Effect of Various Environmental and Social Diverse Locations on Heavy Metal Accumulation in Selected Medicinal Plants of Northern Region of India

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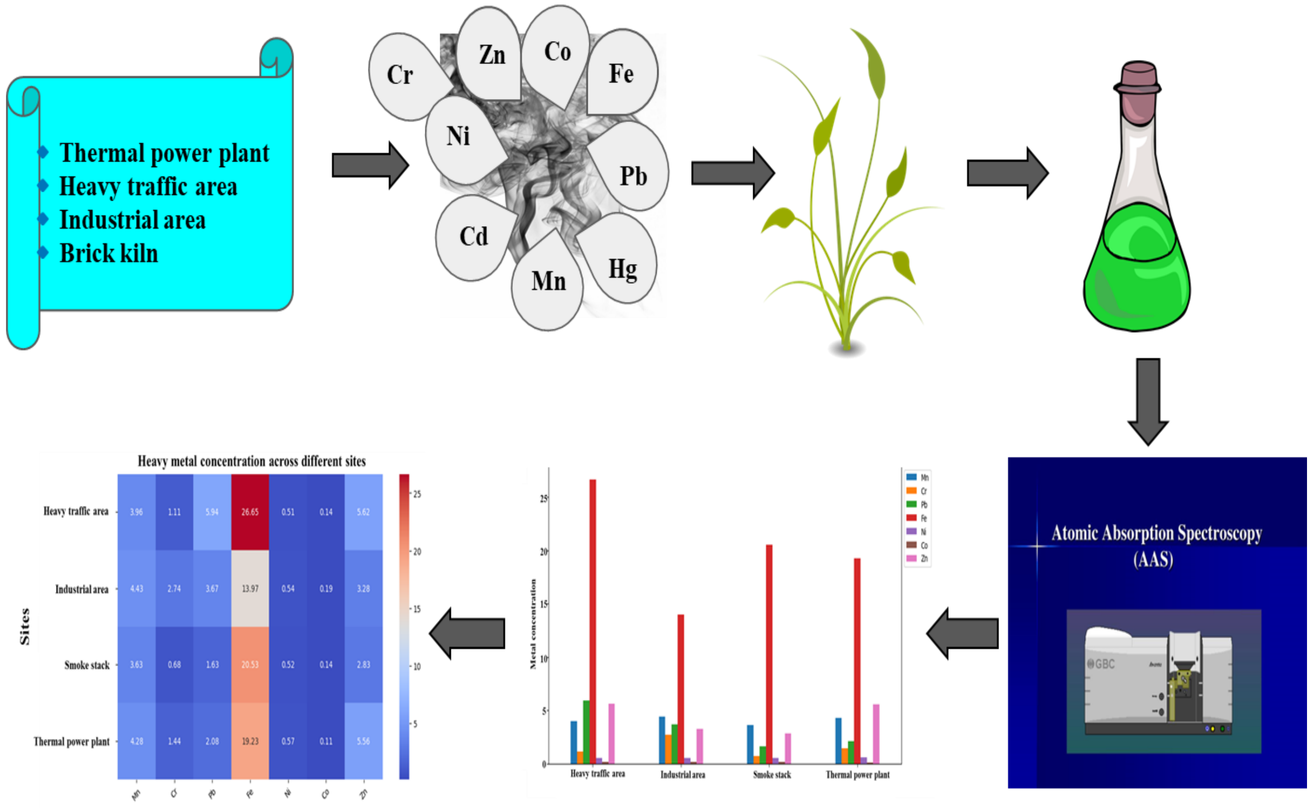
Abstract

This study aims to measure the heavy metal concentration in soil and frequently used medicinal plants collected from various environmental locations, while evaluating the influence of these sites on the mineral composition of the plants. Plant and soil samples of Calotropis procera, Euphorbia hirta, Achyranthes aspera, Cynodon dactylon and Argemone mexicana were collected from four different environmental locations of the northern region of India namely: Thermal power plant (TPP), Industrial area (IA), Brick kiln (BK) and Heavy traffic area (HTA). Essential metals i.e. Mn, Fe, Co, Zn and potentially toxic metals i.e. Cr, Ni, Cd, Hg and Pb were quantified in soil and plant samples using Atomic absorption spectrophotometry (AAS) and subsequently compared.

The highest concentration of iron (Fe) (70.8±0.02 ppm) was found in soil from the thermal power plant area. The most toxic heavy metal lead (Pb) (8.3± 0.01ppm) and cadmium (Cd) (0.1±0.001ppm) were observed highest from Heavy traffic area sites in Calotropis procera and Euphorbia hirta respectively, although found in permissible limit of 10 and 0.3 ppm respectively set by WHO (World Health Organisation) for herbal products. The concentration of Hg remained below detection limit in all tested samples. There is a significant variation in plant location and heavy metal concentration (P≤0.05). Heavy metals were found below permissible limits in all tested samples. However, continuous consumption of some toxic metals can lead to accumulation in the body. Therefore, in order to prevent health risks, it is recommended that cultivation of medicinal plants should be prohibited near environmentally polluted site especially heavy traffic area.

Keywords: Heavy Metal, Medicinal Plants, Atomic adsorption spectrophotometer, Permissible limit, Health risk.



Graphical abstract

Introduction

Herbal medicines have been used for over five millennia across various civilizations as a traditional remedy for ailments. Even today, plant-based treatments remain a key component of primary healthcare in many developing countries, whereas approximately 25% of all contemporary medications are sourced, either directly or through indirect means from higher plant species[25]. Medicinal plants constitute a fundamental source of raw materials for the pharmaceutical sector. Approximately one-fourth of prescription drugs in U.S. include at least one active compound derived from plants. Additionally, herbal remedies play a significant role in traditional medicine, particularly in developing nations like China and India[11,37].

Ayurveda, a traditional medical system originated in India, has been practiced for over two thousand years. This ancient healing system predominantly utilizes herbs and their formulations to promote health and to treat ailments[9]. Herbal medicines are often favoured over modern synthetic drugs due to their added nutritional benefits[26]. Rapid industrialization and unregulated urbanization often result in the accumulation of toxic pollutants including heavy metals and pesticides, across environmental media such as water, soil and air. Heavy metal contamination in agricultural soils poses a persistent problem in the scientific community. These metals enter into crop through root absorption, foliar uptake and the decomposition of specific compounds[1]. Natural sources of soil contamination include rock weathering, volcanic eruption and wildfires[4].

