

Impact of Arsenic on *Pisum sativum* and Its Amelioration Using Phosphate

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ABSTRACT

The study examines the impact of arsenic on seed germination and seedling growth of *Pisum sativum* (pea) across different concentrations of Arsenic from 0 to 30 ppm and evaluates the mitigating role of phosphate for arsenic toxicity. The germination experiment was carried out with six seeds per Petriplate in triplicate across six arsenic concentrations (with and without phosphate), yielding 36 plates for each kind of medium. The toxic heavy metal significantly reduced germination rates (70.6-2.3%), root (4.83-0.76cm) and shoot lengths (3.04-0.5cm), fresh cotyledon (0.48-0.53g), root (0.13-0.002g) and shoot (0.11-0.01g) weight and dry root (0.44-0.012g), shoot (0.10-0.03g) and cotyledon weights (0.33-0.36g) of plant with severe impacts at 20 ppm and above. Amendment with 40 ppm Phosphate greatly improved all the growth indices viz germination percentage (73.7-11.8%), root (6.45-1.38cm) and shoot (4.45-1.94cm) length, fresh cotyledon (0.53-0.57g) root (0.28-0.041g) and shoot (0.28-0.10g) weight and dry root (0.05-0.02g), shoot (0.14-0.047g) and cotyledon weights (0.415-0.43g) enhancing plant resilience to arsenic stress from 0-30 ppm concentration. However, at higher concentrations, the toxic effect of As was more pronounced, and the addition of 40ppm Phosphate helps the plant to bear the toxicity of As at lower concentrations, highlighting the potential of amendments in managing contamination.

Keywords: Arsenic toxicity, *Pisum sativum*, Phosphate amelioration, Plant stress response, Heavy metal accumulation, Soil remediation.

Highlights

- Peas provide a plant-based protein source, fiber, vitamin E and antioxidants.
- Arsenic interferes with nutrient uptake, causes oxidative stress, and can lead to growth inhibition and reduced yield.
- Phosphate application improved germination percentage and enhanced root and shoot growth under arsenic stress.
- Phosphate also improves the nutrient status, aiding in the overall resilience of plants to toxic elements.

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INTRODUCTION

Arsenic is a significant environmental pollutant that adversely affects soil quality and agricultural productivity, leading to considerable health risks through the food chain. Its sources are both natural, such as the weathering of arsenic-rich minerals, and anthropogenic, including the use of arsenic-based pesticides, mining activities, and various industrial processes. In India, the issue is particularly acute in areas where arsenic-contaminated groundwater is used for irrigation. The first identification of arsenic contamination in groundwater occurred in Chandigarh in 1976, followed by a second instance in West Bengal in 1984 (Shaji *et al.*, 2021). Since then, numerous states, including West Bengal, Bihar, Uttar Pradesh, Jharkhand, Arunachal Pradesh, Assam, and Punjab, have reported similar arsenic-related challenges (Mukherjee *et al.*, 2009; Chakraborti *et al.*, 2018). The water contamination results in elevated arsenic levels in soil, threatening agricultural systems and human health. In West Bengal and parts of Bihar, soil arsenic concentrations often exceed 10 mg/kg, with some reports indicating levels as high as 20 mg/kg (Dube *et al.*, 2024). The reliance on arsenic-rich groundwater for rice cultivation is a key factor exacerbating these elevated levels in both soil and crops. Similar challenges have been observed in Assam and Uttar Pradesh, where soil arsenic concentrations frequently surpass 5 mg/kg (Das *et al.*, 2009). Globally, the variability of soil arsenic levels is striking. In Bangladesh, groundwater contamination is particularly severe; soil arsenic concentrations in agricultural areas can range from 10 to 50 mg/kg, depending on local conditions and agricultural

