CORRELATION OF ELEMENTS ACCUMULATION IN ROOTS AND FRUIT OF BLACK PEPPER (*Piper nigrum* L.) UNDER ARSENIC SPECIES STRESS

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ABSTRACT

Arsenic species contamination in agricultural soils threatens crop growth and food safety, yet its impact on nutrient accumulation in plants remains unclear. This study examines the accumulation, translocation, and correlation of essential elements in black pepper (*Piper nigrum* L.) roots and fruits under stress from arsenite, arsenate, monomethylarsonic acid, and dimethylarsinic acid. After ten months, roots and fruits were analyzed using inductively coupled plasma mass spectrometry. Statistical analyses, including variance analysis, linear mixed models, and Pearson correlation, assessed arsenic-induced nutrient changes. Results showed significant effects on Zn, Cd, K, Ca, and Cu uptake and translocation, with roots retaining 2.5 to 7.3 times higher concentrations than fruits. The Fe/Mn ratio in roots (143.87) was significantly higher than in fruits (2.06, $p < 0.001$), while K was preferentially translocated to fruits (root-to-fruit ratio = 0.45). A strong correlation between Mg and P ($r = 0.78$, $p < 0.05$) was observed in roots, while Mg and Cu remained stable in fruits across treatments ($r = 0.64$, $p < 0.05$). These findings highlight arsenic species's role in nutrient balance through selective ion retention and translocation, providing insights for risk assessment and mitigation in contaminated environments.

MỐI TƯƠNG QUAN GIỮA SỰ TÍCH LŨY CÁC NGUYÊN TỐ TRONG RỄ VÀ QUẢ CÂY HỒ TIÊU (*Piper nigrum* L.) DƯỚI TÁC ĐỘNG CỦA CÁC DẠNG ASEN

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TỪ KHÓA

Stress do dạng asen
Tích lũy dinh dưỡng
Vận chuyển kim loại
Cây hồ tiêu
Phân tích ICP-MS

TÓM TẮT

Sự ô nhiễm dạng asen trong đất nông nghiệp đe dọa sự sinh trưởng của cây trồng và an toàn thực phẩm, nhưng tác động của nó đến quá trình tích lũy dinh dưỡng trong cây vẫn chưa được hiểu rõ. Nghiên cứu này đánh giá sự tích lũy, vận chuyển và mối tương quan giữa các nguyên tố thiết yếu trong rễ và quả cây hồ tiêu (*Piper nigrum* L.) dưới ảnh hưởng của arsenite, arsenate, monomethylarsonic acid và dimethylarsinic acid. Sau mười tháng, rễ và quả được phân tích bằng quang phổ khối plasma cảm ứng. Các phân tích thống kê, bao gồm phân tích phương sai, mô hình tuyến tính hỗn hợp và tương quan Pearson, được áp dụng để đánh giá sự thay đổi dinh dưỡng do dạng asen gây ra. Kết quả cho thấy stress do dạng asen ảnh hưởng đáng kể đến sự hấp thu và vận chuyển Zn, Cd, K, Ca và Cu, với rễ giữ lại hàm lượng cao hơn từ 2,5 đến 7,3 lần so với quả. Tỷ lệ Fe/Mn trong rễ (143,87) cao hơn đáng kể so với quả (2,06, $p < 0{,}001$), trong khi K có xu hướng ưu tiên vận chuyển lên quả (tỷ lệ rễ/quả = 0,45). Mối tương quan chặt chẽ giữa Mg và P ($r = 0{,}78$, $p < 0{,}05$) được quan sát trong rễ, trong khi Mg và Cu duy trì mối quan hệ ổn định trong quả ($r = 0{,}64$, $p < 0{,}05$). Những phát hiện này nhấn mạnh vai trò của asen trong điều hòa dinh dưỡng thông qua cơ chế giữ ion chọn lọc và vận chuyển nguyên tố, cung cấp thông tin quan trọng cho việc đánh giá rủi ro và đề xuất các chiến lược giảm thiểu ô nhiễm trong môi trường trồng trọt.

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1. Introduction

Arsenic (As) pollution in agricultural soils is a significant environmental issue, particularly in areas with naturally elevated As levels in groundwater and soils or where anthropogenic activities exacerbate As accumulation [1] - [3]. Arsenic appears in several chemical forms, with the inorganic As - arsenite (As(III)) and arsenate (As(V)) - exhibiting more toxicity than the organic forms, including monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) [4], [5]. Consequently, inorganic arsenic has been designated as "carcinogenic to humans," whereas organic arsenic has been classified as "possibly carcinogenic to humans" by the International Agency for Research on Cancer [6], [7]. As(V), owing to its structural resemblance to phosphate, is readily absorbed by phosphate transporters, but As(III) infiltrates plant cells via aquaporins, hence disturbing cellular metabolism and nutritional equilibrium [8]. Comprehending the interactions between arsenic and critical plant nutrients is crucial for evaluating its effects on plant development and food safety.

Black pepper (*Piper nigrum* L.), referred to as the "king of spices," is a commercially significant spice crop used globally [9]. Besides its use as a gourmet spice, it serves as a significant component in traditional medicine in several developing countries, owing to its great effectiveness and affordability [10], [11]. Due to customer demand, the plant is cultivated in regions vulnerable to arsenic pollution, rendering it subject to arsenic stress [12]. The accumulation of metals in plant is affected by intricate interactions among nutrients (P, K, Ca, Mg, Fe, Cu, Zn, Mn, etc.) and arsenic species, which impact plant development and physiological activities [13]. A previous study indicates that plants exposed to arsenic stress mostly obtain arsenic in the roots instead of transferring it to consumable portions as a detoxifying strategy [14]. The level to which various As species influence metal accumulation and transport of nutrients in black pepper is still unclear.