Pollution also arises from the release of impurities concentrated in clay minerals with high absorption capacity, found within sedimentary rock layers, their predecessor sediments and aquatic system[8]. Multiple variables influence the presence of heavy metal contamination within agricultural soils. These encompass the application of fertilizers and pesticides, atmospheric deposition stemming from urban waste, emissions originating from industrial activities and processes associated with metal production.

Plants indiscriminately take up heavy metals from the soil, leading to the accumulation of both non-essential metals such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb), as well as essential metals like manganese (Mn), chromium (Cr), cobalt (Co), iron (Fe), zinc (Zn) and copper (Cu), in plants and vegetables[27].

Currently, the analysis of heavy metals such as Pb, Cd, Hg, Ni, Cu, Mn, As, Fe and Cr in medicinal plants has gained significant scientific interest, as these plants are widely used as an alternative form of medication across the world[17,36,47]. Increased concentrations of toxic metals may be observed in medicinal formulations, particularly when metals such as lead (Pb) and mercury (Hg) are employed as active constituents in certain Chinese and Mexican remedies. Additionally, elevated levels can arise when medicinal plants are grown in contaminated environments such as those proximate to roadways, metal mining operations, or smelting facilities[45].

Medicinal plants grown in polluted areas may contain toxic heavy metals, creating health risks for humans and animals who consume them. Soil type and its composition play a crucial role in determining heavy metal concentrations in medicinal plants[1,4,8,17,27,36]. Therefore, systematic monitoring of heavy metal concentrations in both medicinal plants and soils across different environmental conditions is essential, along with efforts to enhance scientific awareness to mitigate risks of metal toxicity. This study analyses the concentration of heavy metals in medicinal herbs cultivated in various polluted environments including a thermal power station, industrial area, heavy traffic zone and brick kiln. The objective is to assess the safety of herbal medicines by examining how different pollution sources influence heavy metal uptake during plant growth[7].

Heavy metals may enter into the human body through contaminated food, air or water, leading to gradual bioaccumulation. Key heavy metals posing significant risks to biotic components in natural, terrestrial and aquatic ecosystems include cadmium (Cd), arsenic (As), copper (Cu), zinc (Zn), lead (Pb), chromium (Cr) and nickel (Ni), as documented in contemporary environmental research. Since plants are sessile, their growth and development depend entirely on their surrounding environmental conditions[32].

To ensure optimal therapeutic efficacy, it is essential to assess the quality of raw herbal materials for potential metal contamination. The World Health Organization recommends that medicinal plants used as raw materials for finished products, should undergo evaluation for the heavy metal content[49]. Various countries have established distinct national standard for the permissible levels of heavy metals in raw herbal materials. For example, India’s AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy) guidelines specify maximum allowable limits of 3.0, 10.0, 0.3 and 1.0 ppm for arsenic, lead, cadmium and mercury respectively[18].

India and China possess rich traditional knowledge systems and extensively utilize medicinal plants, cultivating diverse species domestically, particularly in rural regions, to ensure their appropriate and sustainable use. Heavy metals concentration in medicinal plants is commonly quantified in parts per million (PPM) and can be assessed through advanced trace element detection methodologies, including Atomic Absorption Spectrometry (AAS) employing the relative method and Instrumental Neutron Activation Analysis (INAA)[26].

Medicinal herbs can become contaminated at different phases, encompassing their growth, development and processing stages. Upon collection and processing into dosage forms, the heavy metals contained within these plants enter the human body where they accumulate in various organs. This accumulation can affect normal functions of the central nervous system and severe health issues such as kidney damage, chronic toxicity symptoms, liver damage and various conditions such as different types of cancers, skin eruptions and gastrointestinal ulcers. A 1990 investigation into Ayurvedic medicinal products in India revealed that 41% of samples contained arsenic, while 64% were contaminated with lead and mercury.

Similarly, a 2004 study of Ayurvedic formulations from South Asia, sold in Boston, detected toxic heavy metal levels in 20% of products, highlighting significant health risks and recommending mandatory heavy metal testing for consumer safety[5,11,15,16,22]. The study aims to asses contamination of heavy metal in commonly used medicinal herbs including *Calotropis procera, Euphorbia hirta, Cynodon dactylon, Achyranthes aspera and Argemone Mexicana,* collected across various contaminated sites.

Sampling locations were selected based on the presence of contaminated soil and atmospheric pollution sources, such as industrial emissions. These plants were selected for their significant therapeutic applications in traditional medicines and modern pharmaceutical preparations. Given that these herbs are often collected indiscriminately by untrained individuals from polluted environments and supplied to markets or raw drug vendors, there is a risk of harmful minerals entering the human body upon consumption. Therefore, the objective is to evaluate the presence and concentration of toxic heavy metals, including mercury (Hg), cadmium (Cd), lead (Pb), iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), chromium (Cr) and nickel (Ni) in the selected medicinal plants.

The novelty of this research lies in the assessment of contamination in medicinal plants collected from two highly polluted sites, a thermal power plant and a brick kiln. A review of existing literature revealed limited studies conducted in this specific context. Although considerable efforts have been made for analysing heavy metal accumulation in medicinal and edible plants grown in contaminated soils, the examination of medicinal plants in such severely polluted environments remains insufficiently explored.

Material and Methods

Criteria for Selection of Medicinal Plants for the Study: The medicinal plants selected for this study include *Calotropis procera*, *Euphorbia hirta*, *Achyranthes aspera*, *Cynodon dactylon* and *Argemone mexicana*. Plants were chosen primarily based on their significant role in Ayurveda, extensive use in modern pharmaceutical preparations, diverse therapeutic properties and their adaptability to various ecological conditions. These herbs have long been employed to treat a wide range of conditions such as inflammation, skin infections, respiratory problems and wounds. In this study, table 1 presents a general description of these medicinal plants, their medicinal properties and a summary of related studies.

