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Original Article

Estimation of Trace and Toxic Metals from Cannabis Sativa Plant in District, Kech Baluchistan, Pakistan

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ABSTRACT

Background: Cannabis sativa, a plant indigenous to the Kech district in Baluchistan, Pakistan, is traditionally used for medicinal purposes. Understanding its trace and toxic metal content is crucial for evaluating its safety and therapeutic potential.

Objective: The primary aim of this study is to estimate the levels of trace and toxic metals in various parts of Cannabis sativa plants grown in the Kech district and assess the potential health risks associated with their consumption.

Methods: The leaves, stems, and roots of Cannabis sativa were analyzed for metals including Iron (Fe), Copper (Cu), Zinc (Zn), Potassium (K), Magnesium (Mg), Calcium (Ca), Sodium (Na), Cadmium (Cd), Lead (Pb), and Nickel (Co). Flame Atomic Absorption Spectroscopy was employed to quantify the metal content.

Results: The plant exhibited high levels of Fe (20.34 ppm in leaves), Zn (24.14 ppm in leaves), Ca (9.29 ppm in leaves), Cu (14.40 ppm in leaves), K (10.56 ppm in leaves), Mg (13.47 ppm in leaves), and Na (12.25 ppm in leaves). Conversely, lower concentrations of Cd (0.65 ppm in leaves), Co (2.29 ppm in leaves), As (0.53 ppm in leaves), and Pb (1.60 ppm in leaves) were detected. The leaves contained higher concentrations of these metals compared to the stems and roots.

Conclusion: The presence of beneficial trace metals like Fe, Cu, Zn, and Ca in significant amounts suggests Cannabis sativa's potential for treating diseases related to deficiencies of these metals. However, the low levels of toxic metals indicate minimal health risks, affirming its safety for medicinal use.

Keywords: Cannabis sativa, Flame Atomic Absorption Spectroscopy, Medicinal Plant, Trace and Toxic Metals.

INTRODUCTION

Plants, the cornerstone of our environment, have long been esteemed as vital sources of therapeutic agents. In the realm of traditional medicine, they stand as foundational elements, with nearly 80% of the global populace, predominantly in developing nations, depending on plant-based treatments for healthcare needs (WHO). This reliance underscores the integral role of medicinal plants in meeting basic health requirements in these regions. Pakistan, with its rich biodiversity, reports about 6,000 species of sophisticated plants, of which 12% are recognized for their medicinal virtues. These plants are repositories of active chemical constituents like phytochemicals, minerals, and vitamins, essential for various bodily functions including nervous system operation, enzyme activation, and fluid regulation, thanks to elements like calcium, magnesium, zinc, manganese, chromium, sodium, and potassium (3,4,5).

In recent decades, therapeutic plants have gained attention in pharmacological studies, being potential sources of novel phytochemicals with beneficial properties and paving the way for drug development. The traditional system of medicine, hence, continues to be indispensable across societies for its crucial role in healthcare (6,7). Cannabis sativa, a notable herbal plant grown worldwide and belonging to the Cannabaceae family, exemplifies this. Used in various regions for its oil and medicinal properties, Cannabis sativa has a complex mixture of compounds, including cannabinoids, terpenoids, nitrogenous compounds, non-cannabinoids, phenols, flavonoids, alkaloids, and steroids. Notable among these are psychoactive compounds such as tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBND) (9,10).

The Cannabis genus encompasses species like C. Sativa, C. indica, and C. ruderalis, found across Russia, China, India, Iran, and Pakistan. In Pakistan, where it's commonly known as 'Bhang' in local parlance, its cultivation is relatively limited, yet it naturally

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proliferates in regions like Sialkot, Layyah, Mianwali, Muzafargarh, D. G Khan Islamabad, Quetta, Kalat, and the Mekran Division (11,12,13). C. sativa, particularly, is rich in cannabinoids, with about 104 identified types, making it a focus of interest. Its seeds are nutritionally rich, containing carbohydrates, proteins, insoluble fiber, and a variety of vitamins and minerals like potassium, phosphorus, sulfur, calcium, magnesium, iron, and zinc, along with vitamins B6, B1, B2, B3, D, E, and C (14,15).

Historically, C. sativa extracts and tinctures have been used for a range of ailments including pain, whooping cough, asthma, and as a sedative/hypnotic agent. Modern applications include treatment for glaucoma, AIDS, eye problems, muscle spasticity, insomnia, convulsions, depression, hypertension, and pain management. Significantly, cannabinoids from C. sativa have also been shown to inhibit and prevent cancer cell growth (16, 18). The plant's history is deeply intertwined with human civilization, with its use dating back to ancient cultures in Africa, Asia, China, India, Egypt, and the Arab world, where it served as a medicinal herb, painkiller, and antiseptic (19,20,21,22).

