

Influence of integrated fertilization on growth and lead content of pepper under lead stress

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Abstract: To investigate the effect of fertilization on Pb content in vegetables, pepper was planted in L₁₆ 4⁵ pot orthogonal experiment. The effects of inorganic fertilizers (N, P, K) and organic fertilizers (sheep manure) on the growth and Pb content in various parts of pepper under Pb stress were analyzed. The results showed that: (1) The Pb content in pepper fruit ranged from 0.011 mg·kg⁻¹ to 0.085 mg·kg⁻¹, which did not exceed the limit value (0.1 mg·kg⁻¹) in the National Standard for Food Safety-Limit of Contaminants in Food (GB2762-2017); (2) The effect order of fertilization on pepper fruit weight was P₂O₅ > sheep manure > N > K₂O; The horizontal combination of factors that promoted the maximum fruit weight of pepper was N (0.15 g·kg⁻¹), P₂O₅ (0.225 g·kg⁻¹), K₂O (0.15 g·kg⁻¹) and sheep manure (9 g·kg⁻¹); (3) The order of fertilizer effects on Pb content in pepper fruit was Pb²⁺ > K₂O > N = sheep manure > P₂O₅; the factor level combination that resulted in the maximum Pb content in pepper fruits was N (0.15 g·kg⁻¹), P₂O₅ (0 g·kg⁻¹), K₂O (0.45 g·kg⁻¹), sheep manure (6 g·kg⁻¹) and Pb²⁺ (350 mg·kg⁻¹); (4) Based on the soil fertility characteristics of Urumqi, the recommended optimal fertilizer application rate was: high phosphorus fertilizer P₂O₅ (0.225 g·kg⁻¹), low potassium fertilizer K₂O (0.15 g·kg⁻¹), medium nitrogen fertilizer N (0.3 g·kg⁻¹) (or low nitrogen fertilizer N (0.15 g·kg⁻¹) + high organic fertilizer sheep manure (9 g·kg⁻¹), which can achieve high yield while ensuring that the Pb content in the fruits does not exceed the standard. Strengthening control of effective and reasonable fertilization methods in Urumqi agricultural land is helpful to reduce the Pb content in vegetables.

Keywords: fertilization; Pb; Stress; Pepper; Urumqi

Introduction

Soil is the basis for plant growth, and the soil-plant system is the basic structural unit of the biosphere, which provides strong productivity for human beings. Soil is often limited by a low level of available nutrients, so fertilization is often necessary to replenish them. But, fertilization can introduce heavy metals into the soil [1]. Excessive uses of fertilizers increase the availability of naturally occurring heavy metals lead (Pb) in the form of fertilizers and pesticides by dropping the pH of the soil. It harming the soil's physical properties which in turn affects the plant's growth [2]. Pb is harmful to human health through ingestion, inhalation and skin contact, which can cause hearing loss, heart disease, neurologic disorder, developmental abnormalities, diabetes, neurobehavioral disorders, hematologic, immunologic disorders, and many types of cancer in humans [2].

Lead from anthropogenic sources typically builds up mostly in the top layer of soil, and as depth increases, its concentration declines and only limited amounts of the lead in soil are thought to be soluble and hence available for plant uptake because of its high binding [3]. Due to the detrimental effects on soil microbiology, food safety and crop growth, lead accumulation in soils is a severe concern in agricultural production [4].

Lead's speciation in soil has a significant impact on its bioavailability and, consequently, its toxicity to plants and microorganisms[9]. According to Hemida, even brief contact with metal produces a noticeable decline in microbial activity and Pb- toxicity causes respiratory problems in microorganisms. To combat the poisonous effects of lead, many plants and microorganisms have evolved detoxifying processes. with organic and colloidal components[6–8].

Nitrogen fertilizer is the most widely used fertilizer in agricultural production and plant cultivation, and can directly affect plant yield and quality. Lou conducted a root box test and found that ammonium nitrogen fertilizer could reduce the pH value of the foundation soil, improve the activity of Pb in the rhizosphere soil, and promote the absorption of Pb by maize, while the effect of nitrate nitrogen is the opposite[9]. Phosphorus fertilizer is the second macronutrient necessary for plant growth. Seaman found that phosphorus compounds could reduce the absorption of Pb by plants by enhancing the adsorption and complexation of soil colloid surfaces[10]. However, Basta showed that the application of ammonium phosphorus fertilizer and calcium-phosphorus fertilizer would reduce soil pH value, and the decrease of pH value would increase the solubility and mobility of heavy metals, improve their bioavailability and toxicity, and promote the absorption of heavy metals by plants[11]. Potash fertilizer is also one of the essential nutrient elements for plant growth, mainly used to improve crop quality[12]. The research by Tu et al. showed that K^+ could compete with Pb^{2+} for adsorption sites on the surface of soil particles to increase the content of water-soluble and exchangeable Pb, thus increasing the content of its available state[13,14]. Organic fertilizer can not only improve soil fertility but also affect the form of heavy metals in soil and their absorption by plants[15,16]. Studies have shown that both the available Pb content and wheat absorption were significantly negatively correlated with the application of organic fertilizer, while the available Pb content in soil was significantly positively correlated with the amount of Pb absorbed by wheat[17,18]. The application of organic fertilizer could inhibit the absorption of Pb in soil by wheat[19]. Therefore, it is of great significance for the safe and sustainable development of agricultural products to understand the effects of nitrogen, phosphorus, potassium and organic fertilizers on the content of heavy metals in soil and plants[20,21].

