

## Article

# Effect of Peanut Intercropping on Arsenic Uptake and Remediation Efficiency of Plants in Arsenic-Contaminated Soil

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**Abstract:** Phytoremediation is an economically viable and environmentally friendly technique among various arsenic-contaminated soil remediation technologies. Field plot experiments were conducted to investigate the effects of peanut intercropping with sunflower, lucerne, and jute on the growth and development of intercropped crops and the efficiency of arsenic (As) remediation in polluted soil within the intercropping system. The results indicate that intercropping peanuts with other crops can enhance the biomass and yield of the crops. The land equivalent ratios (LER) of the three intercropping patterns were 1.03, 1.70, and 1.17, respectively. The intercropping pattern also influences the absorption and accumulation of As in crops. Total arsenic accumulation in peanuts intercropped with jute reached  $493 \mu\text{g} \cdot \text{plant}^{-1}$ , which was significantly higher by 29.5% compared to peanut monoculture. Additionally, the translocation factor (TF) and bioaccumulation factor (BCF) of peanut seeds were significantly higher in peanut-jute intercropping compared to other treatments, but the As content of peanut seeds in all treatments complied with national food safety standards (GB2762-2022,  $0.5 \text{ mg} \cdot \text{kg}^{-1}$ ). Intercropping of peanuts altered the pH and Eh values of rhizosphere soil, further influencing the percentage content of various forms of As in the soil, and reducing the mobility and effectiveness of As. The metal removal equivalent ratios (MREER) for the three intercropping patterns were 1.30, 2.11, and 1.26, respectively. The intercropping of peanuts and lucerne resulted in an MREER of 2.11. It indicates that peanut intercropping has a significant promotion and high restoration efficiency on the growth and development of lucerne. Therefore, among the three patterns, the peanut intercropping lucerne pattern has the best effect in applying to contaminated soil, and can better realize the integration of economic and ecological benefits.

**Keywords:** phytoremediation; intercropping; arsenic; peanuts; cash crops



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## 1. Introduction

It has been reported that annual arsenic emissions amount to 24,000 tons, approximately 60% of the arsenic emissions are attributed to copper smelting and coal combustion, and only 2% of arsenic emissions occur through natural events [1]. During this process, Arsenic released into the environment enters the soil through atmospheric deposition or irrigation with contaminated water. Arsenic in soil can pose a threat to human health through the amplification effect of the food chain [2,3]. However, arsenic contamination is a widespread problem on all continents of the planet, particularly in regions such as Southeast Asia, Africa, North America, and South America, where high concentrations

of arsenic are prevalent, China has also become one of the global hotspots for arsenic contamination [4,5].

Peanut (*Arachis hypogaea* L.) is recognized as one of the principal oilseed crops globally. The fruits of this plant are rich in unsaturated fatty acids, proteins, carbohydrates, and vitamins. The consumption of peanuts and their by-products has been associated with various health benefits, including the enhancement of human health, a reduction in the risk of diseases, and the inhibition of cancer cell growth [6–8]. Currently, the area dedicated to peanut cultivation in China encompasses 4.6 million hectares, representing 40% of global production [9]. China is the largest producer of peanuts worldwide. However, peanuts are particularly vulnerable to heavy metal contamination, and the elevated concentrations of heavy metals in agricultural soils present a substantial risk to the safety of peanut products. It has been reported that 100% of the 255 peanut samples collected were contaminated with heavy metals, with 61.5% of these samples being contaminated by arsenic [10]. Zhang et al. [11] found that among 34 soil samples and paired peanut samples collected, soil As and Cd posed the greatest ecological contamination risk, and the average concentration of soil As was  $61.5 \text{ mg} \cdot \text{kg}^{-1}$ , and the As accumulated in the seeds posed the highest level of human health risk. Research indicates that the heavy metal content in food primarily originates from soil, contaminated water, and the application of pesticides and herbicides [12,13]. Furthermore, a significant correlation has been observed between the heavy metal content in soil and the heavy metal contamination of peanuts [14]. Therefore, achieving safe production of edible peanut parts in regions with severe As contamination in China holds urgent practical significance.

Phytoremediation is a cost-effective and environmentally sustainable remediation technology that employs the synergistic interactions of plants and microorganisms to remediate contaminated water bodies and soil, in contrast to alternative methods such as chemical and physical remediation techniques [15,16]. Particularly, the application of intercropping has significantly facilitated the popularization and application of this technology. In the existing studies on the phytoremediation of soil heavy metals, most of the studies have focused on intercropping of cash crops with hyperaccumulative crops [17,18] and very few studies have used intercropping of different cash crops to explore their actual remediation effects. Sunflower (*Helianthus annuus* Linn.) is a widely cultivated oil-producing economic crop globally, rich in nutrients, and a crop of both economic and ornamental value. However, sunflowers have a high accumulation capacity for heavy metals [19], which adversely affects both their biomass and oil quality. This characteristic considerably restricts their cultivation on land contaminated with heavy metals [20]. Lucerne (*Luffa aegyptiaca* Mill.) is an annual green vegetable widely grown in China with good suitability for the growing environment, and it has been reported that different varieties of lucerne have different levels of absorption of heavy metals such as chromium (Cr), cadmium (Cd), lead (Pb) and zinc (Zn) [21]. Jute (*Corchorus capsularis* L.), similar to lucerne, exhibits a substantial biomass and a rapid growth rate, which facilitates multiple harvests throughout the growing season. This characteristic enables the root system to dilute the transfer of heavy metals to the aboveground parts of the plant [22]. Sunflower, lucerne, and jute are common economic crops in China, and their application in arsenic-contaminated farmlands is relatively acceptable by farmers.

However, there are fewer studies on the remediation effects of sunflower, lucerne, and jute on As-contaminated land, resulting in the feasibility of intercropping sunflower, lucerne, and jute with peanuts for the management of As-contaminated farmland is not clear. Therefore, we conducted field experiments to investigate the remediation effects of sunflower, lucerne, and jute intercropped with peanuts in both monoculture and intercropping systems on arsenic-contaminated farmland, additionally, we explored whether

intercropping could promote the growth of these crops. The aim of this study is to provide insights into achieving simultaneous soil remediation and agricultural production in arsenic-contaminated soils, thereby facilitating the promotion and application of this intercropping model within phytoremediation technologies.

