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Health Risk Assessment of Heavy Metals in Selected Culinary and Medicinal Herbs: A Case Study of Rose, Thyme, Turmeric, Chamomile, and Fennel

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Abstract: Plants are a significant source of heavy metals, with humans consuming large quantities of plants daily. Evaluating the levels of certain heavy metals (Pb, Cr, Cd, and Ni) is crucial for determining the potential risks associated with commonly used medicinal plants. This study aimed to estimate the concentrations of heavy metals in Curcuma longa, Rose rubiginosa, Thymus vulgaris, Foeniculum vulgare, and Matricaria chamomilla, utilizing Atomic Absorption Spectrophotometer. The results revealed that the concentrations of the studied heavy metals varied across the plant species. The concentrations of Pb, Cd, Cr, and Ni in the studied plants ranged between $2.5*10^{-5}$ - 0.44, 0.24 – 0.43, 0.40 – 0.84, and 4.83 – 4.82 respectively. While the Pb and Cr concentrations remained under the permissible limits set by WHO/FAO (Pb = 10 and Cr = 2 $mg.kg^{-1}$) in all studied herbs, Cd and Ni showed exceedance in concentration in all herbs except Cd in Chamomile was below the addressed limit. Furthermore, the relationship between the studied heavy metals among the herbs in soil was mainly determined by the origin (natural or anthropogenic) and other factors such as environmental factors, genetic makeup as well as plant uptake mechanism and affinity for certain heavy metals. Moreover, the Tue key analysis at the p-value $\leq$ 0.05 showed no significant differences among the studied herbs for the studied heavy metals. On the other hand, the Health Risk Index has shown that HQ and HI for all studied metals were < 1, which means pose no risk to human health. Additionally, the carcinogenic risks were all under acceptable limits.

Keywords: *Curcurma Longa; Rose Rubiginosa; Thymus Vulgaris; Foeniculum Vulgare; Matricaria Chamomilla; Medicinal Plant; Heavy Metal; Lead; Chrome; Cadmium; Nickle; Risk Assessment.*

1. Introduction

As naturally occurring elements of the Earth's crust, heavy metals (HMs) are often found in all elements of the environment [1]. Also, HMs can enter the environment through vehichle exhaust, industrial processes, municipal waste, fertilizers and pesticides used in agriculture [2]. The primary issue with heavy metals is their inability to break down into organic contaminants [3]. The HMs that are most frequently linked to human toxicity include cobalt (Co), manganese (Mn), mercury (Hg), arsenic (As), cadmium (Cd), and lead (Pb) (Bolan et al., 2017). Herbal plants are a significant class in many traditional medical systems, and both in industrialized and developing nations, their usage in primary healthcare interventions has grown in recent years. The World Health Organization (WHO) believes that between 70 and 80 percent of the world's population still largely use herbal remedies, which are derived from plants, as a source of medication [4]. HM accumulation in plants is influenced by many factors, such as pollution of the soil and water during agriculture, fertilization, manufacturing processes, and different anthropogenic activities. In addition, the climate, atmospheric deposition, soil metal concentrations, and particular soil characteristics where the plants are cultivated all have an impact on the uptake and bioaccumulation of these metals [5]. One of the primary routes through which soil trace elements enter the food chain is via plant absorption [6]. One of the main environmental problems is the accumulation of HMs in plants because plants take HMs from the soil up the food chain and into human bodies [7]. Because of their low rates of renal excretion, HMs may be harmful to humans even at extremely low doses [8]. Numerous earlier studies have demonstrated that the consumption of herbs containing excessive amounts of toxic metals can result in a variety of poisoning incidents and health issues in humans[9, 10]. Emissions from heavy traffic release heavy metals like Cd, Pb, zinc (Zn), and nickel (Ni), which contaminate nearby areas and water bodies. Agricultural activities contribute to soil pollution through the use of various agricultural chemicals like fertilizers and pesticides that contain metals including As, Cu, Fe, Mn and Zn. Cadmium contamination is especially linked to phosphate-based fertilizers. These metals build up in soil over time with continued use of contaminated fertilizers[11], potentially causing serious health effects including cancer after long-term exposure [12]. Due to these risks, organizations like WHO have set guidelines for acceptable metal content in herbs used for consumption [13], and recommend comprehensive qualitative and quantitative testing before use in order to determine the level of HMs [14]. Testing methods typically require breaking down plant samples, with techniques like atomic absorption spectrometry (AAS) being used to measure trace metals [15]. The aim of this study is to evaluate concentrations of specific HMs in five different herbs and medicinal plants commonly used in Iraq and a flavouring or traditional remedies. The study also assesses the health risks associated with these heavy metals by calculating estimated daily intsak (EDI) and performing non-carcinogenic risk assessment.

