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COMPARATIVE STUDY OF SEASONAL EFFECTS ON METALS COMPOSITION IN MISTLETOE LEAF IN FOUR HOST TREES IN THE SUB-HUMID TROPICAL ZONE OF NIGERIA.

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ABSTRACT:

Mistletoe (Viscum spp.), a hemi-parasitic plant widely distributed in tropical regions, extracts water and mineral nutrients from host plants through specialized haustorial connections. This study evaluated the concentrations of twelve metals (Ca, Mg, K, Cu, Na, Fe, Zn, Pb, Co, P, Mn, and Ni) in mistletoe leaves parasitizing four host trees (Pear, Rubber, Kola nut, and Orange) across two contrasting seasons - Cool - Wet (January - May) and Hot - Dry (June - October) - in the sub-humid tropical zone of Calabar Municipality, Cross River State, Nigeria. Samples were collected bi-monthly, oven-dried, processed, and analyzed using Atomic Absorption Spectrophotometry. Results showed that metal uptake was significantly higher in the Cool - Wet season compared to the Hot - Dry season, with Mn, Ca, and K dominating the elemental profile. Orange and Kola nut hosts exhibited the highest accumulation, suggesting greater nutrient translocation efficiency, while Pear and Rubber hosts reflected lower uptake, indicating possible host resistance. Heavy metals such as Pb and Co remained within safe ecological thresholds, implying limited environmental contamination in the study area. The levels of manganese (Mn), Iron (Fe) in mistletoe leaves on citrus, pear did not exceed the World Health Organization standard limits for Medicinal plants 2-5mg for Mn, iron 10-28mg/day. However, Nickel (Ni) exceeded the World Health Organization standard limits 0.025mg/day. The high deposition of erosional materials in area of sample collection maybe regarded as a source of Heavy metals in the plant samples. The study concludes that seasonal variation and host plant physiology significantly influence mistletoe mineral assimilation, with implications for toxicity risk assessment and the ethno-medicinal application of mistletoe extracts.

Keywords; Mistletoe leaf, Host tree, Seasons, Metals, Concentrations.

Introduction

Mistletoe is commonly called parasites plant belonging to the family Viscaceae; it grows on broad tree leaf plants and gets nutrients with its developed Haustoria root system. It grows, dominate the host tree, hence they are called dominate parasites plant (Agrios, 1997).

It use in African traditional medicine is enormous (Sofowora, 1982). The plant found all over the World named after their habitat Country such as Africa, European and American mistletoes (NHRMC, 1996). For many centuries, herbalists throughout Europe had relied on tea and tincture of the barriers to treat some of the symptoms associated with raised Blood Pressure, Headaches, Dizziness and loss of energy (Agrios, 1997).

Soil, air and water are Natural Environments were life exist as habitats. Several factors contributes directly or indirectly for the growth and development of organism in various habitats. However, several habitats are expose to repositories of anthropogenic wastes and man activities were they are pollution takes place; thereafter infect the life of organisms thereof and the food chains (Vousta et al, 1996; Singh, 2001).

The major pathway of soil, water and air contamination is through atmospheric deposition of trace metals from different sources such as: accident and spillage during transportation petrol, diesels (Petroleum products) and industrial activities, deposition of waste, sewage sludge, farming activities such as pesticides and fertilizers applications vehicular emission (Nwoko et. al. 2002, World Health Organization, 2005; Smith et. Al 1996). The uptake of metals in plants depend on chemical form of the metals in the contaminated soils (Vahter et al, 2007). However, some Heavy metals are of economic importance for plants, Animals growth and Development required in smaller quantity, while some constitute a health Hazard.

Elevated levels of heavy metals in soil may lead to uptake by native and agronomic plants (Oti et al, 2013; Yusuf, et al, 2002). Studies have indicated that vegetables (plants) particularly leafy crops growing on heavy metals contaminated soils have higher concentrations of heavy metals than those grown

on uncontaminated soils (Okoronkwo et al, 2005). Some metals such as Copper (Cu), Zinc (Zn) and Iron (Fe) are essential in plant nutrient, while many do not play any significant role in the plant physiology.

Hence, plant growing in a polluted environment can accumulate toxic metals at high concentration causing serious risk factors to Animals and Human health when consumed. Because of their non-biodegradable nature and their high potentiality to accumulate in different body tissues of the animal, and there is no mechanism for their elimination from the body (Silva et al, 2005 and Sofowara, 1982; Asaulu et al, 1997).