Table 1
Medicinal plants, medicinal properties of the plants used in this study

Medicinal Plant	Common name	Medicinal Properties	Relevant Studies and Findings
Calotropis procera[10]	Aak Tree	Anti-inflammatory, analgesic, wound healing, antimicrobial, anticancer, anthelmintic and hepatoprotective properties.	Known for its latex, which contains bioactive compounds with anti-inflammatory and wound-healing properties. Also used in Ayurveda for treating skin diseases and digestive disorders.
Euphorbia hirta[39]	Spurge	Antiviral, antimalarial, anti-asthmatic, diuretic, anti-inflammatory and wound healing.	Traditionally used in tropical medicine to treat asthma, bronchitis and gastrointestinal disorders. Rich in flavonoids and tannins, which contribute to its pharmacological activity.
Achyranthes aspera[42]	Chaff-flower	Antifungal, antibacterial, anti-inflammatory, hepatoprotective, diuretic and immunomodulatory effects.	Used in Ayurvedic medicine for treating kidney stones, rheumatism and hypertension. Studies highlight its antimicrobial and hepatoprotective potential.
Cynodon dactylon[28]	Dhub Grass	Antioxidant, anti-diabetic, hepatoprotective and anti-inflammatory.	Commonly used in traditional medicine for wound healing and diabetes management. Research shows its role in reducing oxidative stress and supporting liver health.
Argemone mexicana[35]	Mexican Poppy	Antibacterial, antimalarial, analgesic, anti-inflammatory and hepatotoxic effects.	Despite its medicinal benefits, studies have shown that excessive use can lead to toxicity due to alkaloid content. Used in traditional medicine for skin infections and pain relief.

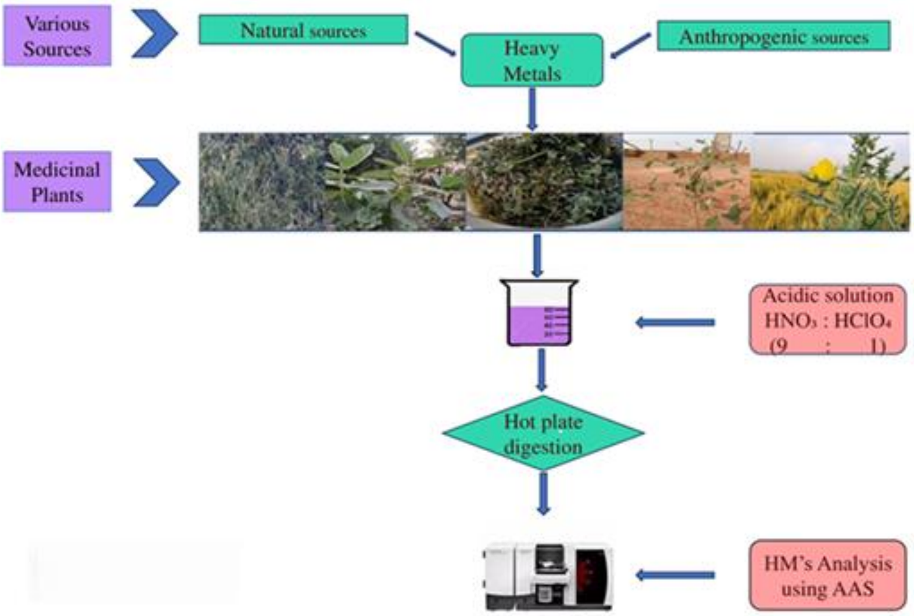


Figure 1: Flow chart showing the complete process

Whole plant samples of five medicinal plants, *Calotropis procera*, *Euphorbia hirta*, *Achyranthes aspera*, *Cynodon dactylon* and *Argemone mexicana*, were collected from four environmentally distinct locations of the northern region of India:

A. Thermal power plant
B. Heavy traffic area
C. Industrial area
D. Brick kiln

Sampling was done from four distinct locations namely, Hisar, Jhajjar, Rohtak and Bahadurgarh areas of Haryana. These sites were chosen based on their high levels of soil and environmental contamination caused by pollution-emitting industries, heavy traffic near highways and other pollution sources. The discharge from these locations contains heavy metals such as lead (Pb), cadmium (Cd) and mercury (Hg), which significantly affect surrounding vegetation. Heavy traffic contributes to lead and cadmium contamination primarily through fuel combustion and tire wear. Industrial emissions release toxic metals like chromium (Cr) and cadmium (Cd), which accumulate in both soil and plants.

Additionally, fly ash emissions from brick kilns lead to aerosol deposition in nearby areas, further contaminating the soil and plant life. These factors make the selected locations ideal for investigating heavy metal accumulation in medicinal plants and facilitate the assessment of how collection sites for raw herbal materials influence heavy metal toxicity in these plants, particularly as untrained individuals gather medicinal plants from arbitrary locations without prior evaluation and supply to the market.

Sample collection and preparation: Plant and soil samples were collected from within the premises of a thermal power plant, an industrial area and a region approximately 50 meters near a brick kiln and highways. Soil specimens were systematically obtained from the upper 0–15 cm soil layer, with each representative sample consisting of an aggregated mixture of several subsamples collected across the respective sampling locations. All collected plant and soil samples were sealed in clean polyethylene bags and transported to the laboratory for further processing. The collected plant samples were thoroughly washed with tap water, followed by distilled water, to remove dust and surface contaminants.

The washed plants were then shade-dried, compressed and manually ground using a mortar before being sieved through a 20-mm (RETSCH ISO 3310-2/ 400mm diameter) size mesh. Similarly, soil samples were placed in glass Petri dishes, dried in an oven at 100°C for 48 hours, ground and sieved to ensure uniformity. Both plant and soil samples were stored in sealed containers until further analysis.

Heavy metal estimation: Soil samples were digested according to Agarwal et al[2], using 5ml (6 parts of HNO_3 and 1 part of $HClO_4$) for digestion mixture[2]. After cooling, the sample was filtered using Whatmann filter paper no. 41 and rinsed with 1% HNO_3. A final volume of 10 ml was made and stored until further analysis.

Plant sample: To assess heavy metal content, 5 g of powdered plant samples were digested using the wet digestion method[29] in an acid mixture of nitric acid HNO_3 and $HClO_4$ (9:1). The samples were heated on a hot plate namely, (MC02, digital spinot, Tarson) with the acid mixture until a clear solution was obtained.

After cooling, the sample was filtered through Whatmann filter paper no. 42. The final volume was adjusted to 50 mL using ultrapure water and stored in glass vials washed with 10%(v/v) nitric acid solution until further analysis.

All experiments were conducted in triplicate to ensure accuracy and precision. Heavy metal analysis was performed using a double-beam Atomic absorption spectrophotometer (AAS4141). Standard reference solutions for Fe, Mn, Co, Zn, Hg, Pb, Cr, Ni and Cd procured from Merck, Germany, were prepared in the range of 1 to 10 ppm for accurate calibration. A calibration curve was generated by plotting concentration against measured absorbance in ppm.