This study hypothesizes that arsenic stress alters element accumulation and translocation in black pepper, with roots retaining higher element concentrations than fruits. The effects vary by arsenic species, with As(III) and As(V) inhibiting element uptake, while MMA and DMA may enhance translocation. The study aims to quantify element accumulation in roots and fruits and assess correlations between arsenic species and nutrient elements. The findings provide insights into arsenic species-induced nutrient imbalances and metal regulation, contributing to improved risk assessment and mitigation strategies for arsenic contamination in agriculture.

2. Materials and methods

2.1. Chemical and reagents

The ICP-MS Calibration standard (XXI) containing 29 elements at 10 mg/L in HNO_3 5% were purchased from CPAchem (Stara Zagora, Bulgaria). Total Phosphorus 1000 mg/L calibration standard, nitric acid (HNO_3, 70%, purified by redistillation), and hydrogen peroxide (H_2O_2, 30% wt.% in H_2O) were obtained from Merck (Darmstadt, Germany). Standard 1000 mg/L solutions of As(III), As(V), MMA, and DMA were prepared using sodium (meta)arsenite ($NaAsO_2$) (Sigma-Aldrich), sodium arsenate dibasic heptahydrate ($Na_2HAsO_4\cdot 7H_2O$) (Sigma-Aldrich), disodium methylarsenate hexahydrate ($Na_2CH_3O_3As\cdot 6H_2O$) (Chem Service), and dimethylarsinic acid ($C_2H_7AsO_2$) (Fluka), respectively. The SRM-1570a was purchased from the U.S. National Institute of Standards and Technology (NIST). All solution preparation used ultrapure water (UPW,18.2 MΩ/cm) obtained using the Milli-Q Integral water purification system (Darmstadt, Germany).

2.2. Reseach methods

2.2.1. Pepper cultivation

Black pepper (*Piper nigrum* L.) plants were cultivated in a greenhouse under controlled conditions (25–27°C, 70% humidity) using a pot experiment with 17 dm^3 pots containing 10 kg of substrate. The substrate used for plant growth consisted of a peat-soil-coconut fiber mixture (3:12:8, v/v) to ensure proper aeration, water retention, and nutrient availability. The plants were irrigated with tap water on non-treatment days and Yoshida nutrient solution was applied weekly to maintain consistent nutrient availability. As species (As(III), As(V), MMA, DMA) were applied at 20, 50, 100, 200, and 250 μg/L, replacing the blank nutrient solution at the flowering stage, except for the control. Based on the controlled greenhouse environment and space limitations, each treatment had two pots per concentration, with three plants per pot. Despite the limited replication, the accuracy and reliability of the results were ensured through advanced statistical methodologies (Linear Mixed Models (LMM), ANOVA and Pearson correlation analysis. Additionally, samples from each pot were homogenized before analysis to enhance data reliability and minimize within-group variability. Nutrient solution was applied weekly, and tap water was used on the remaining days. After 10 months from the initial arsenic treatment, plants were harvested, separated into roots and fruits, washed, rinsed, blotted dry, and snap frozen in liquid nitrogen. The samples were then ground in liquid nitrogen using a mortar and pestle before digestion for further analysis.

2.2.2. Sample analysis

- *Sample preparation:* The sample preparation procedure was conducted in accordance with the research of Bui *et al.* [15], with some modifications. A mass of 1.0 ± 0.001 g of homogenized material was accurately weighed and placed into a polytetrafluoroethylene vessel. A volume of 10 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2 was introduced into the vessel and allowed to stand for 30 minutes at ambient temperature. The vessel was securely sealed and positioned in a Mars X-press plus microwave digesting system (CEM, Matthews, NC, USA). The sample underwent digestion following the subsequent cycle: 0-15 min: elevate to 200°C, maintain for 10 min; 25-35 min: raise from 120 °C to 160 °C, sustain for 10 min; 45-55 min: ascend from 160°C to 180 °C, retain for 30 min. The sample is then cooled to room temperature and diluted to 25 mL with UPW.

Table 1. *Method validation parameters for elemental analysis using NIST SRM 1570a*

Analyte	R^2	LOD (mg/kg)	LOQ (mg/kg)	NIST SRM 1570a			Repeatability (Reproducibility) (mg/kg)		
				Measured (mg/kg)	Certified (mg/kg)	Recovery (%)	0.1	5	10
Mg	0.9996	0.05	0.15	9012±8	9000	100.1	3.0 (5.9)	1.0 (9.0)	4.8 (7.9)
P	0.9994	0.01	0.04	5202±54	5187±67	100.3	2.7 (8.6)	5.6 (6.5)	2.5 (5.0)
K	0.9992	0.09	0.30	29064±112	29000±260	100.2	1.9 (7.7)	5.1 (5.2)	2.6 (8.4)
Ca	0.9989	0.06	0.20	15089±354	15260±660	98.9	1.1 (5.7)	2.7 (7.0)	5.8 (6.5)
Cu	0.9996	0.01	0.03	12.46±1.22	12.22±0.86	102.0	1.6 (7.6)	2.9 (9.0)	3.1 (5.1)
Zn	0.9991	0.03	0.09	80.6±1.5	82.3±3.9	97.9	3.9 (8.5)	4.9 (8.6)	2.4 (7.8)
As	0.9990	0.03	0.10	0.071±0.009	0.068±0.012	104.4	4.8 (8.7)	4.5 (7.2)	3.3 (5.9)
Mn	0.9994	0.03	0.10	75.1±0.9	76.0±1.2	98.8	5.2 (8.1)	5.0 (5.4)	3.9 (8.5)
Co	0.9996	0.01	0.03	0.371±0.024	0.393±0.030	94.4	4.3 (7.2)	5.0 (8.7)	1.5 (5.6)
Sr	0.9993	0.03	0.09	56.02±0.29	55.54±0.50	100.9	3.1 (5.0)	2.3 (6.1)	3.3 (4.5)
Cd	0.9999	0.01	0.03	2.712±0.034	2.876±0.058	94.2	3.7 (8.4)	5.7 (5.1)	2.8 (7.8)
Pb	0.9991	0.02	0.07	0.17±0.04	0.2	85.0	2.0 (6.8)	3.0 (6.8)	4.2 (8.7)
Fe	0.9991	0.02	0.05	295±4	293±6*	100.7	4.9 (4.2)	5.5 (8.6)	3.0 (6.3)
Ba	0.9994	0.02	0.05	13.8±0.4	13.2±1.3*	104.5	5.4 (5.4)	5.7 (8.2)	1.2 (7.4)
Cr	0.9995	0.03	0.10	1.71±0.08	1.92±0.04*	89.1	5.1 (6.4)	3.7 (4.4)	1.3 (4.3)