The current study aims to explore and identify the trace and toxic metals present in Cannabis sativa, further substantiating its application in traditional medicine. This endeavor not only seeks to understand its pharmacological potential but also to authenticate its usage as a traditional drug, thus bridging the gap between historical practices and modern medical research. Through this exploration, the study contributes to the broader understanding of C. sativa's role in healthcare, particularly in contexts where traditional medicine remains a primary healthcare resource.

MATERIAL AND METHOD

In the conducted study, fresh samples of Cannabis sativa, comprising leaves, stems, and roots, were meticulously collected. A total of 5000 grams of these samples were gathered using metal-free, washed plastic containers to prevent any potential contamination, ensuring the purity of the sample. The collection took place in a cultivated area, specifically chosen for its relevance to the study. Post-collection, the samples underwent a drying process. This was carried out by exposing them to sunlight for three days on flat, white plastic materials, which were previously screened to be metal-free, further emphasizing the precautionary measures taken to maintain sample integrity.

Once dried, the samples were transferred back into clean plastic containers and subsequently stored in a refrigerator to preserve their quality. For the preparation of the samples for analysis, each component – leaves, stems, and roots – weighing 5000 grams, was finely crushed into a smooth consistency using a mortar and pestle. This process was crucial for ensuring uniformity in the sample size and texture, which is essential for accurate analysis.

The crushed samples were then placed in digestion flasks. An acid mixture, comprising equal ratios of nitric acid (HNO3), perchloric acid (HCCO4), and hydrofluoric acid (HF), was added to each flask. This mixture was subject to constant stirring to ensure even distribution and effective digestion of the plant material. The samples on hot plates underwent a preliminary heating phase, followed by an overnight placement in a fume cupboard, facilitating a thorough digestion process.

Upon cooling, the digests were meticulously filtered and subsequently made up to mark in 100ml volumetric flasks using deionized water, a step critical for achieving the desired concentration for analysis. The prepared sample solutions were then returned to the refrigerator in the laboratory, where they were stored until the time of analysis. The final stage involved the use of Atomic Absorption Spectrophotometers (AAS) for the precise determination of trace and toxic metals in the samples, a method renowned for its accuracy and reliability in such analyses (23). This comprehensive methodological approach ensured the integrity and accuracy of the data collected, paving the way for insightful findings in the study.

RESULTS

The results obtained from the analysis of cannabis sativa leaves, stem and roots are presented in table 1, 2.

Table 1 Level of Trace-Metals in Three parts of Cannabis sativa Plants

Trace-Metals (ppm)	Leaves	Stem	Roots
Ca	9.29	9.13	8.75
Fe	20.34	12.34	18.20
K	10.56	10.23	7.55
Mg	13.47	13.32	7.56
Na	12.25	11.32	3.43
Zn	24.14	12.46	22.78
Cu	14.40	8.77	12.56
Co	2.29	1.85	1.16

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Table 1 presents the levels of various trace metals measured in parts per million (ppm) within three different components of the Cannabis sativa plant: the leaves, stem, and roots. The metals analyzed include Calcium (Ca), Iron (Fe), Potassium (K), Magnesium (Mg), Sodium (Na), Zinc (Zn), Copper (Cu), and Cobalt (Co). The results indicate varying concentrations of these metals across the different parts of the plant. For instance, Calcium levels are relatively similar in leaves and stems but slightly lower in roots. Iron shows a higher concentration in leaves and roots compared to the stem. Potassium and Magnesium levels are also notably higher in the leaves and stem than in the roots. Sodium shows a significant decrease in concentration in the roots. Zinc has the highest concentration in the leaves, with a notable decrease in the stem and a similar level in the roots as in the leaves. Copper is found in the highest concentration in the leaves, with lower levels in the stem and roots. Cobalt, the least concentrated of the measured metals, follows a decreasing trend from leaves to roots. This table provides valuable insights into the distribution of trace metals within different parts of the Cannabis sativa plant, which is essential for understanding its medicinal properties and potential health implications.