Selection of Heavy Metals for the Study: The selection of heavy metals (Mn, Cr, Pb, Fe, Cd, Ni, Co, Zn and Hg) for this study was based on their environmental relevance, associated health risks and significant contributions to industrial and vehicular pollution. While metals such as iron (Fe), manganese (Mn), zinc (Zn) and cobalt (Co) are essential for plants and humans in trace amounts, excessive concentrations can be harmful. Others, including hexavalent chromium (Cr VI), mercury (Hg), lead (Pb) and cadmium (Cd), are non-essential and pose serious toxicological threats even at minimal exposure levels.

Given their ability to accumulate in medicinal plants, professional assessment of these metals is essential to prevent potential health hazards in humans. These metals accumulate in polluted environments due to emissions from industrial activities, vehicular traffic and urban pollution.

The combustion of fossil fuels, metal processing and battery waste contributes significantly to lead (Pb), cadmium (Cd) and mercury (Hg) contamination. Chromium (Cr) and nickel (Ni) pollution arise primarily from cement production, electroplating and industrial waste. Similarly, the natural levels of iron (Fe) and manganese (Mn) in soil increase substantially in industrial zones. Cobalt (Co) and zinc (Zn) enter the environment primarily through industrial waste, vehicle emissions and fertilizer application. Given the potential risks, strict quality control measures and regulatory oversight are necessary to ensure that medicinal herbs are safe for human consumption.

The ability of these heavy metals to bioaccumulate in plants is a critical factor in their selection for analysis, as they may subsequently enter the food chain. Prolonged exposure, typically over a year, to toxic heavy metals such as lead (Pb), mercury (Hg) and cadmium (Cd) may result in chronic poisoning, affecting the liver, kidneys and nervous system.

Mercury (Hg) is particularly neurotoxic, disrupting brain function, while lead (Pb) impairs both brain development and nervous system function. Cadmium (Cd) is associated with renal damage and increase the risk of osteoporosis. Additionally, hexavalent chromium (Cr VI) is a known carcinogen with severe long-term health implications. Given the widespread use of medicinal plants in traditional remedies, it is essential to ensure they are free from heavy metal contamination through rigorous monitoring and quality control.

Statistical analysis: The dataset was derived from triplicate measurements and analysed using One-way analysis of variance (ANOVA) to elucidate the influence of plant location on mineral content, as well as the inherent effect of the plant species on own mineral composition. Differences across locations and among plant specimens were assessed through the least significant difference (LSD) method at a 5% probability threshold ($P \leq 0.05$). Statistical analyses were performed utilizing SPSS-22 software.

Results and Discussion

Heavy metal concentration in soil samples: Table 2 presents the heavy metal concentrations (in ppm) in dry soil samples collected from various sites including the thermal power plant site, heavy traffic area, an industrial area and a brick klin. The analysed elements include manganese (Mn), chromium (Cr), lead (Pb), iron (Fe), cadmium (Cd), nickel (Ni), cobalt (Co), zinc (Zn) and mercury (Hg). Overall, the concentrations of heavy metals in soil samples obtained from the study sites were found to be low.

However, notable differences in metal levels were observed across locations. The highest lead (Pb) concentration was recorded in the heavy traffic area (8.1 ± 0.01 ppm) while the lowest was detected near the brick kiln (4.6 ± 0.06 ppm). The concentration of mercury (Hg) in all analysed soil samples was found to be below the detection limit (BDL).

Table 2
Heavy metal concentration in dry soil samples of all sites(ppm)

Site	Mn	Cr	Pb	Fe	Cd	Ni	Co	Zn	Hg
Thermal power plant	11.80 ±0.9	5.2 ±0.01	6 ±1.3	70.8 ±0.02	3.6 ±0.7	2.1 ±0.3	1.0 ±0.04	10.25± 0.2	BDL
Heavy traffic area	10.6 ±0.2	4.8 ±0.23	8.1 ±0.01	65.2 ±0.4	3.5 ±0.03	3.2 ±0.02	1.6 ±0.02	15.46±0. 32	BDL
Industrial area	10.82 ±0.5	7.9 ±0.4	5 ±0.2	50.4 ±1.2	3.4 ±0.4	2.0 ±0.09	1.5 ±0.013	7.42 ±0.5	BDL
Brick kiln area	8.70 ±0.4	3.5 ±0.2	4 ±0.06	38.6 ±0.05	2.0 ±0.36	1.70 ±0.01	1.7 ±0.01	6.11 ±0.15	BDL

*Data represents the mean of three replicates (n=3) ± standard error
* BDL=Below detection limit

The maximum cadmium (Cd) level (3.6 ± 0.7 ppm) was found at the thermal power plant, with lower concentrations observed near the brick kiln (2.0 ± 0.36 ppm). Iron (Fe) content was highest at the thermal power plant site (70.8 ± 0.02 ppm) and lowest near the brick kiln (38.6 ± 0.05 ppm), suggesting that power plants contribute significantly to Fe pollution. Among all sites, manganese (Mn) concentrations peaked at the thermal power plant (11.80 ± 0.9 ppm) and were lowest near the brick kiln (8.70 ± 0.4 ppm). Chromium (Cr) showed the highest concentration in the industrial area (7.9 ± 0.4 ppm) and the lowest near the brick kiln (3.5 ± 0.2 ppm).

Nickel (Ni) levels were slightly higher in the heavy traffic area (3.2 ± 0.02 ppm) compared to other locations. Cobalt (Co) concentrations remained relatively stable, ranging from 1.0 ± 0.04 ppm at the thermal power plant site to 1.7 ± 0.01 ppm near the brick kiln. Zinc (Zn) levels were highest in roadside soil (15.46 ± 0.32 ppm) and lowest in samples collected near the brick kiln (6.11 ± 0.15 ppm). The findings indicate that the concentration of cadmium (Cd), Iron (Fe) and manganese (Mn) in soil observed at thermal power plant may be a result of atmospheric emission via the stack, including volatized elements and fly ash and their deposition on nearby land and water bodies.