There have been occurrences of Pb exceeding the standard in locally produced vegetables in Urumqi. Wang planted radishes through the "3414" fertilization test when the Pb content of the soil in Urumqi city was not beyond the standard, and the results showed that improper fertilization would lead to excessive Pb of the edible part of the radish. Therefore, exploring the effect of fertilization on the growth and Pb content of real estate vegetables in Urumqi City can provide the theoretical basis for safe and reasonable fertilization. In this study, the common solanaceous vegetable pepper was taken as the object, and the effects of different levels of nitrogen, phosphorus and potassium fertilizer and organic fertilizer on the growth and Pb content of pepper under Pb stress were studied by pot orthogonal experiment in the shed[22].

2. Materials and methods

2.1. Test materials

The pepper variety tested was the improved pig large intestine produced by Xi an Qinshu Agriculture Company. The tested soil was chernozem soil purchased from Mingzhu Flower Market, Urumqi City, and the organic fertilizer was pure sheep dung fermented at high temperature from Bafang Zehui Company. The basic physical and chemical properties of soil and organic manure (sheep manure) were shown in Table 1. The nitrogen fertilizer used in the experiment was urea (N 46 %), the phosphate fertilizer was potassium dihydrogen phosphate (P_2O_5 52 %), the potassium fertilizer was potassium sulphate (K_2O 54%), and Pb was added as an external compound $Pb(NO_3)_2$ (containing Pb^{2+} 62%).

Table 1. Basic physicochemical properties of soil and organic fertilizer (sheep manure).

Sample	Total nitrogen /g·kg ⁻¹	Total phosphorus /g·kg ⁻¹	Total potassium /g·kg ⁻¹	Available nitro- gen /mg·kg ⁻¹	Available phospho- rus /mg·kg ⁻¹
Soil	9.36	1.84	1.87	80.91	42.16
Organic fertilizer	12.79	2.392	2.73	95.37	61.96

Sample	Available potassium /g·kg ⁻¹	Organic matter /g·kg ⁻¹	Conductivity /ms·cm ⁻¹	Pb /mg·kg ⁻¹	pH
Soil	0.11	127.58	2.37	15.74	7.21
Organic fertilizer	0.18	227.36	1.75	2.19	7.09

2.2. Experimental design

An orthogonal experiment L₁₆4⁵ (5 factors, 4 levels, a total of 16 treatments) was used to design and analyze the effects of the combined action of four fertilizers and Pb²⁺ on the Pb absorption of pepper. The five factors were N, P₂O₅, K₂O, sheep manure and Pb²⁺. The four levels are blank, low, medium and high. The orthogonal experimental design is shown in Table 2.

Table 2. Orthogonal experimental design.

Sample	Factor level					Concentration				
	A	B	C	D	E	N	P ₂ O ₅	K ₂ O	Sheep ma- nure	Pb ²⁺
	N	P ₂ O ₅	K ₂ O	Sheep ma- nure	Pb ²⁺	/g·kg ⁻¹	/g·kg ⁻¹	/g·kg ⁻¹	/g·kg ⁻¹	/mg·kg ⁻¹
1	1	1	1	1	1	0	0	0	0	0
2	1	2	2	2	2	0	0.075	0.15	3	30
3	1	3	3	3	3	0	0.150	0.30	6	100
4	1	4	4	4	4	0	0.225	0.45	9	350
5	2	1	2	3	4	0.15	0	0.15	6	350
6	2	2	1	4	3	0.15	0.075	0	9	100
7	2	3	4	1	2	0.15	0.150	0.45	0	30
8	2	4	3	2	1	0.15	0.225	0.30	3	0
9	3	1	3	4	2	0.30	0	0.30	9	30
10	3	2	4	3	1	0.30	0.075	0.45	6	0
11	3	3	1	2	4	0.30	0.150	0	3	350
12	3	4	2	1	3	0.30	0.225	0.15	0	100
13	4	1	4	2	3	0.45	0	0.45	3	100
14	4	2	3	1	4	0.45	0.075	0.30	0	350
15	4	3	2	4	1	0.45	0.150	0.15	9	0
16	4	4	1	3	2	0.45	0.225	0	6	30

Potted plants were used in this experiment. To avoid the interference of rain, the experiment was conducted in the greenhouse behind the agricultural science building of Xinjiang Agricultural University. The seedlings will be raised on April 7, 2022. On May 14, 7 kg of soil was loaded into each basin, and the corresponding fertilizer and Pb(NO₃)₂ of each treatment were evenly mixed into the soil according to Table 2 (fertilizer was applied in the form of base fertilizer (60%) + top fertilizer (40%), and Pb(NO₃)₂ was applied in one time during the application of base fertilizer), and 1 L of distilled water was poured and left to stand. On May 25, the pepper seedlings were transplanted into pots with 3 plants per pot, and the seedlings were irregularly thinned to 1 plant per pot. Irrigate each basin with 1 L distilled water every 5 days for 22 times until harvest. The peppers received uniform natural light during the growth period and were harvested on September 9, 2022. The soil pH value was measured after the peppers were harvested.

2.3. Measurements and methods

After harvesting the peppers, they were divided into four parts: roots, stems, leaves, and fruits. The samples were first rinsed clean and then washed 2-3 times with deionized water, dried surface moisture, and weighed fresh. The plant samples were then killed at 105 °C for 30 min, dried in an oven at around 65 °C, ground and sieved, and stored for later use.

The total Pb content of plant and soil samples was determined by System 5000 graphite furnace atomic absorption spectrometer. basic physical and chemical properties of soil were determined by conventional methods of soil agrochemical analysis.

2.4. Data analysis

Microsoft Excel 2010, IBM SPSS Statistics 25.0 and Orthogonal Design Assistant II v3.1 were used for statistical analysis of the data. Pb content was calculated as fresh weight, with Mean±SD value. Plot with Origin 2018 and Sigmaplot 10.0 software.

