnesium and calcium may have better water retention capacity. For instance, it has been recorded in [21] that the soils rich in calcium and magnesium can retain more water compared to the soils devoid of these minerals. Also about the effect of pH: It is said by [22] that a near neutral pH is optimum for good plant growth, which here has been proven by indicating that between pH 6.8 and 7.2, good plant-fungal growth occurs. These findings represent an important building block in understanding how minerals and environmental conditions affect the soil and fungi in the area of Nukhayb/Ratbah of Anbar Governorate.

Table 2 Results for pH, Mineral Composition, and Water Retention Capacity:

Sample	pH	Magnesium (Mg)	Calcium (Ca)	Sodium (Na)	Potassium (K)	Water Retention Capacity (%)
Terfeziaceae (Near Soil)	7.2	1.5	3.2	0.6	0.4	35%
Terfeziaceae (Far from Soil)	7.1	1.4	3.1	0.5	0.3	32%
Soil (Near Terfeziaceae)	6.8	1.8	4.0	1.2	0.5	40%
Soil (Far from Terfeziaceae)	7.0	1.6	3.7	1.0	0.6	38%

Cadmium (Cd) and lead (Pb) accumulations in soil and plant tissues have an adverse effect on plant growth through several ways. For Cd: Plants absorb cadmium more quickly than lead, which leads to its accumulation in plant tissues. Cadmium disrupts the absorption of essential nutrients such as calcium, magnesium, and potassium, which disrupts vital processes and leads to poor growth, yellowing of leaves, and reduced photosynthetic efficiency. Other effects include cadmium inhibiting enzymes in photosynthesis and respiration, causing oxidative stress and damage to cellular structures. Although plants absorb lead from the soil, lead is less mobile than cadmium and tends to accumulate in the roots. High levels of lead cause root damage, which limits the absorption of water and nutrients. Other effects include lead inhibiting metabolic processes and affecting the transport of water and nutrients within the plant. In addition, cadmium was found in higher concentrations within the soil, especially that further from the fungi, than within the fungi, which indicates that cadmium is probably not absorbed as efficiently by fungi as are other metals. Concentrations of lead were higher in soil further from fungi, which could indicate the mobility of lead within soils.

In terms of environmental effects, the pH and composition of the soil were important: the soil in the area was close to neutral-between 6.8 and 7.2-which is optimal for plant growth-and the water-holding capacity of the soil near the fungi was superior to that far away, helping to increase the plants' resistance to pollution.

Conclusion

1- The results showed the high concentrations of heavy metals, including lead and cadmium in the soil nearby to the pollution sites, whereas concentrations were relatively lower in the *Terfezia Tirmania*.

2- The heavy metals were more concentrated in the soil far from the fungus, indicating the effect of different pollution factors on the distribution of metals. Although the fungus may contribute to reducing the concentrations of heavy metals around its environment, the accumulation of metals in it was low for cadmium and lead.

3- *Terfezia Tirmania* can be considered an excellent biomarker for soil heavy metal pollution because of its bioaccumulation capability, which helps in assessing the level of environmental pollution in polluted areas.

4- This study gives credibility to the use of fungi in the early detection of environmental pollution with heavy metals, especially in industrial areas, the long-term experiments are needed to evaluate the performance of fungi in removing heavy metals and their long-term environmental impact.

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دراسة تأثير تلوث الرصاص والكادميوم على التربة وفطريات الكمأ البري (Terfezia Tirmania) في منطقة النخيب/قضاء الرطبة/محافظة الأنبار/العراق باستخدام تقنيات التحليل الطيفي.

نور عباس صدخان (1)
قسم علوم الحياة، الجامعة المستنصرية، صندوق بريد 46079، العراق - بغداد
kcm4125@uomustansiriyah.edu.iq

د. زيد عثمان أحمد (2)
قسم علوم الحاسوب، الجامعة المستنصرية، العراق - بغداد
zied_othman@uomustansiriyah.edu.iq

د. ريام ناجي عجمي (3)
قسم علوم الحياة، الجامعة المستنصرية، صندوق بريد 46079، العراق - بغداد
reyam80a@uomustansiriyah.edu.iq

مستخلص البحث:
تم إجراء دراسة على ثلاث مناطق أخذ عينات من التربة والفطر *Terfezia Tirmania*، شملت مواقع قريبة من الفطر، بعيدة عنه، ومباشرة حوله. تم جمع عينات التربة من كل موقع باستخدام أدوات معقمة أُعدت خصيصًا لهذا الغرض، ثم وضعت في أكياس بولي إيثيلين مغلقة. تم تحليل العينات من التربة والفطر باستخدام جهاز الامتصاص الذري باللهب لتحديد تركيزات بعض المعادن الثقيلة: الرصاص والكادميوم. أظهرت التحليلات أن أعلى تركيزات للكادميوم كانت في التربة البعيدة عن الفطر (5.13 جزء في المليون)، بينما كانت أقل التركيزات في الفطر نفسه (1.44 جزء في المليون). كما أظهرت نتائج الرصاص أن أعلى التركيزات كانت في التربة البعيدة عن الفطر (1.05 جزء في المليون)، في حين كانت أقل التركيزات في الفطر (0.68 جزء في المليون). أظهرت النتائج أن الفطر لديه قدرة منخفضة على امتصاص الكادميوم، وأن اختلاف التركيزات حسب المسافات المختلفة يعود إلى مصادر التلوث المتنوعة وقابلية الرصاص للتحرك في التربة. لذلك، يمكن استخدام *Terfezia Tirmania* كمؤشر حيوي جيد لتلوث التربة بالمعادن الثقيلة مثل الرصاص والكادميوم، وهي عناصر تثير القلق البيئي.

الكلمات المفتاحية: تلوث المعادن الثقيلة، *Terfezia Tirmania*، مؤشر حيوي، الامتصاص البيئي. مطيافية الامتصاص الذري باللهب.

ملاحظة : هل البحث مستل من رسالة ماجستير او اطروحة دكتوراه ؟ نعم من رسالة ماجستير