Table 3
Heavy metal concentration in medicinal plants collected from all sites(ppm)

Sites	Medicinal plants	Mn	Cr	Fe	Ni	Co	Zn
Thermal power plant	*Calotropis procera*	5.42 ±0.140	1.56 ±0.020	24.09 ±0.064	0.47 ±0.024	0.02 ±0.0031	2.75 ±0.217
	Euphorbia hirta	2.84 ±0.017	1.42 ±0.037	16.01 ±0.032	0.64 ±0.014	0.12 ±0.0033	6.42 ±0.122
	Achyranthes aspera	3.52 ±0.014	0.68 ±0.0202	12.42 ±0.026	0.26 ±0.005	0.04 ±0.0122	4.52 ±0.080
	Cynodon dactylon	4.85 ±0.021	1.82 ±0.0185	33.56 ±0.011	0.39 ±0.017	0.23 ±0.029	5.17 ±0.073
	Argemone mexicana	4.78 ±0.017	1.73 ±0.0240	10.05 ±0.035	1.08 ±0.057	0.14 ±0.058	8.93 ±0.02
Heavy traffic area	*Calotropis procera*	3.67 ±0.021	0.58 ±0.031	41.35 ±0.020	0.55 ±0.015	0.05 ±0.07	7.59 ±0.011
	Euphorbia hirta	2.39 ±0.012	1.12 ±0.020	32.39 ±0.035	0.16 ±0.075	0.20 ±0.029	5.13 ±0.026
	Achyranthes aspera	5.83 ±0.142	0.72 ±0.0371	13.64 ±0.063	0.47 ±0.029	0.14 ±0.075	6.46 ±0.054
	Cynodon dactylon	4.73 ±0.0240	1.51 ±0.173	17.48 ±0.029	0.83 ±0.019	0.23 ±0.0145	5.32 ±0.317
	Argemone mexicana	3.18 ±0.0180	1.64 ±0.0480	28.41 ±0.049	0.52 ±0.0348	0.07 ±0.0173	3.61 ±0.736
Industrial area	*Calotropis procera*	4.32 ±0.018	1.87 ±0.048	15.19 ±0.0317	0.06 ±0.0223	0.03 ±0.0264	3.09 ±0.0305
	Euphorbia hirta	2.18 ±0.012	2.62 ±0.044	11.42 ±0.0176	1.13 ±0.019	0.15 ±0.0134	2.25 ±0.0907
	Achyranthes aspera	6.41 ±0.0176	2.84 ±0.058	13.19 ±0.392	0.57 ±0.037	0.40 ±0.0200	3.10 ±0.0173
	Cynodon dactylon	5.70 ±0.038	3.56 ±0.808	17.89 ±0.069	0.24 ±0.017	0.18 ±0.115	4.58 ±0.0343
	Argemone mexicana	3.54 ±0.015	2.80 ±0.0260	12.18 ±0.0180	0.72 ±0.0120	0.21 ±0.0284	3.40 ±0.014
Brick kiln	*Calotropis procera*	2.60 ±0.013	0.65 ±0.024	25.40 ±0.0633	0.69 ±0.026	0.20 ±0.0193	2.25 ±0.030
	Euphorbia hirta	6.34 ±0.026	0.46 ±0.0212	18.30 ±0.0317	0.51 ±0.019	0.17 ±0.025	1.89 ±0.023
	Achyranthes aspera	2.26 ±0.011	1.07 ±0.0231	20.18 ±0.1308	0.87 ±0.082	0.05 ±0.034	3.25 ±0.021
	Cynodon dactylon	4.60 ±0.020	0.35 ±0.0472	16.50 ±0.2334	0.41 ±0.009	0.10 ±0.0112	4.18 ±0.014
	Argemone mexicana	2.34 ±0.012	0.89 ±0.0711	22.25 ±0.146	0.12 ±0.0021	0.16 ±0.0025	2.56 ±0.017

*Data represents the mean of three replicates (n=3) ± standard error

Table 4
Concentration of some toxic heavy metals in studied medicinal plants

Sites	Medicinal plants	Pb	Cd	Hg
Thermal power plant	*Calotropis procera*	2.71±0.121	0.01±0.03	BDL
	Euphorbia hirta	0.66±0.0115	0.03± 0.0012	BDL
	Achyranthes aspera	3.26±0.038	BDL	BDL
	Cynodon dactylon	2.52±0.02	0.02± 0.0011	BDL
	Argemone mexicana	1.27±0.0185	0.01± 0.0004	BDL
Heavy traffic area	*Calotropis procera*	8.34±0.0102	BDL	BDL
	Euphorbia hirta	3.07±0.054	0.1± 0.0012	BDL
	Achyranthes aspera	7.97± 0.031	0.02± 0.031	BDL
	Cynodon dactylon	5.62± 0.026	BDL	BDL
	Argemone mexicana	4.68± 0.0122	0.2± 0.012	BDL
Industrial area	*Calotropis procera*	2.21± 0.024	0.03± 0.014	BDL
	Euphorbia hirta	4.54± 0.012	0.02± 0.012	BDL
	Achyranthes aspera	3.48± 0.088	BDL	BDL
	Cynodon dactylon	2.32± 0.036	0.02± 0.004	BDL
	Argemone mexicana	5.81± 0.014	0.01± 0.0002	BDL
Brick kiln	*Calotropis procera*	1.45± 0.026	0.02± 0.001	BDL
	Euphorbia hirta	2.05± 0.030	BDL	BDL
	Achyranthes aspera	1.61± 0.036	0.02± 0.017	BDL
	Cynodon dactylon	1.84± 0.025	0.01± 0.019	BDL
	Argemone mexicana	1.19± 0.0135	BDL	BDL

***Pb** = Lead (ppm) ***Cd** = Cadmium (ppm) ***Hg** = Mercury (ppm) ***BDL** = Below Detection Limit

Agarwal had reported soil contamination with heavy metal from four major thermal power plant of North India. The soil was analysed for the presence of Cd, Ni, Pb and As and all were found in detectable level[2].

The concentration of mercury (Hg) is not detected in soil samples. Chromium (Cr) concentration varied among all sites with the highest level in the industrial area. This could be due to industrial and agricultural runoff. Various cadmium compounds are used in the chemical industry and in the production of pesticides and herbicides utilized in agricultural practices[19].

Nickel (Ni) and cobalt (Co) concentrations exhibited minor variations across sites. Nickel's behaviour is frequently indicative of its linkage to anthropogenic sources, as substantiated by previous studies. A study has determined that airborne particles generated from vehicle brake wear and tire abrasion contain significant quantities of nickel (Ni). Slight variations in cobalt (Co) concentrations suggest relatively consistent behaviour across the analysed samples. The mobility and distribution of cobalt are governed by several factors, including soil properties and redox conditions[44].

The high concentration of Pb in the Heavy traffic area (Table 2) depicts that environment is polluted with vehicular emission and fuel combustion because of heavy traffic on the road. Lead has toxicological properties, is prevalent in many electronic devices. It serves as a primary component in lead-acid batteries, widely utilized in automotive applications including car batteries and tires. Through processes such as corrosion, lead from these sources can contaminate soil, posing environmental and health risks. Zinc displays high concentration in heavy traffic area. Zinc (Zn) is incorporated into brake linings due to its effective heat-conducting properties. Consequently, it is released into the environment through mechanical abrasion of vehicle components, combustion of engine oil and wear of motor vehicle tires[14,31,46].