## 2. Materials and Methods

### 2.1. Test Site and Basic Physical and Chemical Properties of Soil

Field experiments were conducted from 25 August 2023 to 3 January 2024 in Longgang District, Shenzhen, Guangdong Province, China. The location is characterized by a subtropical monsoon climate, with an annual average temperature of 22.3 °C and an annual average rainfall of 1933 mm. Soil samples from the 0–20 cm surface layer were collected using the five-point sampling method prior to crop planting. These samples were then taken back to the laboratory for air-drying and subsequent analysis. According to the Chinese Soil Classification System, the soil at the test site is classified as sandy loam, with silt accounting for 71%, sand 19%, and clay 10%. Additionally, the pH value is 6.68, alkali-hydrolyzable nitrogen is 74.1 mg·kg<sup>-1</sup>, available phosphorus is 47.7 mg·kg<sup>-1</sup>, rapidly available potassium is 123.7 mg·kg<sup>-1</sup>, and total arsenic 264.7 mg·kg<sup>-1</sup>, which exceeds the risk management standard for As contamination in farmland soils in China (GB15618-2018) [23].

### 2.2. Field Trial Design and Yield Measurement

The test plant peanut was purchased from the shade-tolerant variety “Guihua No. 57” which is suitable for intercropping in Guangxi, the sunflower used was the annual plant variety “DuLe Kui No. 1”, and the lucerne and jute were selected from the common vegetable varieties in Chaoshan area.

Field experiments commenced on 25 August 2023. The experiment included seven treatments: monocropping of peanut (MA), monocropping of sunflower (MH), monocropping of lucerne (ML), monocropping of jute (MC), intercropping of peanut and sunflower (AH), intercropping of peanut and lucerne (AL), and intercropping of peanut and jute (AC). Each treatment was repeated three times and each plot size was 6 m<sup>2</sup> (2 × 3) and was arranged in a completely randomized manner, with a 50 cm isolation zone designed between adjacent plots to avoid mutual influence between plots water management, fertilizer application, and field management of all plots were carried out in accordance with local crop farming patterns.

In monocropping, the row spacing and plant spacing for peanuts and sunflowers is 40 cm, while the plant spacing for lucerne and jute is 40 cm with a row spacing of 60 cm. In intercropping, the intercropping ratio between the two plants is 1:1. The row spacing and plant spacing for peanut intercropping with sunflowers are the same as those for monocropping. For peanut intercropping with lucerne and peanut intercropping with jute, the plant spacing is 40 cm and the row spacing is 60 cm. Simultaneously with the collection of samples, the biomass and yield of each crop in each plot were fully harvested and measured.

### 2.3. Sample Collection and Analysis

Upon maturity, the entire plant was harvested and brought back to the laboratory for separation. Samples of lucerne were collected on 30 November 2023, while samples of peanut, sunflower, and jute were collected on 5 January 2024. This study divided peanuts into roots, stems, leaves, shells, and seeds, and sunflowers into roots, stems, leaves, flowers, and seeds; lucerne into roots, stems, leaves, and fruits; and jute into roots, stems, and leaves. The separated samples were rinsed three times with deionized water, then placed in an

oven at 105 °C for 30 min to inactivate enzymes, and subsequently dried at 70 °C until constant weight was achieved to determine the dry weight of each plant part. The samples were then ground in a sample mill for further analysis.

Collect rhizosphere soil samples simultaneously with plant specimens. The collected rhizosphere soil was air-dried in a well-ventilated area, and stones and plant residues were removed. The soil was then sieved and stored in sealed bags for the determination of relevant rhizosphere soil indicators.

### 2.3.1. Plant Sample Analysis

Accurately weigh 0.2 g of pulverized plant samples, place them into polytetrafluoroethylene digestion tubes, add 10 mL of nitric acid, and subject them to preliminary digestion in a graphite furnace. The samples were then sealed and placed in a microwave digestion apparatus (Mars 6; CEM Corporation, Matthews, NC, USA) for digestion. After digestion, the samples were acidified in a graphite furnace until the liquid volume was reduced to 1–2 mL. The cooled solution was transferred to a 25 mL volumetric flask (Hunan Xiangbo, Changsha, China) diluted to volume, filtered, and stored in a 30 mL vial for subsequent analysis. The concentration of As in the digested solution was measured using an atomic fluorescence spectrophotometer (AFS-933, Beijing Jitian, Beijing, China). A standard curve was prepared during the measurement process using a purchased arsenic standard solution (CFGG-AA-060033-0201). Quality control was ensured by using rice flour reference material (GBW(E)-100349) and blank samples, with a recovery rate of  $92.35\% \pm 3.27$  for the rice flour reference material.

### 2.3.2. Soil Sample Analysis

Determination of rhizosphere soil pH and Eh. Weigh 10 g of rhizosphere soil sieved through a 2 mm mesh into a centrifuge tube (Hunan Xiangbo, China), add 25 mL of pure water, and place it on a shaker for thorough shaking for 1 h. Afterward, allow it to settle for 3 h. Measure the pH and Eh values of the supernatant using a portable pH meter (SX620, Sanxin Instrument Co., Ltd., Shanghai, China) and an Eh meter (SX630, Sanxin Instrument Co., Ltd., Shanghai, China), respectively.

Different As species in the rhizosphere soil. Determination of different forms of Arsenic in rhizosphere soil using the Wenzel [24] Five-Step extraction method. Including non-specifically adsorbed arsenic (F1), specifically adsorbed arsenic (F2), amorphous iron and aluminum oxide-bound arsenic (F3), crystalline iron and aluminum oxide-bound arsenic (F4), and residual arsenic (F5), with quantification by atomic fluorescence spectrophotometry (AFS-933, Beijing Jitian, China).

## 2.4. Data Calculation and Statistical Analysis

### 2.4.1. Data Calculation

#### (1) Total As accumulation

$$\text{Total As accumulation} = C_p \times B_p$$

where  $C_p$  represents the heavy metal content in various parts of the plant, and  $B_p$  represents the corresponding biomass of each part. Reflects the ability of plants to extract and accumulate heavy metals from soil. Total arsenic accumulation is also a measure of the plant's bioconcentration amount (BCA).

#### (2) Bioaccumulation Factor (BCF)

$$\text{BCF} = \frac{C_{\text{shoot}}}{C_{\text{soil}}} [\text{CC1}]$$

where  $C_{shoot}$  and  $C_{soil}$  represent the heavy metal content in various parts of the plant and the heavy metal content in the soil, respectively. Reflects the ability of plant extracts to remove heavy metals from soil.

(3) Transfer factor (TF)

$$TF = \frac{C_{shoot}}{C_{root}} [CC2]$$

where  $C_{shoot}$  represents the heavy metal concentrations in various parts of the plant above-ground (e.g., stems, leaves),  $C_{root}$  indicates the heavy metal concentration in the plant roots. Reflecting the capacity of a certain heavy metal to be translocated from the underground parts of plants to various aboveground organs.

(4) Land equivalent ratio (LER)

$$LER = \left( \frac{Y_{ic}}{Y_{mc}} \right) + \left( \frac{Y_{ip}}{Y_{mp}} \right) [CC3]$$

where  $Y_{ic}$  and  $Y_{ip}$  represent the yields of the two crops in the intercropping system, where  $Y_{mc}$  and  $Y_{mp}$  represent the yields of the two crops in the monocropping system, respectively. Reflects whether intercropping patterns confer yield advantages. When  $LER = 1$ , it indicates that the intercropping system has the same resource use efficiency as the corresponding monocropping system.  $LER > 1$  indicates that the intercropping pattern has a yield advantage, while  $LER < 1$  indicates that the intercropping pattern has a yield disadvantage.