3. Results and discussion

3.1. Effects of fertilization on plant height and fruit weight of pepper under Pb stress

As can be seen from Fig. 1, The highest pepper plant height in the 8th group was 49.87 cm, while the lowest pepper plant height in the 11th group was 27.83 cm; the highest number of pepper fruits in the 2nd group was 20, and the lowest number of pepper fruits in the 13th group was 7; the highest pepper fruit weight in the 12th group was 165.47 g, and the lowest pepper fruit weight in the 13th group was 67.50 g. Most of the soil pH was weakly acidic except for groups 1, 3 and 4.

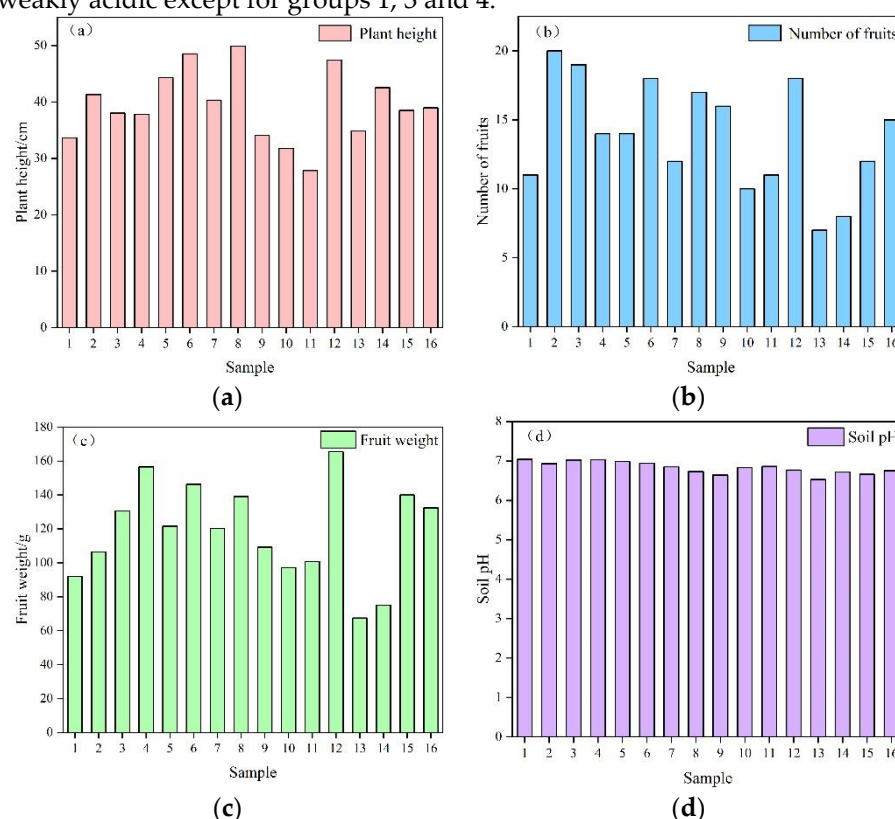


Figure 1. Pepper growth indexes and soil pH table of each group.

According to Table 3 and Fig. 2, the factors affecting the plant height of chilli peppers in order of importance were: A (N) > B (P₂O₅) > C (K₂O) > E (Pb²⁺) > D (sheep manure), and N fertilizer has the greatest influence on pepper plant height. It can be seen from the mean

level K value that the level combination of factors that promote pepper plant height to reach the maximum is: A2 B4 C2 D1 E3, namely N ($0.15 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.225 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.15 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($0 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($100 \text{ mg}\cdot\text{kg}^{-1}$); The level combination of factors contributing to the minimum plant height of pepper was as follows: A3 B3 C4 D3 E4, namely N ($0.3 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.15 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.45 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($6 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($350 \text{ mg}\cdot\text{kg}^{-1}$).

Table 3. Range analysis of fertilization on pepper plant height, fruit weight and soil pH.

Sample	Parameter	A	B	C	D	E
		N	P_2O_5	K_2O	Sheep manure	Pb^{2+}
Plant height	K1	37.708	36.717	37.242	40.983	38.433
	K2	45.758	41.033	42.892	38.475	38.675
	K3	35.267	36.175	41.125	38.258	42.216
	K4	38.717	43.525	36.191	39.733	38.125
	R	10.491	7.350	6.701	2.725	4.091
	Main and secondary order: A(N)>B(P_2O_5)>C(K_2O)>E(Pb^{2+})>D(Sheep manure)					
		Highest level A2 B4 C2 D1 E3				
		Minimum level A3 B3 C4 D3 E4				
Fruit weight	K1	121.355	97.530	117.808	113.173	116.813
	K2	131.550	106.237	133.402	103.215	117.077
	K3	118.155	122.913	113.222	120.438	127.420
	K4	103.767	148.148	110.395	138.002	113.518
	R	27.783	50.618	23.007	34.787	13.902
	Main and secondary order: B(P_2O_5)>D(Sheep manure)>A(N)>C(K_2O)>E(Pb^{2+})					
		Highest level A2 B4 C2 D4 E3				
		Minimum level A4 B1 C4 D2 E4				
Soil pH	K1	6.973	6.805	6.897	6.845	6.815
	K2	6.882	6.855	6.843	6.763	6.792
	K3	6.775	6.815	6.745	6.870	6.783
	K4	6.665	6.820	6.810	6.817	6.905
	R	0.308	0.050	0.152	0.107	0.122
	Main and secondary order: A(N)>C(K_2O)>E(Pb^{2+})>D(Sheep manure)>B(P_2O_5)					
		Highest level A1 B2 C1 D3 E4				
		Minimum level A4 B1 C3 D2 E3				