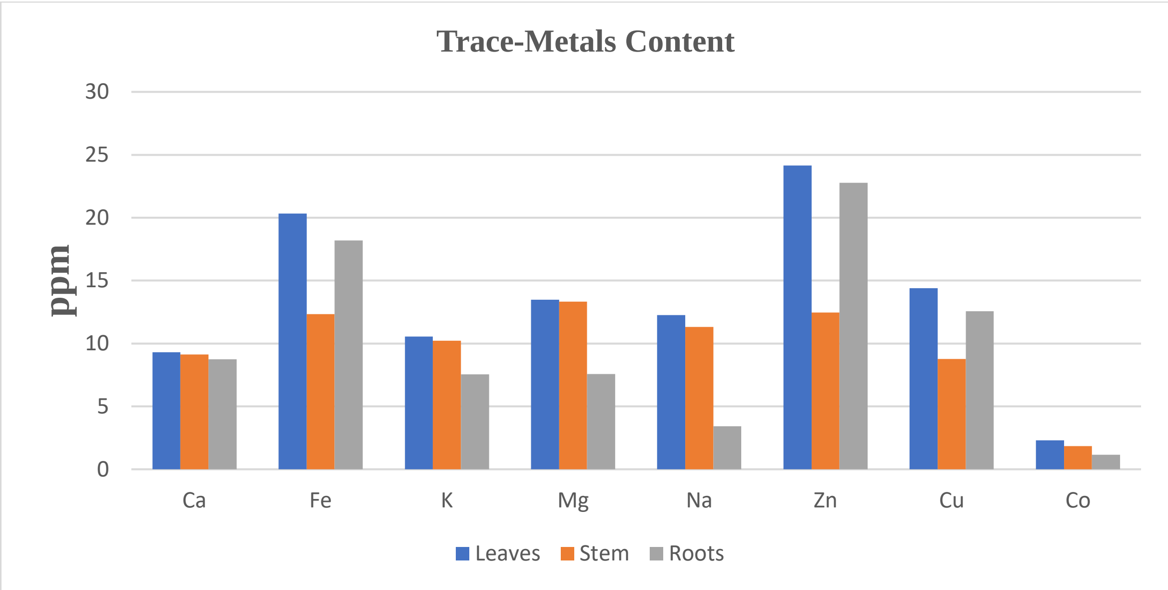

Figure 1 Level of Trace-Metals in Three Parts of Plant (Cannabis sativa)

Table 2 Level of Toxic Metals in Three Parts of Cannabis sativa Plant

Elements (ppm)	Leaves	Stem	Roots
Lead	1.60	1.26	1.21
Cadmium	0.65	0.63	0.24
Chromium	6.65	3.46	1.18
Arsenic	0.53	0.22	0.21

Table 2 illustrates the concentration of toxic metals in different parts of the Cannabis sativa plant, specifically the leaves, stem, and roots, with values given in parts per million (ppm). The toxic elements analyzed include Lead, Cadmium, Chromium, and Arsenic. The data shows that Lead has the highest concentration in the leaves, with slightly lower levels in the stem and roots. Cadmium is present in all three parts, but its concentration is notably lower in the roots. Chromium exhibits a significant decrease from leaves to roots, with the highest concentration in the leaves and the lowest in the roots. Arsenic, although present in all parts, is found in the smallest amounts compared to the other metals, showing a similar trend of decreasing concentration from leaves to roots. This table provides important insights into the distribution and levels of toxic metals in Cannabis sativa, which is crucial for assessing its safety and potential health risks associated with its use.

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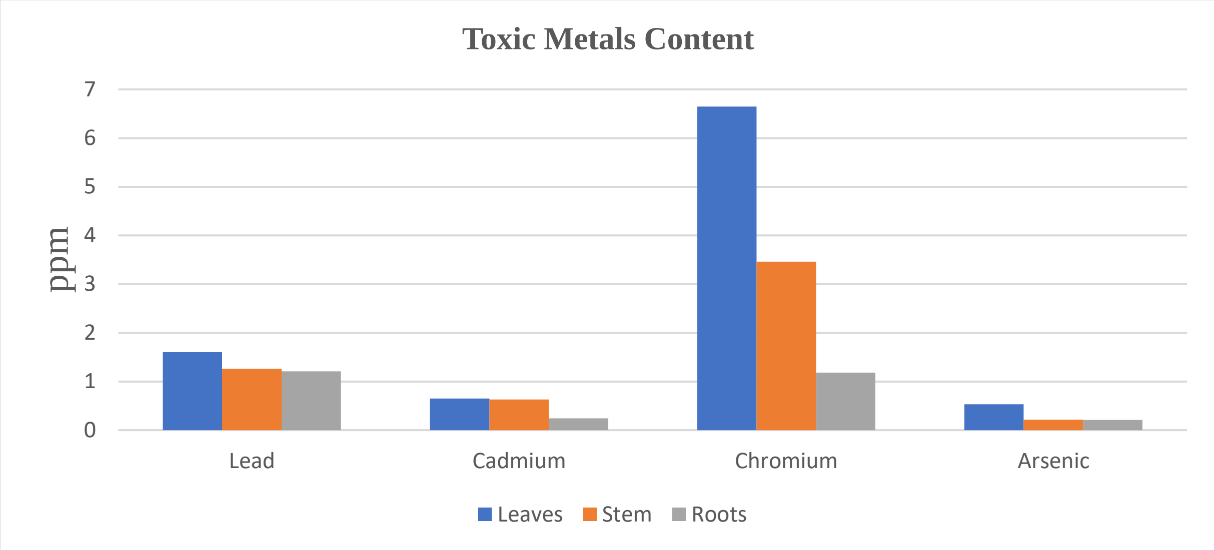

Figure 2 Level of toxic Metal in Three Parts of Plant (Cannabis Sativa)

Level of calcium was the highest in leaves of the plant (9.29 ppm) and the lowest content was observed in stem of the plant (8.75ppm).