The concentrations of Mn, Pb, Zn and Fe in the soil were comparatively higher than those of other heavy metals. The variation in heavy metal concentrations across different sites can be attributed to localized industrial activities, automobile emissions and combustion byproducts.

The elevated levels of specific metals, such as Fe at thermal power plant sites and Cr in the industrial area, highlight potential contamination risks. If these metals become bioavailable, they could pose environmental and health hazards.

Heavy metal concentration in medicinal plants: Table 3 and table 4 present the heavy metals concentration detected in five medicinal plants species: *Calotropis procera, Euphorbia hirta, Achyranthes aspera, Cynodon dactylon and Argemone mexicana*, each collected from all four sites (Heavy Traffic area, Thermal Power Plant, Industrial area, Brick kiln). The heavy metals determined in the plant samples were identical to those analyzed in the soil.

Manganese (Mn): The study observed significant variation in manganese (Mn) concentrations, with the highest content found in *Calotropis procera* (5.42 ppm) and the lowest in *Euphorbia hirta* (2.84 ppm) at the thermal power plant site. The highest Mn concentration among all sites was recorded in *Achyranthes aspera* (6.41 ppm) from the industrial area (Table 3).

Mercury (Hg): The concentration of mercury (Hg) in all collected plant samples from all investigated sites was observed below the detection limit (BDL).

Lead (Pb):

- The highest lead (Pb) concentration was detected in *Calotropis procera* (8.34 ± 0.0102 ppm) from heavy traffic areas. Barthwal et al[6] also investigated the various heavy metals in medicinal plants and found the highest lead (Pb) concentration in the root of *Calotropis procera* collected from traffic areas.
- The lowest concentration was found in *Euphorbia hirta* (0.66 ± 0.030 ppm) from the thermal power plant site.
- Pb levels were relatively higher in plants collected from heavy traffic area and industrial area, likely due to heavy vehicular emissions and industrial pollution (Fig. 2).

Cadmium (Cd): Cadmium (Cd), a toxic heavy metal, can significantly reduce plant productivity, with concentrations ranging from 5 to 30 mg/kg[24]. In this study, Cd concentrations in medicinal plants ranged from:

- ± 0.0012 ppm (maximum) to 0.01 ± 0.0003 ppm (minimum).

Nickel (Ni) and Cobalt (Co):

- Nickel (Ni) concentrations varied from 0.06 ± 0.0223 ppm to 1.13 ± 0.019 ppm.
- Cobalt (Co) concentrations ranged from 0.02 ± 0.0081 ppm to 0.40 ± 0.02 ppm.

Zinc (Zn):

- The highest zinc (Zn) concentration was detected in *Argemone mexicana* (8.93 ± 0.02 ppm) from thermal power plant followed by *Calotropis procera* (7.59 ± 0.011 ppm) from site heavy traffic area.
- The lowest Zn concentration was recorded in *Euphorbia hirta* (1.89 ± 0.023 ppm) from the brick kiln area.

Iron (Fe):

- Iron (Fe) exhibited highest concentration among all heavy metals and showed variability in the range of (10.05 ±0.035 to 41.35 ±0.020ppm).
- The highest iron (Fe) concentration was recorded in *Calotropis procera* samples from the heavy traffic area whereas the lowest Fe concentration was observed in *Argemone mexicana* samples from the thermal power plant site. A comparable pattern of Fe concentration was noted in stem samples of *Acacia nilotica* and leaf samples of *Withania somnifera* collected from Jhunjhunu and Bahadurgarh, consistent with findings reported by Kulhari et al[24].

Chromium (Cr):

- The highest Cr concentration (3.56 ± 0.808 ppm) was found in *Cynodon dactylon* (industrial area),
- The lowest Cr concentration (0.35 ± 0.0472 ppm) was recorded in *Cynodon dactylon* (brick kiln).

Identifying the Most Polluted Site: To identify the most polluted area, we calculated the total heavy metal concentration (THMC) for each site by summing the concentrations of all metals present.

- The site with the highest THMC value is considered the most polluted (Table 5)
- Among all the sites, the heavy traffic area displays the highest level of pollution, which is reflected in its elevated THMC value.

In medicinal plants, the highest THMC values were found in traffic areas and hence the most polluted sites and were observed in the order: HTA> TPP > BK> IA.

Statistical validation: This study represents the comparative analysis of plant location to the heavy metal accumulated in medicinal plants. The data were subjected to statistical analysis by using One-way Analysis of Variance (ANOVA). The result showed that there was a significant difference observed in heavy metal concentration and location of the plant species ($p < 0.05$).

Table 5
Total heavy metal concentration (THMC) for each site

Site	Concentration (ppm)
Heavy traffic area	50.205714
Thermal power plant	38.022857
Brick kiln	34.226286
Industrial area	32.955429

The World Health Organization[49] (WHO) reports that approximately 80% of the population in developing countries relies on plant-based therapeutics for their primary healthcare needs. A significant proportion of these resources is harvested from wild populations; only a few are cultivated. There are various nutritional minerals accumulated in the plants, but not all are beneficial for human health; some are detrimental such as Pb, Cd, As, Hg, Co etc. The accumulation of these plants is highly dependent upon their availability in the soil[3].

The dataset provides a detailed analysis of heavy metal concentrations in five medicinal plant species collected from all four sites of northern region of India: *Calotropis procera, Euphorbia hirta, Achyranthes aspera, Cynodon dactylon* and *Argemone Mexicana.* An analysis of metal concentrations in medicinal plants indicated a wide distribution range for iron (Fe), lead (Pb), zinc (Zn) and manganese (Mn). In contrast, narrower distribution ranges were observed for chromium (Cr), cobalt (Co), cadmium (Cd) and nickel (Ni).