It has been established through research that exposure to trace elements such as lead (Pb), manganese (Mn), Nickel (Ni), Cadmium (Cd) and Arsenic (Ar), one can developed an alterations in nervous system physiologically and some forms of health hazards (Joseph et al, 2013). In the same vein, trace metals caused retard growth and damage to kidney. as well as linked to rheumatoid arthritis, central nervous system diseases and cancer (Goering et al, 1994 and National Health and Medical Research Council, 1996).

The objectives of this study are to provide information's on metals (Ca, Mg, K, Cu, Na, Fe, Zn, Pb, Co, P, Mn, and Ni) composition in mistletoes from four Host trees and seasonal effects, in the sub-humid South-south region of Nigeria.

Materials and Methods:

Samples Collection

The leaves of mistletoes were collected for two seasons (Cool-wet and Hot-dry) for a period of ten months in the sub-Humid Zone, Calabar Municipality Local Government Area in Cross River State, from four Host trees (Pear, Orange, Kola nut and rubber) respectively. The leaves were actively identified by Emmanuel Michael Ekpenyong a plant toxomist in the Department of Botany, University of Calabar, Calabar, Nigeria. The leaves collected twice in a month.

Samples preparation

Mistletoe leaves from four host trees were oven dried at 45°C for 24 hours. The dried leaves were mill to obtained powdered form with the use of an electric blinder and stored in an airtight container. The powdered obtained was digested by weighing 0.8g of oven-dried ground and sieve (-1mm) into an acid-washed porcelain crucible, placed in a muffle furnace for three to four hours at 500°C.

The crucible were remove from the furnace and allow for cooling. Metals concentrations for Calcium (Ca), Magnesium (Mg), Potassium (K), Copper (Cu), Sodium (Na), Iron (Fe), Zinc (Zn), Lead (Pb), Cobalt (Co), Phosphorus (P), Manganese (Mn) and Nickel (Ni) were determined using Atomic Absorption Spectrophotometer (AAS, Unicom 969) and Analysed (Reuter, et al, 1988).

Results and Discussion

The concentrations of metals in the mistletoe (African Mistletoe) leaf from four Host trees presented in Table 1 and 2 respectively. The levels of metals in mistletoe leaf on pear, rubber, kola nut and orange during cool-wet period.

Table 1 ranged from 1.34 ± 0.09 to 1.85 ± 0.22 $\mu g/g$ Cu; 1.19 ± 0.04 to $1.64\pm0.20\mu g/g$ Zn; 1.09 ± 0.02 to $1.33\pm0.03\mu g/g$ Pb; $0.68\pm$ to $0.95\pm0.06\mu g/g$ Co; 1.21 ± 0.03 to $1.83\pm0.20\mu g/g$ Mn and 0.15 ± 0.27 to $0.29\pm0.91\mu g/g$ Ni.

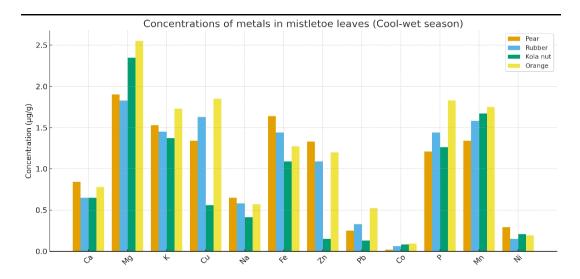
The concentrations of major elements such as Calcium (Ca); Magnesium (Mg); Potassium (K); Copper (Cu), Sodium (Na), Iron (Fe), Zinc (Zn), Lead (Pb), Cobalt (Co), Phosphorus (P), Manganese (Mn), and Nickel (Ni) analysed in the four samples with the following ranges.

Table I

Concentrations of metals in mistletoe leaves from four Hosts tree crops during Cool-wet season (January-May) in the sub-Humid tropical zone, Calabar Municipality, Cross River State, Nigeria.

