Risk of Heavy Metal Contamination in Three Seawater-Cultivated Vegetables

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Abstract

To utilize a saline coastal mud flat, seawater-cultivated vegetables were bred and planted in Jiangsu Province, P. R. China. Due to ubiquitous heavy metal pollution in coastal areas and high bioaccumulation potential of heavy metals in halophytes, food safety of seawater-cultivated vegetables should be a public concern. In the present study we collected three seawater-cultivated vegetable species (Suaeda glauca, Salicornia europaea and Portulaca oleracea), soils, seawater and freshwater runoff from five locations in Jiangsu Province. Concentrations of Cu, Zn, Cr, Cd, Pb and Ni in these samples were determined. The results showed that all soil samples showed a low level of heavy metals and complied with the Chinese Environmental Quality Standard for Soils, but seawater and freshwater runoff surrounding sampling sites displayed a high level of Cu and Pb, suggesting a high risk of heavy metal contamination. Levels of Cr and Pb in all investigated plant species were sometimes higher than the limits in the Chinese Standard of Food Safety, suggesting potential risk to food safety. Overall, seawater-cultivated vegetables might accumulate heavy metals and subsequently endanger the health of human beings when planting on saline soils.

Keywords: food safety, heavy metal, ICP-MS, pollution, seawater-cultivated vegetable

Introduction

Saline and sodic soils are typical stressful soil types in arid and semi-arid coastal regions and in regions of poor natural drainage. There are approximately 954 million hectares of saline and sodic soil worldwide. Due to high salinity, high sodicity and sometimes high pH value, these soils have poor physical and chemical properties and are difficult for common plants to grow in, thus becoming more and more barren [1].

Coastal mud flats generally show high saline content and sodicity. Different from traditional saline and sodic soil, coastal mud flat is located in intertidal area and often receives runoff and seawater rich of nutrients. It is neither dry nor barren, since it is efficient sediment traps that facilitate the accumulation and storage of organic matters from water column and from below ground production [2]. Thus, coastal mud flats are potential land resources for agriculture and ecological landscape construction [3].

Jiangsu Province has the largest area of coastal mud flats in China, approximately equal to 5,000 km². To utilize these areas, seawater-cultivated vegetables were introduced, which can endure high salinity and...
have rich nutrition and good taste [4]. For example, sea asparagus (*Sarcocornia ambigua*), seepweed (*Suaeda salsa*) and froggrass (*Salicornia europaea*) have been widely cultivated in Jiangsu Province and already sold as vegetables in supermarkets in America, Mexico, Netherlands, Saudi Arabia, Egypt, Israel, Eritrea, Australia, Canada, New Zealand, South Korea, Japan and China [4,5].

Halophytes are tolerant to not only high salinity but also heavy metal pollution. Many studies have shown that halophytes could accumulate high levels of heavy metals [6-9]. Coastal mud flats are facing severe

| No. | Site name                  | Site code | Geographical coordinates       |
|-----|---------------------------|-----------|--------------------------------|
| 1   | Tiaozini, Dongtai County  | TZN       | 32°45′43″N, 120°57′31″E        |
| 2   | Jinhai farm, Dafeng District| JH        | 32°59′36″N, 120°48′42″E        |
| 3   | Shuntai farm, Sheyang County| ST       | 33°41′33″N, 120°22′47″E        |
| 4   | Liuduo, Sheyang County    | LD        | 34°04′53″N, 120°14′30″E        |
| 5   | Binhai port, Binhai County| BH        | 34°18′41″N, 120°15′45″E        |

**Table 1.** Information on sampling sites.

Information on sampling sites is shown in Table 1. Values are means±SD. Means followed by the different superscript letters significantly differ from each other using the Student’s *t*-test (*P*<0.05). ND: not detected.