(5) Metal removal equivalent ratio (MRER)

$$MRER = \left( \frac{BCA_{ic}}{BCA_{mc}} \right) + \left( \frac{BCA_{ip}}{BCA_{mp}} \right) [CC4]$$

where  $BCA_{ic}$  and  $BCA_{ip}$  represent the BCA of the two crops in the intercropping system,  $BCA_{mc}$  and  $BCA_{mp}$  represent the BCA of the two crops in the monocropping system, respectively. Reflecting the advantage of intercropping systems in heavy metal removal. When  $MRER > 1$ , it indicates that the intercropping pattern has an advantage in heavy metal removal, while when  $MRER < 1$ , it indicates that the intercropping pattern has a disadvantage in heavy metal removal.

#### 2.4.2. Statistics and Analysis

Statistical analysis was conducted using IBM SPSS Statistics 27.0, with all treatments replicated three times ( $n = 3$ ), data are presented as mean  $\pm$  standard deviation. Determine significant differences among treatments using One-way ANOVA and Independent Samples *t*-test, and visualize results with origin 2024b.

### 3. Results

#### 3.1. Biomass and Yield

The biomass changes of various crops under different treatments are shown in Table 1. Compared to monoculture, the root, stem, and leaf biomass of peanuts significantly increased when intercropped with sunflower and jute. The root biomass increased by 29.6% and 28.7% compared to MA ( $p < 0.05$ ), respectively. The stem biomass increased by 114.1% and 75.2% ( $p < 0.05$ ) compared to MA, and the leaf biomass increased by 130.0% and 43.9% ( $p < 0.05$ ) compared to MA. When intercropped with sunflower and lucerne, peanut seed yield decreased by 10.3% and 15.5%, respectively, compared to monocropping, but these differences were not statistically significant. In the AC treatment, peanut shell, and seed yields significantly increased by 66.3% and 46.9%, respectively, compared to monocropping ( $p < 0.05$ ).

**Table 1.** Biomass and yield of each crop under different treatments.

| Treatment | Biomass of Different Parts (g·plant <sup>−1</sup> ) |                |                |                |                 | Yield (g·plot <sup>−1</sup> ) | LER  | MRER |
|-----------|-----------------------------------------------------|----------------|----------------|----------------|-----------------|-------------------------------|------|------|
|           | Root                                                | Stem           | Leave          | Seed/Fruit     | Shell/Flower    |                               |      |      |
| MA        | 1.08 ± 0.1 b                                        | 10 ± 0.65 c    | 4.94 ± 0.34 c  | 28.27 ± 3.12 b | 10.95 ± 1.22 bc | 1130.93 ± 124.88 a            | -    | -    |
| AH-A      | 1.4 ± 0.11 a                                        | 21.41 ± 2.38 a | 11.36 ± 1.29 a | 25.36 ± 1.78 b | 12.65 ± 2.05 b  | 507.2 ± 35.55 c               | 1.03 | 1.30 |
| AL-A      | 1.17 ± 0.11 b                                       | 11.53 ± 1.7 c  | 5.56 ± 0.84 ab | 23.9 ± 4.57 b  | 9.03 ± 0.83 c   | 382.4 ± 73.17 c               | 1.70 | 2.11 |
| AC-A      | 1.39 ± 0.09 a                                       | 17.52 ± 1.05 b | 7.11 ± 1.13 b  | 41.52 ± 4.15 a | 18.21 ± 1.54 a  | 664.27 ± 66.47 b              | 1.17 | 2.26 |
| MH        | 5.48 ± 0.93 a                                       | 16.14 ± 1.07 a | 11.85 ± 1.67 a | 29.79 ± 5.8 a  | 15.93 ± 2.03 a  | 1191.47 ± 232.17 a            | -    | -    |
| AH-H      | 2.96 ± 0.27 b                                       | 10.42 ± 0.79 b | 9.6 ± 1.58 a   | 34.48 ± 6.43 a | 9.82 ± 1.5 b    | 689.53 ± 128.59 b             | -    | -    |
| ML        | 0.73 ± 0.16 b                                       | 32.79 ± 1.73 b | 43.62 ± 8.3 a  | 9.17 ± 0.6 b   | -               | 293.5 ± 19.24 b               | -    | -    |
| AL-L      | 1.06 ± 0.09 a                                       | 42.14 ± 2.29 a | 54.19 ± 9.52 a | 25.02 ± 1.44 a | -               | 400.25 ± 22.98 a              | -    | -    |
| MC        | 7.07 ± 0.91 a                                       | 29.67 ± 4.27 a | 6.41 ± 0.51 a  | -              | -               | 205.01 ± 16.42 a              | -    | -    |
| AC-C      | 8.44 ± 0.97 a                                       | 26.34 ± 0.99 a | 7.42 ± 1.33 a  | -              | -               | 118.77 ± 21.34 b              | -    | -    |

Biomass of each crop under different treatments. (All values are expressed as mean ± standard error (n = 3). The independent samples *t*-test was used to determine if there was a significant difference between the two means of the single and intercrops. Different letters indicate significant ( $p < 0.05$ ) differences between treatments according to Duncan's test. M denotes monoculture, A, H, L, and C denote peanut, sunflower, lucerne, and jatropha, respectively).

The biomass of roots, stems, and flowers in sunflower intercropped with peanuts was significantly reduced by 46.0%, 35.4%, and 38.4%, respectively, compared to sole sunflower cropping. Although the seed biomass increased by 15.7% in intercropped sunflowers, this increase did not reach a statistically significant level. Compared to monoculture, intercropping peanuts with lucerne resulted in significant increases in root, stem, and fruit biomass by 45.2%, 28.5%, and 172.8%, respectively ( $p < 0.05$ ). However, intercropping jute with peanuts did not show significant differences in biomass across all plant parts.

Due to the reduction in the planting area, the yield per plot of peanuts and sunflowers in intercropping systems was significantly lower than that in monoculture systems. In contrast, the yield of lucerne increased by 172.7%, reaching a significant difference compared to monoculture. In addition, the LER values for the three intercropping patterns with peanuts were all greater than 1, with the LER for peanut intercropping with lucerne reaching 1.70, indicating that intercropping patterns have a yield advantage.