2. Methodology

2.1 Sampling

A total of 25 samples of dried herbal medicines, representing five commonly used varieties, were obtained from five local markets in Erbil in April 2024 for heavy metal (Pb, Cd, Cr, and Ni) analysis. Table 1 lists the common and scientific names of the herbs studied.

2.2 Chemicals And Reagents

All glassware was cleaned by soaking it in 10% nitric acid overnight, followed by rinsing with deionized water. The acids used for wet digestion (69.0% HNO3, 30% H2O2) were of suprapure quality (E. Merck) [16]. Double-deionized water was utilized for all dilutions. Heavy metal concentrations were measured using an AAS (Agilent Technologies, Model 280FS AA).

Table 1: Medicinal herbs analyzed for heavy metal content, including local names, scientific names, codes, and sample numbers.

Scientific name	Local name	English name	Code given	Number of samples
Curcurma longa	Zardachu	Turmeric	1	5
Rose rubiginosa	Gullabagh	Rose	2	5
Thymus vulgaris	Jatra	Thyme	3	5
Foeniculum vulgare	Razyana	Fennel	4	5
Matricaria chamomilla	Baybun	Chamomile	5	5

2.3 Sample Preparation and Digestion

The dried herbs were ground into a powder, and the powdered samples were subjected to acid digestion using nitric acid and hydrogen peroxide. Approximately 0.5 g of each sample was used for the digestion process. The digestion was performed with 4.0 mL of nitric acid (69.0% HNO3) and 2.0 mL of hydrogen peroxide (30% H2O2). The mixture was heated to 130°C for 4 hours, then diluted to a final volume of 25 mL with deionized water. Blank samples was also processed the same processes [16].

2.4 Heavy Metal Detection and Quantification

After sample digestion, by using the AAS (Agilent Technologies, Model 280FS AA spectrophotometer) the concentration of HMs in the samples been measured and quantified. ASTM testing method - D3559 for lead, D1687 for chromium, D3557 for cadmium, and D1886 for nickel were applied. All measurements were reported in milligrams per kilogram.

2.5 Index-Based Health Risk Assessment

To evaluated health risks from heavy metal consumption, three indexed were employed: hazard index (HI), target hazard quotient (THQ), and EDI (estimated daily intake). These methods followed the work of [17] to assess potential threats to consumer health.

2.5.1 Estimated Daily Intake (EDI)

The EDI results of Cd, Pb, Cr, and Ni (in mg/kg/day/person) for consumption of medicinal herbs was calculated used Equation no.1, which was modified from the tudy by [18].

(1) $$\text{EDI} = \frac{C * IR}{bwt}$$

C is the metals concetration in medicinal herbs in mg/kg.
IR is the daily ingestion rate/capita, it is 0.5 g·d^{1-} [19].
IR represents the daily average ingestion rate of medicinal herbs, and expressed in kg/day/person.
bwt denotes body weight, according to [20] and average human weight of 60 kg is used in the equation.

2.5.2 Target Hazard Quotient

Non-carcinogenic risk for metals is assessed by comparing the level of exposure over a specific time period (such as lifetime) to a reference dose established for a similar exposure duration. This non-cancer risk can be expressed as a THQ [21].