- *Instrument conditions*: Elemental concentrations were quantified using an iCAP™ TQ ICP-MS (Thermo Scientific, USA) under optimal conditions: RF power of 1550 W, plasma Ar flow of 14 L/min, auxiliary Ar flow of 0.8 L/min, and carrier Ar flow of 1.0 L/min. The sample depth was established at 9.0 mm, the spray chamber temperature at 2 °C, and the sample flow rate at 500 µL/min. Nickel sampler and skimmer cones were used. To guarantee enhanced sensitivity, the production of oxides (less than 1%) and the presence of doubly charged ions (less than 3%) were reduced. The elements P, As, Mn, Co, Fe, and Cr were examined in Reaction Cell Mode (with an O_2 flow of 0.5 mL/min) to mitigate mass spectrometry noise and enhance precision.

- *Method validation*: The characteristics of limit of detection (LOD), limit of quantification (LOQ), repeatability, and reproducibility were conducted in accordance with the research by Bui *et al.* [15]. The NIST SRM 1570a was analyzed to confirm the optimum sample preparation. The findings of the validation of the elemental analysis method are shown in Table 1. Consequently, the improved approach was suitable for its intended purpose.

2.3. Data analysis

Statistical analyses were conducted to evaluate the effects of arsenic species stress on nutrient accumulation in black pepper. ANOVA was used to compare elemental concentrations across As treatments, with As species as the fixed factor and elemental concentrations in roots and fruits as dependent variables. Tukey's HSD test identified significant differences between treatments. LMM assessed the impact of As concentration in irrigation water on elemental accumulation, with As concentration as the fixed effect and plant replicate as the random effect to account for variability. Pearson correlation analyzed relationships between nutrient elements in roots and fruits to determine interactions influenced by arsenic stress. For statistical analysis, results below the LOD were assigned a value of LOD/2. All statistical analyses were performed in R software using the 'lme4', 'stats', and 'ggplot2' packages to ensure accurate and reliable interpretations of nutrient dynamics under As exposure.

3. Result and Discussion

3.1. Effect of As species stress on elements accumulation in roots and fruits

Utilizing the modified analytical method, elemental composition in pepper roots and fruits was examined under different experimental conditions. The concentration data (Table 2 and Table 3) highlight the significant impact of As species stress on nutrient uptake and translocation, particularly in roots, which regulate ion concentrations to mitigate As toxicity. ANOVA results showed significant variations in Zn, Cd, K, Ca, Fe, Cu and As levels in both roots and fruits ($p <$ 0.001), demonstrating the substantial influence of As on elemental distribution. Zn and Cd (F-statistic: 13.2 and 12.8, Table 4) were highly affected, suggesting their sequestration in root cells to protect antioxidant enzymes from metal stress [16]. K and Ca were significantly influenced by As ($p < 0.001$), likely owing to alterations in intracellular ion equilibrium and absorption processes. K, Mg, and P concentrations were significantly higher in fruits than in roots ($p < 0.01$), suggesting preferential translocation to support reproductive growth. Conversely, Fe was predominantly sequestered in roots, with its concentration being 7.7 times higher than in fruits, indicating a potential detoxification mechanism via Fe-As complex formation. The K/Ca ratio in fruits (77.87) was more than twice that in roots (34.79, $p < 0.05$), suggesting that potassium is actively transported via the phloem, while calcium remains largely in the roots due to its structural role. As(III) and As(V) may hinder K transport by affecting root membrane permeability, while DMA and MMA may enhance Ca mobility by modifying cell wall structure and Ca ion channels [16].

Table 2. *Concentration (mean ± standard deviation, mg/kg) of element in root under As species stress*