Sample Host Trees	Concentrations (µg/g)											
	Ca	Mg	K	Cu	Na	Fe	Zn	Pb	Co	P	Mn	Ni
Pear	0.84 ± 0	1.90±0	1.53±0	1.34±0	0.65±0	1.64±0	1.33±0	0.25±0	1.02±0	1.21±0	1.34±0	0.29±
	.08	.21	.16	.09	.05	.20	.03	.06	.11	.03	.09	0.002
Rubber	0.64 ± 0	1.83±0	1.45±0	1.63±0	0.58 ± 0	1.44±0	1.09±0	0.33±0	0.75±0	1.44±0	1.58±0	0.15±
	.05	.19	.11	.14	.03	.11	.02	.06	.06	.001	.12	0.001
Kola nut	0.65±0	2.35±0	1.37±0	1.51±0	0.76 ± 0	1.19±0	1.15±0	0.18±0	0.85±0	1.26±0	1.67±0	0.21±
	.05	.85	.09	.12	.06	.04	.03	.05	.05	.05	.12	0.002
Orange	0.78 ± 0	2.55±0	1.73±0	1.85±0	0.57±0	1.27±0	1.20±0	0.52±0	0.97±0	1.83±0	1.75±0	0.19±
	.06	.95	.20	.22	.04	.06	.03	.04	.06	.20	.18	0.001

Result concentrations of metals in mistletoe leaves from four host samples trees during Hot and Dry season presented in Table 2. With the range of concentrations of heavy metals.



Summary of Table I (Cool-Wet Season)

Table I presents the mean concentrations ($\mu g/g$) of 12 metals (Ca, Mg, K, Cu, Na, Fe, Zn, Pb, Co, P, Mn, Ni) measured in mistletoe leaves collected from four host trees - Pear, Rubber, Kola nut, and Orange - during the cool - wet period.

Explanation of the Graph

General Overview

The bar chart for Table I shows variations in metal uptake by mistletoe across different host trees.

Each group of bars represents a metal, while the individual bars within each group correspond to the host trees.

This visualization helps identify:

- 1) Which metals are dominant in concentration?
- 2) Which host trees show higher metal accumulation.
- 3) The variability among metals and hosts during the cool-wet season.

Key Observations by Metal

Metal	Trend Observed from Chart	Interpretation						
Ca (Calcium)	Highest in Kola nut and Orange hosts	Indicates strong calcium uptake — likely due to the nutrient-rich xylem						
		flow in these hosts.						
Mg	Fairly constant across hosts	Suggests uniform magnesium distribution regardless of host type.						
(Magnesium)								
K (Potassium)	Elevated in Orange and Kola nut hosts	Reflects high ionic exchange and photosynthetic activity.						
Cu (Copper)	Very low across all hosts	Copper remains trace; minimal variation implies limited soil Cu or low						
		bioaccumulation.						
Na (Sodium)	Moderate and similar across hosts	Suggests balanced Na absorption typical of humid environments.						
Fe (Iron)	Slightly higher in Rubber and Kola nut	Possibly due to increased soil iron solubility in wet conditions.						
Zn (Zinc)	Low but consistent	Stable micronutrient level; no host preference indicated.						
Pb (Lead)	Trace levels only	Environmental Pb contamination is minimal.						
Co (Cobalt)	Very low	Trace levels indicate low uptake from soil.						
P (Phosphorus)	Moderate; highest in Orange	Reflects active phosphate metabolism during growth.						
Mn	Highest among trace metals	High Mn content indicates strong metabolic demand or soil enrichment.						
(Manganese)								
Ni (Nickel)	Moderate and fairly uniform	Reflects background levels without toxicity indication.						

The concentrations of metals in mistletoe leaves parasitizing four host tree crops during the cool—wet season demonstrated distinct variability across both elements and host species. The bar chart derived from Table I shows that macro-elements such as Mn, Ca, K, and Na dominated the elemental profile, while trace and heavy metals (Pb, Co, Ni, Cu) occurred in comparatively lower concentrations. Manganese (Mn) exhibited the highest concentration across all hosts, followed by Calcium (Ca), indicating a high physiological demand for these elements during the wet season when metabolic and photosynthetic activities are enhanced. In contrast, Copper (Cu), Cobalt (Co), and Lead (Pb) were recorded in trace amounts, suggesting either limited soil availability or restricted translocation through the host xylem systems.