### 3.2. Arsenic Content in Various Parts of Crops

Under intercropping conditions, the As content in peanut roots decreased by 56.3%, 36.9%, and 27.5% compared to monocropping ( $p < 0.05$ ; Figure 1a). In AL treatment, the arsenic content in peanut stems was significantly reduced by 47.3% compared to monocropped peanuts. In the AH and AC treatments, although the As content in peanut stems increased, the difference did not reach a significant level. When peanuts were intercropped with seed sunflowers, the As content in the leaves of peanuts decreased significantly by 210.6%, 213.7%, and 268.5% compared to the MA, AL, and AC treatments, respectively. Intercropping reduced the accumulation of As in peanut shells, with significant differences observed between AH and AL treatments. The trend in As concentration in peanut seeds was AC > MA > AH > AL, with the As concentration in peanut seeds under the AC treatment being 0.24 mg·kg<sup>−1</sup>, significantly different from other treatments ( $p < 0.05$ ). However, the As content in peanut seeds under all treatments met the national food safety standard (GB2762-2022, 0.5 mg·kg<sup>−1</sup>) [25].

As shown in Figure 1b, intercropping significantly reduced the As content in the roots, leaves, and seeds of sunflower, with reductions of 20.6%, 32.4%, and 34.9%, compared to MH. Under AL treatment, the As content in the roots of lucerne significantly decreased by 24.52% compared to ML, while the As content in the leaves significantly increased by 123.4% ( $p < 0.05$ , Figure 1c). The As content in the fruits of Lucerne decreased from 0.55 mg·kg<sup>−1</sup> to 0.45 mg·kg<sup>−1</sup>, although no significant difference was observed. However, the As content in the fruits of lucerne under AL treatment met the national food safety

standards. Intercropping jute with peanuts reduced the As content in the roots, stems, and leaves of jute by 21.7%, 13.9%, and 51.2%, respectively, with significant differences observed in the leaf levels.

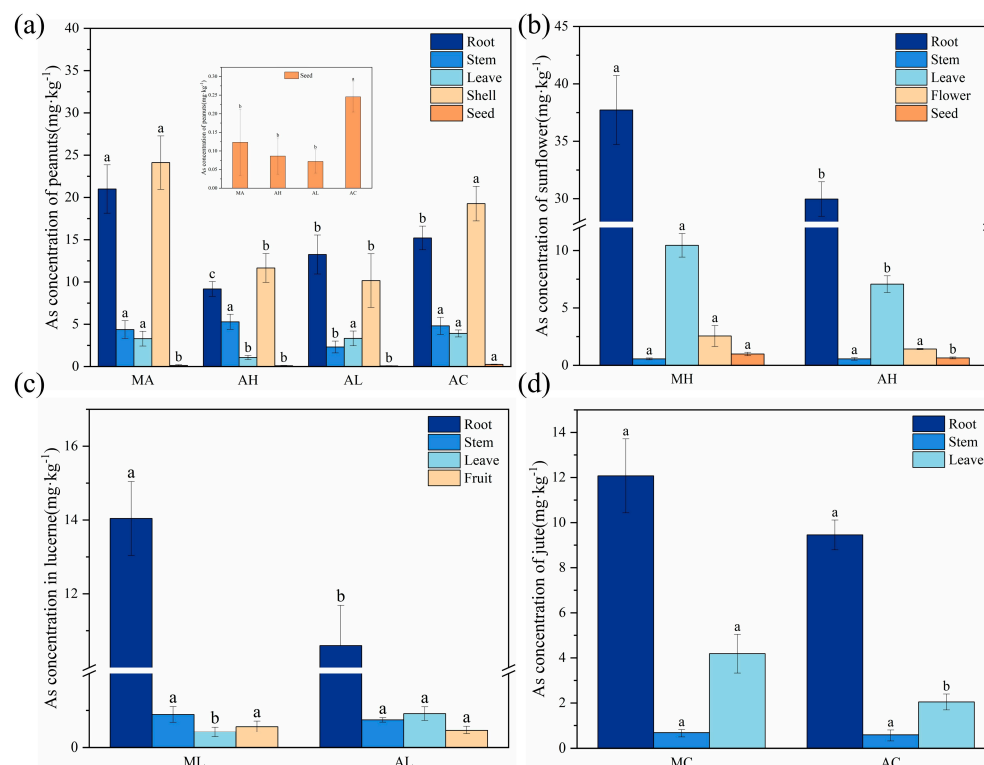


**Figure 1.** As concentration in various parts of peanut (a) sunflower (b) lucerne (c) and jute (d) under different treatments. (All values are expressed as mean  $\pm$  standard error ( $n = 3$ ). The independent samples  $t$ -test was used to determine if there was a significant difference between the two means of the single and intercrops. Different letters indicate significant ( $p < 0.05$ ) differences between treatments according to Duncan's test. M denotes monoculture, A, H, L, and C denote peanut, sunflower, lucerne, and jute).

### 3.3. Total Arsenic Accumulation in Plants

The total As accumulation in peanut was highest under AC treatment ( $492.6 \mu\text{g}\cdot\text{plant}^{-1}$ ), which was significantly higher than that in MA, AH, and AL by 29.5%, 42.4%, and 69.3%, respectively ( $p < 0.05$ ; Figure 2a). Significant differences were observed among the treatments. The trend in total arsenic accumulation in peanut plants was generally similar to that in the shells and seeds. Intercropping reduced the total As accumulation in both sunflower and jute by 51.4% and 16.1%, respectively, compared to monocropping (Figure 2b). Notably, the total As accumulation in lucerne intercropped with peanut ( $102.23 \mu\text{g}\cdot\text{plant}^{-1}$ ) was significantly higher by 39.9% ( $p < 0.05$ ) than that in monocropped lucerne, which can be attributed to the effective increase in biomass of lucerne in this intercropping system.

### 3.4. TF and BCF

The transfer factor of As in different parts of peanuts under various treatments is shown in Figure 3a. The TF in peanut stems under the AH treatment was significantly higher than those under MA, AL, and AC treatments, increasing by 64.1%, 70.1%, and 45.3%, respectively ( $p < 0.05$ ). Conversely, the TF in peanut leaves under the AH treatment was significantly lower than those under MA, AL, and AC treatments, decreasing by 35.9%, 117.4%, and 124.3%, respectively. The translocation factors of peanut shells and kernels were the lowest when peanuts were intercropped with lucerne, with values of 0.75 and 0.005, respectively. For sunflowers, the transfer factor in flowers and seeds under

monoculture and intercropping conditions was slightly reduced compared to MH but did not reach significant levels of difference. However, the transfer factor in leaves was significantly reduced by 14.9% compared to MH (Figure 3b). The TF of lucerne under AL treatment where higher in all parts compared to ML, with significant differences observed in the leaves. Intercropping jute with peanuts significantly reduced the As transfer factor in the leaves by 37.6% compared to MC, while no significant difference was found in the stem transfer factor.