As species	As conc. (μg/L)	Mg	P	K	Ca	Fe	Cu	Zn	Mn	As	Cr	Co	Sr	Cd	Ba	Pb
Control	0	686.96±64.11	1891.43±220.79	5670.88±650.96	103.52±11.37	33.39±2.16	2.41±0.16	2.96±0.22	27.99±4.16	0.1±0.01	0.57±0.04	< LOD	0.1±0.01	0.01±0.01	0.1±0.01	0.07±0.01
As(III)	20	1237.40±118.58	3185.86±376.60	10044.08±1151.15	100.93±13.40	88.58±13.26	3.42±0.28	2.96±0.37	29.65±1.69	1.43±0.08	2.07±0.29	< LOD	0.14±0.01	< LOD	0.13±0.01	0.09±0.01
	50	1157.28±98.52	2863.03±321.49	9264.82±463.61	114.16±12.11	157.77±19.65	3.39±0.27	3.34±0.21	31.17±2.84	3.39±0.31	0.67±0.07	< LOD	0.2±0.03	< LOD	0.07±0.01	0.05±0.01
	100	1283.58±107.20	3519.59±335.35	11433.74±650.12	151.77±10.85	156.10±13.73	4.16±0.54	3.19±0.25	40.46±4.25	5.89±0.67	0.62±0.08	< LOD	0.21±0.02	< LOD	0.09±0.01	0.06±0.01
	200	770.67±82.15	3810.15±402.43	7261.61±850.99	57.36±7.76	124.15±6.49	2.86±0.15	2.46±0.24	37.59±5.22	4.78±0.34	0.62±0.08	< LOD	0.13±0.02	< LOD	0.03±0.01	0.02±0.01
	250	1022.61±66.70	5268.63±296.57	11634.11 1454.38	71.15±3.71	114.85±11.66	3.03±0.33	1.77±0.13	36.26±3.05	5.56±0.31	0.41±0.03	< LOD	0.12±0.01	< LOD	0.04±0.01	0.03±0.01
As(V)	20	787.57±92.72	2288.65±134.60	12363.84±911.21	106.16±6.87	136.99±11.72	4.71±0.50	4.78±0.57	85.78±7.51	1.25±0.18	0.61±0.06	0.01±0.01	0.17±0.02	0.01±0.01	0.3±0.03	0.13±0.01
	50	922.89±134.23	2530.8±333.76	8333.90±813.39	84.65±11.53	136.15±17.82	4.30±0.48	5.68±0.55	61.67±3.69	2.39±0.27	0.41±0.03	< LOD	0.2±0.01	0.01±0.01	0.16±0.01	0.18±0.03
	100	1209.33±158.77	2396.77±293.60	8968.83±1320.57	136.47±7.01	133.13±19.84	5.81±0.42	8.82±1.32	115.7±11.5	4.43±0.59	0.45±0.03	< LOD	0.25±0.02	0.03±0.01	0.17±0.02	0.18±0.01
	200	1230.35±117.93	2555.30±244.41	13582.44±921.57	124.98±13.08	109.76±13.47	5.56±0.34	4.12±0.56	91.75±12.8	5.55±0.39	0.99±0.11	0.01±0.01	0.18±0.02	< LOD	0.21±0.03	0.16±0.01
	250	1023.53±135.43	4308.78±476.98	12735.74±1727.48	137.59±9.35	161.53±9.70	5.71±0.74	5.45±0.32	117.79±16.79	6.03±0.4	1.56±0.11	0.02±0.01	0.26±0.02	0.01±0.01	0.43±0.04	0.17±0.01
DMA	20	1743.88±143.49	5381.58±596.01	10573.50±1320.52	190.83±13.93	137.71±20.35	3.75±0.37	3.62±0.32	81.81±6.73	0.93±0.12	0.47±0.07	0.01±0.01	0.35±0.02	0.01±0.01	0.16±0.02	0.13±0.01
	50	3112.71±318.09	7245.96±832.85	9756.19±796.69	414.60±40.82	218.32±21.49	4.81±0.43	5.7±0.58	223.2±24.28	2.05±0.24	0.65±0.06	0.02±0.01	0.64±0.06	0.01±0.01	0.41±0.06	0.25±0.03
	100	2469.87±242.44	5984.90±710.89	11031.98±1382.86	469.52±65.38	247.35±27.49	4.49±0.55	5.3±0.64	254.09±36.49	3.71±0.42	0.70±0.07	0.02±0.01	0.64±0.04	0.01±0.01	0.75±0.09	0.11±0.01
	200	2514.51±260.96	7103.56±497.89	8609.66±496.78	463.01±47.04	238.23±14.79	5.57±0.63	6.3± 0.85	224.86±12.61	4.26±0.61	0.62±0.06	0.01±0.01	0.84±0.09	0.02±0.01	0.19±0.03	0.12±0.01
	250	3055.49±389.73	2390.50±221.43	9539.09±514.82	633.10±79.60	218.57±13.80	5.94±0.86	7.42±0.89	213.7±16.89	3.96±0.38	0.74±0.06	0.02±0.01	0.64±0.04	0.02±0.01	0.46±0.04	0.18±0.02
MMA	20	695.49±77.35	2082.17±279.28	8204.47±1160.77	94.60±8.04	78.45±9.71	2.44±0.18	2.57±0.27	39.22±3.45	0.11±0.01	1.3±0.15	< LOD	0.2±0.02	< LOD	0.14±0.01	0.08±0.01
	50	1098.52±151.12	2311.58±242.09	9977.17±1300.32	126.39±13.24	72.56±9.96	4.36±0.26	3.27±0.37	47.8±4.36	0.22±0.01	1.55±0.19	< LOD	0.2±0.03	< LOD	0.14±0.02	0.06±0.01
	100	997.96±68.45	2304.82±168.67	9259.63±921.33	97.83±5.67	105.45±9.14	3.23±0.48	2.77±0.39	42.26±3.07	0.25±0.02	0.87±0.08	< LOD	0.24±0.04	< LOD	0.11±0.01	0.05±0.01
	200	825.40±117.80	2267.43±163.75	7748.40±623.67	124.56±15.70	68.40±6.02	3.67±0.34	2.76±0.4	38.42±4.51	0.39±0.03	0.61±0.04	< LOD	0.25±0.04	< LOD	0.12±0.02	0.05±0.01
	250	741.81±77.79	2533.59±273.35	6837.14±413.65	98.52±9.12	99.52±11.90	2.71±0.26	2.93±0.43	48.7±6.44	0.54±0.05	0.69±0.05	< LOD	0.17±0.02	< LOD	0.12±0.02	0.04±0.01

Table 3. *Concentration (mean ± standard deviation, mg/kg) of element in fruit under As species stress*