**Table 2.** Metal concentration in *Suaeda glauca* and nearby soil (mg/kg, dry weight).

| Site   | Cu     | Zn     | Cr     | Cd     | Pb     | Ni     |
|--------|--------|--------|--------|--------|--------|--------|
| Soil   |        |        |        |        |        |        |
| TZN    | 12.8±1.0<sup>a</sup> | 59.3±2.8<sup>bc</sup> | 29.3±4.8<sup>bc</sup> | ND     | 4.89±0.76<sup>a</sup> | 14.4±1.0<sup>a</sup> |
| JH     | 11.4±0.4<sup>a</sup>  | 53.0±2.4<sup>bc</sup> | 25.8±3.6<sup>a</sup>  | ND     | 5.07±0.96<sup>a</sup> | 13.0±0.4<sup>a</sup> |
| ST     | 23.0±0.2<sup>a</sup>  | 66.0±0.4<sup>a</sup> | 34.3±3.9<sup>a</sup>  | ND     | 5.46±0.90<sup>a</sup> | 24.7±0.2<sup>a</sup> |
| LD     | 13.9±1.0<sup>a</sup>  | 51.9±1.1<sup>a</sup> | 29.5±2.4<sup>a</sup>  | ND     | 3.95±0.60<sup>a</sup> | 15.6±0.9<sup>a</sup> |
| BH     | 23.3±0.4<sup>a</sup>  | 76.3±2.3<sup>a</sup> | 39.1±0.6<sup>a</sup>  | ND     | 7.67±0.38<sup>a</sup> | 25.0±0.4<sup>a</sup> |
| Root   |        |        |        |        |        |        |
| TZN    | 25.9±1.6<sup>a</sup>  | 1.8±0.3<sup>c</sup>  | 10.5±2.1<sup>a</sup>  | ND     | 0.06±0.00<sup>b</sup> | 0.1±0.0<sup>b</sup> |
| JH     | 22.3±0.8<sup>a</sup>  | 2.3±0.2<sup>b</sup>  | 7.4±1.2<sup>a</sup>   | 0.13±0.06<sup>a</sup> | 0.09±0.03<sup>a</sup> | 0.2±0.0<sup>a</sup> |
| ST     | 16.9±1.7<sup>a</sup>  | 3.7±0.5<sup>a</sup>  | 0.9±0.2<sup>a</sup>   | 0.00±0.00<sup>a</sup> | 0.04±0.02<sup>c</sup> | 0.1±0.1<sup>b</sup> |
| LD     | 12.7±0.8<sup>a</sup>  | 2.6±0.3<sup>b</sup>  | 0.7±0.1<sup>a</sup>   | 0.00±0.00<sup>a</sup> | 0.02±0.02<sup>c</sup> | 0.0±0.0<sup>b</sup> |
| BH     | 11.0±1.4<sup>a</sup>  | 1.5±0.1<sup>c</sup>  | 1.1±0.5<sup>a</sup>   | 0.03±0.06<sup>a</sup> | 0.06±0.01<sup>c</sup> | 0.1±0.0<sup>c</sup> |
| Stem   |        |        |        |        |        |        |
| TZN    | 8.8±0.2<sup>b</sup>   | 25.8±1.4<sup>bc</sup> | 0.2±0.1<sup>c</sup>   | 0.30±0.05<sup>a</sup> | 1.38±0.42<sup>a</sup> | ND     |
| JH     | 11.6±1.0<sup>a</sup>  | 21.5±1.8<sup>a</sup> | 0.3±0.1<sup>a</sup>   | 0.16±0.06<sup>a</sup> | ND     | 0.6±0.1<sup>a</sup> |
| ST     | 10.1±0.2<sup>b</sup>  | 28.0±2.3<sup>bc</sup> | 0.3±0.1<sup>a</sup>   | 0.10±0.00<sup>a</sup> | 0.88±0.90<sup>a</sup> | ND     |
| LD     | 9.7±1.3<sup>c</sup>   | 33.4±4.8<sup>b</sup> | 0.4±0.1<sup>a</sup>   | 0.09±0.00<sup>a</sup> | 0.50±0.57<sup>a</sup> | 0.2±0.1<sup>b</sup> |
| BH     | 8.1±0.4<sup>c</sup>   | 14.7±1.3<sup>a</sup> | 0.3±0.2<sup>a</sup>   | 0.15±0.05<sup>a</sup> | 0.78±0.77<sup>a</sup> | 0.2±0.1<sup>b</sup> |
| Leaf   |        |        |        |        |        |        |
| TZN    | 9.1±0.5<sup>a</sup>   | 55.8±1.6<sup>c</sup> | 0.3±0.0<sup>c</sup>   | 0.62±0.06<sup>a</sup> | 0.90±0.28<sup>a</sup> | ND     |
| JH     | 17.3±0.7<sup>b</sup>  | 41.8±2.9<sup>b</sup> | 0.6±0.1<sup>a</sup>   | 0.10±0.00<sup>a</sup> | 0.73±0.73<sup>a</sup> | 0.8±0.1<sup>a</sup> |
| ST     | 11.1±1.0<sup>a</sup>  | 74.5±8.0<sup>a</sup> | 1.5±0.1<sup>a</sup>   | 0.19±0.01<sup>a</sup> | 1.01±0.04<sup>a</sup> | 0.2±0.1<sup>b</sup> |
| LD     | 12.3±0.4<sup>a</sup>  | 53.1±2.0<sup>a</sup> | 0.1±0.2<sup>bc</sup>  | ND     | 0.64±0.18<sup>a</sup> | ND     |
| BH     | 13.5±1.4<sup>a</sup>  | 64.6±1.3<sup>a</sup> | 1.0±0.2<sup>a</sup>   | 0.10±0.00<sup>a</sup> | 1.85±0.28<sup>a</sup> | 0.0±0.1<sup>b</sup> |
pollution of heavy metals [10-13]. Seawater-cultivated vegetables are newly bred food cultivars. The risk of heavy metal pollution in seawater-cultivated vegetables could not be ignored. To the best of our knowledge, there are no reports evaluating levels of heavy metals in seawater-cultivated vegetables, which, however, is a big concern to human beings.