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practices (Marghade *et al.*, 2023). The consequences of arsenic toxicity on agriculture are significant, as it inhibits plant growth and reduces crop yields, with rice being particularly prone to arsenic uptake. This accumulation in crops poses serious health risks to humans, as chronic exposure through contaminated food and water is linked to various health issues, including skin lesions, cancers, and developmental disorders (Chen *et al.*, 2021). Moreover, arsenic contamination adversely impacts plant growth, agricultural productivity, and induces oxidative stress while challenging the antioxidant defense mechanisms in plants. As a toxic metalloid, arsenic disrupts essential physiological processes, resulting in reduced seed germination and stunted seedling growth (Saha *et al.*, 2008). The model legume, *P. sativum* (pea), demonstrates particular sensitivity to arsenic exposure. Phosphate, an essential macronutrient, plays a crucial role in plant metabolism, particularly in energy transfer and photosynthesis. It is a vital component of ATP (adenosine

triphosphate), which fuels various cellular processes, including nutrient uptake and nucleic acid synthesis. According to recent developments, biotechnological and nutrient-based treatments are essential for reducing arsenic toxicity and improving soil health, providing long-term solutions for crop resilience in contaminated soils (Chauhan *et al.*, 2024). Phosphate has the potential to alleviate stress in plants by enhancing root development and nutrient absorption, thereby increasing overall resilience. Additionally, by increasing antioxidant activity and lowering oxidative stress in plant tissues, phosphate has been demonstrated to lessen the impacts of harmful substances like arsenic (Mishra *et al.*, 2022). Actually, research has shown that phosphate improves the plant's antioxidant defense mechanisms in addition to competing with arsenate for absorption sites, which lowers arsenic buildup (Sinha *et al.*, 2023). It has been demonstrated that phosphate-arsenate rivalry reduces arsenate adsorption in soils, hence restricting its bioavailability (Sato *et al.*, 2023). Additionally, molecular research suggests that boosting the availability of phosphorus can inhibit particular arsenic transporters, which lowers the absorption and translocation of arsenic in plants (Geng *et al.*, 2023). This nutrient not only supports plant growth under optimal conditions but also enhances resilience against environmental stresses, making it vital for sustainable agriculture and food security. This research explores the impact of varying arsenic concentrations (0 to 30 ppm) on the seed germination and seedling growth of *P. sativum*, while also assessing the effectiveness of phosphate amendments in alleviating arsenic toxicity. The findings indicate that exposure to arsenic significantly impairs seed germination rates, root and shoot elongation, and both fresh and dry plant weights, with severe effects observed at concentrations of 20 ppm and above.

Based on the above context, the study is guided by the following hypothesis:

Phosphate application mitigates the toxic effects of arsenic on the germination and early seedling growth of *P. sativum* by reducing arsenic uptake and improving physiological performance.

Accordingly, the specific objectives of the study are: -

1. To assess the impact of varying arsenic concentrations (0–30 ppm) on *P. sativum* seed germination and early seedling development.
2. To evaluate the effectiveness of phosphate in reducing the toxicity caused by arsenic.
3. To evaluate the growth characteristics and germination performance of arsenic-only and arsenic + phosphate treatments.

MATERIAL AND METHOD

Plant material and treatment details

The certified seeds of Pea (*Pisum sativum*) PB89 were procured from the Directorate of Agriculture, Boileuganj, Shimla, and ICAR- National Bureau of Plant Genetic Resources, Regional

Station, Shimla, India, respectively. The metal stress of Arsenic was created by using Sodium arsenate (Na_3AsO_4). A stock solution of arsenic (1000 ppm) was prepared and used to make different concentrations of arsenic (5, 10, 20, 25, and 30 ppm) using distilled water and a control (without Arsenic).

Seed germination experiment

To assess the phytotoxicity of heavy metals most common methodology of Petri plates lined by filter paper was used, following (Bae *et al.*, 2014). The germination experiment was conducted in sterilized Petri plates (9 cm diameter) lined with autoclaved Whatman No.1 filter papers. The papers were moistened with respective concentrations of As. Seeds were surface sterilized with a 0.01% solution of mercuric chloride (HgCl_2) for two minutes before the experiment, then thoroughly washed several times with distilled water to remove all traces of disinfecting solution. Seeds were then soaked in respective concentrations (0, 5, 10, 20, 25 and 30 ppm) for 4 hours. Seven seeds were placed in each Petri plate. The moisture level of filter papers was maintained by adding 5 ml of the respective concentration of As periodically during the experiment. The experiment was replicated thrice. The germination assay was kept in a BOD incubator at a temperature of $25 \pm 20^\circ\text{C}$ for 8 days.

The seeds were observed for germination after 24 hours of experiment setup, and the parameters of germination were observed every day. On the 8th day of the experiment, the mean germination percentage, length of plumule and radicle, fresh weight of root, shoot, and cotyledon were observed. Root, shoot, and cotyledon were kept for drying at 50°C in an oven overnight to observe the dry weight.