As species	As conc. (μg/L)	Mg	P	K	Ca	Fe	Cu	Zn	Mn	As	Cr	Co	Sr	Cd	Ba	Pb
Control	0	686.96±64.11	1891.43±220.79	5670.88±650.96	103.52±11.37	33.39±2.16	2.41±0.16	2.96±0.22	27.99±4.16	0.1±0.01	0.57±0.04	< LOD	0.1±0.01	0.01±0.01	0.1±0.01	0.07±0.01
As(III)	20	1237.40±118.58	3185.86±376.60	10044.08±1151.15	100.93±13.40	88.58±13.26	3.42±0.28	2.96±0.37	29.65±1.69	1.43±0.08	2.07±0.29	< LOD	0.14±0.01	< LOD	0.13±0.01	0.09±0.01
	50	1157.28±98.52	2863.03±321.49	9264.82±463.61	114.16±12.11	157.77±19.65	3.39±0.27	3.34±0.21	31.17±2.84	3.39±0.31	0.67±0.07	< LOD	0.2±0.03	< LOD	0.07±0.01	0.05±0.01
	100	1283.58±107.20	3519.59±335.35	11433.74±650.12	151.77±10.85	156.10±13.73	4.16±0.54	3.19±0.25	40.46±4.25	5.89±0.67	0.62±0.08	< LOD	0.21±0.02	< LOD	0.09±0.01	0.06±0.01
	200	770.67±82.15	3810.15±402.43	7261.61±850.99	57.36±7.76	124.15±6.49	2.86±0.15	2.46±0.24	37.59±5.22	4.78±0.34	0.62±0.08	< LOD	0.13±0.02	< LOD	0.03±0.01	0.02±0.01
	250	1022.61±66.70	5268.63±296.57	11634.11 1454.38	71.15±3.71	114.85±11.66	3.03±0.33	1.77±0.13	36.26±3.05	5.56±0.31	0.41±0.03	< LOD	0.12±0.01	< LOD	0.04±0.01	0.03±0.01

As species	As conc. (μg/L)	Mg	P	K	Ca	Fe	Cu	Zn	Mn	As	Cr	Co	Sr	Cd	Ba	Pb
As(V)	20	787.57±92.72	2288.65±134.60	12363.84±911.21	106.16±6.87	136.99±11.72	4.71±0.50	4.78±0.57	85.78±7.51	1.25±0.18	0.61±0.06	0.01±0.01	0.17±0.02	0.01±0.01	0.3±0.03	0.13±0.01
	50	922.89±134.23	2530.8±333.76	8333.90±813.39	84.65±11.53	136.15±17.82	4.30±0.48	5.68±0.55	61.67±3.69	2.39±0.27	0.41±0.03	< LOD	0.2±0.01	0.01±0.01	0.16±0.01	0.18±0.03
	100	1209.33±158.77	2396.77±293.60	8968.83±1320.57	136.47±7.01	133.13±19.84	5.81±0.42	8.82±1.32	115.7±11.5	4.43±0.59	0.45±0.03	< LOD	0.25±0.02	0.03±0.01	0.17±0.02	0.18±0.01
	200	1230.35±117.93	2555.30±244.41	13582.44±921.57	124.98±13.08	109.76±13.47	5.56±0.34	4.12±0.56	91.75±12.8	5.55±0.39	0.99±0.11	0.01±0.01	0.18±0.02	< LOD	0.21±0.03	0.16±0.01
	250	1023.53±135.43	4308.78±476.98	12735.74±1727.48	137.59±9.35	161.53±9.70	5.71±0.74	5.45±0.32	117.79±16.79	6.03±0.4	1.56±0.11	0.02±0.01	0.26±0.02	0.01±0.01	0.43±0.04	0.17±0.01
DMA	20	1743.88±143.49	5381.58±596.01	10573.50±1320.52	190.83±13.93	137.71±20.35	3.75±0.37	3.62±0.32	81.81±6.73	0.93±0.12	0.47±0.07	0.01±0.01	0.35±0.02	0.01±0.01	0.16±0.02	0.13±0.01
	50	3112.71±318.09	7245.96±832.85	9756.19±796.69	414.60±40.82	218.32±21.49	4.81±0.43	5.7±0.58	223.2±24.28	2.05±0.24	0.65±0.06	0.02±0.01	0.64±0.06	0.01±0.01	0.41±0.06	0.25±0.03
	100	2469.87±242.44	5984.90±710.89	11031.98±1382.86	469.52±65.38	247.35±27.49	4.49±0.55	5.3±0.64	254.09±36.49	3.71±0.42	0.70±0.07	0.02±0.01	0.64±0.04	0.01±0.01	0.75±0.09	0.11±0.01
	200	2514.51±260.96	7103.56±497.89	8609.66±496.78	463.01±47.04	238.23±14.79	5.57±0.63	6.3± 0.85	224.86±12.61	4.26±0.61	0.62±0.06	0.01±0.01	0.84±0.09	0.02±0.01	0.19±0.03	0.12±0.01
	250	3055.49±389.73	2390.50±221.43	9539.09±514.82	633.10±79.60	218.57±13.80	5.94±0.86	7.42±0.89	213.7±16.89	3.96±0.38	0.74±0.06	0.02±0.01	0.64±0.04	0.02±0.01	0.46±0.04	0.18±0.02
MMA	20	695.49±77.35	2082.17±279.28	8204.47±1160.77	94.60±8.04	78.45±9.71	2.44±0.18	2.57±0.27	39.22±3.45	0.11±0.01	1.3±0.15	< LOD	0.2±0.02	< LOD	0.14±0.01	0.08±0.01
	50	1098.52±151.12	2311.58±242.09	9977.17±1300.32	126.39±13.24	72.56±9.96	4.36±0.26	3.27±0.37	47.8±4.36	0.22±0.01	1.55±0.19	< LOD	0.2±0.03	< LOD	0.14±0.02	0.06±0.01
	100	997.96±68.45	2304.82±168.67	9259.63±921.33	97.83±5.67	105.45±9.14	3.23±0.48	2.77±0.39	42.26±3.07	0.25±0.02	0.87±0.08	< LOD	0.24±0.04	< LOD	0.11±0.01	0.05±0.01
	200	825.40±117.80	2267.43±163.75	7748.40±623.67	124.56±15.70	68.40±6.02	3.67±0.34	2.76±0.4	38.42±4.51	0.39±0.03	0.61±0.04	< LOD	0.25±0.04	< LOD	0.12±0.02	0.05±0.01
	250	741.81±77.79	2533.59±273.35	6837.14±413.65	98.52±9.12	99.52±11.90	2.71±0.26	2.93±0.43	48.7±6.44	0.54±0.05	0.69±0.05	< LOD	0.17±0.02	< LOD	0.12±0.02	0.04±0.01