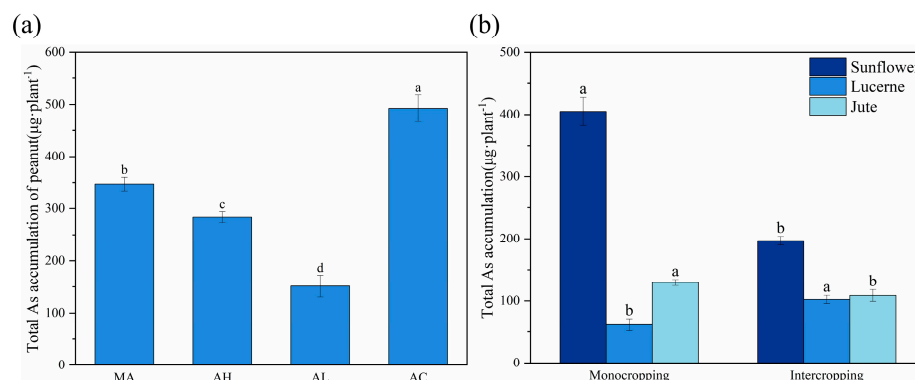


**Figure 2.** Total As accumulation of peanut (a) sunflower, lucerne and jute (b). (All values are expressed as mean  $\pm$  standard error ( $n = 3$ ). The independent samples  $t$ -test was used to determine if there was a significant difference between the two means of the single and intercrops. Different letters indicate significant ( $p < 0.05$ ) differences between treatments according to Duncan's test).

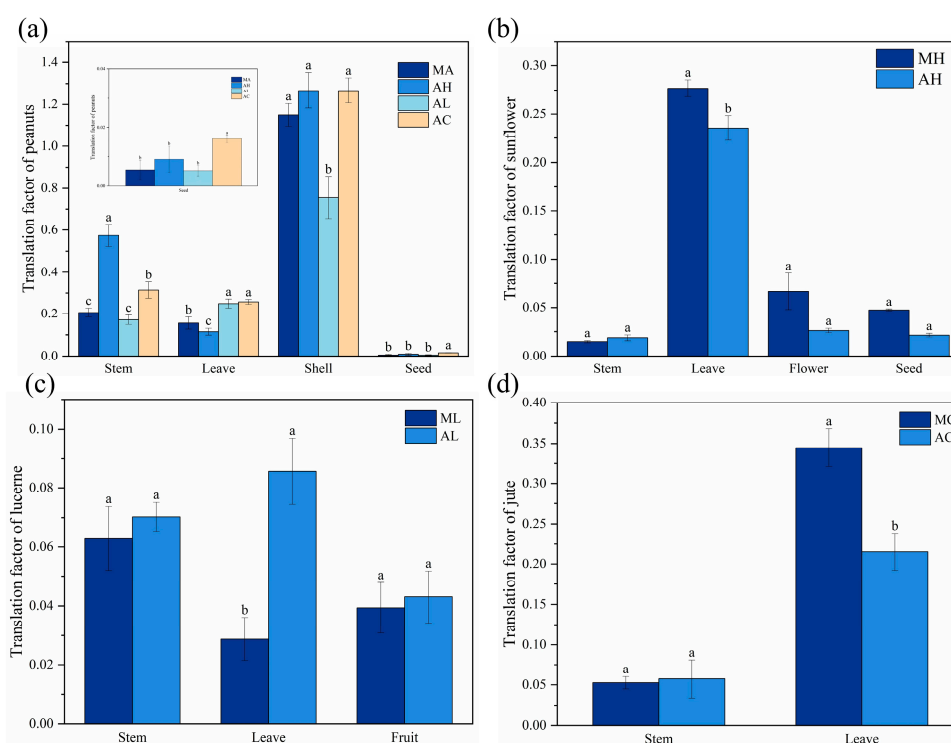


**Figure 3.** Translation factor of peanut (a) sunflower (b) lucerne (c) and jute (d) under different treatments. (All values are expressed as mean  $\pm$  standard error ( $n = 3$ ). The independent samples  $t$ -test was used to determine if there was a significant difference between the two means of the single and intercrops. Different letters indicate significant ( $p < 0.05$ ) differences between treatments according to Duncan's test. M denotes monoculture, A, H, L, and C denote peanut, sunflower, lucerne, and jute).

As shown in Figure 4a, the As bioaccumulation factor in peanut roots under intercropping was significantly lower than that under monocropping. The As bioaccumulation factor in peanut stems under the AL treatment and in peanut leaves under the AH treatment exhibited significant differences compared to other treatments. The BCF of peanut shells under intercropping was reduced by 51.7%, 57.8%, and 20.1% ( $p < 0.05$ ) compared to monocropping, respectively. Notably, the BCF of peanut seeds was significantly higher in the AC treatment compared to other treatments, with an increase ranging from 14.9% to 72.9%. The BCF of roots, leaves, and seeds in intercropped sunflowers was significantly lower than those in monocropped sunflowers, while the BCF of stems and flowers showed a decrease compared to MH but did not reach a significant difference (Figure 4b). The BCF of lucerne roots, stems, and fruits, as well as jute roots, stems, and leaves, decreased after intercropping compared to monocropping. However, the As BCF in lucerne leaves significantly increased by 55.2% ( $p < 0.05$ , Figure 4c,d) compared to ML.