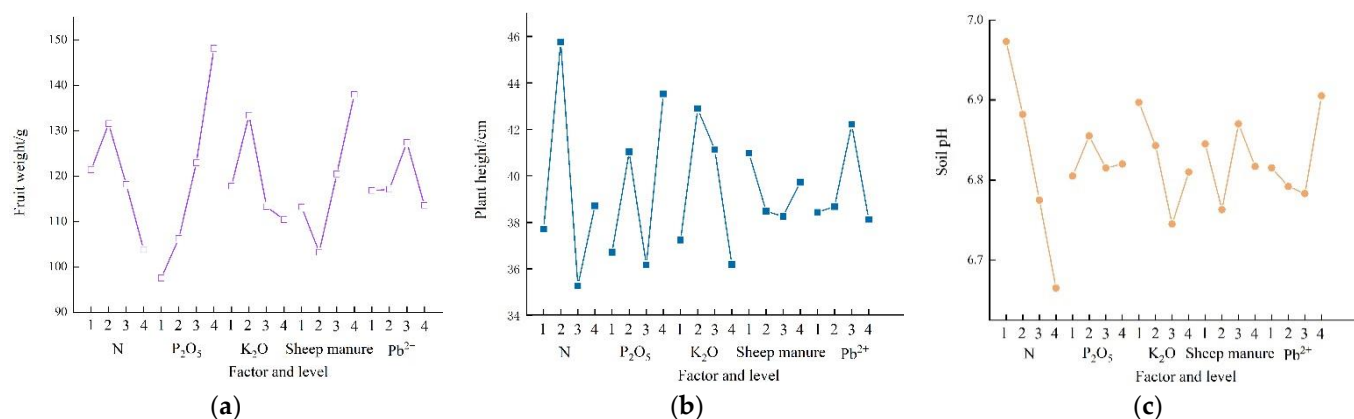


Figure 2. Effects of fertilization on pepper plant height, fruit weight and soil pH.



Table 2. O_5 > D (sheep manure) > A (N) > C (K_2O) > E (Pb^{2+}), phosphorus fertilizer had the greatest effect on pepper fruit weight. The horizontal combination of factors that promote the maximum fruit weight of pepper is: A2 B4 C2 D4 E3, namely N ($0.15\text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.225\text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.15\text{ g}\cdot\text{kg}^{-1}$), sheep manure ($9\text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($100\text{ mg}\cdot\text{kg}^{-1}$); The horizontal combinations of factors that promoted pepper fruit weight to reach the minimum value were: A4 B1 C4 D2 E4, namely N ($0.45\text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0\text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.45\text{ g}\cdot\text{kg}^{-1}$), sheep manure ($3\text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($350\text{ mg}\cdot\text{kg}^{-1}$).

Soil pH was measured after harvest. The main and secondary influencing factors on soil pH were: A (N) > C (K_2O) > E (Pb^{2+}) > D (sheep manure) > B (P_2O_5), and N fertilizer had the greatest influence on soil pH. It can be seen from the mean level K value that the level combination of factors that promote soil pH to reach the maximum value is: A1 B2 C1 D3 E4, namely N ($0\text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.075\text{ g}\cdot\text{kg}^{-1}$), K_2O ($0\text{ g}\cdot\text{kg}^{-1}$), sheep manure ($6\text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($350\text{ mg}\cdot\text{kg}^{-1}$); The level combination of factors that promoted soil pH to the minimum value was: A4 B1 C3 D2 E3, namely N ($0.45\text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0\text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.3\text{ g}\cdot\text{kg}^{-1}$), sheep manure ($3\text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($100\text{ mg}\cdot\text{kg}^{-1}$).

3.2. Analysis of Pb content in pepper

The results of Pb content determination in pepper were shown in Table 4. As can be seen from Table 4, the Pb content range of different parts of pepper in each treatment was generally in the order of root ($0.104\sim1.086\text{ mg}\cdot\text{kg}^{-1}$) > stem ($0.032\sim0.298\text{ mg}\cdot\text{kg}^{-1}$) > leaf ($0.022\sim0.072\text{ mg}\cdot\text{kg}^{-1}$), indicating that Pb was mainly concentrated in the root. Pb content in fruits ranged from $0.011\text{ mg}\cdot\text{kg}^{-1}$ to $0.085\text{ mg}\cdot\text{kg}^{-1}$, among which the highest Pb content in fruits treated 16 was $0.085\text{ mg}\cdot\text{kg}^{-1}$, but it did not exceed the limit ($0.1\text{ mg}\cdot\text{kg}^{-1}$) in the “National Standard for Food Safety-Limit of Pollutants in Food” (GB 2762-2017).

Table 4. Pb content in pepper (fresh weight).

Sample	Pb ²⁺ content/mg·kg ⁻¹			
	Root	Stem	Leaf	Fruit
1	0.126±0.014	0.249±0.023	0.052±0.018	0.058±0.004
2	0.104±0.021	0.092±0.014	0.040±0.006	0.034±0.005
3	0.191±0.002	0.130±0.028	0.039±0.005	0.035±0.010
4	0.610±0.017	0.181±0.012	0.069±0.013	0.083±0.002
5	0.455±0.026	0.290±0.005	0.046±0.009	0.085±0.009
6	0.144±0.018	0.212±0.029	0.039±0.004	0.050±0.010
7	0.131±0.036	0.195±0.029	0.044±0.010	0.043±0.003
8	0.104±0.001	0.230±0.019	0.022±0.012	0.046±0.013
9	0.149±0.038	0.128±0.026	0.031±0.014	0.038±0.002
10	0.106±0.004	0.133±0.015	0.030±0.008	0.061±0.010
11	0.295±0.000	0.298±0.025	0.038±0.008	0.067±0.002
12	0.148±0.022	0.209±0.020	0.045±0.007	0.011±0.003
13	0.746±0.103	0.102±0.024	0.056±0.007	0.042±0.009
14	1.086±0.022	0.121±0.030	0.072±0.011	0.033±0.008
15	0.112±0.033	0.032±0.009	0.060±0.003	0.040±0.012
16	0.202±0.012	0.054±0.010	0.067±0.009	0.035±0.014
Min~Max	0.104~1.086	0.032~0.298	0.022~0.072	0.011~0.085