The LMM analysis confirmed the significant impact of arsenic on metal accumulation of Zn, Cd, K, Ca, and Cu in both roots and fruits ($p < 0.05$, Table 4). This aligns with the concept that plants primarily absorb As through roots and limit its translocation to fruits to mitigate toxicity. While species-specific differences exist, previous studies on rice and hyperaccumulator plants (*Arabidopsis thaliana*, *Pteris vittata*) have reported similar trends, supporting the general mechanism of restricted arsenic translocation as a detoxification strategy, which is also observed in black pepper [17], [18]. K levels decreased significantly as As concentration increased (coef: -5.67, $p < 0.05$), suggesting that As stress induces K ion leakage, leading to electrochemical imbalance and restricted uptake [18]. In fruit, P (p = 8.80E-01), Mg (p = 2.72E-01), and Ba (p = 9.59E-01) exhibited no significant variation with increasing As treatment levels ($p > 0.1$), indicating that plants activate intrinsic regulatory mechanisms to maintain nutrient homeostasis in roots and fruits [18]. Notably, Ca exhibited a significant increase in fruits under As treatment (p = 2.66E-02), indicating a potential role in structural reinforcement under stress conditions. Consequently, As stress significantly impacts nutrient distribution, with certain elements (Mg, K, P) being preferentially translocated to fruits, while Fe and toxic elements (Zn, Cd) are retained in roots to mitigate potential toxicity. The LMM analysis further highlights that while As significantly influences the accumulation of several elements, intrinsic plant mechanisms likely regulate Mg, P, and K in fruits to maintain physiological stability. The observed variations emphasize the complex ion regulation mechanisms in black pepper plants under arsenic stress.

Table 4. *ANOVA, t-test and linear mixed model (LMM) results showing the impact of As species stress on elements accumulation in roots and fruits*

Elements	ANOVA				Linear Mixed Model			
	Root		Fruit		Root		Fruit	
	F-statistic	*p*-value	F-statistic	*p*-value	Coef	*p*-value	Coef	*p*-value
Zn	13.2	6.02E-05	14.6	3.32E-05	-0.007	4.70E-01	0.0005	8.07E-01
Cd	12.8	7.03E-05	5.1	7.81E-03	6.33	4.30E-01	-10.0	9.39E-01
K	10.6	2.15E-04	6.42	2.79E-03	-5.67	7.60E-04	-0.31	9.03E-01
Ca	10.3	2.60E-04	16.3	1.68E-05	0.12	2.15E-01	0.38	2.66E-02
Cu	8.3	8.08E-04	12.7	6.80E-05	-0.006	5.10E-01	0.002	2.00E-01
Sr	8.1	8.85E-04	24.6	1.16E-06	0.0002	5.50E-01	0.0004	3.71E-02
Co	7.8	1.07E-03	10.4	2.42E-04	-5.8	3.05E-01	8.52E-06	2.85E-01
Cr	6.6	2.40E-03	0.46	7.64E-01	0.0006	7.40E-01	-0.0007	4.61E-01
Ba	4.2	1.50E-02	3.92	2.11E-02	0.003	1.49E-01	1.9E-05	9.59E-01
As	3.9	2.00E-02	5.62	5.08E-03	0.12	2.48E-09	0.01	1.40E-07
Mn	3.7	2.60E-02	24.3	1.25E-06	-0.009	2.35E-01	0.14	4.82E-02
Fe	2.7	7.00E-02	32.1	1.84E-07	-1	3.50E-01	0.07	2.23E-01
P	2.3	1.02E-01	6.92	1.97E-01	-0.92	1.18E-01	0.46	8.80E-01
Pb	2.2	1.10E-01	19.6	5.29E-06	-0.0005	3.20E-01	-1.2E-04	8.49E-02
Mg	0.6	6.90E-01	26.1	7.70E-07	-0.18	1.39E-01	0.86	2.72E-01

3.2. Correlation between elements in roots and fruits

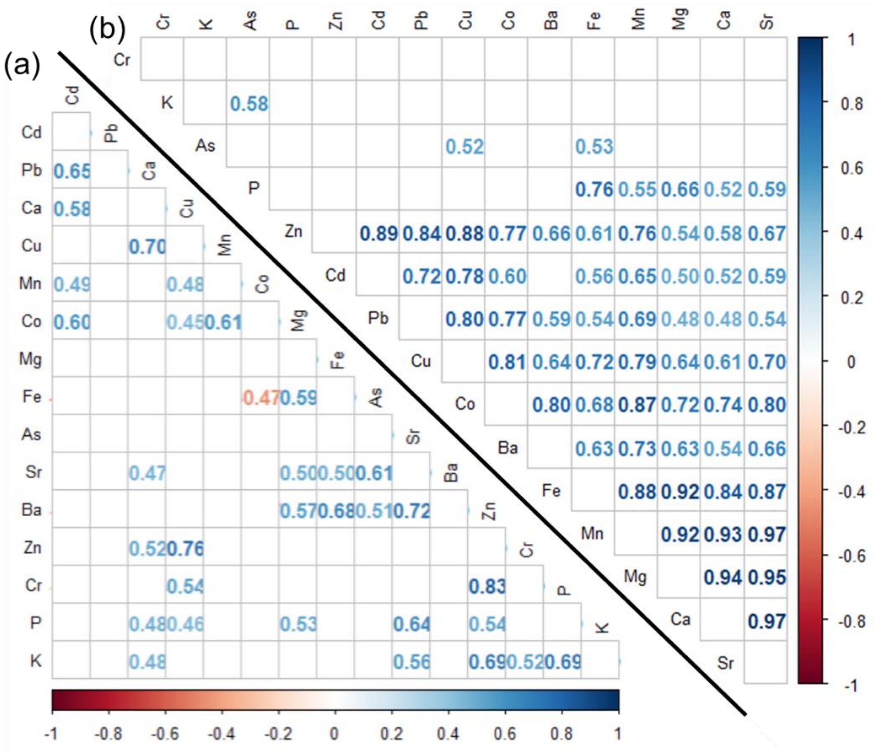

Figure 1. *Pearson correlation matrix of metal concentrations in (a) roots and (b) fruits of black pepper under As species stress. Bold numbers denote statistically significant correlations (p < 0.05)*