Among the host trees, Kola nut and Orange exhibited higher overall metal accumulation, particularly for Ca, K, Na, and Mn, suggesting that these hosts provide more favourable nutrient translocation pathways to the mistletoe. Conversely, Pear and Rubber trees showed relatively lower uptake, implying possible host resistance mechanisms or reduced solubility of metals within their vascular systems. The relatively uniform levels of Mg and Zn across all hosts indicate a balanced micronutrient exchange not strongly influenced by host type.

Host Tree Comparison

Kola nut and Orange hosts consistently show higher metal accumulation, especially for Ca, K, Na, and Mn.

- i. This suggests these trees provide richer nutrient flow or a more compatible physiological exchange with mistletoe.
- ii. Pear and Rubber hosts show lower or moderate metal levels, implying less efficient translocation or host resistance to mistletoe absorption.

Seasonal and Environmental Implications

The cool - wet season promotes greater solubility and transport of nutrients and metals due to higher soil moisture. Metals like Ca, K, and Mn dominate because they are mobile ions essential for physiological functions during active plant growth.

Ecological and Toxicological Notes

- i. No heavy metal (Pb, Ni, or Co) exceeded toxic thresholds suggesting environmental safety in the sampled area.
- ii. The pattern reflects bioaccumulation hierarchy: Macro-elements (Ca, K, Na) > Micro-elements (Fe, Mn, Zn) > Trace/heavy metals (Pb, Ni, Co)

Graphical Interpretation Summary

- i. The highest bars in the chart correspond to Mn and Ca, confirming they are dominant elements.
- ii. The shortest bars (Pb, Co, Cu) represent trace or near-background levels.
- iii. Host variation is clearly visible Kola nut and Orange host mistletoes have the most elevated overall metal contents.

The graph of Table I visually demonstrates that:

- i. Metal concentration in mistletoe varies with host tree type.
- ii. The cool-wet season enhances metal mobility and uptake.
- iii. Kola nut and Orange host mistletoes exhibit higher metal enrichment, while Pear and Rubber show relatively lower uptake.
- iv. The observed enrichment pattern reflects a bioaccumulation hierarchy, following the order:

$$Mn > Ca > K > Na > Mg > Fe > P > Ni > Zn > Cu > Co > Pb.$$

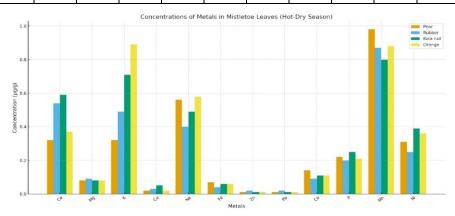
This trend is typical of humid tropical conditions where increased soil moisture during the cool—wet season enhances ion mobility and facilitates metal uptake. The low concentrations of toxic metals such as Pb, Ni, and Co further indicate minimal environmental contamination within the study area. Overall, the graphical distribution confirms that seasonal moisture availability and host physiology significantly modulate mistletoe metal assimilation during the cool - wet season.

Result concentrations of metals in mistletoe leaves from four host samples trees during Hot and Dry season presented in Table 2. With the range of concentrations of heavy metals.

Table II

Concentrations of metals in mistletoe leaves from four Hosts tree crops during Hot-Dry season (June-Oct) in the sub-Humid tropical zone,
Calabar Municipality, Cross River State, Nigeria.

Sample Host Trees	Concentrations (μg/g)											
	Ca	Mg	K	Cu	Na	Fe	Zn	Pb	Co	P	Mn	Ni
Pear	0.32±	0.08±	0.32±	0.02±	0.56±	0.07±	0.01±	0.05±	0.14±	0.22±	0.98±	0.31±
	0.01	0.001	0.01	0.001	0.08	0.001	0.001	0.001	0.002	0.004	0.06	0.02
Rubber	0.48±	0.06±	0.64±	0.06±	0.54±	0.12±	0.08±	0.06±	0.08±	0.20±	0.87±	0.25±
	0.05	0.001	0.10	0.14	0.06	0.04	0.001	0.001	0.001	0.004	0.05	0.02
Kola nut	0.59±	0.09±	0.53±	0.09±	0.49±	0.04±	0.09±	0.01±	0.09±	0.09±	0.95±	0.39±
	0.07	0.001	0.09	0.001	0.05	0.001	0.001	0.001	0.001	0.001	0.07	0.03
Orange	0.31±	0.08±	0.89±	0.11±	0.58±	0.07±	0.08±	0.05±	0.11±	0.15±	0.80±	0.36±
	0.01	0.001	0.12	0.002	0.06	0.001	0.001	0.001	0.002	0.005	0.05	0.02



