Nutrition and Safety Evaluation of Hydroponic-cultured Pea Sprout under Lead and Cadmium Stress

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Abstract. Heavy metals can bring health risks through the enrichment of crops and food chain, but there are few reports on their impact on the nutritional quality of crops. In this paper, the hydroponic-cultured pea seedlings under lead and cadmium stress were used to evaluate the heavy metal effects on growth, safety and nutrients variation. Lead has little effect on the sprout growth, and it mainly concentrates in the roots and seldom can be transferred to the edible parts. It would not bring health and safety hazards to the edible parts of stems and leaves but decrease the mineral nutrients in peasprout slightly. Cadmium significantly affects both growth and nutrition value of pea sprout, not only the root is abundant, but also the transfer rate of root and stem, which is about 13%. The nutrient content of Ca and Zn in stem and leaf is decreased significantly under cadmium stress.

1. Introduction
The sprouts are cultured using the soilless cultivation technology, which dispenses with any nutrient solution or pesticides and has attracted wide attention. The most studied sprouts are pea seedlings[1], soybeans[2], radishes[3], and oil sunflower[4]. Among them, pea seedlings, which are also called “Dragon Beards” and “Butterfly Vegetable”.boast delicate fragrance and rich nutrition. Due to its ornamental features, rapid growth and short growth cycle, small soil coverage, convenience for cultivation and management, the pea seedling has become a member of family ornamental gardening in recent years.

Heavy metal pollution is still a severe problem. Heavy metals can enter crops through soil, atmosphere, and water, especially through sewage irrigation systems that are widely adopted. Heavy metals have physiological effects on crops through the enrichment of crops such as aquatic plants [5,6], endanger people’s health through the food chain, and incur food hygiene and safety issues. Existing studies on the response of crops and plants under heavy metal pollution focus on physiological changes including growth biomass, growth mechanism, etc. However, there are few reports on its changes in nutrition. Therefore, this paper conducts tests that culture pea seedlings in simulated lead and cadmium-polluted waste water with different concentrations, evaluated the growth indicators including heavy metal enrichment and mineral nutrients of the seedlings to provide a reference for the cultivation and selection of pea sprouts.

2. Materials and methods

2.1 Planting methods
Selected healthy pea seeds were washed and soaked with 10% sodium hypochlorite for 10-20 minutes
followed by rinse with distilled water several times. Seven seedling trays were filled with different groups of culture solutions: distilled water, 5, 10 and 20 mg/L lead nitrate solution, and 5, 10 and 20 mg/L cadmium nitrate solution. Culture paper was spread on the mesh holes, made the liquid level slightly higher than the culture paper, scattered the soaked seedsevenly on the cultivation paper of each seedling tray, labelled and sent the seedlings to the artificial incubator. The temperature of the incubator was set at 20 °C. The germination of peas was daily observed, and culture solution of each group was replenished every 2 days.

2.2 Biomass measurement
The germination rate of peas in each group was counted on the fifth day. The height of the pea seedlings in each group was measured once every 2 days since the seventh day, and on the 15th day, 30 seedlings from each group were weighed. The freshly measured pea seedlings were separated from rhizomes, and the weights of roots, stems and leaves were measured separately, and then were placed in an oven for 8-12 hours. The temperature of the oven was set at 120 °C to ensure that the water completely evaporated. The dry weight of the pea seedlings, and the moisture content was calculated. The tests of each group were repeated three times.

2.3 Determination of mineral elements
The dried samples were beaten with a powder machine. 0.5 g powder from each group was sampled into a 50 ml small beaker, 16 ml of concentrated nitric acid and 4 ml of perchloric acid were added to the samples which were then sealed and allowed to stand overnight. The samples underwent nitrolysis on a hot plate for 4-8 h the next day. The temperature of the hot plate was set at 240 °C. When the acid in the beaker was completely evaporated to dryness and white crystals were precipitated in the beaker, the nitrolysis was completed. After that, distilled water was added to dissolve the white crystals and make up the volume in a 50 ml volumetric flask. A fixed-volume sample was taken to determine the concentration of each mineral ion by atomic absorption spectrophotometer.

2.4 Statistical analysis
The data were analysed by SPSS software (ver. 21.0, SPSS Inc., Chicago, IL, USA). Chi-square test was used to test the significance of differences.

