

Investigation of Heavy Metals Levels in Thymus Vulgaris Collected from Two Cities in Libya

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دراسة مستوى المعادن الثقيلة في نبات الزعتر (Thymus Vulgaris) النامي برياً في مدينتين في ليبيا

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Abstract:

Thymus Vulgaris is an indigenous plant in Libya, and it is widely used for its medicinal properties. There is a growing concern that this plant may be contaminated with heavy metals. This study aimed to determine the levels of eleven heavy metals (As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, Zn) in Thymus Vulgaris growing in two Libyan cities: Gharyan and Qasar Ben Ghesheer. Samples of the aerial section of the plant (leaves and stems) were collected and digested using cold and hot digestion methods. The digested contents were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). The mean and standard deviation were computed. The mean levels of heavy metals were compared to a set of acceptable limits for these elements. All heavy metals were detected in all samples in both cities and in both plant parts. Overall, only three heavy metals, Barium (Ba = 18.38 mg kg⁻¹), Lead (Pb = 14.63 mg kg⁻¹), Strontium (Sr = 75.35 mg kg⁻¹), exceeded the acceptable limits. The levels of these contaminants varied significantly between the two cities and the two plant parts, with Pb being notably higher in Gharyan and Sr higher in the stems. The plants in both cities are contaminated by heavy metals. Although most heavy metals were within acceptable levels, their long half-life (up to 30 years) makes them easily accumulated in the human body, posing a risk, especially for those individuals who use this plant for extended periods. To reduce the impact of pollution on medicinal plants strict regulations should be applied, and close monitoring is necessary.

Keywords Heavy metals, Medicinal plants, Thymus vulgaris, Libya.

الملخص:

الخلفية: نبات الزعتر (Thymus Vulgaris) هو نبات أصلي في ليبيا، ويُستخدم على نطاق واسع لخصائصه الطبية. هناك مخاوف من احتمال تلوث هذا النبات بالمعادن الثقيلة. الهدف: هدفت هذه الدراسة إلى تحديد مستويات أحد عشر معدناً ثقيلًا (الزرنيخ، الباريوم، الكاديوم، الكروم، النحاس، الحديد، المنغنيز، النيكل، الرصاص، السترونتيوم، الزنك) في نبات الزعتر الذي ينمو في مدينتين ليبيايتين: غريان وقصر بن غشير. المنهجية: تم جمع عينات من الجزء الهوائي للنبات (الأوراق والساقان) وهضمها باستخدام طريقتي الهضم البارد والساخن. وتم تحليل المحتويات المهضومة بواسطة مطياف الكتلة البلازما المقترن بالحث (ICP-MS). وتم حساب متوسط تركيز المعادن الثقيلة والانحراف المعياري للقراءات، وقورنت متوسطات مستويات المعادن الثقيلة بمجموعة من الحدود المقبولة لهذه العناصر. النتائج: تم الكشف عن جميع المعادن الثقيلة في جميع العينات في كلتا المدينتين وفي كلا جزأي النبات. بشكل عام، تجاوزت ثلاثة معادن ثقيلة فقط الحدود المقبولة،

وهي: الباريوم ($Ba = 18.38$ مج/كغ-١)، والرصاص ($Pb = 14.63$ مج/كغ-١)، والسترونشيوم ($Sr = 75.35$ مج/كغ-١). وتفاوتت مستويات هذه الملوثات بشكل كبير بين المدينتين وبين جزأي النبات، حيث كان الرصاص (Pb) أعلى بشكل ملحوظ في غريان، والسترونشيوم (Sr) أعلى في السيقان. الاستنتاج: إن النباتات في كلتا المدينتين ملوثة بالمعادن الثقيلة. وعلى الرغم من أن معظم المعادن الثقيلة كانت ضمن المستويات المقبولة، فإن نصف عمرها الطويل (الذي يصل إلى ٣٠ عاماً) يجعلها تتراكم بسهولة في جسم الإنسان، مما يشكل خطراً، خاصة على الأفراد الذين يستخدمون هذا النبات لفترات طويلة. للحد من تأثير التلوث على النباتات الطبية، يجب تطبيق لوائح صارمة، وتعد المراقبة الدقيقة المستمرة ضرورية.

الكلمات المفتاحية: المعادن الثقيلة، النباتات الطبية، نبات الزعتر، ليبيا.