The impacts of different concentrations of arsenic were studied on the following morphological growth parameters

Germination percentage and percent inhibition

The Germination percentage of the seed was calculated using the equation:

$$GP = (n / N) \times 100 \quad \text{-----(1)}$$

where 'n' is the number of grown seeds and N is the total number of tested seeds.

Percent inhibition of germination was calculated with the following formula given by Baruah. (Baruah *et al.*, 2019)

$$\text{Percent inhibition of germination} = 100 - \text{GI of treatment} / \text{GI of control} * 100 \quad \text{-----(2)}$$

Length of root and shoot

A ruler was used to measure the length of the root and the shoot. The roots and shoots were separated by cutting them down with the help of a sharp blade. They were lying down on a white sheet of paper, and a ruler was placed parallel to them to measure the length.

Fresh and dry weight of root, shoot, and cotyledon

The fresh weight of the whole plant was done by keeping it on a weighing balance. The roots, shoots, and cotyledons were

separated by cutting them with the help of blades. Dry weight was measured by oven drying plant material at 50 °C overnight.

Germination index

Germination index (GI) was determined by the formula given by (AOSA 1983):

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}} \text{-----(3)}$$

Vigor index

Seed vigor index was calculated as follows (Maisuria and Patel, 2009):

$$\text{Vigor index} = \text{Root length} + \text{Shoot length} \times \text{Seed germination \%} \text{-----(4)}$$

Tolerance index

The tolerance index (T.I.) was calculated using the formula given by (Iqbal and Rahmati 1992).

$$T. I. = \frac{\text{Mean root length in the metal solution}}{\text{Mean root length in control}} \times 100 \text{-----(5)}$$

Relative germination rate

The relative germination rate (RGR) and the root and shoot length were measured, as well as the ratio between them.

$$RGR = \frac{\text{Germination percentage in the metal concentration}}{\text{Germination Percentage in the control}} \text{-----(6)}$$

Percent Phytotoxicity

Percent phytotoxicity was calculated on the 8th day after germination using the formulae of Chou & Linn 1976.

$$\text{Percent Phytotoxicity} = \frac{\text{Radical length in control} - \text{Radical length in treatment}}{\text{Radical length of control}} \times 100 \text{-----(7)}$$

STATISTICAL ANALYSIS

The seed germination and seedling growth data were analysed by using Student’s t-test done in XLSTAT and two-way Analysis of Variance (ANOVA) done by using SPSS software 29 (2022), followed by Tukey-Kramer Significance Difference (HSD) test between the means of treatment to determine the significant difference. All values in this experiment are the mean of three in petriplates.

RESULTS AND DISCUSSION

Germination Percentage

Table 1 describes the mean germination percentage and inhibition of *P. sativum* at varying concentrations of As. With an increase in concentration of metal, the germination of the plant decreased from 70.63% to 2.37% and the inhibition ranged from 10.1% to 96.63%. However, with phosphate amendment increase in germination percentage was observed at each concentration of As (73.79 % to 11.87%) and a decrease in inhibition (7.6% - 83.9%) from control to higher arsenic doses. The improvement in germination with 40ppm of phosphate at each level of As concentration was statistically significant (p = 0.0003; p ≤ 0.05) according to Student’s t-test (Table 1). The results suggest that

while phosphorus can alleviate some of the negative effects of arsenic, it does not completely counteract the severe impact of high arsenic levels on germination. Phosphate plays a crucial role in enhancing germination percentages in plants exposed to toxic conditions. Because phosphate (PO₄³⁻) and arsenate (AsO₄³⁻) directly compete for the same uptake transporters, phosphorus lessens the toxicity of arsenic during germination. By lowering arsenate entrance into seeds and seedlings, this competition improves germination rates and lessens oxidative stress. It facilitates energy transfer and metabolic processes, which are vital for seedling development. Studies have shown that phosphate can mitigate the effects of heavy metal toxicity by promoting root growth and improving nutrient uptake (Bechtaoui *et al.*, 2021), thereby enhancing overall plant health and germination rates. For instance, research by Khan *et al.*, (2022) demonstrated that phosphate application significantly improved germination rates in *Phaseolus vulgaris* under cadmium stress, indicating its potential as a mitigating agent in contaminated soils. This highlights the importance of phosphate in supporting plant resilience during germination in adverse environmental conditions.