3. Results and discussion
3.1 Influence of lead and cadmium on germination rate of pea sprouts
Table 1 shows that the germination rate of peas cultivated in the lead group is close to that of the control group, and there is no statistical difference from the control group. The cadmium ion culture group had a different budding rate from the control group, and with the increase of cadmium ion concentration, the budding rate decreases significantly. The germination rate of peas in the control group reaches 94.67%, while the germination rate of peas cultivated with 20mg/L cadmium ion solution is only 85%. The budding rates of pea seedling treated with lower concentration of lead is a little higher than the control group, probably due to the weakly acidic environment resulting from lead nitrate which is suitable for the germination of peas.

| Group | Sum | Budding number | Budding rate | $\chi^2$ | P |
|-------|-----|----------------|--------------|---------|---|
| Control | 300 | 284 | 94.67 | -- | -- |
| Pb5 | 300 | 290 | 96.67 | 1.45 | $\geq 0.05$ |
| Pb10 | 300 | 286 | 95.33 | 0.14 | $\geq 0.05$ |
| Pb20 | 300 | 282 | 94.00 | 0.12 | $\geq 0.05$ |
| Cd5 | 300 | 271 | 90.33 | 4.06 | <0.05 |
| Cd10 | 300 | 264 | 88.00 | 8.42 | <0.05 |
| Cd20 | 300 | 255 | 85.00 | 15.35 | <0.05 |

Table 1. Budding rate of pea seeds after 5 days of cultivation
3.2 Influence of lead and cadmium on biomass of pea sprouts

Table 2. Biomass of pea after 15 days of hydroponic growth

| Groups | Fresh weight (g) | Fresh root weight (g) | Dry weight (g) | Root/shoot ratio | Water content (%) |
|--------|------------------|-----------------------|----------------|------------------|------------------|
| Control | 4.52±0.21        | 1.88±0.03             | 0.42±0.02      | 0.41±0.02        | 90.7±1.23        |
| Pb5    | 4.62±0.18        | 1.83±0.06             | 0.42±0.02      | 0.39±0.01        | 90.9±1.56        |
| Pb10   | 4.35±0.12        | 1.74±0.06             | 0.41±0.03      | 0.40±0.03        | 90.8±1.33        |
| Pb20   | 4.15±0.09        | 1.72±0.12             | 0.37±0.05      | 0.41±0.02        | 91.0±2.15        |
| Cd5    | 3.62±0.11        | 1.18±0.15             | 0.33±0.03      | 0.33±0.02        | 90.9±1.78        |
| Cd10   | 2.96±0.15        | 0.65±0.12             | 0.26±0.02      | 0.22±0.02        | 92.6±1.96        |
| Cd20   | 2.17±0.05        | 0.38±0.06             | 0.21±0.02      | 0.18±0.01        | 90.3±0.85        |

Table 2 shows that the effect of lead ions on the biomass of peas is minimal. Even when the concentration of lead ions in the water reaches 20 mg/L, its fresh weight, dry weight, root weight, rhizome ratio and water content are very close to those of the control group. Cadmium ions significantly reduced the fresh weight, dry weight, root weight and root/shoot ratio of peas. When the cadmium ion concentration reaches 20 mg/L in the environment, the root/shoot ratio is only 0.18, showing a significant metal toxicity effect.

3.3 Influence of lead and cadmium on the growth height of pea sprouts

Figure 1 shows that a low concentration of lead ions has almost no effect on the growth of pea sprouts, and only high concentrations of lead ions have a slight effect on the later growth of peas. The effect of cadmium ions on peas is very significant throughout the growth process, and the effect is more significant as the concentration increases. When the growth height of the peas in the control group reaches 30.89 cm in 15 days, the height of the peas grown under the cadmium ion environment at 20 mg/L is only 19.73 cm.