(2) $$\text{THQ} = \frac{\text{C} \times \text{IR} \times \text{Ef} \times \text{Ed} \times \text{t}}{\text{AT} \times \text{BW} \times \text{RfD}}$$

The heavy metal concentration in the exposure media is given as C (mg/l or mg/kg). The intake rate is denoted by IR (l/day or kg/day), while the exposure frequency is indicated by EF (365 days per year). The exposure duration (ED) is 70 years, which corresponds to the average lifespan. BW denotes body weight in kilograms, and AT is the average duration for non-carcinogens, computed as 365 days each year multiplied by the number of exposure years, assuming a 70-year lifespan. The oral reference doses (RfDs) for Cr, Ni, Cd, and Pb are 0.003, 0.02, 0.001, and 0.004 mg/kg/day [22]. If the Hazard Quotient (HQ) is less than 1, the local population is considered safe. If the HQ is one or higher, it is considered unsafe for human health [23].

2.5.3 Hazardous Index (HI)

When multiple heavy metals are present, the risk to human health is greater than with a single heavy metal. To evaluate the overall impact of numerous heavy metals on health, we employ the total hazard index (HI), which represents the cumulative non-carcinogenic risks associated with exposure to these metals. The formula is as follows.

$$\text{HI} = \sum_{i=1}^{m} \text{THQi} \quad (3)$$

THQi – represents target hazard coefficient of heavy metal i.
The HI value smaller than one (<1.0) indicates that there is no clear non-carcinogenic damage to human body, whereas, HI value greater than one (>1.0) indicated very high risk of harm to human health [24].

2.6 Cancer risk assessment

The Incremental Lifetime Cancer Risk (ILCR) was used to quantify the potential for cancer risks associated with the examined herbs through ingestion of carcinogenic heavy metals [25].

$$\text{ILCR} = \text{CDI}\times\text{CSF} \quad (4)$$

CSF represents the cancer slope factor, while the probability of lifetime cancer risk is represented by the variable CR. The CSF values listed below were utilized in our calculations: The US EPA has set values of 0.38, 0.0085, 0.5, and 0.84 $mg.\ kg^{-1}.day^{-1}$ for Cd, Pb, Cr, and Ni, respectively. CR of these four metals in the same herbal medicine was also summed up to give the total CR of a single herbal medicine. A CR of 10-4 indicates that there is a one in 10,000 chance that someone may get cancer. Also for 10^{-6} is one chance may getting cancer in 1000000 [26]. Total cancer risk values beyond 1×10^{-4} are considered intolerable, risks below 1×10^{-6} are not thought to have a substantial impact on health, and risks in the range of 1×10^{-4} to 1×10^{-6} are usually considered as satisfactory [18].

2.7 Data Analysis

Statistical data analysis (One-way ANOVA and Pearson's Correlation) was carried out using Microsoft Office Excel 2021 and GraphPad Prism software version 10.2. A p-value of ≤ 0.05 was deemed statistically significant [27].

3. Findings and Discussion

3.1 Concentrations of Metals in Herbs

The means of the studied heavy metals are presented in Table 1 and compared to WHO/FAO permissible limit. Pb ranged between $2.5*10^{-5}$ to 0.44 $mg.kg^{-1}$ in which the lowest concentration observed in Thyme and Chamomile whereas the highest concentration was observed in Turmeric. The WHO has determined a maximum permissible level of Pb (10 ppm) in all plant parts [28]. According to the norm, Pb concentrations in the studied herbs have remained under the limit of the WHO/FAO. In contrast, the Haripur basin in Pakistan was found to have a Pb content ranging from 3.15 to 10.63 ppm [29], in the Kisii Region of Southwest Kenya, from 0.15 to 0.41 ppm [30]; and in Northwestern India, from 0.25 to 2.64 ppm [31]. The post-hoc analysis has shown that the Pb concentration is not significantly different among the studied herbs $p = 0.681$ (Figure 1). The correlation matrix Figures 5,6, and 8 has indicated that Pb has a positive relationship with Cd this indicates that both heavy metals can coexist in soil and can be taken together by plants [32]. However, Pb is inversely correlated with Cr and Ni in all studied herbs indicating the behavior of Pb with Cr and Ni is significantly different in soil environments in which solubility and chemical properties of the heavy metals in soil could be the main reason for the inverse relationship between them [33]. Pb is extremely harmful to humans, particularly to the neurological and urinary systems. Lead poisoning commonly manifests as anemia, convulsions, colic, headache, chronic nephritis, hypertension, and problems of the central nervous system [34].