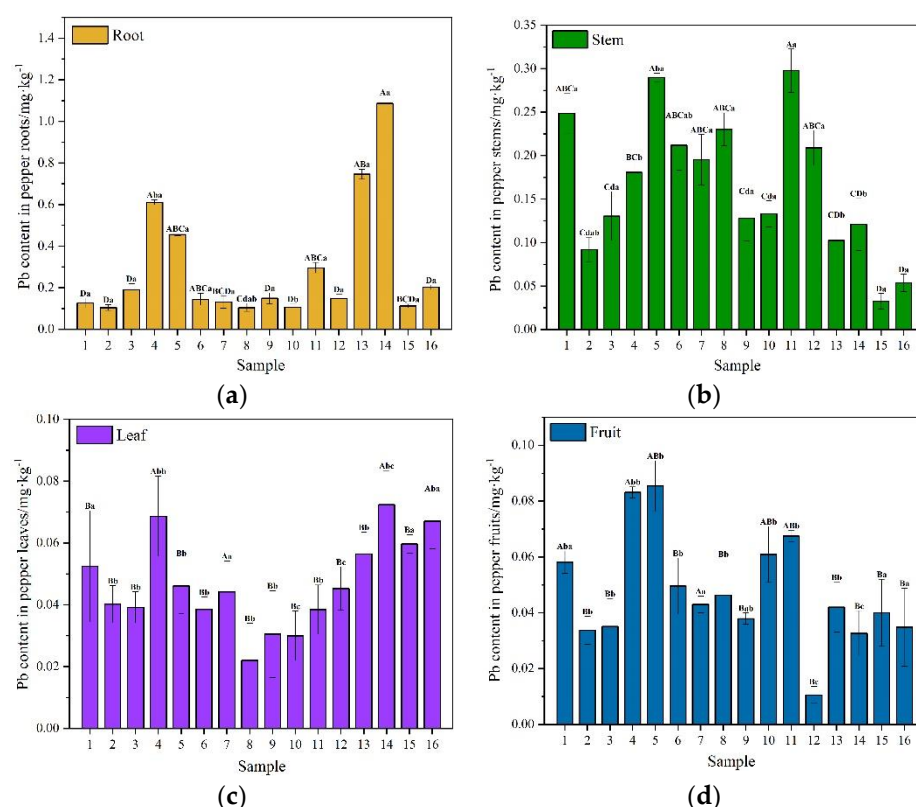


Figure 3. Analysis of variance between pepper parts and treatments.

The variance analysis of Pb content in different parts of pepper applied with different kinds of fertilizer is shown in Figure 3. The difference in Pb content in different parts of pepper under different treatments shows that there is no significant difference in Pb content in different parts of pepper in groups 1, 7, 15 and 16. Pb content in the roots and stems of capsicum in groups 3, 5 and 11 was significantly different from that in the leaves and fruits. The Pb content in the roots of pepper in groups 2 and 6 was significantly different from that in the leaves and fruits. Pb content in the roots of capsicum seedlings in groups 4 and 13 was significantly different from that in stems, leaves and fruits. The Pb content in the roots of group 14 was significantly higher than that in stems, leaves and fruits, and the Pb content in stems, leaves and fruits also showed significant differences, which may be because high levels of Pb^{2+} were enriched in pepper roots through cation adsorption, oxidation and reduction reactions of exchange complexes and because heavy metals in roots were not easily transported upward from stems. The content of Pb in roots was significantly different from that in stems, leaves and fruits. A large number of studies have shown that roots have the ability to absorb a large amount of Pb while limiting its transport to higher parts of the plant.

In addition, the difference between groups in the same part of pepper under different treatments was as follows: the Pb content of group 14 in the root was significantly different from that of group 7 and group 15; Pb content in the stems of group 11 was significantly different from that in the stems of group 4, which may be due to the application of high concentration organic fertilizer, which resulted in the chelation of Pb^{2+} with organic matter and other metal oxides, which restricted its mobility and reduced the absorption and transport capacity of Pb in the stems of pepper. Pb content in leaf group 7 was significantly different from that in groups 1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13 and 15. Pb content in fruit group 7 was significantly different from that in groups 2, 3, 8, 9, 12, 13, 14, 15 and 16, which may be because potassium plays an important role in plant energy metabolism, acting as a cofactor or activator of many enzymes in carbohydrate and protein metabolism. In

addition to helping the roots to absorb Pb from the soil and transfer it from the stem to the leaves, it can also accelerate the process of Pb absorption by the leaf surface itself.

3.3 Effects of fertilization on Pb content in pepper under Pb stress

Table 5. Range analysis table of the effect of fertilization on Pb content in pepper under Pb stress.