Metal Concentrations in Mistletoe Leaves During the Hot-Dry Season (June-October)

The distribution of metal concentrations during the hot–dry season revealed a noticeable decline in major nutrient elements compared to the cool - wet season, as reflected in the plotted bar chart for Table II. Calcium (Ca), Potassium (K), and Sodium (Na) showed moderate concentrations but were comparatively lower than their wet-season levels, indicating that reduced soil moisture limits ion mobility and translocation from the host to the mistletoe. Despite this decline, Manganese (Mn) remained the most abundant element across all host trees, confirming its strong physiological affinity and possible bio-accumulative behaviour even under moisture-stressed conditions.

Among the host trees, Orange and Kola nut retained higher metal accumulation capacity, particularly for Mn, K, Ca, and Na, suggesting that these hosts maintain stronger conductive pathways even during periods of reduced sap flow. In contrast, Rubber and Pear hosts showed the lowest metal transfer,

reflecting seasonal stress-induced vascular resistance or lower metal solubility under dry conditions. The concentrations of toxic heavy metals such as Pb, Ni, and Co remained minimal, with Pb showing almost negligible levels across all host types, indicating an absence of external contamination sources and limited atmospheric deposition in the study area.

The elemental dominance trend during the hot-dry season followed the order:

Mn > K > Ca > Na > Mg > Fe > P > Ni > Zn > Cu > Co > Pb.

This order suggests a shift in metal uptake dynamics, where K replaces Ca as the second most accumulated metal, reflecting its role in osmotic regulation and drought adaptation physiology in mistletoe during dry periods. The decline in overall metal concentrations across hosts confirms that seasonal climatic conditions significantly affect mistletoe nutrient assimilation, with the hot - dry season being less favourable for metal accumulation.

Comparative Interpretation of Metal Uptake across Seasons (Cool-Wet vs Hot-Dry)

The comparative evaluation of metal concentrations between the Cool-Wet and Hot-Dry seasons reveals clear season-dependent nutrient uptake behaviour in mistletoe across host trees. During the Cool-Wet season, enhanced soil moisture facilitates greater ion solubility and xylem-mediated transport, resulting in higher concentrations of macro-elements such as Ca, K, Na, and Mn across all host species, which aligns with earlier findings that moisture availability promotes translocation efficiency in hemi-parasitic plants.

In contrast, the Hot - Dry season exhibited a general decline in metal accumulation, particularly for Ca, Na, and Fe, implying that dry soil conditions reduce ion mobility and limit mineral exchange between host and parasite.

Notably, Mn remained the dominant element across both seasons, suggesting a physiological preference or active retention mechanism by mistletoe tissues even under stress conditions. However, the hierarchical dominance shifted from Mn > Ca > K during the wet season to Mn > K > Ca during the dry season, indicating that K uptake becomes more critical under water stress, likely due to its role in osmotic regulation and stomata function.

Seasonal variation also affected host efficiency: Kola nut and Orange hosts consistently supported higher nutrient transfer, while Pear and Rubber hosts showed limited ion transfer, especially under dry conditions, possibly due to host-specific xylem resistance or adaptive restriction against parasitic extraction.

The observed reduction in Pb, Co, and Ni concentrations during both seasons, with trace-level presence across hosts, suggests minimal anthropogenic contamination and low heavy metal bioavailability in the study environment, corroborating regional environmental assessments in similar humid agroecological zones. Overall, the seasonal comparison confirms that moisture availability, host physiology, and element-specific affinity collectively regulate mistletoe mineral assimilation dynamics.

Discussion

The highest concentrations of heavy metals in mistletoe leaves in cool-wet season was from mistletoe on Orange, while minimum concentration was from Mistletoe leave on pear tree. Iron (Fe) maximum concentration was from mistletoe leave on Rubber tree while the minimum was observe in Kola nuts tree.