Table 2: Means and WHO/FAO Permissible limits of the studied heavy metals

Medicinal Plant	English name	Heavy metals (Mean ± Std. Dev)			
		Pb	Cd	Cr	Ni
Curcurma longa	Turmeric	0.44±0.99	0.31±0.35	0.42±0.37	4.83±4.89
Rose rubiginosa	Rose	0.39±0.88	0.43±0.33	0.51±0.35	5.82±3.59
Thymus vulgaris	Thyme	$2.5*10^{-5}$±0	0.33±0.21	0.40±0.40	4.49±3.57
Foeniculum vulgare	Fennel	0.35±0.56	0.4±0.38	0.74±0.74	5.53±5.19
Matricaria chamomilla	Chamomile	$2.5*10^{-5}$±0	0.24±0.47	0.84±0.34	5.52±7.09
Permissible limits WHO/FAO ($mg.kg^{-1}$)		10	0.3	2	1.5

Cadmium concentrations were between 0.24 to 0.43 $mg.kg^{-1}$ in which the lowest concentration was in Chamomile and the highest in Rose. In this study, all the samples contained Cd levels that surpassed the WHO's permissible limit of 0.3 ppm. Except Chamomile remained under the standard. Comparable findings were observed in a study by [29], where Cd ranged from 0.15 to 0.41 ppm (mg/kg). Another study in India found Cd concentrations between 0.25 to 2.64 ppm (mg/kg) [35]. in contrast, a much higher level of Cd was reported in the study conducted by [36], In which Cd levels ranged from 8.81 to 10.25 ppm (mg/kg). The post-hoc analysis has shown that the Cd concentration is not significantly different among the studied herbs p = 0.921 (Figure 2). Considering Cd has no physiological function in the human body but can sometimes cause food poisoning. Cd poisoning can lead to kidney failure, osteopenia, anemia, and ultimately death, a condition known as itai-itai disease [37]. Cd has shown a strong and moderate negative relationship with Cr and Ni in Turmeric, Rose, and Fennel respectively. The strong negative correlation indicates manmade interaction in the soil or distinct sources. Chemical forms play a critical role in determining the behavior of heavy metals, multiple oxidation forms of Cr is significantly affect its solubility and interaction with other heavy metals, especially Cd, similar relationship results were also obtained by [38] and [39]. Furthermore, Cd presented a very strong relationship with Cr and Ni in Chamomile with a possible reason of shared source or pathways of accumulation in the plant in which Cd and Ni can come together in the event of application of certain fertilizer and industrial effluents in soil. The statement was also backed by [33] And [40].