Scheme	Parameter	A	B	C	D	E
		N	P ₂ O ₅	K ₂ O	Sheep manure	Pb ²⁺
Root	K1	0.258	0.369	0.192	0.373	0.112
	K2	0.208	0.360	0.205	0.312	0.147
	K3	0.175	0.182	0.383	0.238	0.307
	K4	0.537	0.266	0.398	0.254	0.611
	R	0.362	0.187	0.206	0.135	0.499
Main and secondary order: E(Pb ²⁺)>A(N)>C(K ₂ O)>B(P ₂ O ₅)>D(Sheep manure)						
Highest level A4 B1 C4 D1 E4						
Minimum level A3 B3 C1 D3 E1						
Stem	K1	0.163	0.192	0.203	0.194	0.161
	K2	0.232	0.140	0.156	0.180	0.117
	K3	0.192	0.164	0.152	0.152	0.163
	K4	0.077	0.169	0.153	0.138	0.222
	R	0.155	0.052	0.051	0.056	0.105
Main and secondary order: A(N)>E(Pb ²⁺)>D(Sheep manure)>B(P ₂ O ₅)>C(K ₂ O)						
Highest level A2 B1 C1 D1 E4						
Minimum level A4 B2 C3 D4 E2						
Leaf	K1	0.050	0.046	0.049	0.053	0.041
	K2	0.038	0.045	0.048	0.039	0.045
	K3	0.036	0.045	0.041	0.045	0.045
	K4	0.064	0.051	0.050	0.050	0.056
	R	0.028	0.006	0.009	0.014	0.015
Main and secondary order: A(N)>E(Pb ²⁺)>D(Sheep manure)>C(K ₂ O)>B(P ₂ O ₅)						
Highest level A4 B4 C4 D1 E4						
Minimum level A3 B2 C3 D2 E1						
Fruit	K1	0.053	0.056	0.053	0.036	0.051
	K2	0.056	0.045	0.043	0.047	0.037
	K3	0.044	0.046	0.038	0.054	0.035
	K4	0.038	0.044	0.057	0.053	0.067
	R	0.018	0.012	0.019	0.018	0.032
Main and secondary order: E(Pb ²⁺)>C(K ₂ O)>A(N)=D(Sheep manure)>B(P ₂ O ₅)						
Highest level A2 B1 C4 D3 E4						
Minimum level A4 B4 C3 D1 E3						

According to the R-value in Table 5, the main and secondary influencing factors of different fertilizers on Pb content in pepper roots were as follows: E (Pb²⁺) > A (N) > C (K₂O) > B (P₂O₅) > D (sheep manure), and Pb²⁺ had the greatest influence on Pb content in pepper roots. It can be seen from the mean level K value that the combination of factors that promote the maximum Pb content in pepper root is as follows: A4 B1 C4 D1 E4,

namely N ($0.45 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.45 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($0 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($350 \text{ mg}\cdot\text{kg}^{-1}$); The level combination of factors to promote the minimum Pb content in pepper root was as follows: A3 B3 C1 D3 E1, namely N ($0.3 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.15 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($6 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($0 \text{ mg}\cdot\text{kg}^{-1}$).

The main and secondary influencing factors were A (N) > E (Pb^{2+}) > D (sheep manure) > B (P_2O_5) > C (K_2O), and N fertilizer had the greatest influence on Pb content in pepper stem. The level combination of factors that promoted the maximum Pb content in pepper stems was as follows: A2, B1, C1, D1, E4, namely N ($0.15 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($0 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($350 \text{ mg}\cdot\text{kg}^{-1}$); The level combination of factors that promote the minimum Pb content in pepper stems is as follows: A4 B2 C3 D4 E2, namely N ($0.45 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.075 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.3 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($9 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($30 \text{ mg}\cdot\text{kg}^{-1}$).

The main and secondary influencing factors on Pb content in pepper leaves were as follows: A (N) > E (Pb^{2+}) > D (sheep manure) > C (K_2O) > B (P_2O_5), and N fertilizer had the greatest effect on Pb content in pepper leaves. The combination of factors that promote the maximum Pb content in pepper leaves is as follows: A4 B4 C4 D1 E4, namely N ($0.45 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.225 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.45 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($0 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($350 \text{ mg}\cdot\text{kg}^{-1}$); The combination of factors that promote the minimum Pb content in pepper leaves is as follows: A3 B2 C3 D2 E1, namely N ($0.3 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.075 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.3 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($3 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($0 \text{ mg}\cdot\text{kg}^{-1}$).

The main and secondary influencing factors on Pb content in pepper fruit were as follows: E (Pb^{2+}) > C (K_2O) > A (N) = D (sheep manure) > B (P_2O_5), and Pb^{2+} had the greatest influence on Pb content in pepper fruit. The combination of factors that promote the maximum Pb content of pepper fruit is as follows: A2 B1 C4 D3 E4, namely N ($0.15 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.45 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($6 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($350 \text{ mg}\cdot\text{kg}^{-1}$); The combination of factors that promote the minimum Pb content of pepper fruit is as follows: A4 B4 C3 D1 E3, namely N ($0.45 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.225 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.3 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($0 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($100 \text{ mg}\cdot\text{kg}^{-1}$).

3.4. Effect of fertilization level on Pb content in pepper under Pb stress

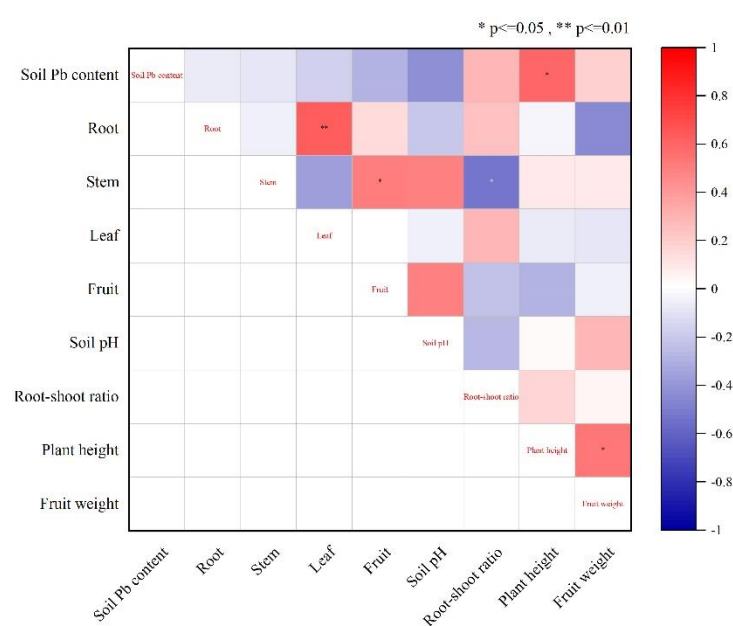


Figure 4. Correlation analysis of various physical and chemical indicators between soil and pepper.