Introduction:

Heavy metal pollution represents a significant global threat to human and ecological health, primarily driven by rapid urbanization and industrial growth. These contaminants enter the environment through both natural processes and anthropogenic activities, and their presence is a major environmental concern due to their persistence and toxicity [1]. Plants, including those used for medicinal purposes, readily absorb heavy metals from their surrounding environment, soil, water, and air. The amount picked up by the plant depends on combinations of chemical, biological, and environmental parameters [1,2]. When these contaminated plants are ingested, the metals can enter the human food chain and accumulate in body tissues over time, leading to serious health complications [3]. This risk is particularly pronounced because many heavy metals possess long biological half-lives, meaning that even low-level exposure over prolonged periods can pose a chronic health risk [4].

Thymus vulgaris is a small perennial shrub that grows up to 15-30 cm high. It is a widely cultivated herb of the Lamiaceae family and a popular medicinal plant in Libya, where it is extensively used in food, hot drinks, and as a remedy for various illnesses [5]. It is also known for its pharmacological properties, including antitussive, anthelmintic, antispasmodic, and antioxidant effects, with volatile oils like thymol and carvacrol [5,6]. Given its widespread and frequent consumption in primary healthcare, it is crucial to assess its contamination levels to ensure consumer safety. Therefore, continuous monitoring of heavy metal concentrations in commonly consumed medicinal plants like *Thymus vulgaris* is essential to mitigate health risks and guide effective protective measures [7].

Gharyan and Qasar Ben Ghesheer are agriculturally significant cities located in the western-south region of Libya, near the capital, Tripoli. The region's agricultural dependency, combined with potential environmental factors such as proximity to traffic or the use of contaminated water/fertilizers in farming, elevates the risk of heavy metal exposure in both wild and cultivated medicinal plants. *T. vulgaris* is a native plant that thrives in the mountainous areas of Gharyan. The purpose of this study was to determine the levels of eleven key heavy (As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, and Zn) in *Thymus vulgaris* growing in these two Libyan cities. The assessment included the analysis of both the plant's stems and leaves to provide a thorough understanding of inorganic chemical contaminants and their potential health implications for local consumers.

Materials and Methods:

Sample collection and preparation:

Samples of fresh, naturally growing aerial parts of *Thymus vulgaris* were collected in July 2024 from two Libyan cities: Gharyan and Qasar Ben Ghesheer. The identity of the collected plant material was botanically confirmed by an expert prior to laboratory analysis. The samples were appropriately labelled, transported to the laboratory, and stored in plastic bags at room temperature until processing.

In the laboratory, the plant material was separated into distinct leaf and stem fractions. The samples were washed twice with distilled water to remove external contaminants, air-dried, and then oven-dried at 65°C for 24 hours. The dried material was subsequently ground into a fine powder using a stainless-steel mill.

Reagents and glassware:

Analytical-grade chemicals were used for all sample preparation and analysis. Reagents included distilled water, nitric acid (65%), hydrogen peroxide (30%), and sulfuric acid (95%). Before use, all laboratory glassware was cleaned with deionized water, submerged overnight in a 10% nitric acid solution, and air-dried to prevent cross-contamination.

Samples Digestion:

The dried and ground plant powder was subjected to a standardized acid digestion method combining dry-ashing and wet acid treatment. A 5.0 g portion of the powder was accurately weighed into a porcelain crucible and pre-treated with 0.5 mL of 95% sulfuric acid (H_2SO_4) for few hours at room temperature. The crucible was then placed in an oven. The temperature was steadily raised to 480 °C and maintained for four to five hours until the material was completely converted into white ash.

Following cooling, 2 mL of 65% nitric acid (HNO₃) and 1 mL of 30% hydrogen peroxide (H₂O₂) were added to the resulting ash. The content was gently heated to 90 °C using an electric heater to completely remove the residual acid and moisture. An additional 2 mL of 65% HNO₃ was added, and the drying step on the electric heater was repeated.

The dried residue was then continuously washed and quantitatively transferred with distilled water into a volumetric flask. The mixture was filtered using a Whatman Grade 1: 11-µm medium flow filter paper. The obtained filtrate was then brought to a final volume of 50 mL with distilled water. The final solution was stored in amber glass bottles at 24 °C pending heavy metal detection.

Instrumental Analysis and Data Processing:

The concentrations of eleven heavy metals (As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, and Zn) were determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). All analyses were performed in once.

Data analysis was performed using Microsoft Excel software. The mean and standard deviation (SD) of the heavy metal concentrations for each element were calculated for the two geographical locations and the two plant parts (leaves and stems). The resulting mean concentrations were compared to established international acceptable limits.

Results:

Since there is no single, universally recognized permissible limit for heavy metals in medicinal plants, acceptable limits were compiled from various literature sources and are presented in Table 1 for reference.