Root and Shoot Length

Fig. 1(a and b) describes the root and shoot length of *P. sativum* under varying concentrations of As with and without the amendment of phosphate. Arsenic exposure significantly reduced root and shoot lengths, with roots decreasing from 4.83 cm to 0.76 cm and shoots from 3.04 cm to 0.5 cm at 25 ppm As. Phosphate addition improved these measurements, with roots growing from 6.45 cm to 1.38 cm and shoots from 4.45 cm to 1.94 cm at 25 ppm As. Phosphate reduced the inhibition of root length from 84.3% to 79% and shoot length from 83.5% to 57% at 25 ppm. At 30 ppm As, roots showed no growth, while phosphate partially alleviated shoot growth. Statistical analysis confirmed a significant improvement in shoot length with phosphate amendment (p = 0.00013). Fig. 2 (a and b) shows the growth of *P. sativum* at different concentrations of As and As+P. The growth of root hairs is notably enhanced with As+P treatment

Table 1: Effect of As applied either singly or in combination with phosphate on germination of *P. sativum* at various concentrations

Concentrations (ppm)	Mean Germination Percentage		% Inhibition of Germination	
	As	As+P	As	As+P
0ppm (Control)	70.63±17.0	73.79±15.8	-	-
5 ppm	63.48±16.4	68.25±16.1	10.1	7.6
10 ppm	58.72±16.5	62.69±16.0	16.9	15.0
20 ppm	23.01±9.1	46.02±13.4	67.4	37.6
25 ppm	17.45±8.25	38.88±13.3	75.3	47.3
30 ppm	2.37±1.12	11.87±4.08	96.6	83.9

P = 40 ppm K₂HPO₄; values are means of three replicates. “Means ± SE”; The mean values at different concentrations of As and As+P are statistically significant according to Multivariate Tukey (HSD) and Student’s t-test between two groups. HSD Value for Root length is 1.01, and Shoot length is 0.40.

as compared to As. Phosphorus alleviates the detrimental effects of arsenic, promoting better root hair development and improving nutrient uptake. This improvement underscores the role of phosphorus in mitigating arsenic toxicity. Arsenate stress reduces root and shoot length by hijacking high-affinity phosphate transporters (PHTs) that transport phosphate (PO_4^{3-}), interrupting growth. When phosphate is provided externally, it competes with arsenate for these absorption sites, limiting arsenic input. This reduction of As uptake aids in the restoration of normal cell division and elongation, resulting in improved root and shoot development. Empirical investigations in barley reveal that additional phosphate inhibits PHT expression and lowers As accumulation, increasing plant longevity (Zvobgo *et al.*, 2018). Due to competitive inhibition between As(V) and phosphate at uptake sites, phosphate application greatly decreased arsenic toxicity in rice by increasing root and shoot length and biomass (Chattopadhyay *et al.*, 2021). More broadly, phosphorus administration has been proven to reduce As levels in both roots and shoots, which correlates with improved morphological growth.

Fresh weight and Dry weight (Root, Shoot, and Cotyledon)

The data depicted in Table 2 demonstrate that arsenic exposure markedly reduces root and shoot fresh weights, with root weight dropping from 0.13 g at 0 ppm to 0.002 g at 25 ppm and shoot weight from 0.11 g to 0.01 g with an increase in concentration