The correlation analysis of various physical and chemical indicators of soil and pepper is shown in Fig. 4. The significant relationship between each index is as follows: the Pb content of pepper roots is significantly positively correlated with the Pb content of

leaves, and the Pb content of pepper stems and fruits is significantly positively correlated. We speculate that this might be due to while the stem transports water and nutrients, the transport of Pb is also bidirectional, that is, the Pb absorbed by the roots and leaves is transported to the fruit through the stem, resulting in the accumulation of Pb in the fruit. The ratio of pepper stem to root and shoot showed a significant negative correlation; soil Pb content was significantly positively correlated with plant height, and plant height was significantly positively correlated with fruit weight; it may be because Pb can promote the absorption of nutrients in peppers within a certain concentration range, which is beneficial to the growth and development of pepper plant height and fruit weight.

Table 6. Linear relationship between fertilization and Pb content in pepper under Pb stress.

Sample	Root		Stem		Leaf		Fruit	
	r	P	r	P	r	P	r	P
N	0.628	0.372	-0.585	0.415	0.400	0.600	-0.891	0.109
P ₂ O ₅	-0.713	0.287	-0.272	0.728	0.674	0.326	-0.813	0.187
K ₂ O	0.925	0.075	-0.804	0.196	-0.126	0.874	0.103	0.897
Sheep ma- nure	-0.907	0.093	-0.990	0.010**	-0.063	0.937	0.906	0.094
Pb ²⁺	0.993	0.007**	0.874	0.126	0.979	0.021*	0.744	0.256

Note: "*" means significant at the 0.05 level, "**" means extremely significant at the 0.01 level.

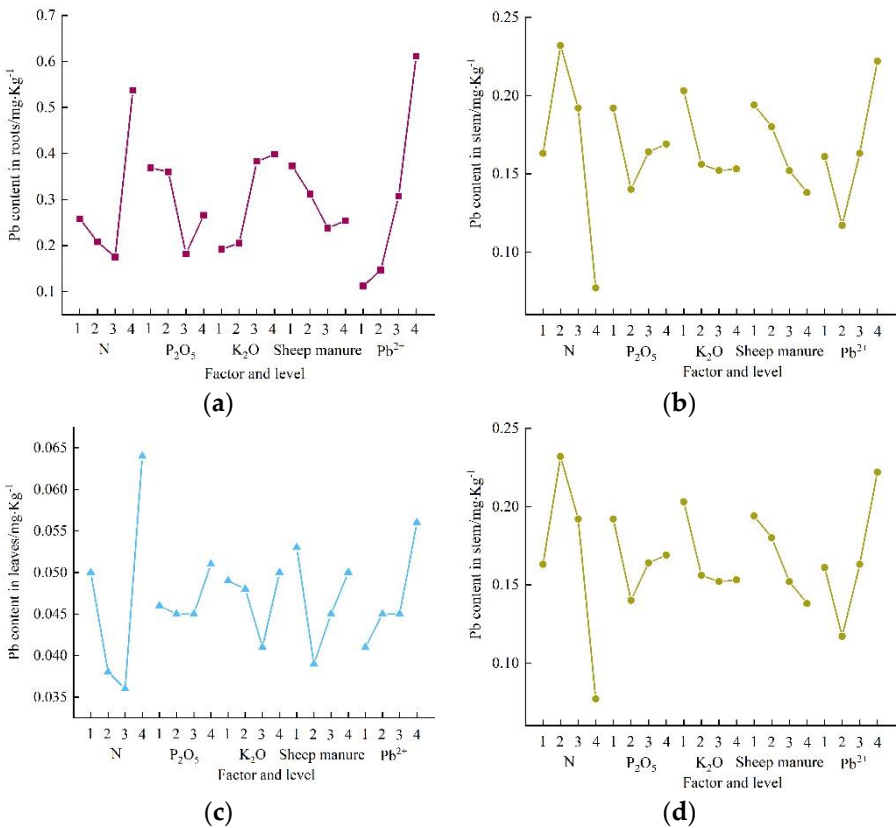


Figure 5. Effects of fertilization on Pb content in pepper under Pb stress.

The linear relationship between Pb content in different parts of pepper and different fertilizer applications is shown in Table 6. The variation of lead content in each part of pepper as shown in Fig. 5. According to Table 6 and Fig. 5 we can find that there was an extremely significant negative correlation between sheep manure and Pb content in pepper stems ($r = -0.990$, $P = 0.010$). Pb²⁺ was positively correlated with Pb content in root

($r = 0.993$, $P = 0.007$), and Pb content in leaf ($r = 0.979$, $P = 0.021$). There was no significant correlation between Pb content in different parts of pepper and other fertilizers.

4. Conclusions

Fertilization is one of the important agricultural measures to ensure the increase of agricultural production and income, and it also affects the adsorption and resolution of heavy metals in soil, the physicochemical properties of rhizosphere soil and the absorption of heavy metals by crops [2]. Pb is a non-essential element for plants, thus it is hazardous even in low quantities. It easily passes from the soil and atmosphere to plants [2].

Nitrogen is an essential element for plant growth and is contained in the composition of vitamins and energy systems in plants. In this experiment, there was a significant positive correlation between nitrogen fertilizer and plant height of pepper, that is, the increase of nitrogen fertilizer would promote the growth of plant height [24]. The Werf study showed that the average growth rate and plant height of plants increased when the nitrogen supply of plants increased, which was consistent with the results of this experiment. However, nitrogen application is considered to be the main driving factor of soil acidification, because the input of nitrogen fertilizer will make the nitrogen in the soil easy to nitrate, and the generated NO_3^- will produce H^+ , which is easy to leach out of the soil with base ions [25]. Wu showed that the nitrification of nitrogen in the soil would produce a large number of protons and lead to a decrease in soil pH, while the addition of fertilizer nitrogen in the soil would lead to more significant soil acidification [26]. In this experiment, there was a significant negative correlation between nitrogen fertilizer and soil pH value, that is, the increase of nitrogen fertilizer led to the decrease of soil pH value. The decrease in soil pH can increase the content of extractable heavy metal elements in soil, improve the bioavailability of heavy metals, and enable plants to enrich more heavy metals [27]. Therefore, attention should be paid to the decrease in soil pH value caused by nitrogen fertilizer application.