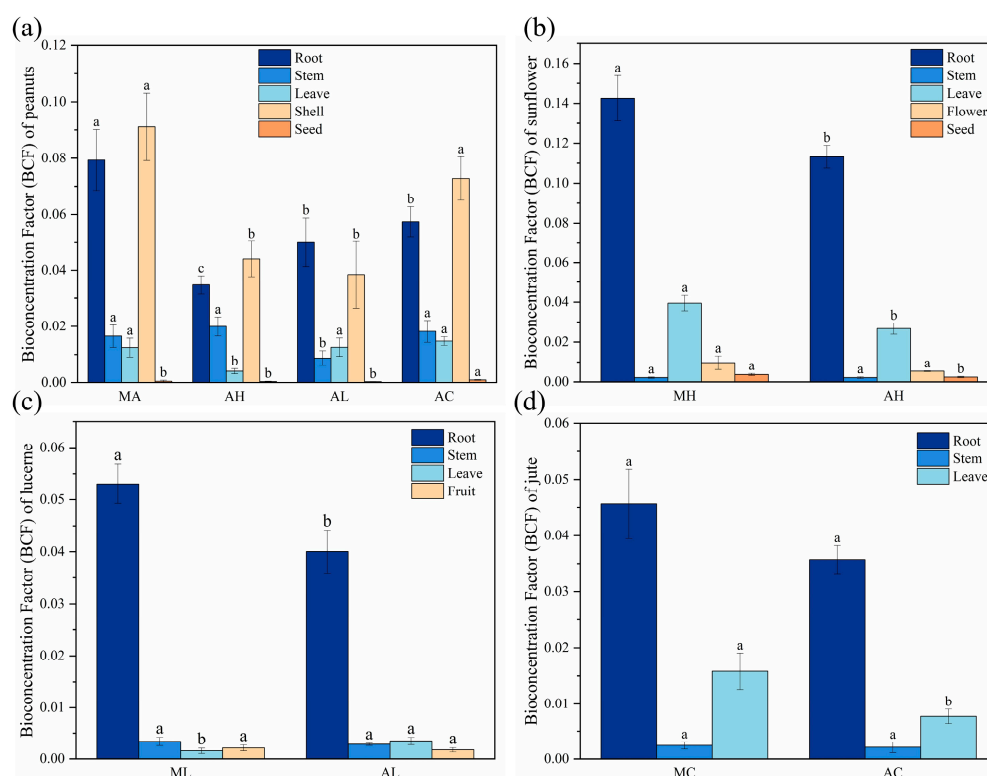


**Figure 4.** Bioconcentration Factor (BCF) of peanut (a) sunflower (b) lucerne (c) and jute (d) under different treatments. (All values are expressed as mean  $\pm$  standard error ( $n = 3$ ). The independent samples  $t$ -test was used to determine if there was a significant difference between the two means of the single and intercrops. Different letters indicate significant ( $p < 0.05$ ) differences between treatments according to Duncan's test. M denotes monoculture, A, H, L, and C denote peanut, sunflower, lucerne, and jute).

### 3.5. The pH and Eh of Rhizosphere Soil

Intercropping altered the pH and Eh of peanut rhizosphere soil (Figure 5a), when intercropped with jute, the pH of peanut rhizosphere soil was 6.71, which was significantly higher by 3.9% ( $p < 0.05$ ) compared to MA. The pH of peanut rhizosphere soil increased slightly during AH and AL treatments but did not differ significantly from that in the MA treatment. The Eh of peanut rhizosphere soil decreased by 16.4% and 14.4% in the AH and AC treatments, respectively, compared to monocropped peanuts. The pH of rhizosphere soil in intercropping systems of sunflower and peanut increased significantly by 1.6% compared to MH, while Eh decreased significantly by 4.1% (Figure 5b). The pH

values of rhizosphere soil from intercropped lucerne and jute were significantly lower than those from monocropping, measuring 6.89 and 6.59, respectively. The Eh values showed a decreasing trend after intercropping but did not differ significantly from those in monocropping (Figure 5c,d).

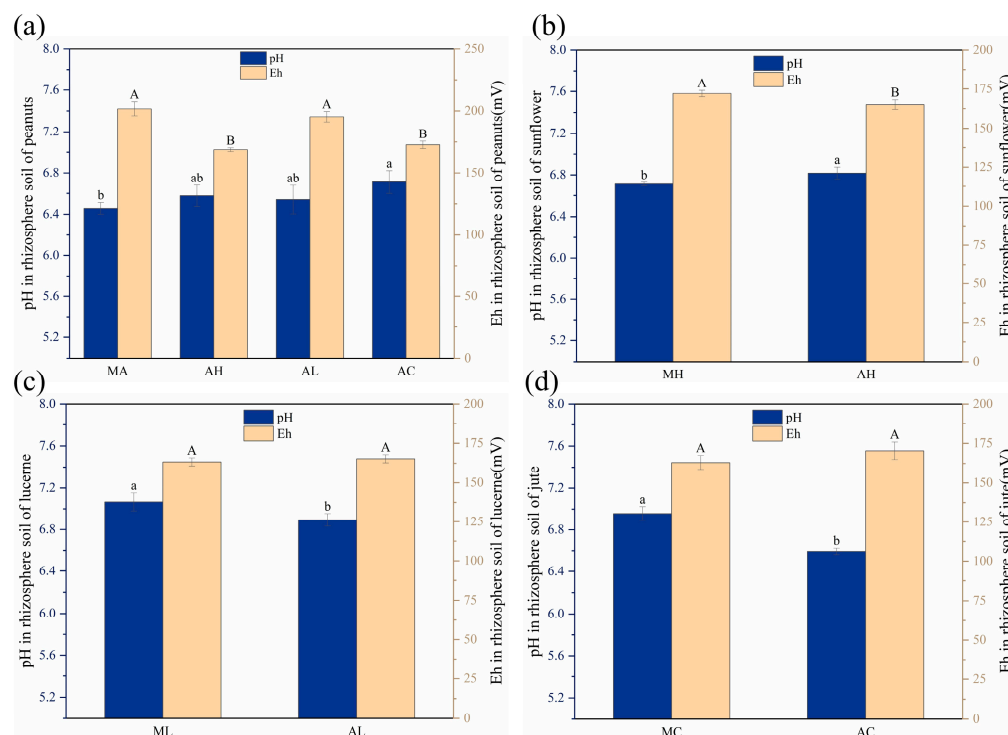


**Figure 5.** pH, Eh of rhizosphere soil for peanut (a) sunflower (b) lucerne (c) and jute (d) under different treatments. (All values are expressed as mean  $\pm$  standard error ( $n = 3$ ). The independent samples  $t$ -test was used to determine if there was a significant difference between the two means of the single and intercrops. Different letters indicate significant ( $p < 0.05$ ) differences between treatments according to Duncan's test. M denotes monoculture, A, H, L, and C denote peanut, sunflower, lucerne, and jute).

### 3.6. Different As Species of Rhizosphere Soil

The changes in arsenic (As) speciation in the rhizosphere soil of peanuts under different treatments are shown in Figure 6a, compared to the MA treatment, the percentages of F1 and F2 in the AH and AL treatments decreased by 2.2%, 44.0%, 14.4%, and 40.1%, respectively. In contrast, the AC treatment increased the percentages of F1 and F2 by 57.5% and 9.0%, respectively, compared to MA. Under the AH, AL, and AC treatments, the percentage of F4 content decreased by 17.3%, 19.0%, and 6.6%, respectively, compared to the MA treatment. Conversely, the percentage of F5 content increased from 24.5% in the MA treatment to 38.8% and 41.1% under the AH and AL treatments, respectively. When sunflower is intercropped with peanuts, the percentages of F1, F2, F3, and F4 all decrease, while the percentage of F5 As content increases to 38.3% compared to MH (Figure 6b). When intercropping with lucerne, the percentages of F1 and F2 in the rhizosphere soil decreased to 2.1% and 6.3%, respectively, while the percentages of F3 and F4 increased to 10.6% and 13.6%, respectively (Figure 6c). When jute is intercropped with peanuts, the percentages of F2, F3, and F4 increase to 7.3%, 9.3%, and 47.8%, respectively, while the percentages of F1 and F5 decrease to 3.0% and 32.6%, respectively (Figure 6d).