DISCUSSION

In this study, the analysis of trace and toxic metals in Cannabis sativa revealed significant findings pertinent to medical and environmental sciences. The presence of calcium in considerable amounts across all plant parts, especially in leaves and stems, highlights its potential role in treating bone-related ailments such as rheumatism and osteoporosis. Calcium's pivotal role in bone health, nerve signal transmission, muscle function, hormone release, heartbeat regulation, and blood clotting were well-documented (24), suggesting the plant's efficacy in these areas.

Iron, predominantly concentrated in the leaves, suggests the plant's utility in addressing iron deficiency and anemia, particularly in rural populations where such deficiencies are prevalent. The high iron content in leaves (20.34ppm) aligns with traditional uses of Cannabis sativa in treating anemia (24). Potassium, vital for membrane potential regulation, signal transduction, insulin secretion, and immune responses, was found in higher concentrations in the leaves and lower in the stems. This disparity in distribution may influence the plant's effectiveness in treating potassium deficiency disorders.

The study also noted magnesium's highest concentration in leaves and lowest in roots. Given magnesium's crucial role in muscle function, energy production, and protein synthesis, its deficiency leading to symptoms like anorexia, fatigue, and memory issues (25), the plant's leaves may offer therapeutic benefits. Sodium, essential for metabolite transport in cells, showed higher levels in leaves, indicating potential utility in addressing sodium deficiency-related conditions such as mood changes and muscle cramps (26). Zinc, crucial for cell growth, bone metabolism, and sensory functions, exhibited the highest concentration in leaves (24.14ppm) and lowest in stems. This distribution is significant given zinc deficiency's implications like weight loss and impaired taste and vision (27,28). Copper, vital for growth but toxic at high levels, showed a balanced distribution across the plant parts, suggesting a moderate risk of toxicity (29).

However, the study also identified concerning levels of toxic metals. Lead, known for its limited uptake by plants, was found in higher concentrations in the aerial parts of Cannabis sativa, indicating a strong bio-accumulative nature and potential airborne contamination sources (30). The presence of lead in all parts, especially in leaves, raises concerns regarding its safety for human consumption, given the established safe limit of 25 ug/kg body weight (32).

Cadmium, with no essential biological role, showed the lowest concentration in roots and highest in leaves. Its presence, mainly through inhalation, ingestion, and dermal absorption, is a matter of concern for human health (31). Chromium and arsenic, found in higher concentrations in leaves, also pose potential risks. The elevated levels of chromium, particularly in leaves, might be attributed to the humus-rich soil in the cultivation area, raising questions about environmental factors influencing metal accumulation in plants.

The study's strengths lie in its comprehensive analysis of trace and toxic metal concentrations in different parts of Cannabis sativa, providing valuable insights into its pharmacological potential and environmental interactions. However, limitations include the lack of longitudinal data to understand how these concentrations might vary over time or under different environmental conditions.

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Additionally, the study does not account for potential variances in metal concentrations due to cultivation practices or genetic differences among Cannabis sativa strains.

While Cannabis sativa shows promise in treating various deficiencies and ailments due to its rich concentration of beneficial trace metals, the presence of toxic metals cannot be overlooked. This dichotomy underscores the need for careful consideration in the medicinal use of Cannabis sativa, balancing its therapeutic benefits against potential health risks. Future research should focus on exploring the environmental factors influencing metal accumulation in Cannabis sativa and assessing the long-term health implications of its use, considering both its beneficial trace metals and harmful toxic metals.

CONCLUSION

The objective of this study was to establish standards that significantly contribute to the quality control of Cannabis sativa, a plant with notable medicinal utility. The analysis of heavy metals in this plant suggests that, for the most part, the highest concentrations of these metals are found in the leaves, followed by the stem and roots. However, certain metals displayed greater concentration in the roots. Importantly, all parts of the Cannabis sativa plant contained metal concentrations within the permissible limits set by the World Health Organization (WHO) and other international regulatory bodies.

Therefore, it can be concluded that the utilization of Cannabis sativa in the treatment of various diseases is safe concerning its metal content. Its use in traditional medicine aligns well with local empirical data, reinforcing its value in folk medical systems. This conclusion underscores the plant's potential as a safe and effective component in herbal medicinal products, provided that ongoing quality control and monitoring of metal content are maintained.

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