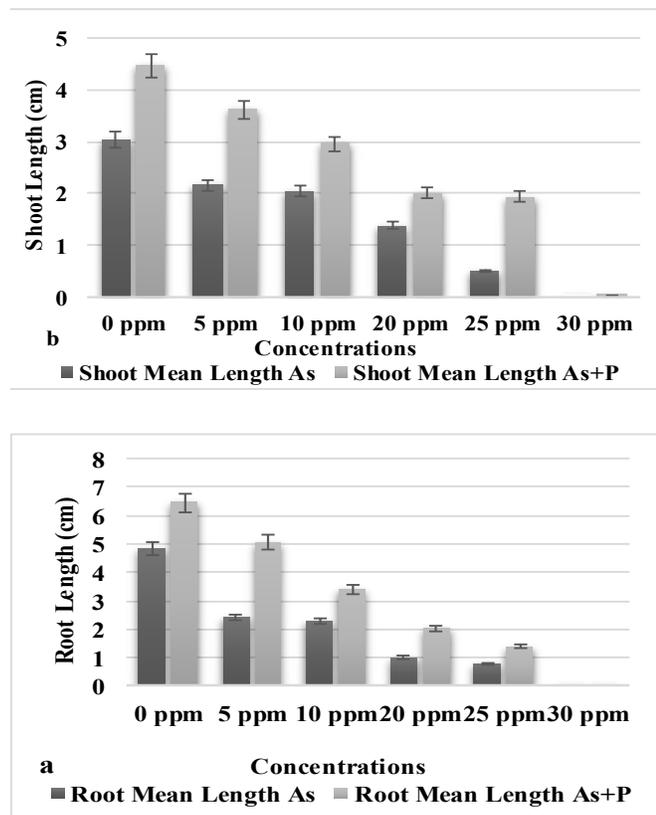


Fig. 1: Effect of different concentrations of As, either singly or in combination with Phosphate, on Root length (a) and shoot length (b) of *P. sativum*

of heavy metal. Phosphorus addition mitigates these effects to some extent, improving root and shoot weights compared to arsenic; the improvements are more pronounced at lower arsenic concentrations. At a higher concentration of 30 ppm, no root and shoot growth was observed. Cotyledon fresh weights increase with arsenic exposure and are notably higher with phosphorus addition, indicating a greater improvement in cotyledon growth with phosphorus. The data presented in Table 3 show that arsenic exposure significantly decreases root and shoot dry weights, with root weight reducing from 0.044 g to 0.002g and shoot weight from 0.10 g to 0.047g. Phosphorus addition partially alleviates these effects, improving root and shoot weights, particularly at lower arsenic concentrations. Cotyledon dry weights increase with both arsenic and phosphorus, with phosphorus showing a greater enhancement, particularly at higher arsenic levels same results were observed by Khan *et al.*, 2022 and Srivastava and Sharma 2014) in *Arabidopsis thaliana* and *Vigna mungo*. Phosphate application improves fresh and dry weight of roots, shoots, and cotyledons under arsenic stress because phosphate directly competes with arsenate at the phosphate uptake sites, reducing arsenate entry into the plant. With less arsenate absorbed, metabolic processes and ATP production recover, leading to better growth and biomass accumulation. Cotyledon weight also increases because reduced stress slows the mobilization of stored reserves, allowing greater retention of dry matter. Recent research confirms phosphorus as a critical driver of legume development. Khajuria and Debbarma (2023) discovered that phosphorus fertilizer considerably improves overall biomass and vigor in *P. sativum*. Similar findings were observed for *Vicia faba* and *Phaseolus vulgaris*, with enough P supply improving growth, physiology, and yield (Papakaloudis & Dordas, 2023).

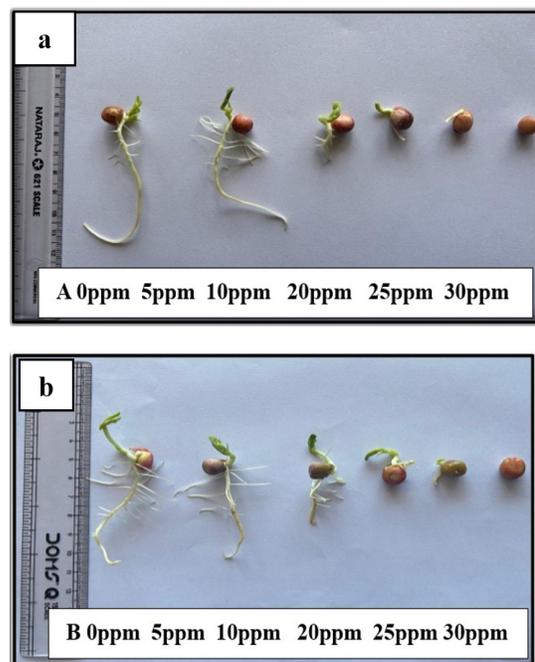


Fig. 2: Photograph showing growth of *P. sativum* at different concentrations of As (a) and As+P (b) at the 8th day

Phosphate Mitigation of Arsenic Toxicity in Plants

Table 2: Effect of As applied either singly on the fresh weight of root, shoot, and cotyledon on *P. sativum*