Chromium concentrations were in the range of 0.42 in Thyme to 0.84 in Chamomile. Following the WHO/FAO permissible limit of 2.0 $mg.kg^{-1}$, Cr has shown no evidence in all studied herbs. Cr is necessary for human physiological processes; nevertheless, its buildup results in low blood glucose, gastrointestinal and cardiovascular problems, and other issues [28]. Like Pb and Cd, Cr concentration is not significantly different among the studied herbs p = 0.493 (Figure 3). Ni concentrations were highest compared to the other studied heavy metals ranging between 4.49 to 5.82 $mg.kg^{-1}$. Thyme has indicated the lowest concentration of Ni while Rose showed the lowest concentration. Notably. WHO's permissible limit of nickel in medicinal plants is 1.5 mg/kg, every sample in this study showed Ni concentrations surpassing the permissible limit. Ni concentrations between 0.09 and 1.6 ppm were reported by [29], while in research done in Baghdad, Iraq, the range of Ni showed to be between 8.81 to 10.25 ppm, above the WHO-established limit [36]. The most prevalent condition associated with Ni is allergic contact dermatitis; additionally, Ni is considered a potential carcinogen that may affect the lungs and nasal cavities [41]. Furthermore, the post-hoc analysis has shown that the Ni concentration is not significantly different among the studied herbs p = 0.992 (Figure 4). Nickle's relationship with Cr is positive in all studied herbs except for Rose which was negative. The concurrence of Ni with Cr is more likely during metal smelting which releases both metals resulting in their concurrence in soil and plant. A similar relationship was also obtained by [40]. The variations in heavy metals concentrations among the studied plants could be due to differences in plant species characteristics, soil composition, environmental factors, the mineral content of the soil in which they are grown, fertilizer application, irrigation water, and weather [42]. The uptake mechanism of the herb, along with agricultural practices and other environmental factors, significantly influence the heavy metal content in the soil. The plant's uptake mechanism, such as certain proteins, can make the herb more prone to absorbing specific heavy metals. [43]. Additionally, agricultural activities like fertilizer application, irrigation with high nickel content water, and certain pesticides can also increase nickel concentrations in the herb. Other soil characteristics, such as organic matter content, pH, and moisture, can also facilitate increased heavy metal uptake by the herb [44, 45]. The herb's phytoremediation ability to absorb specific heavy metals from the soil can substantially contribute to elevated heavy metal concentrations in the plant [46]. Furthermore, the herb's genetic makeup can play a crucial role, allowing it to naturally take up high concentrations of heavy metals without a pre-determined mechanism to boost the uptake [43].

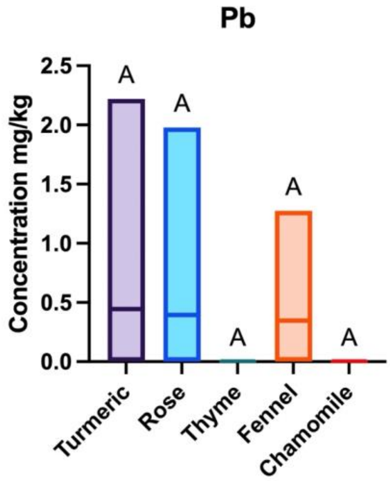

Figure 1: Tukey analysis among the studied herbs.

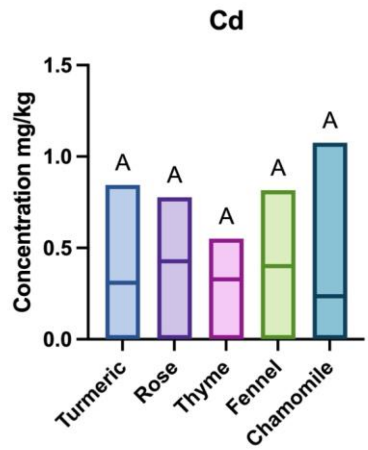

Figure 2: Tukey analysis among the studied herbs.

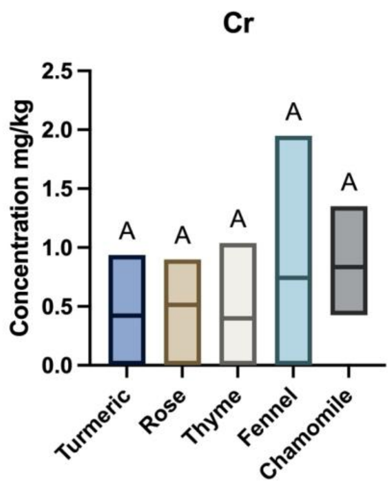

Figure 3: Tukey analysis among the studied herbs.