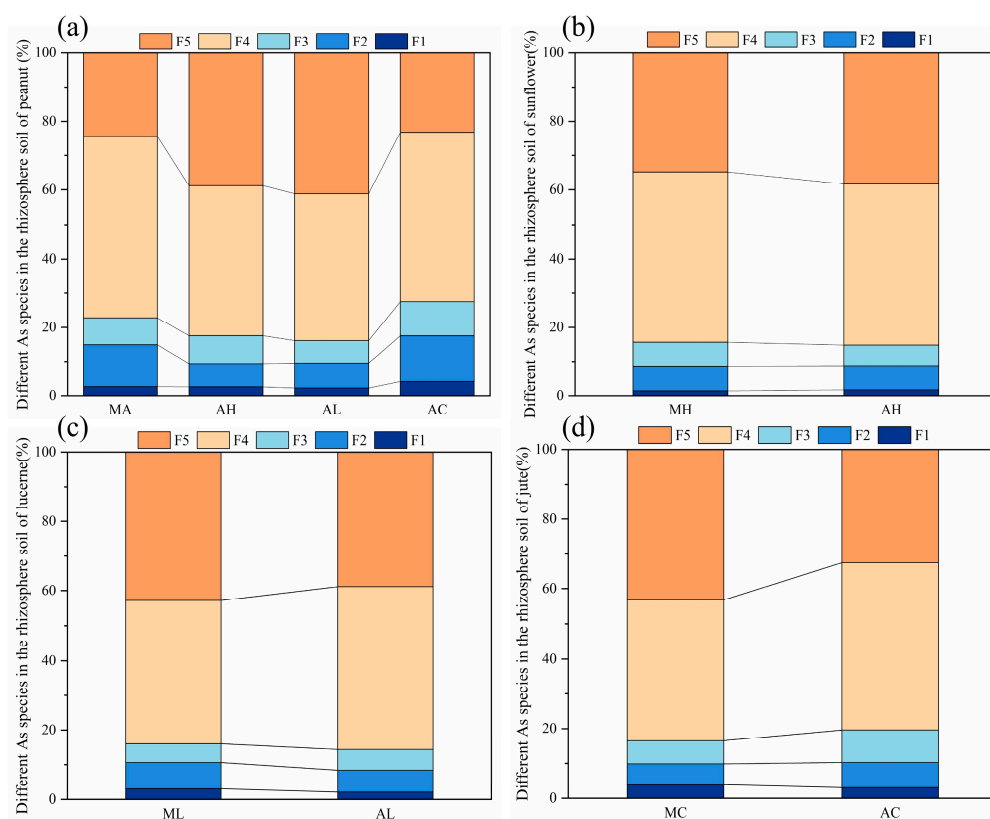


**Figure 6.** Different As species in the rhizosphere soil of peanut (a) sunflower (b) lucerne (c) and jute (d) under different treatments. F1–F5 means different As species in rhizosphere soil, including Non-specifically sorbed (F1), specifically sorbed (F2), amorphous and poorly-crystalline hydrous oxides of Fe and Al (F3), well-crystallized hydrous oxides of Fe and Al (F4), residual phases (F5). (M denotes monoculture, A, H, L, and C denote peanut, sunflower, lucerne, and jute).

### 3.7. Correlation Analysis

The correlation analysis between As content in various parts of the peanut and the physicochemical indicators of the rhizosphere soil is shown in Figure 7. The As concentration in peanut roots was significantly positively correlated with Eh and F2 content in the rhizosphere soil ( $p < 0.05$ ), extremely significantly positively correlated with F4 content ( $p < 0.01$ ), and extremely significantly negatively correlated with F5 content ( $p < 0.01$ ). The As content in leaves showed a significant positive correlation with the F2 content in rhizosphere soil ( $p < 0.05$ ), while the As content in shells exhibited a highly significant positive correlation with both F2 and F4 contents ( $p < 0.01$ ) and a highly significant negative correlation with F5 content ( $p < 0.01$ ). The content of As in seeds showed significant positive correlations with F2 and F3 ( $p < 0.05$ ) and a highly significant positive correlation with F1 ( $p < 0.01$ ). The total arsenic accumulation in peanuts was significantly positively correlated with rhizosphere soil F1, F2, and F3 ( $p < 0.01$ ) and significantly negatively correlated with F5 ( $p < 0.01$ ), indicating that the arsenic forms in rhizosphere soil are a key factor affecting arsenic uptake by peanuts.

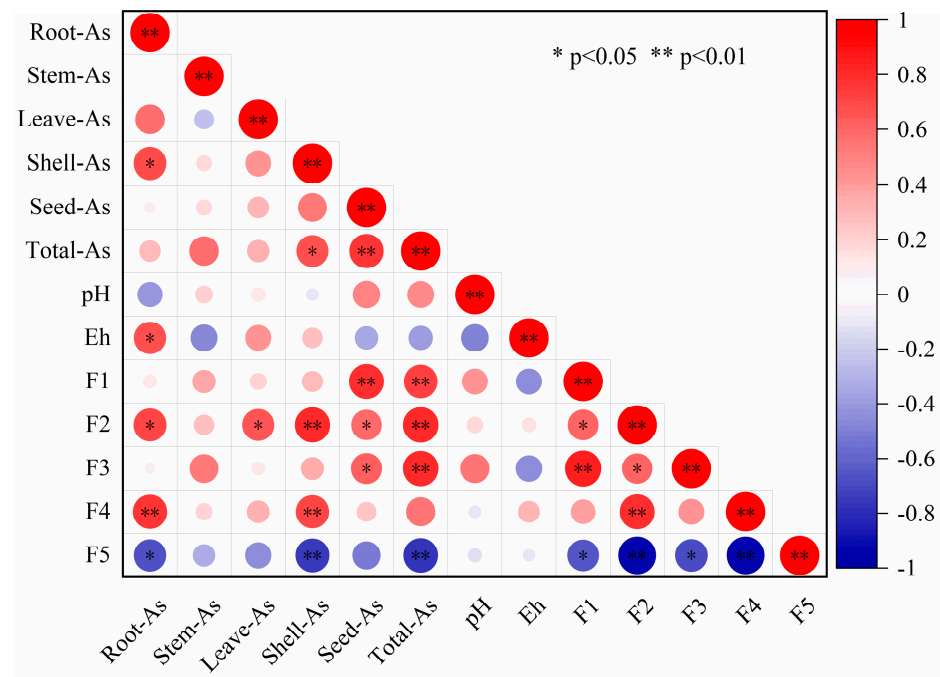


**Figure 7.** Correlation analysis between As content in various parts of peanut and rhizosphere environmental factors. (\*, \*\* indicate significant differences at  $p < 0.05$ ,  $p < 0.01$ ).