In the present study, we collected three species of common seawater-cultivated vegetables (Suaeda glauca, S. europaea and Portulaca oleracea) and surrounding soils, runoff and seawater from five places in Jiangsu Province. Then, concentrations of Cu, Zn, Cr, Cd, Pb and Ni in different tissues of plants, water samples and soils were determined. These results should be useful for evaluating the risk of heavy metal pollution in seawater-cultivated vegetables.

### Experimental

#### Sample Collection

During August 2017, plants of S. glauca, S. europaea and P. oleracea were collected. The geographical coordinates of sampling sites are listed in Table 1. Healthy plants were carefully dug out from soils. After washing with dd-H2O to remove contaminated particles and air-drying, plants were sealed in sample bags and then transported to a laboratory at 4°C. Meanwhile, surface soil (0-10 cm) near the plants was also collected.

To track contamination sources, three freshwater runoff samples and three seawater samples within 100 m of each plant sampling location were also collected. The liquids were filtered through 0.22 μm membranes and then preserved in 7% HNO3 until analyses.
Sample Pretreatment

For each species, 10 plants were randomly selected from each location. Next, leaf, stem and root were separated. Five to fifteen plants were pooled as one sample and three samples were prepared for each location as replicates.

Plant samples and soil samples were dried completely at 80°C overnight, and then ground into powders. For each sample, approximately 0.1 g of material was taken out, precisely weighed and placed in digestion tubes. After 5 ml of 65% HNO₃ solution was added, samples were digested using a high-performance microwave digestion system (Ethos ONE, Milestone, Italy). Samples were heated to 165°C in 10 min, kept at 165°C for 15 min and then naturally cooled. Afterward, digestion solution was accurately diluted to 100 ml using deionized water.

Determining Heavy Metal Concentration

Seawater samples were diluted for 10-fold before analyses. Concentrations of Cu, Zn, Cr, Cd, Pb and Ni in sample solutions were determined using inductively coupled plasma mass spectrometry (ICP-MS; NexIon300X, Perkin Elmer, USA) using both standard and kinetic energy discrimination (KED) modes. The operating conditions are as follows: sample uptake rate = 0.5 mL/min, flush delay = 35 s, read delay = 90 s, wash time = 40 s, RF power = 1600 W, sample running time = 3 min/sample, plasma gas flow = 17.0 L/min, auxiliary gas flow = 4.1 L/min, nebulizer gas flow = 0.98 L/min. Standard metal solution was analyzed in the same condition, and then calibration curves were generated using NexION software. Each sample was run for three times as technical repeats.