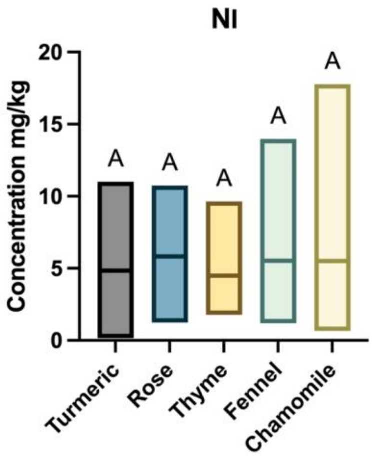

Figure 4: Tukey analysis among the studied herbs.

Note: Different letters indicate significant differences at $p \leq 0.05$

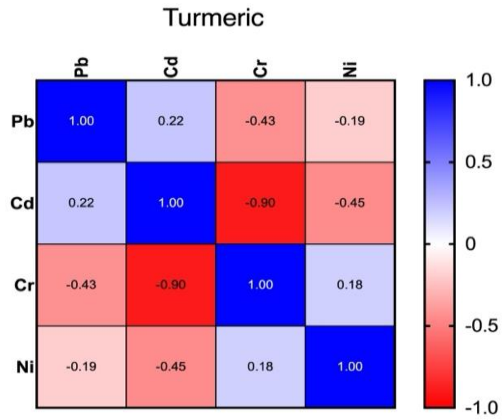

Figure 5: Correlation between studied metals in Turmeric.

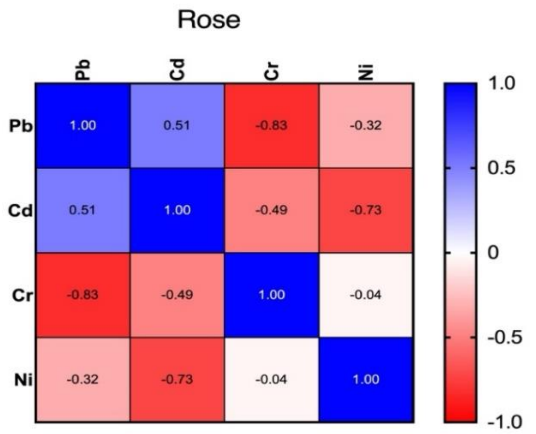

Figure 6: Correlation between metals metals in Rose.

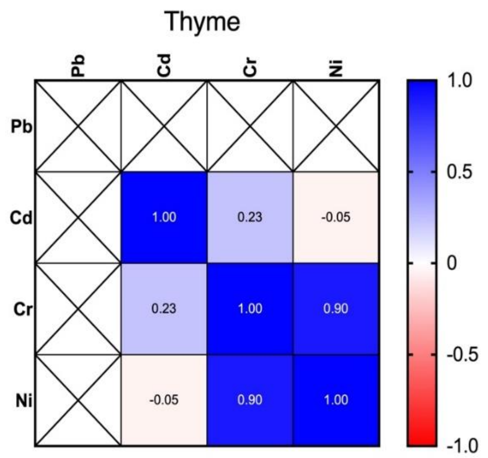

Figure 7: Correlation between studied metals in Thyme.

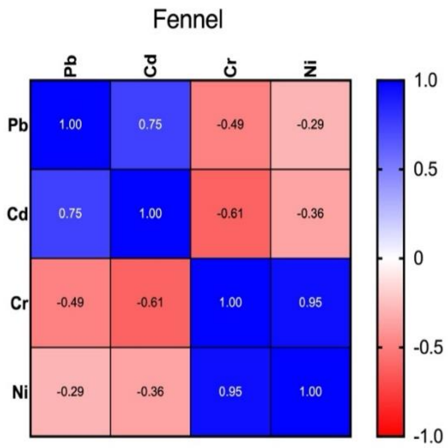

Figure 8: Correlation between studied metals in Fennel.