Pearson correlation analysis indicated that in roots (Figure 1a), Mg-P, K-Ca, Cu-Zn, and Fe-Mn exhibited substantial correlations ($r > 0.70$, $p < 0.05$), signifying the relative intake and accumulation of these elements. Mg and P were intimately associated, reflecting the regulatory function of magnesium in energy metabolism and photosynthesis, alongside the importance of phosphorus in ATP generation and nucleic acid construction. Besdies, K-Ca and Cu-Zn exhibited a strong correlation, indicating their shared functions in regulating osmotic pressure, as well as ion control and transport across tissues [18], [19]. The correlations among components in fruits (Figure 1b) were markedly different from those in roots. In fruits, the Mg-Cu and Mg-Sr correlations remained stable across arsenic treatments, suggesting Mg's essential role in enzymatic stability and metabolic processes, irrespective of stress conditions [16]. Furthermore, Fe and P exhibited a strong correlation in the As(III) treatment group ($r = 0.47$, $p < 0.05$), indicating competition for Fe and P absorption via the phosphate transport system since As(V) and P possess similar chemical structures [20]. Besides, Fe-Mn exhibited a robust correlation in roots but weaker in fruits ($r = 0.42$, $p > 0.05$), indicating that Fe sequestration occurs via Fe-As precipitation, limiting its translocation [20]. This elucidates the alteration in the Fe/Mn ratio since Fe is sequestered in the roots, while Mn is readily translocated to the top portions of the plant to sustain photosynthetic enzyme activity. Furthermore, As exhibited a moderate correlation with the elements ($r = 0.52$, $p < 0.05$), indicating that As transport levels were governed by endogenous regulatory mechanisms, which assisted plants in minimizing As accumulation in fruits to mitigate toxicity [18]. The redistribution of elements in fruits was higher than in roots, as roots primarily absorb and sequester metals, while both xylem and phloem contribute to element movement in fruits. Endogenous regulation and plant hormones further influence ion transport, underscoring their role in managing nutrient interactions under arsenic stress [21].

3.3. Impact of As species stress on the control of elemental ratios in roots and fruits

Table 5. *Results of t-test evaluating elemental ratios in roots and fruits*

Element Ratio	Tissue	Range	Mean	*t*-statistic	*p*-value
Fe/Mn	Root	38.60 - 405.96	143.87	6.27	3.98E-06
	Fruit	0.93 - 4.31	2.06		
K/Ca	Root	10.55 - 72.87	34.79	-5.28	1.25E-05
	Fruit	16.14 - 126.34	77.87		
Mg/P	Root	0.16 - 0.30	0.23	-3.39	2.68E-03
	Fruit	0.21 - 1.43	0.41		
Cu/Zn	Root	0.48 - 1.80	1.06	-0.02	0.98
	Fruit	0.72 - 2.13	1.06		

The variations in Fe/Mn, K/Ca, Mg/P, and Cu/Zn ratios between roots and fruits indicate the micronutrient regulatory mechanism under As species stress (Table 5). The *t*-test results showed significant differences in Fe/Mn, K/Ca, and Mg/P ratios ($p < 0.01$), while Cu/Zn remained unchanged ($p = 0.98$). The Fe/Mn ratio was significantly higher in roots (143.87) than in fruits (2.06, $p < 0.05$), indicating Fe sequestration in roots via insoluble Fe-As complexes, while Mn remained mobile for enzymatic and photosynthetic functions [20], [22]. The K/Ca ratio in fruit (77.87) was almost twice that in roots (34.79, $p < 0.05$), reflecting K mobility in the phloem and Ca retention in roots for structural support [18]. In response to stress, plants may preferentially transfer potassium to preserve ionic equilibrium in fruit, whereas calcium aids in safeguarding roots from oxidative damage. The Mg/P ratio in fruit (0.41) was considerably elevated compared to that in roots (0.23, $p < 0.05$), indicating Mg's role in photosynthesis and ATP synthesis, while P competed with As(V) for uptake. The Cu/Zn ratio exhibited no significant difference between roots and fruits ($p > 0.05$), indicating stable antioxidant enzyme protection against As stress. The disparity in micronutrient ratios between roots and fruits illustrates the stringent ion management system of pepper plants under arsenic stress, which enhances physiological activities and protects reproductive organs from harmful impacts.

4. Conclusion

The research demonstrated that As stress markedly influenced the accumulation and control of elements in the roots and fruits of black pepper (*Piper nigrum* L.). The absorption and distribution of Zn, Cd, K, Ca, and Cu varied considerably across the As species treatment groups, with Zn and Cd being sequestered in the roots to mitigate toxicity, whilst K and Ca were influenced by alterations in ion transport. Fe was sequestered in the roots owing to the development of insoluble Fe-As complexes, while Mn, K, and Mg were preferentially transferred to the fruits. The alterations in the association between root and fruit components indicated the endogenous regulatory mechanism and the function of xylem and phloem in ion transport. The disparities in Fe/Mn, K/Ca, and Mg/P ratios between roots and fruits indicated that Fe was sequestered in the roots to mitigate toxicity, while Mn was readily translocated to the fruits, K was preferentially conveyed via the phloem, and Ca was deposited in the roots to preserve cellular integrity. These findings elucidate the micronutrient absorption process in pepper plants and the influence of As on nutritional equilibrium, offering valuable data for study on plants in As species-contaminated settings.

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REFERENCES

[1] Kiran, R. Bharti, and R. Sharma, "Effect of heavy metals: An overview," *Materials Today: Proceedings*, vol. 51, pp. 880-885, 2022.

[2] L. D. B. Suriyagoda, K. Dittert, and H. Lambers, "Mechanism of arsenic uptake, translocation and plant resistance to adccumulate arsenic in rice grains," *Agriculture, Ecosystems & Environment*, vol. 253, pp. 23-37, 2018.