Table 4. Metal concentration in Portulaca oleracea and nearby soil (mg/kg, dry weight).

| Site | Cu      | Zn      | Cr      | Cd      | Pb      | Ni      |
|------|---------|---------|---------|---------|---------|---------|
|      | Soil    |         |         |         |         |         |
| TZN  | 18.6±0.4 | 58.9±2.3 | 29.9±0.3 | ND      | 5.60±0.14 | 20.3±0.4 |
| JH   | 14.5±0.1 | 56.7±6.3 | 28.2±5.0 | 0.85±0.18 | 8.50±0.36 | 19.9±0.4 |
| ST   | 22.4±0.5 | 71.9±6.1 | 33.0±3.9 | 0.63±0.12 | 10.60±0.62 | 25.6±0.9 |
| LD   | 17.8±0.7 | 59.5±3.3 | 38.0±7.0 | 0.53±0.10 | 7.90±0.74 | 24.0±1.5 |
| BH   | 24.8±0.4 | 68.1±1.2 | 40.2±1.5 | 0.54±0.12 | 10.10±0.69 | 26.4±0.4 |
|      | Root    |         |         |         |         |         |
| TZN  | 33.5±3.5 | 6.4±1.0 | 4.9±2.5 | 0.21±0.02 | 0.10±0.05 | 0.4±0.2 |
| JH   | 37.5±4.6 | 2.0±1.8 | 5.5±2.4 | 1.01±0.24 | ND      | 0.2±0.0 |
| ST   | 34.4±6.4 | 5.4±3.6 | 3.8±1.9 | 0.36±0.31 | 0.10±0.32 | 0.2±0.1 |
| LD   | 28.4±0.0 | 63.1±0.0 | 2.8±0.0 | 0.19±0.00 | 1.30±0.00 | 1.6±0.0 |
| BH   | 54.7±0.2 | 60.7±1.8 | 3.2±0.6 | 0.10±0.35 | 2.00±0.49 | 1.7±0.1 |
|      | Stem    |         |         |         |         |         |
| TZN  | 14.7±0.7 | 47.2±2.3 | 2.1±0.4 | 0.73±0.05 | 1.87±0.51 | 1.7±0.2 |
| JH   | 18.9±0.6 | 52.8±0.5 | 1.8±0.1 | 0.67±0.03 | 1.77±0.53 | 1.5±0.2 |
| ST   | 18.0±0.2 | 38.7±1.7 | 2.3±0.2 | 0.99±0.04 | 1.41±0.58 | 2.0±0.0 |
| LD   | 13.2±1.4 | 47.6±9.4 | 1.6±0.2 | 0.70±0.08 | 2.03±0.90 | 1.7±0.3 |
| BH   | 19.1±1.3 | 45.7±1.8 | 1.7±0.7 | 0.22±0.39 | 0.76±0.86 | 0.6±0.8 |
|      | Leaf    |         |         |         |         |         |
| TZN  | 15.0±0.7 | 54.6±2.0 | 0.4±0.5 | 0.13±0.06 | 1.11±1.29 | 0.5±0.3 |
| JH   | 20.9±0.7 | 61.4±4.9 | 0.6±0.1 | 0.10±0.00 | 1.05±0.84 | 1.1±0.4 |
| ST   | 20.6±0.9 | 62.1±2.2 | 0.9±0.7 | 0.19±0.10 | 1.33±0.26 | 1.4±0.1 |
| LD   | 17.3±0.7 | 60.3±4.5 | 0.4±0.6 | 0.00±0.06 | 2.09±0.12 | 0.1±0.1 |
| BH   | 21.8±0.3 | 56.0±1.7 | 0.8±0.2 | 0.03±0.08 | 1.00±0.54 | 0.6±0.1 |

Information on sampling site is shown in Table 1. Values are means±SD. Means followed by the different superscript letters significantly differ from each other using the Student’s t-test (P<0.05). ND: not detected.
Data Analyses

Content of metals was calculated as mg of metal per kg of dry plant tissue or soil. All data are expressed as mean±SD. The homogeneity of data was tested using Levene’s test. Student’s t-tests were conducted to determine differences between samples.

Results and Discussion

Heavy metal concentrations in plants, soils, seawater and freshwater runoff from five sampling sites are presented in Tables 2-5.