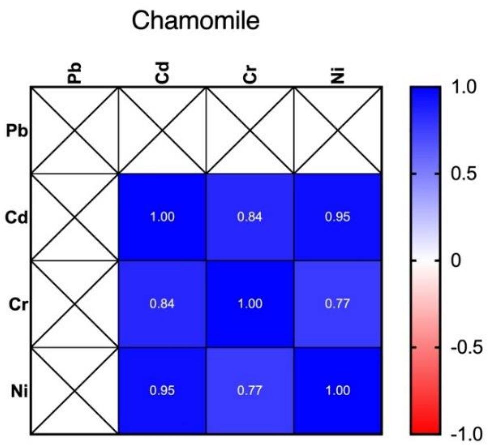

Figure 9: Correlation between studied metals in Chamomile.

3.2 Non-carcinogenic risk assessment

Humans can encounter various environmental pollutants, including heavy metals, through several pathways like skin contact, inhalation, and the food chain. However, oral ingestion is the most common route of exposure compared to other methods [47]. Consequently, this study opted for an assessment of oral health risks. The results in Table 3, indicate significant variability in the EDI values for the analyzed medicinal plants. Thyme and Chamomile exhibited the lowest Pb-EDI values of 2.083 E-10 mg/kg, while Turmeric had the highest Pb-EDI at 3.666E-06 mg/kg. For Cd, the lowest EDI was found in Chamomile at 2.00E-06 mg/kg, whereas the highest was recorded in Rose at 3.583E-06 mg/kg. In terms of Cr, the lowest EDI value was 3.333E-06 mg/kg in Thyme, while Chamomile had the highest at 7.00E-06 mg/kg. Finally, for Ni, Thyme again showed the lowest EDI at 3.741E-05 mg/kg, while Rose had the highest value at 4.85E-05 mg/kg.

The THQ values for all metals across the samples were below 1, indicating no associated risk from these metals in the studied medicinal herbs. Although the THQ values were below the recommended tolerable limits, it's important to understand that these figures are not conclusive. There is still a potential for health risks, especially with prolonged exposure. Rose showed the highest THQ value for Cd and Ni, and Turmeric showed the highest value of THQ for Pb, while the highest THQ value for Cr was found in Chamomile, suggesting that excessive consumption could pose a risk.

The graph depicting the Hazard Index (HI) illustrates the cumulative risk of heavy metal exposure across the studied herbs. Similarly, the HI for all samples was also below 1, suggesting that there are no risks to human health. Turmeric and rose have the highest HI values, indicating a greater combined risk from multiple metals. This is particularly concerning as the HI can serve as an indicator of potential health risks when these herbs are consumed in combination or over extended periods.

Table 3: The results of EDI and HQ of heavy metals in selected medicinal herbs

Medicinal Plant	**EDI (Pb)**	**EDI (Cd)**	**EDI (Cr)**	**EDI (Ni)**
Curcurma longa (Turmeric)	3.666E-06	2.583E-06	3.50E-06	4.025E-05
Rose rubiginosa (Rose)	3.25E-06	3.583E-06	4.25E-06	4.85E-05
Thymus vulgaris (Thyme)	2.083E-10	2.75E-06	3.333E-06	3.741E-05
Foeniculum vulgare (Fennel)	2.916E-06	3.333E-06	6.166E-06	4.608E-05
Matricaria chamomilla (Chamomile)	2.083E-10	2.00E-06	7.00E-06	4.60E-05
HQ				
Curcurma longa (Turmeric)	9.15E-04	2.584E-03	1.166E-03	2.012E-03
Rose rubiginosa (Rose)	8.1E-04	3.58E-03	1.417E-03	2.425E-03
Thymus vulgaris (Thyme)	5.208E-08	2.75E-03	1.11E-03	1.87E-03
Foeniculum vulgare (Fennel)	7.3E-04	3.33E-03	2.06E-03	2.30E-03
Matricaria chamomilla (Chamomile)	5.208E-08	2.001E-03	2.334E-03	2.3E-03