Concentration (ppm)	Root Mean Weight (g)		Shoot Mean Weight (g)		Cotyledon Mean Weight (g)	
	As	As+P	As	As+P	As	As+P
0 ppm (Control)	0.13±0.017 (100)	0.28±0.08 (100)	0.11±0.01 (100)	0.28±0.08 (100)	0.48±0.04 (100)	0.53±0.07 (100)
5 ppm	0.12±0.021 (92.3)	0.18±0.06 (64.2)	0.09±0.07 (81.8)	0.23±0.01 (82.1)	0.45±0.02 (93.7)	0.47±0.06 (88.6)
10 ppm	0.1±0.008 (77)	0.10±0.01 (35.7)	0.05±0.01 (45.4)	0.15±0.01 (53.5)	0.41±0.03 (85.4)	0.43±0.04 (81)
20 ppm	0.07±0.06 (53.8)	0.08±0.03 (28.5)	0.06±0.01 (54.5)	0.11±0.02 (39.2)	0.46±0.02 (95)	0.49±0.04 (92)
25 ppm	0.002±0.001 (1.5)	0.041±0.01 (14.2)	0.01±0.001 (9)	0.10±0.02 (35.7)	0.51±0.02 (106)	0.54±0.07 (101.8)
30 ppm	0±0 (0)	0±0 (0)	0±0 (0)	0±0 (0)	0.53±0.009 (110)	0.57±0.03 (107.5)
HSD VALUE	0.2		0.27		0.30	

P = 40 ppm K₂HPO₄; values are means of three replicates. "Means ± SE", the mean values at different concentrations of As are statistically significant according to Multivariate Tukey (HSD) and Student's t-test between two groups. Values in parentheses represent relative values, respective control taken as 100

Table 3: Effect of As applied in combination with phosphate on the root, shoot, and cotyledon dry weight of *P. sativum*

Concentration (ppm)	Root Mean Dry Weight (g)		Shoot Mean Dry Weight (g)		Cotyledon Mean Dry Weight (g)	
	As	As+P	As	As+P	As	As+P
0 ppm (Control)	0.044± 0.007 (100)	0.05± 0.007 (100)	0.10± 0.034 (100)	0.14± 0.012 (100)	0.33± 0.013 (100)	0.415± 0.01 (100)
5 ppm	0.037±0.006 (84)	0.045±0.009 (90)	0.09±0.033 (90)	0.12±0.018 (85.7)	0.30±0.018 (90)	0.39±0.015 (95.1)
10 ppm	0.025±0.005 (56.8)	0.03±0.006 (60)	0.07±0.012 (70)	0.08±0.009 (57.1)	0.31±0.015 (94)	0.40±0.018 (96.3)
20 ppm	0.024±0.07 (54.5)	0.01±0.003 (20)	0.01±0.002 (10)	0.04±0.005 (28.5)	0.33±0.02 (100)	0.41±0.02 (100)
25 ppm	0.012±0.014 (27.2)	0.02±0.003 (40)	0.03±0.006 (30)	0.047±0.006 (33.5)	0.35±0.02 (106)	0.42±0.02 (102)
30 ppm	0±0 (0)	0±0 (0)	0±0 (0)	0±0 (0)	0.36±0.13 (109)	0.43±0.10 (104)
HSD VALUE	0.121		0.21		0.243	

P = 40 ppm K₂HPO₄; values are means of three replicates "Means ± SE"; The mean values at different concentrations of As+P are statistically significant according to Multivariate Tukey (HSD) and Student's t-test between two groups. Values in parentheses represent relative values, respective control taken as 100.

Growth indices

Table 4 shows the effect of As and As + P treatments on plant Germination Index (GI), Vigor Index (VI), and Relative Germination Rate (RGR), Tolerance index and Percent phytotoxicity. The data shows a decline in the Germination Index as arsenic concentration increases. In the control group (0 ppm), the Germination Index was highest (19.6 for As and 21.2 for As + P). At 5 ppm, there's a noticeable drop, with values of 16.9 and 18.6, respectively. By 20 ppm, the index falls to 4.4 (As) and 16 (As + P), indicating significant toxicity. At 30 ppm, germination is severely affected, especially for As (0),