Rational application of phosphorus fertilizer can increase crop yield, improve crop quality, promote flowering and fruit of jacket vegetables, and improve results. Zhang showed that after applying phosphorus fertilizer, the yield of pepper increased with the increase of phosphorus application, and the yield was the highest at the highest phosphorus application [28,29]. In this experiment, there was a significant positive correlation between phosphorus fertilizer and pepper fruit weight, that is, the increase of phosphorus fertilizer would increase pepper fruit weight, possibly because phosphorus would participate in the metabolism and transportation of pepper carbohydrates, which is conducive to the growth of pepper fruit.

Organic fertilizer can not only improve soil fertility but also affect the form of heavy metals in soil and their absorption by plants [16]. In this experiment, the content of organic fertilizer was negatively correlated with the Pb content in the roots of pepper and significantly negatively correlated with the Pb content in the stems [9]. Pb intake studies in plants revealed that roots have the ability to absorb large amounts of Pb while limiting their translocation to higher parts of the plant [2]. This may be because organic fertilizer has a fixed effect, including the adsorption of heavy metals by macromolecules of solid organic matter and clay minerals in the soil, limiting its mobility and reducing the availability of heavy metals. Moreover, after entering the root, ions in the soil are transported to the xylem through both ectoplasmic and symplast pathways, and then to the aboveground part. However, the low permeability of the ectoplasmic barrier to heavy metal ions makes it difficult for Pb to transfer to the stem of pepper.

According to the statistics of soil census data, the total nitrogen content of cultivated soil in Urumqi city was $1.13 \text{ g}\cdot\text{kg}^{-1}$, the total phosphorus content was above $0.8 \text{ g}\cdot\text{kg}^{-1}$ and the total potassium content was about $19.28 \text{ g}\cdot\text{kg}^{-1}$, showing the phenomenon of nitrogen deficiency, phosphorus deficiency and potassium enrichment [30]. Pb content in soil is relatively low, generally $< 100 \text{ mg}\cdot\text{kg}^{-1}$. In view of the fact that the Pb content of all pepper fruits treated in this experiment is not beyond the standard, the optimal fertilizer

application can be recommended according to the factor level combination (A2 B4 C2 D4) that promotes the maximum fruit weight of pepper: N ($0.15 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.225 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.15 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($9 \text{ g}\cdot\text{kg}^{-1}$); Or according to the combination of treatment 12 with the maximum fruit weight (A3 B4 C2 D1), namely N ($0.3 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.225 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.15 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($0 \text{ g}\cdot\text{kg}^{-1}$), both of which were treated with high-level phosphorus fertilizer and low-level potassium fertilizer. medium level of nitrogen fertilizer (or low level of nitrogen fertilizer + high level of organic fertilizer) can achieve a higher yield and not exceed the standard of Pb in fruit.

5. Conclusions

(1) The Pb content in pepper fruit ranged from $0.011 \text{ mg}\cdot\text{kg}^{-1}$ to $0.085 \text{ mg}\cdot\text{kg}^{-1}$, which did not exceed the limit ($0.1 \text{ mg}\cdot\text{kg}^{-1}$) in the National Standard for Food Safety - Limit of Pollutants in Food (GB 2762-2017).

(2) The effect order of fertilization on fruit weight of pepper was $\text{P}_2\text{O}_5 > \text{sheep manure} > \text{N} > \text{K}_2\text{O} > \text{Pb}^{2+}$; The horizontal combination of factors that promoted pepper fruit weight to reach the maximum value were N ($0.15 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0.225 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.15 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($9 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($100 \text{ mg}\cdot\text{kg}^{-1}$).

(3) The effects of fertilization on Pb content in pepper fruit were as follows: $\text{Pb}^{2+} > \text{K}_2\text{O} > \text{N} = \text{sheep manure} > \text{P}_2\text{O}_5$; The level combination of factors that promoted the maximum Pb content in pepper fruit was N ($0.15 \text{ g}\cdot\text{kg}^{-1}$), P_2O_5 ($0 \text{ g}\cdot\text{kg}^{-1}$), K_2O ($0.45 \text{ g}\cdot\text{kg}^{-1}$), sheep manure ($6 \text{ g}\cdot\text{kg}^{-1}$), Pb^{2+} ($350 \text{ mg}\cdot\text{kg}^{-1}$). There was a significant negative correlation between sheep manure and Pb content in pepper stem ($r = -0.990$, $P = 0.010$). There was a significant positive correlation between Pb^{2+} and Pb content in the root ($r = 0.993$, $P = 0.007$) and in the leaf ($r = 0.979$, $P = 0.021$). There was no significant correlation between Pb content in different parts of pepper and other fertilizers.

(4) Recommended optimal fertilizer application: high-level phosphorus fertilizer P_2O_5 ($0.225 \text{ g}\cdot\text{kg}^{-1}$), low-level potassium fertilizer K_2O ($0.15 \text{ g}\cdot\text{kg}^{-1}$), medium-level nitrogen fertilizer N ($0.3 \text{ g}\cdot\text{kg}^{-1}$) (or low-level nitrogen fertilizer N ($0.15 \text{ g}\cdot\text{kg}^{-1}$) + high-level organic manure sheep manure ($9 \text{ g}\cdot\text{kg}^{-1}$)). Can achieve high yield and fruit Pb does not exceed the standard.

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