The analysis results for the heavy metal concentrations in *Thymus vulgaris* are summarized in Tables 2, 3, and 4. These tables present the overall mean concentrations, the concentrations categorized by plant part (leaves vs. stems), and the concentrations categorized by collection site (Gharyan vs. Qasar Ben Ghesheer), respectively.

Table (1): The acceptable limits of heavy metals in the literature.

Heavy metal	Permissible limit (mg kg ⁻¹)	Reference
As	0.2-5	[7-9]
Ba	0.7	[2]
Cd	0.3-4	[7, 9, 10]
Cr	0.006-18	[8]
Cu	0.4-150	[8, 11]
Fe	20-2486	[8, 11]
Mn	γ-100	[8, 11]
Ni	0.1-5	[8]
Pb	3-10	[7-10]
Sr	1.6	[2]
Zn	1-160	[8]

The overall mean concentrations of heavy metals, irrespective of plant part or collection site, are presented in Table 2. All targeted heavy metals were detected, showing varied concentrations. Only three metals—Barium (Ba), Lead (Pb), and Strontium (Sr), had concentrations that exceeded the acceptable limits identified from the literature (Table 1). The overall mean concentrations for these contaminants were Ba = 18.38 mg kg⁻¹, Pb = 14.63 mg kg⁻¹ and Sr = 75.35 mg kg⁻¹. These values exceeded the acceptable limits of 0.7mg kg⁻¹, 10 mg kg⁻¹ & 1.6 mg kg⁻¹ respectively.

Table (2): overall mean concentration of heavy metals (mg kg⁻¹) in *Thymus vulgaris* Samples

Heavy metal	Mean (mg kg ⁻¹)	SD
As	0.11	0.08
Ba	18.38	7.53
Cd	0.04	0.04
Cr	1.03	0.29
Cu	19.78	17.39
Fe	282.38	81.87
Mn	11.15	2.35
Ni	0.85	0.31
Pb	14.63	25.42
Sr	75.35	10.8
Zn	21.00	6.82

To investigate the internal distribution of heavy metals, mean concentrations were compared between the leaves and stems. As shown in Table 3, all elements were detected in both plant parts. Eight elements (As, Ba, Cr, Fe, Mn, Ni, Pb, and Zn) accumulated at higher levels in the leaves than in the stems. Conversely, the remaining elements (Sr, Cu, and Cd) were found in higher concentrations in the stems. Regarding contamination, Ba and Sr exceeded acceptable limits in both leaves and stems, whereas Pb only exceeded the limit in the leaves (26.60 mg kg⁻¹), being significantly lower in the stems (2.65 mg kg⁻¹). Notably, Sr accumulated much higher in the stems (82.65 mg kg⁻¹) than in the leaves (68.05 mg kg⁻¹).

Table (3): The mean concentrations of heavy metals according to the studied plant parts of *Thymus vulgaris*

Heavy metal	Leaves		Stems	
	Mean (mg kg ⁻¹)	SD	Mean (mg kg ⁻¹)	SD
As	0.17	0.04	0.04	0.00
Ba	19.05	12.66	17.70	2.83
Cd	0.02	0	0.06	0.06
Cr	1.10	0.42	0.95	0.21
Cu	5.70	1.13	33.85	10.68
Fe	325.30	60.1	239.45	95.53
Mn	12.75	0.92	9.55	2.33
Ni	0.95	0.35	0.75	0.35
Pb	26.60	36.91	2.65	1.48
Sr	68.05	0.92	82.65	11.67
Zn	21.35	9.26	20.65	7.28

When comparing heavy metal concentrations based on geographical location, eight elements (Ba, Cd, Cu, Fe, Mn, Pb, Sr, and Zn,) were found at higher mean concentrations in samples from Gharyan than those from Qasar Ben Ghesheer. In contrast, two elements (As, and Cr) showed higher levels in samples collected from Qasar Ben Ghesheer. The levels of Ni were comparable between the two locations (Table 4).

Contamination was observed in both cities, with Ba and Sr exceeding permissible limits in both locations. Crucially, the Lead (Pb) concentration in Gharyan samples (28.20 mg kg⁻¹) was significantly higher than its acceptable limit (10 mg kg⁻¹). Conversely, Pb level in Qasar Ben Ghesheer (1.05 mg kg⁻¹) was within the permissible range. More details are presented in Table 4.