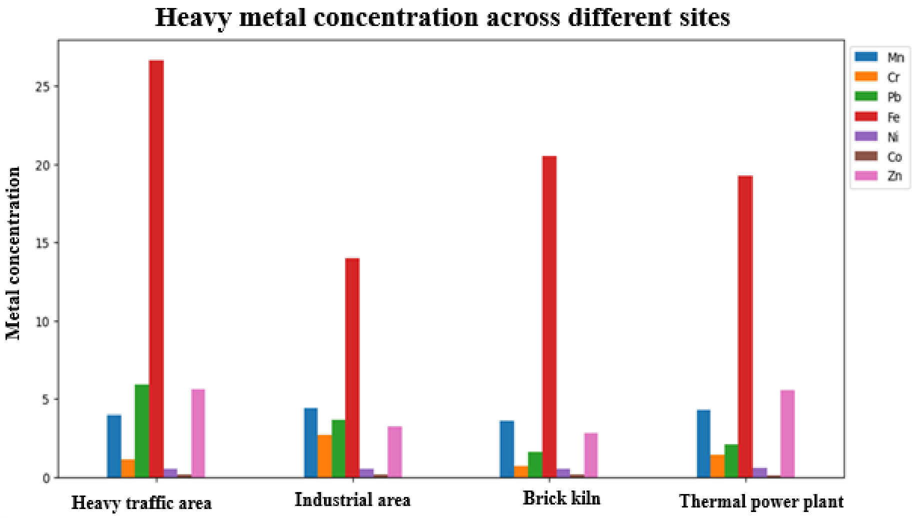


Figure 2: Heavy metal concentration (ppm) in medicinal plants and their comparison across all locations. Our study reveals that the plant sample collected from heavy traffic area having comparatively high metal concentration indicated the most polluted site

The mean concentrations of elements (Mn, Cd, Cr, Fe, Co, Ni, Pb, Hg and Zn) in plants showed the highest values in heavy traffic area whereas the lowest were found in the industrial area. The maximum amount of Mn is recorded in *Calotropis procera* from the industrial area (Table 3). The highest mean concentration of manganese (Mn) was observed in plants located within industrial area. Among the species examined, *Achyranthes aspera* exhibited the greatest bioaccumulation of Mn. The primary sources of manganese in soil are fertilizers, sewage sludge and ferrous smelters[21]. Manganese is a critical trace element that serves as a cofactor in a variety of enzymatic reactions. In comparison to other heavy metals, it exhibits relatively lower toxicity, prolonged exposure to Mn dust and fumes at concentrations above 5 mg/m³ and may result in neurological impairments[24].

The FAO/WHO has set a permissible manganese limit of 2 ppm for edible plants, whereas the WHO has not defined a specific limit for medicinal plants. Lead and cadmium are considered as significant heavy metal pollutant. Prolonged exposure to lead (Pb) is associated with deleterious effects on liver, lung and renal systems. Similarly, elevated exposure to mercury (Hg) in its metallic, inorganic, or organic forms may lead to permanent neurological damage and compromised renal function. Cadmium (Cd) exposure, both acute and chronic, induces toxicological effects, adversely impacting liver and renal tissues, as well as the vascular and immune systems[12,13,20,40].

Although the World Health Organization (WHO) has not established specific permissible limits for mercury (Hg) concentration in raw herbal materials, various national regulatory frameworks have defined acceptable permissible limits. These include: a limit of 1 ppm for Hg in India, as stipulated by the AYUSH (India) guidelines; and limits of 0.5 ppm and 0.2 ppm for Hg in China and Canada respectively[38]. In the current study, lead (Pb) concentrations ranged from (0.66±0.0115ppm) to (8.34± 0.0102ppm) in thermal power plant and heavy traffic area respectively. Results show that cadmium in most of the plants examined is either low or BDL; however, *Argemone mexicana* contained 0.2± 0.012 ppm and 0.01 ± 0.0012 ppm in heavy traffic area and thermal power plant respectively. In the analysed samples, mercury (Hg) concentrations were consistently below the detection limit. According to the World Health Organization (WHO) and the Department of AYUSH (India), the permissible limits for cadmium (Cd) and lead (Pb) in raw herbal materials are 0.3 ppm and 10 ppm respectively. All measured concentrations of Pb and Cd in the samples complied with WHO standards. Medicinal plants collected from areas with heavy traffic density exhibited the greatest accumulation of lead and cadmium. This may be due to its buildup in the soil from fertilizer use, fuel combustion, contamination from vehicular activities and flue gas emissions containing these toxic elements[36].

The main contributors to cadmium (Cd) accumulation in soil and plant systems encompass the application of phosphate fertilizers, emissions from non-ferrous smelters, activities associated with lead and zinc mining, the usage of sewage sludge and the combustion of fossil fuels. Previous studies have quantified trace elements Ca, Mg, Na and heavy metals Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn in *Solanum nigrum* and *Withania somnifera* samples from industrial zones in Islamabad, Pakistan and Indian herbal markets, reporting similarly elevated Cd levels ranging from 0.65 - 0.97 mg kg^{-1} [34]. These findings indicate that industrial and vehicular activities significantly contribute to accumulation of heavy metals in medicinal plants, necessitating continuous monitoring to ensure their safety for medicinal use.

The trace presence of Ni and Co indicates relatively low contamination by these specific metals. Nickel (Ni) and cobalt (Co) are vital trace minerals, required in minute quantities for physiological functioning in the human body. Nickel is directly bound by proteins and is integral to the process of insulin synthesis within the pancreas. Cobalt, on the other hand, is another vital mineral required in minute amounts as a critical constituent of vitamin B12 (cobalamin), playing a crucial role in erythropoiesis (red blood cell formation). Both elements are required in micromolar concentrations for optimal homeostasis[38,43,50]. The lowest concentration of Ni (0.06 ± 0.0223 ppm) was found in *C. procera* and the highest was found to be (1.13 ± 0.019 ppm) *in E. hirta* collected from industrial area.

Cobalt levels in raw herbal samples varied from 0.02 ± 0.0031 ppm in *Calotropis procera* sourced from a thermal power plant to 0.40 ± 0.02 ppm in *Achyranthes aspera* obtained from an industrial area. The FAO/WHO have not established a specific permissible limit for Ni in medicinal plants. However, they have set a maximum allowable concentration of 1.63 ppm for nickel in edible plants[38]. The mean concentrations of elements, particularly Ni, in plants, were highest at the thermal power plant, while the lowest concentrations were found in the heavy traffic area. Similarly, Co showed the highest mean concentration in the industrial area. The concentration of heavy metal exhibited variation across different plant species collected from same site.

In this study, zinc (Zn) was detected across all analyzed plant samples, with concentrations ranging from 1.89 ± 0.023 ppm to 8.93 ± 0.02 ppm. Elevated concentrations were observed for *A. Mexicana* of thermal power plant, *C. procera* and *A. aspera* in heavy traffic area. Zinc is a critical micronutrient, serving as a cofactor in various enzymatic processes and playing an essential role in cellular metabolic processes, immune function, neurological development, behavioral responses, tissue repair and bone formation[33]. The mean concentrations of Zn in plants showed the highest values in the heavy traffic area. Zinc, identified as the predominant traffic-emitted element in roadside environments, exhibits a strong correlation with traffic areas (HM concentration in Hamedan, Iran). These concentrations remained within the permissible limit of 27.4 ppm for edible plants.