![Fig. 1 Growth heights of pea sprout in 15 days under Lead (A) and Cadmium (B) stress](image)

3.4 Lead and cadmium enrichment and population safety evaluation

Figure 2 shows that a large amount of lead ions and cadmium ions are detected in the roots of peas, and as the concentration of lead and cadmium ions in the water increased, the enrichment of lead and cadmium in peas increased. Only cadmium ions can be detected in the leaves and peas of peas. The concentration of cadmium ions in the stems and leaves is about 15% that of the roots, and the detection rate of lead ions is almost zero. Thus, as far as the edible parts of stems and leaves are concerned, lead ions in the water environment will not be transferred to the stems and leaves through the roots of peas, and lead ions will incur no health and safety risks. According to FAO/WHO standards, the daily intake of Cd is 57 ~ 71μg/d[7]. If the dry weight / fresh weight ratio is set at 1/10 and the daily intake of fresh weight peas is set at 250g, the cadmium ions in a 5mg/L solution in 250g peas reach about 50μg, which will endanger human health. Thus, the cadmium ion concentration in the environment of hydroponic-cultured peas should be less than 5mg/L, and the daily consumption of
hydroponic peas containing cadmium in sewage should be less than 250g.

3.5 Influence of lead and cadmium on pea mineral nutrients

Figure 3 shows that there is no significant correlation between potassium and magnesium ions and lead and cadmium ions in the environment. The potassium and magnesium contents of peas in the control and experimental groups are similar. In the cadmium stress group, the calcium and zinc ions in pea stems and leaves decrease significantly. The calcium and zinc ions in the control group are about twice that in the 20 mg/L group, and the zinc ions decreased linearly with the increase of cadmium ion concentration. The effects of cadmium ions on calcium and zinc ions in peas are much greater than those of lead ions. Although lead ions stayin the roots of pea seedlings after being enriched in the growing process, they would not be transferred to the edible parts of pea stems and leaves under lead stress, the calcium and zinc ions in stems and leaves have decreased to some extent. Therefore, although lead is safe from the health perspective since it could not enrich in the edible parts as known from part 2.4, lead ions are also harmful in terms of nutrition.

Calcium and zinc are two essential elements for plant growth. There are two main mechanisms of interaction between calcium and other zinc ions on the membrane surface. First, the addition of calcium and zinc can reduce the electronegativity of the membrane surface. The decrease of the electronegativity will reduce the activity of toxic cations on the membrane surface and increase the activity of anions on the membrane surface[8]; The polarization ability can replace the calcium ion on the surface of the membrane through the electrostatic effect. When the activity of the surface calcium ion cannot meet the needs of normal elongation, the increase of calcium can alleviate its deficiency. At the same time, calcium ions can interact with toxic cations, thereby alleviating the phytotoxicity of metal cations. The mechanism of this remission has always been considered to be that calcium competes with toxic cations for active sites on plant cell membranes, which is also a key assumption in biological ligand models. This means that toxicity is caused by the binding of toxic cations to the membrane surface active site, and the reduction of toxicity must also require the binding of calcium to this site. In fact, there are many binding sites on the membrane surface, and only a few of them are active sites. The combination of calcium and zinc with inactive sites leads to a decrease in the electronegativity of the membrane surface and a decrease in the surface activity of the toxic cation membrane, thereby reducing toxicity[9]. This mechanism may be the main mechanism for calcium to alleviate toxicity. At the same time, calcium and zinc are essential elements for growth. When the surface calcium and zinc cannot meet the growth, the addition of calcium and zinc can alleviate its own deficiency. Calcium ions, zinc ions and cadmium ions have similar electronegativity, and their
binding sites on the surface of geometric cell membranes must occupy the same sites. When cadmium ions preempt the binding site, calcium and zinc ions will not be able to bind to the cell membrane surface, and the cells will show a lack of calcium and zinc. However, the difference between the electronegativity of potassium and magnesium ions and cadmium ions is large, and the combination of potassium and magnesium ions is different from the cell membrane surface sites of cadmium ions [10,11].

4. Conclusions
Cadmium ions in water will undermine the growth of peas, and a large amount of cadmium ions are also enriched in the roots of peas. About 15% of cadmium ions are transferred to the edible parts of the stems and leaves, and the content of calcium and zinc ions decreased significantly. This effect becomes more significant as the concentration of cadmium ions in the environment increases. When lead ions are present in the environment, the growth of peas is not significant. The roots of peas are enriched with a large amount of lead ions, but they are rarely transferred to the edible parts of the stems and leaves. The content of calcium and zinc ions decreases with the increase of lead ion concentration in the leaves of pea seedlings. Although peas hydroponic-cultured in lead ion solutions do not have the same health and safety hazards as those cultivated in cadmium ion solutions, their nutritional value decreases.

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