Table 4: The detected heavy metals levels in Thyme samples collected from two cities

Heavy metal	Gharyan		Qasar Ben Ghesheer	
	Mean (mg kg ⁻¹)	SD	Mean (mg kg ⁻¹)	SD
As	0.09	0.07	0.12	0.11
Ba	23.85	5.87	12.90	3.96
Cd	0.06	0.06	0.02	0.00
Cr	0.95	0.21	1.10	0.42
Cu	23.95	24.68	15.60	15.13
Fe	337.40	42.99	227.35	78.42
Mn	12.30	1.56	10.00	2.97
Ni	0.85	0.21	0.85	0.49
Pb	28.20	34.65	1.05	0.78
Sr	79.80	15.7	70.90	4.95
Zn	26.85	1.48	15.15	0.49

Discussion:

The present study aimed to assess the bioaccumulation of eleven heavy metals in the tissues of *Thymus vulgaris* collected from the Gharyan and Qasar Ben Ghesheer areas. A critical finding was the consistent detection of all eleven target heavy metals in every plant tissue sample examined, irrespective of the plant section or collection site. This pervasive presence suggests a widespread environmental pollution burden in both geographical areas, potentially originating from diverse sources such as vehicular emissions, agricultural runoff, industrial discharge, or solid waste disposal [2].

The overall contamination levels varied, demonstrating that environmental heavy metal exposure differs significantly between the two cities (Table 4). This variability can be attributed to differences in specific anthropogenic activities in each area. For instance, the highly elevated lead (Pb) concentration in Gharyan (28.20 mg kg⁻¹) compared to Qasar Ben Ghesheer strongly suggests a localized source, such as increased traffic density or proximity to industrial operations in Gharyan.

Crucially, the concentrations of three heavy metals—Barium, Lead, and Strontium, were found to exceed internationally recognized permissible limits for herbal safety (Table 1). This finding is of considerable public health concern, as the consumption of contaminated *T. vulgaris* introduces these toxic elements into the food chain [12]. Given the long biological half-lives of certain heavy metals (up to 30 years), [4] even exposure to levels slightly below acceptable limits poses a risk. The accumulation of these elements from regular consumption will, over time, exceed internal safety thresholds, leading to chronic toxicity. The elevated levels of Ba are associated with kidney diseases, neurological, and cardiovascular disorders [13,14]. Furthermore, the high Pb levels are particularly alarming due to its serious neurotoxic effects and association with mental health issues [12]. Finally, the elevated level of Sr is linked to various health issues, including genetic changes that may cause cancer in the bone and other tissues [14].

We observed a differential accumulation pattern of heavy metals within the plant itself (Table 3). This is a manifestation of the plant's complex uptake system and its phytoremediation and detoxification strategies [15]. Most elements (As, Ba, Cr, Fe, Mn, Ni, Pb, and Zn) were found in higher concentrations in the leaves compared to the stems. This phenomenon likely results from the leaves' extensive surface area, which facilitates greater atmospheric deposition, alongside efficient translocation of systemic elements from the roots to the metabolically active aerial parts. In contrast, metals such as Sr, Cu, and Cd were more concentrated in the stems. This suggests that these elements are either less mobile within the plant's vascular system or, more likely, are sequestered within the stem tissues as an active detoxification strategy to protect the metabolically crucial leaves from heavy metal stress [16]. The plant effectively partitions these pollutants away from the tissues essential for photosynthesis and reproduction.

The findings of this study are subject to several methodological constraints. Primarily, the instrumental analysis was performed without replication, meaning the data are limited to descriptive statistics and lack the power for inferential statistical comparisons between the two cities or between plant parts. Furthermore, by restricting sample collection to a single time-point (July 2024), we could not assess the crucial impact of seasonal or temporal variability on metal accumulation. Finally, because our analysis focused solely on the plant's aerial tissues and did not include the surrounding soil or growth substrate, thereby limiting the ability to definitively determine the environmental source (e.g., atmospheric deposition versus root uptake) of the contamination observed in Gharyan and Qasar Ben Ghesheer.

Building directly on this study's findings and limitations, future research should prioritize several critical areas to fully characterize the risk. Specifically, subsequent studies must incorporate seasonal monitoring to assess temporal variability in metal accumulation allowing for definitive source apportionment (root uptake versus atmospheric deposition). The most critical follow-up is a quantitative human health risk assessment based on local consumption rates, to confirm the severity of the long-term toxicity risk from elements. Finally, the scope should be broadened to include other indigenous and widely consumed plant species to provide comprehensive public health guidance for the region.

Conclusion:

All studied heavy metals are present in *Thymus vulgaris* in the two cities under study, with three elements (Ba, Pb, Sr) exceeding permissible levels. Due to the presence of heavy metal contamination, consumption of this medicinal plant is considered unsafe. The noted intra-plant and regional differences in metal distribution serve as a basis for more focused research in the future as well as the creation of efficient methods for environmental monitoring and clean-up.

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