[3] P. Kumarathilaka, S. Seneweera, Y. S. Ok, A. A. Meharg, and J. Bundschuh, "Mitigation of arsenic accumulation in rice: An agronomical, physico-chemical, and biological approach – A critical review," *Critical Reviews in Environmental Science and Technology*, vol. 50, no. 1, pp. 31-71, 2020.

[4] S. Mitsunobu, M. Toda, N. Hamamura, F. Shiraishi, Y. Tominaga, and M. Sakata, "Millimeter-scale topsoil layer blocks arsenic migration in flooded paddy soil," *Geochimica et Cosmochimica Acta*, Vol. 274, pp. 211-227, 2020.

[5] T. Sarwar, S. Khan, S. Muhammad, and S. Amin, "Arsenic speciation, mechanisms, and factors affecting rice uptake and potential human health risk: A systematic review," *Environmental Technology & Innovation*, vol. 22, 2021, Art. no. 101392.

[6] K. Straif, L. Benbrahim-Tallaa, R. Baan, Y. Grosse, B. Secretan, F. E. Ghissassi, V. Bouvard, N. Guha, C. Freeman, and L. Galichet, "A review of human carcinogens—part C: metals, arsenic, dusts, and fibres," *The Lancet Oncology*, vol. 10, no. 5, pp. 453-454, 2009.

[7] IARC, "Arsenic, Metals, Fibres and Dusts," *IARC Monographs*, vol. 100C, no. 1, p. 219, 2012.

[8] A. S. Bali and G. P. S. Sidhu, "Arsenic acquisition, toxicity and tolerance in plants - From physiology to remediation: A review," *Chemosphere*, vol. 283, 2021, Art. no. 131050.

[9] C. Spence, "The king of spices: On pepper's pungent pleasure," *International Journal of Gastronomy and Food Science*, vol. 35, 2024, Art. no. 100900.

[10] J. A. Baig, S. Bhatti, T. G. Kazi, and H. I. Afridi, "Evaluation of Arsenic, Cadmium, Nickel and Lead in Common Spices in Pakistan," *Biological Trace Element Research*, vol. 187, no. 2, pp. 586-595, 2019.

[11] M. A. Aziz, M. Adnan, A. H. Khan, A. A. Shahat, M. S. Al-Said, and R. Ullah, "Traditional uses of medicinal plants practiced by the indigenous communities at Mohmand Agency, FATA, Pakistan," *Journal of Ethnobiology and Ethnomedicine*, vol. 14, no. 1, 2018, Art. no. 2.

[12] V. Angelova, K. Ivanov, and R. Ivanova, "Heavy Metal Content in Plants from Family Lamiaceae Cultivated in an Industrially Polluted Region," *Journal of Herbs, Spices & Medicinal Plants*, vol. 11, no. 4, pp. 37-46, 2006.

[13] S. Mondal, K. Pramanik, S. K. Ghosh, P. Pal, P. K. Ghosh, A. Ghosh, and T. K. Maiti, "Molecular insight into arsenic uptake, transport, phytotoxicity, and defense responses in plants: a critical review," *Planta*, vol. 255, no. 4, 2022, Art. no. 87.
[14] Y.-J. Peng, C.-Y. Hu, W. Li, Z.-H. Dai, C.-J. Liu, and L. Q. Ma, "Arsenic induced plant growth by increasing its nutrient uptake in As-hyperaccumulator Pteris vittata: Comparison of arsenate and arsenite," *Environmental Pollution*, vol. 322, pp. 121168, 2023.
[15] M. Q. Bui, T. C. Quan, Q. T. Nguyen, T.-T. Tran-Lam, and Y. H. Dao, "Geographical origin traceability of Sengcu rice using elemental markers and multivariate analysis," *Food Additives & Contaminants: Part B*, vol. 15, no. 3, pp. 177-190, 2022.
[16] F.-J. Zhao, S. P. McGrath, and A. A. Meharg, "Arsenic as a Food Chain Contaminant: Mechanisms of Plant Uptake and Metabolism and Mitigation Strategies," *Annual Review of Plant Biology*, vol. 61, pp. 535-559, 2010.
[17] M. A. Farooq, F. Islam, B. Ali, U. Najeeb, B. Mao, R. A. Gill, G. Yan, K. H. M. Siddique, and W. Zhou, "Arsenic toxicity in plants: Cellular and molecular mechanisms of its transport and metabolism," *Environmental and Experimental Botany*, vol. 132, pp. 42-52, 2016.
[18] H. F. Bakhat, Z. Zia, S. Fahad, S. Abbas, H. M. Hammad, A. N. Shahzad, F. Abbas, H. Alharby, and M. Shahid, "Arsenic uptake, accumulation and toxicity in rice plants: Possible remedies for its detoxification: A review," *Environmental Science and Pollution Research*, vol. 24, no. 10, pp. 9142-9158, 2017.
[19] A. A. Meharg and J. Hartley-Whitaker, "Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species," *New Phytologist*, vol. 154, no. 1, pp. 29-43, 2002.
[20] T. Q. Nguyen, T.-T. Tran-Lam, H. Q. Nguyen, Y. H. Dao, and G. T. Le, "Assessment of organic and inorganic arsenic species in Sengcu rice from terraced paddies and commercial rice from lowland paddies in Vietnam," *Journal of Cereal Science*, vol. 102, 2021, Art. no. 103346.
[21] T. Garg, A. Kumar, A. Joshi, A. Awasthi, A. Rana, V. Kumar, and S. Kaur, "The role of phytohormones in reducing the arsenic-induced stress in plants," *South African Journal of Botany*, vol. 168, pp. 296-315, 2024.
[22] R. Rajpoot, R. K. Srivastava, A. Rani, P. Pandey, and R. S. Dubey, "Manganese-induced oxidative stress, ultrastructural changes, and proteomics studies in rice plants," *Protoplasma*, vol. 258, no. 2, pp. 319-335, 2021.