while As + P (6.8) shows slightly better resilience, suggesting phosphorus may mitigate some arsenic effects on germination. Loyavar *et al.*, 2021 found that P application alters root surface charge and restricts arsenate translocation to shoots, which directly improves tolerance indices. The Vigor Index results indicate a strong negative impact of arsenic on seed vigor as concentration increases. The control group shows the highest values (553.7 for As and 604.9 for As + P), while at 5 ppm, the indices drop significantly to 289.7 and 459.9. By 20 ppm, the values fall further, with As at 56.4 and As + P at 184.0, reflecting severe toxicity. At 30 ppm, both treatments exhibit critically

low vigor, with As at just 25.9, emphasizing the detrimental effect of arsenic on seed vitality. Recent research has shown that phosphorus plays an important role in reducing arsenic-induced phytotoxicity by modifying metal mobility, improving nutritional homeostasis, and increasing biochemical defense mechanisms in plants. Phosphorus additions lower arsenic bioavailability in soil by competitive adsorption and changing As-P interactions in the rhizosphere, resulting in decreased arsenate absorption due to competition with transporters. The Relative Germination Rate shows a clear decline with increasing arsenic concentration. At 0 ppm, both treatments start at 1, indicating normal germination. As arsenic levels rise, the rates decrease, especially at 30 ppm, where with As is 0.03, and with P amendment is 0.67. This suggests that phosphate may help mitigate arsenic's negative effects on germination. The Tolerance Index of the plant at 0 ppm was 100%. However, as the concentration increases, the tolerance decreases sharply. With 20 ppm of As, the tolerance was only 20.8%, while phosphate amendment (As + P) helps the plant to tolerate metal toxicity a bit better at 31%. At a higher concentration of As (30 ppm), the tolerance drops to 0, meaning the plants can't handle it at all, while with P, it remains at 0.46%, indicating that phosphate helps the plants cope better with arsenic toxicity. The Percent Phytotoxicity results show increasing toxicity as arsenic levels rise. At 0 ppm, there was no phytotoxicity. However, at 5 ppm of As, phytotoxicity was 49.7%, while with P amendment was 21.8%. As concentrations increase, toxicity rises significantly, reaching 100% at 30 ppm for both treatments. This indicates that phosphate helps to reduce phytotoxicity at lower arsenic levels. This emphasizes the protective role of phosphorus in mitigating arsenic toxicity and significantly mitigates the phytotoxic

effects of heavy metals in plants, improving germination and growth metrics. Additionally, Sinha *et al.*, (2020) reported that phosphorus enhances the metabolic activity of plants under stress, enabling better nutrient uptake. Khan *et al.*, (2024) found that phosphate administration efficiently reduces arsenic mobility and increases antioxidant enzyme activity, lowering oxidative stress in seedlings. Similarly, Zhou *et al.*, (2023) found that phosphorus supplementation boosts germination, seedling development, and chlorophyll stability in As-stressed plants by controlling ROS-scavenging pathways. Chen *et al.*, (2025) found that phosphorus boosts ATP synthesis and supports essential metabolic pathways necessary for early seedling growth under metal stress. Further study supports these findings. Mondal *et al.*, (2022) offered molecular insights revealing how phosphorus regulates arsenic absorption and detoxification pathways, whereas Suriyagoda *et al.*, (2022) demonstrated that phosphorus supplements greatly boost rice seedling development. Leavitt *et al.*, (2025) demonstrated that phosphorus-based agronomic interventions effectively lower arsenic accumulation in crop tissues without compromising growth. Collectively, these current studies substantially support the idea that phosphorus has protective, regulating, and detoxifying properties, reducing arsenic toxicity and greatly enhancing germination, vigor, tolerance indices, and overall plant resilience.

Table 5 shows the strong positive correlations between germination index and vigor index (0.937, $P \leq 0.05$), germination index and relative growth rate (0.994, $P \leq 0.05$), as well as vigor index and tolerance index (1.00, $P \leq 0.05$), at different heavy metal concentrations (0, 5, 10, 20, 25, 30ppm). Whereas, the strong negative correlations were observed between percent phytotoxicity and tolerance index (-1, $P \leq 0.05$), as well as percent phytotoxicity and germination index (-0.924, $P \leq 0.001$).