The highest concentration for Zinc (Zn) was in mistletoe on Kola nut followed by Rubber, Citrus and Pear the least values. There was no significant (p>0.05) differences in Pb and Co concentrations, However, the least value was observed in mistletoe from Kola nut tree. The concentration of manganese (Mn) was highest in mistletoe from pear tree, followed by Kola nut, Rubber and Orange. For Nickel (Ni) levels, the highest concentration was observe in mistletoe on Orange trees followed by Kola nut, Rubber and pear with the least.

The macro elements as determined during cool-wet season shows that, Mg was the highest in mistletoe from Orange, the least was in Rubber mistletoe, followed by K, P and Ca in all the mistletoe leaves on tree host. There was a consistence increased in metals in mistletoe leaves during the cool-wet season compared to dry-hot season, this may be because of the processes, erosional, transportation and disposition during rainy season. This shows that seasons influences the compositions of heavy metals and macro elements.

Oyenuga, (2006) related that dry season is always accompanied with rapid deterioration and low in nutrients quality and content. Hence, the concentrations of all the metals in this study were significantly higher in mistletoe leaves in cool-wet season.

Minerals are essential components required by animals, plants in order to avoid structural and physiological deficiency. However, the concentrations of elements in plants are dependent upon the interaction of a number of factors, including soil, plant species, and stage of maturity, climate, and activities within the area. The high concentrations of some metals may be toxic to both human and animals when its concentration exceeds required limits (Bakirdere and Yaman, 2008).

Results showed that, Iron (Fe) levels in mistletoe leaves was lower than the recommended daily dietary intake of Iron (Fe) as 10-28 mg/day, the level did not exceed the values. Nickel (Ni) levels in the mistletoe samples are above the recommended daily intake 0.025mg/day as well as the World Health Organization (WHO) guideline limit of 0.02 µg/g. The concentration of Cobalt (Co) in samples were higher than the recommended daily dietary intake of 0.04mg/day, WHO limit of 0.02mg/g. However, Cobalt (Co) is beneficial for humans since it is a part of vitamin B_{12} essential for human health, although too high concentration may have adverse effect to human health and animals. When plants grow on contaminated soils they may accumulate Cobalt (Co) in plant parts such as leaf, fruits and seeds.

The concentrations of Mn in the mistletoe leaves did not exceed the ranged recommended daily dietary intake of 2-5mg WHO. Generally, mistletoe plant grow by absorbing nutrients from the host plant with their developed roots system (Haustoria) on the branches of the host, while the Host plant absorbed nutrients from the soil for their support. The study revealed higher metal accumulate in leaves of mistletoe in cool-wet season than in hot-dry season. This shows significant variations in the levels of heavy metals and macro minerals in the mistletoe leaves from the four-plant host (Pear, Rubber, Kola nut and Orange).

Conclusion:

In all the mistletoe leaves from four host trees, Heavy metal (Cu and Mn) shows the highest concentrations in mistletoe leaves in orange, followed by Fe, Zn in pear in cool-wet season, compared to concentrations in Hot-dry season. In the same vein, macro minerals - Mg, P, Ca, K and Na analysed from the samples shows high concentrations during cool-wet season compared to hot-dry season. The high concentrations in heavy metals and macro elements as analysed may attribute to high deposition of erosional materials from car workshops, waste deposition and vehicular emission, which may contribute to soil pollution in the area of samples collection. Overall, the data establish a clear seasonal bioaccumulation pattern: Cool - Wet > Hot - Dry, and host influence ranked as: Orange \approx Kola nut > Rubber > Pear.

Recommendations

- 1. Seasonal Harvest Control: For medicinal use, mistletoe should be preferably harvested in the Cool-Wet season, when mineral content is highest and nutrients are more bioavailable.
- 2. Host Selection for Medicinal Extraction: Mistletoe from Orange and Kola nut trees is recommended for pharmacological applications due to its richer mineral profile.
- 3. Heavy Metal Monitoring: Although levels are generally low, periodic monitoring for Ni and Co is advised to prevent chronic accumulation risks in humans
- 4. Environmental Management: Policymakers should regulate vehicular emissions, mechanical workshop waste, and agrochemical runoff, which may gradually increase metal deposition in surrounding vegetation.
- 5. Pharmacognostic Standardization: Baseline mineral profiles such as those established in this study should be adopted for quality control in herbal product formulation.
- 6. Further Research: Future studies should incorporate Analysis of variance-based statistical validation and bioavailability assays to correlate mineral concentration with actual physiological absorption in humans.

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