The iron content in all plant species examined was found relatively high. As a vital trace element, it plays role in various metabolic pathways involving proteins, lipids and carbohydrates and usually, its dietary deficiencies are observed[30]. The highest mean Fe concentrations were observed in heavy traffic areas compared to other sites, with *Calotropis procera* (41.35 ± 0.020 ppm) and *Argemone mexicana* (10.05 ± 0.035 ppm) from HTA and TPP respectively, exhibiting the highest levels (Fig. 2).

Its biological significance is particularly evident in oxygen transport, where it forms the active center of hemoglobin, the primary oxygen-carrying metalloprotein in living organism. Toxicity occurs when ingestion exceeds 20 mg/kg, causing gastrointestinal toxicity[41]. The maximum permissible limit of iron is 1000µg/day given by Annan et al[3]. However, all iron concentration was below the permissible limit. However, the WHO has not established limits for Fe yet[23].

As the result showed an elevated concentration of chromium (Cr) in plants collected from industrial area due to various industrial activities like processing, smelting of heavy metal, fast urbanisation. Comparative analyses with prior studies reveal similar trends, with investigations of heavy metal bioaccumulation in medicinal plants from the Khetri copper mines and Haridwar reporting copper (Cu) levels three to four times higher in mining areas compared to uncontaminated regions. Medicinal plants in Turkey had significantly higher Pb, Cd and Cr levels in plants growing near industrial zones. The variation in results may be attributed to differences in analytical methods e.g. ICP-OES is a more sensitive technique than AAS.

The heatmap effectively delineates the varying degrees of heavy metal accumulation across the investigated plant sites. Sites associated with darker colours, such as red, exhibit strong correlations with elevated heavy metal concentrations, indicating significant pollution. In contrast, lighter-coloured sites reflect lower contamination levels. This visualization enables precise identification of the most polluted sites, thereby guiding environmental management priorities.

Heatmap analysis showed that darker colour indicated the elevated concentrations of iron (Fe), lead (Pb), chromium (Cr), nickel (Ni) and cobalt (Co), exhibiting a strong positive correlation with high-traffic area. It was suggested that these areas are most contaminated sites. Iron (Fe) showed the darkest colour to a heavy traffic area, compared to other heavy metals. This shows its high interaction with that area and also shows its pollution in that area. (Fig. 3)

This study aimed to identify and spread awareness about the identification of suitable raw material sources for the production of pharmaceutical products because people are not aware of the fact that plants collected by them from random sites might be contaminated which can adversely affect the quality of medicine being produced. This study highlights significant variations in heavy metal accumulation among medicinal plants. While Fe and Zn show elevated concentrations in specific plants, Cd and Hg remain low or undetectable. Pb and Cd concentrations were found highest in heavy traffic area because of heavy vehicular activity.

The patterns suggest that plants absorb metals based on soil contamination levels, raising concerns about their safety for medicinal use. Ni and Co mean concentrations were found highest in thermal power plant and industrial area due to ash deposition on nearby soil and vegetation (Fig. 2). These findings underscore the importance of monitoring medicinal plant contamination, as heavy metals in herbal remedies could pose health risks to consumers.

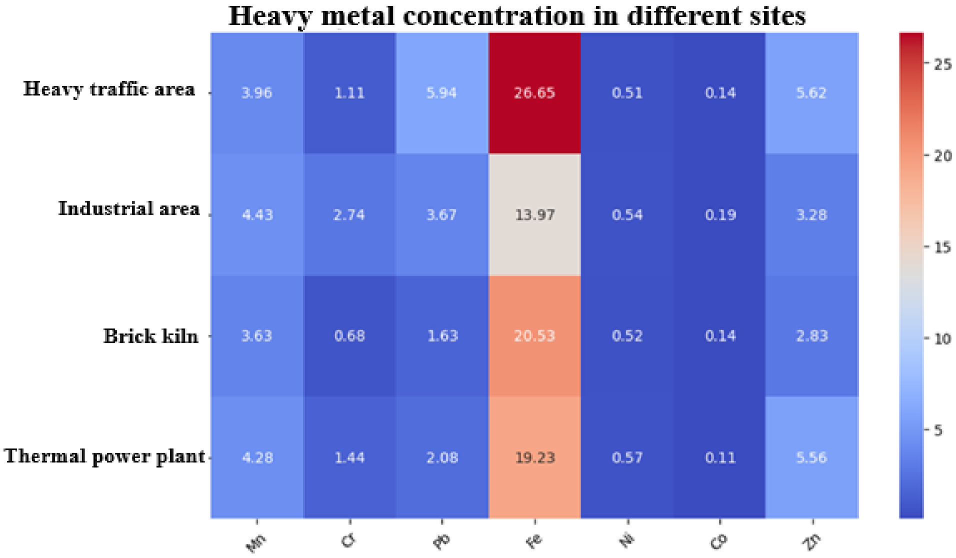


Figure 3: Heatmap showing the correlation between plant site and heavy metal accumulation. Darker colour indicates higher metal concentrations, highlighting polluted sites. The dark colour of Fe, Pb and Zn in heavy traffic area indicates the most polluted site

Conclusion

This study's comparative analysis of environmental pollution across multiple sites identified heavy traffic areas as the most contaminated, with significantly higher concentrations of elemental pollutants compared to other locations, establishing these zones as the most polluted. An evaluation of heavy metal accumulation across various plant species revealed that *Calotropis procera* exhibited the highest capacity for heavy metal uptake.

WHO establishes regulatory limits for permissible concentrations of toxic metals, like 1 ppm for arsenic, 0.3 ppm for cadmium and 10 ppm for lead[48]. Although the concentrations of all heavy metals in analysed medicinal plants are below permissible limits and all these can be good source for making herbal formulations. Since the concentrations of Cd and Pb are found more in traffic area and Cr in industrial area, these can be harmful if we consume them regularly, so it is necessary to implement rigorous quality control measures for plant-derived raw materials. The heat map shows that there is significant difference between metal content and area of plant species collection, similarly, there is significant difference in different species when compared with same heavy metal content($p \leq 0.05$).

This study concludes that it is necessary to raise awareness regarding the preventive collection of medicinal herbs from non-cultivated and alternative sources, which are susceptible to contamination by heavy metals. Thus, regular and systematic assessments are vital for quality control and herbal drug safety.

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