Table 4: Effect of As and As in combination with Phosphate on Germination Index, Vigor Index, Relative Germination Rate, Tolerance Index, and Percent Phytotoxicity of *P. sativum*

Concentration in (ppm)	Germination Index		Vigor Index		Relative Germination Rate		Tolerance Index		Percent Phytotoxicity	
	As	As + P	As	As + P	As	As + P	As	As + P	As	As + P
0ppm(Control)	19.6	21.2	553.7	604.9	1	1	100	100	0	0
5 ppm	16.9	18.6	289.7	459.9	0.89	0.95	50.2	78.1	49.7	21.8
10 ppm	15	16.9	254.1	315.3	0.83	0.89	47.7	52.2	52.2	47.7
20 ppm	4.4	16	56.4	184.0	0.33	0.82	20.8	31.0	79.1	68.9
25 ppm	4.7	10.3	25.9	128.8	0.29	0.69	15.8	21.3	84.1	78.6
30 ppm	0	6.8	0	2.60	0.03	0.67	0	0.46	100	100

Table 5: Correlation matrix between different Growth Indices of *P. sativum* with different concentrations of As (0, 5, 10, 20, 25, 30 ppm)

Growth Indices	Germination Index	Vigor Index	Relative Growth Rate	Tolerance Index	Percent Phytotoxicity
Germination Index	1				
Vigor Index	0.937**	1			
Relative Growth Rate	0.994**	0.914**	1		
Tolerance Index	0.924**	0.990**	0.909**	1	
Percent Phytotoxicity	-0.924**	-0.990**	-0.909**	-1	1

**Correlation is significant at the 0.05 level

Table 6: Correlation matrix between different Growth Indices of *P. sativum* with different concentrations of As+P (0, 5, 10, 20, 25, 30 ppm)

Growth Indices	Germination Index	Vigor Index	Relative Growth Rate	Tolerance Index	Percent Phytotoxicity
Germination Index	1				
Vigor Index	0.936**	1			
Relative Growth Rate	0.978**	0.95**	1		
Tolerance Index	0.937**	1.00**	0.961**	1	
Percent Phytotoxicity	-0.937**	-1.00**	-0.961**	-1.00**	1

**Correlation is significant at the 0.05 level

The strong positive correlations between germination index and vigor index (0.936, $P \leq 0.05$), germination index and relative growth rate (0.978, $P \leq 0.05$), as well as vigor index and tolerance index (1, $P \leq 0.05$), at different heavy metal concentrations (0, 5, 10, 20, 25, 30ppm). Whereas, the strong negative correlations between percent phytotoxicity and tolerance index (-1.00, $P \leq 0.01$), as well as percent phytotoxicity and germination index (-0.937, $P \leq 0.01$), as shown in Table 6.

CONCLUSION

The negative effects of arsenic on *P. sativum*, including stunted development, decreased root-shoot elongation, and large decreases in biomass accumulation, reveal the species' high vulnerability to arsenic toxicity. Arsenic inhibits important metabolic processes, impairs food intake, and causes oxidative stress, all of which affect proper seedling development. However, the use of phosphate significantly reduces these negative effects and promotes superior development performance even when exposed to arsenic. This improvement is primarily due to the competitive interaction between phosphate (PO_4^{3-}) and arsenate (AsO_4^{3-}) ions, which have similar chemical structures and use the same phosphate transporters in plant roots. By outcompeting arsenate at these absorption sites, phosphate efficiently restricts arsenic access into root cells, limiting its translocation to shoots and minimizing physiological and oxidative damage. This study has important practical implications in agriculture, since strategic phosphate treatment can decrease yield losses, preserve crop quality, and provide a viable, low-cost way to manage arsenic-contaminated soils and enhance cropping system productivity. From the perspective of policy, the findings highlight the need for integrated nutrient management recommendations that encourage the prudent application of phosphorus fertilizers in polluted regions, as well as monitoring systems to check arsenic levels in irrigation water and soil. Encouraging extension agencies to promote phosphate-based mitigation strategies and incorporating them into regional agricultural advisories might considerably enhance crop safety and food security in sensitive areas. Finally, the findings give support to long-term management strategies targeted at protecting soil health and agricultural yield in the face of rising environmental pollution.

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AUTHOR CONTRIBUTION

Tara Thakur was involved in research and lab work. The final editing was done by Nisha Rani, and she also supervised the work. All authors read and approve the final version of the manuscript.

CONFLICT OF INTEREST

The author declares that they have no conflict of interest.

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