## 4. Discussion

### 4.1. Effects of Intercropping on Plant Growth and Arsenic Accumulation

Complex field environments significantly influence crop biomass and yield. Our field experiments revealed that the seed biomass yield of peanuts in intercropping systems was lower than that observed in monocropping systems. This reduction is attributed to the intercropping system's impact on light availability, growth space, and competition for water and nutrients among root systems [26], a decrease in the number of peanuts planted per unit area following intercropping is the primary factor leading to reduced peanut yield. The land equivalent ratios of the three intercropping systems in this study were 1.03, 1.70, and 1.17, respectively, indicating that intercropping with peanuts promoted the growth and development of sunflower, lucerne, and jute. The intercropping of sunflower, lucerne, and jute with peanuts forms a high-low field structure, allowing the intercropped plants to fully intercept and utilize light energy, additionally, the increased vegetation coverage reduces soil water evaporation, thereby enhancing the efficiency of irrigation water use [27]. Rhizobia in peanut roots forms a symbiotic relationship with nitrogen-fixing bacteria in the soil, thereby fixing atmospheric  $N_2$  [28]. Protons, organic acids, and phosphatases released by the roots promote the transformation of soil-bound P into a soluble form that is readily available for plant uptake [29,30]. N and P as essential nutrients for plant growth, can enhance the absorption of nutrients and stress resistance in crops by increasing the availability of N and P, thereby conferring a yield advantage in intercropping systems [31,32]. Intercropping lucerne with peanuts significantly enhances crop yield and economic income.

Intercropping affects both crop growth and development and the accumulation of As in crops. Intercropping between AH and AL reduced the biomass and As content of edible parts in peanuts, which was attributed to the competition for As in the soil in addition to the competition for rhizosphere growth resources during intercropping. Additionally, the enhancement of P utilization results in the sharing of transporters for both elements within the plant, leading to concurrent competition during transport [33]. The increase in As content in the edible parts of peanuts during AC treatment, accompanied by a significant

elevation in TF and BCF in the edible parts of peanuts, indicates that peanuts, as the dominant species in intercropping with jute, compete for water and nutrients. This competition is also evidenced by the significant increase in peanut biomass. Arsenic concentration in peanut grains under monoculture and intercropping systems meets national limits for contaminants in food (GB2762-2022) [25], the As content in the edible parts of sunflower, lucerne, and jute also exhibited a similar trend, consistent with previous research findings, for instance, compared to monoculture, the planting spacing of peanuts and maize at 35 cm simultaneously reduced the As content in maize and peanut seeds [34]. Notably, the MRER values for the three intercropping patterns in this study were 1.30, 2.11, and 2.26, respectively, demonstrating significant advantages in heavy metal removal. This is because intercropping generally reduces the As content in various parts of the crops, however, the significant biomass advantage is the primary reason for the increased total As accumulation in peanuts under the AC treatment and in lucerne under the AL treatment, indicating the presence of a “dilution effect”. Biomass influences the efficiency of crop extraction of arsenic-contaminated soils [35,36]. Previous studies have also demonstrated consistent heavy metal removal effects, such as an MRER of 2.34 [37] when using lucerne and cassia intercropping.

#### 4.2. Impact of Soil Physicochemical Properties on Arsenic Uptake by Plants

Root interactions in crop intercropping alter the soil environment [38,39]. In our study, we investigated the effects of intercropping on crop uptake and accumulation of As by examining changes in rhizosphere soil pH, Eh, and As speciation. Intercropping reduced the pH of rhizosphere soil in both lucerne and jute, which was attributed to the soil acidification caused by peanuts as leguminous plants, which simultaneously increased nutrient release [40,41]. Lowering the pH can convert As(III) to the less toxic As(V), which typically binds to clay minerals and iron-manganese oxides in the soil, thereby reducing its uptake by crops [42]. The low availability and mobility of As in rhizosphere soil significantly reduce the uptake of As by crops. This is consistent with previous studies, such as the intercropping of legumes with hyperaccumulator plants, where root exudates containing organic acids reduce rhizosphere soil pH, thereby decreasing the absorption of arsenic by leguminous plants [43]. Typically, a decrease in pH is accompanied by an increase in Eh [44]. This is also reflected in our study: intercropping peanuts with jute significantly increased the pH of rhizosphere soil, while the Eh value was significantly lower compared to monocropping. The reduction in Eh enhances the soil's reducing capacity, which leads to the reduction of As(V) to the more mobile As(III) [45,46]. The toxicity of As(III) is 60 times that of As(V), thereby facilitating the migration of arsenic from the soil to the roots of crops. The increase in As(III) can also be observed in the arsenic speciation content in peanut rhizosphere soil during AC treatment (Figure 6a). Additionally, studies have shown that phosphorus is a major factor regulating arsenic uptake in crops: higher concentrations of phosphorus fertilizer increase arsenic absorption in wheat [47]. This is because phosphate and arsenate often coexist in soil environments, and their similar chemical forms lead to strong competitive adsorption on mineral surfaces [48]. Therefore, the enhanced phosphorus availability during peanut intercropping may also be one of the reasons why the total arsenic accumulation in peanuts under AC treatment was significantly higher than that under other treatments (Figure 2a).

The As forms in the soil can be categorized based on their bioavailability, with F1 non-specifically adsorbed As and F2 specifically adsorbed arsenic being considered as available As [49]. In the AH and AL treatments, intercropping facilitated the transformation of As in the F1 and F2 fractions to residual As in the rhizosphere soil of peanut, sunflower, and lucerne, further confirming the effects of pH and Eh changes on soil arsenic

mobility and availability [50]. Correlation analysis also indicated that the As content in various parts of peanuts was closely related to the As forms in the rhizosphere soil. The total arsenic accumulation in peanuts showed extremely significant correlations with F1, F2, F3, and F5, but pH did not exhibit a significant correlation with As content. Conversely, previous studies have shown that changes in pH are closely related to the availability of As, and thus, rhizosphere pH is the most critical factor affecting As uptake by crops [51]. This may be due because soil is a complex multiphase system, where soil texture and microorganisms also serve as driving factors in altering soil pH and arsenic speciation [52,53]. Future research should also focus on further investigating these influencing factors to elucidate the transformation and migration patterns of As within the “soil-plant” system in intercropping systems.

## 5. Conclusions

The impact of intercropping peanuts with three economic crops on crop growth and phytoremediation efficiency was investigated in field experiments. The study revealed that the root interactions within the intercropping system influenced soil pH, Eh, and the speciation of arsenic, thereby reducing arsenic content in various parts of the crops. Notably, the arsenic content in peanut seeds and lucerne fruits met national food safety standards (GB2762-2022, 0.5 mg·kg<sup>-1</sup>) [25]. However, due to the promoting effects of peanut intercropping systems on crop growth and development, all three intercropping patterns in this study exhibited yield advantages and heavy metal removal advantages. Specifically, the land equivalent ratio and Metal removal equivalent ratio of peanut intercropped with lucerne were 1.70 and 2.11, respectively, enabling simultaneous phytoremediation of As-contaminated land and economic benefits, but the ecological mechanisms influencing As uptake and accumulation in intercropping systems require further in-depth investigation.

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