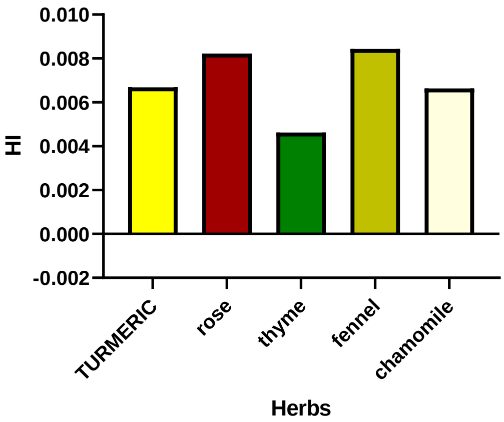

Figure 10: Hazard Index of heavy metals in studied herbs

3.3 Carcinogenic risk assessment

The table shows that the ∑ILCR values for all the metals in medicinal plants are in the range of 10^{-6} and 10^{-4} which is regarded as a safe range. Pb shows minimal cancer risk with ILCR values from 1.77E-12 in Thyme and Chamomile to 3.12E-08 in Turmeric. Cd presents higher variability, with ILCR values from 7.60E-07 in Chamomile to 1.36E-06 in Rose. Cr poses notable risks in Chamomile (3.50E-06) and Fennel (3.08E-06). Nickel (Ni) is the most concerning heavy metal, with incremental lifetime cancer risk (ILCR) values ranging from 3.14E-05 in Thyme to 4.07E-05 in Rose, significantly contributing to the overall cancer risk. The total cancer risk (∑ILCR) varies, with Rose having the highest risk at 4.43E-05 and Thyme the lowest at 3.41E-05. Although the levels are within safe limits, consuming these herbs may still pose a potential cancer risk due to the cumulative effect of the heavy metals present. These findings highlight the need for stringent monitoring and regulation of heavy metals in medicinal herbs to ensure consumer safety and reduce health risks. Regular assessments and the implementation of stringent safety standards are crucial to reducing the cancer risks linked to heavy metal contamination in herbal products. It is important to note that the ILCR values are based on estimates and may vary depending on factors such as the frequency and amount of herb consumption, the bioavailability of the heavy metals, and individual susceptibility.

Table 4: ILCR results and total cancer risk of HMs in medicinal herbs

Medicinal Plant	**Pb**	**Cd**	**Cr**	**Ni**	**∑ILCR**
Curcurma longa (Turmeric)	3.12E-08	9.82E-07	1.75E-06	3.38E-05	3.66E-05
Rose rubiginosa (Rose)	2.76E-08	1.36E-06	2.13E-06	4.07E-05	4.43E-05
Thymus vulgaris (Thyme)	1.77E-12	1.05E-06	1.67E-06	3.14E-05	3.41E-05
Foeniculum vulgare (Fennel)	2.48E-08	1.27E-06	3.08E-06	3.87E-05	4.31E-05
Matricaria chamomilla (Chamomile)	1.77E-12	7.60E-07	3.50E-06	3.86E-05	4.29E-05

4. Conclusion

This study aimed to assess the concentrations of four heavy metals Pb, Cd, Cr, and Ni in five medicinal herbs: turmeric, rose, thyme, fennel, and chamomile. The results showed that the concentrations of Pb and Cr were within the acceptable limits provided by the WHO/FAO. However, the concentrations of Cd and Ni exceeded these limits in all plant samples, except for the Cd level in chamomile. The health assessment indicated that the HQ and HI for all metals were below 1, suggesting no significant risk to human health. The carcinogenic risks were also within acceptable limits. The study highlights the need for continuous monitoring and control of heavy metal levels in medicinal plants to ensure consumer safety. To reduce contamination, best farming practices should be employed. Additionally, more research is needed to determine the long-term health implications of consuming herbal supplements that expose people to low levels of heavy metals.

Author's Contribution

"We confirm that all named authors have read and approved the manuscript. We also confirm that each author has the same contribution to the paper. We further confirm that all authors have approved the order of authors listed in the manuscript."

Conflict of Interest

"*There is no conflict of interest for this paper.*"

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This research has been conducted through the contributions, guidance and support from individual authors. The entier work in this paper incluing the design, sample collection, sample analysis, data collection, data analysis and writing resulted solely from authors contribution.

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