According to the Chinese Environmental Quality Standard for Soils (GB 15618-1995), the highest quality level of agricultural soil (level 1) requires concentrations of Cu, Zn, Cr, Cd, Pb and Ni of less than 35, 100, 90, 0.2, 35 and 40 mg/kg, respectively. In the present study, concentrations of Cu, Zn, Cr, Pb and Ni in all tested soil samples complied with the standard for level 1 soil. The level of Cd in soils surrounding the collection sites of *P. oleracea* in JH, ST, LD and BH was higher than the standard for level 1 soil, but still lower than the standard of level 3 soil (Cd content <1.0 mg/kg).

As generally considered, soils are considered as contaminated when content of Cd is ≥3 mg/kg of soil [14-16]. These results indicated that soil in sampling locations was not polluted by heavy metals, but Cd concentration was a little higher at JH, ST, LD and BH than other sites.

As the Chinese standards for irrigation water quality (GB 5084-2005) requests, concentration of Cu, Zn, Cr and Cd should not exceed 0.5, 2.0, 0.1 and 0.01 mg/L, respectively. In the present study, Cr and Cd were not detected in most seawater and freshwater samples. Concentrations of Zn and Ni were only at μg/L level, which should not be a big concern. In comparison, Cu level in all seawater samples was quite high and even exceeded 1.0 mg/L in BH and LD. Obviously, seawater samples collected in the present study were polluted by Cu. Pb level in freshwater sample of TZN was almost 10-fold higher than other places, which should be abnormal, suggesting that freshwater runoff near these farms might be polluted.

As required by the Chinese Standard of Food Safety: Limitation of Pollutants (GB2762-2012), contents of Pb, Cd, Ni and Cr in fresh vegetables should not exceed 0.1, 0.1, 1.0 and 0.5 mg/kg, respectively. Water content in *S. glauca*, *S. europaea* and *P. oleracea* stem and leaf was all approximately 90% [17-19]. Taking these data together, we defined the safe limits of Pb, Cd, Ni and Cr in dry weight of seawater-cultivated vegetables of 1.0, 1.0, 10.0 and 5.0 mg/kg, respectively. In the present study, levels of Cd and Ni in all plant samples were lower than the safe limits. However, levels of Pb and Cr exceeded the safe limits in some samples. For example, content of Pb in *P. oleracea* stem and leaf from LD and *P. oleracea* root from BH all exceeded 2.0 mg/kg, with levels of Pb in *P. oleracea* stem from TZN, JH, and *S. glauca* leaf from BH higher than 1.5 mg/kg. Levels of Cr in *P. oleracea* root from JH and in *S. glauca* root from TZN and JH were higher than 5.0 mg/kg. Pb and Cr are both severe contaminants. Seawater-cultivated vegetables with unsafe levels of Pb and Cr might endanger health. Two aspects might explain these results. Firstly, a high concentration of Pb in freshwater runoff at TZN was observed, suggesting that irrigation on these

| Site | Cu     | Zn     | Cr     | Cd     | Pb     | Ni     |
|------|--------|--------|--------|--------|--------|--------|
| TZN  | 289.4±78.8a | 3.7±1.1 | ND     | ND     | 0.02±0.06a | 2.51±0.89a |
| JH   | 577.7±106.7b | 4.2±0.8 | ND     | ND     | 0.05±0.04b | 1.98±0.26b |
| ST   | 991.3±128.5c | 5.6±1.8 | ND     | ND     | ND     | 3.80±0.82c |
| LD   | 1290.6±48.9d | 3.4±0.5 | ND     | ND     | 0.13±0.06d | 4.46±0.37d |
| BH   | 1370.5±58.6d | 3.2±0.2 | ND     | ND     | 0.64±0.19d | 4.97±0.20d |

| Site | Cu     | Zn     | Cr     | Cd     | Pb     | Ni     |
|------|--------|--------|--------|--------|--------|--------|
| TZN  | 43.8±0.8a | 16.1±5.2a | ND     | 0.02±0.03 | 3.39±2.50a | 2.15±1.10a |
| JH   | 43.9±3.0a | 14.4±6.1a | ND     | ND     | 0.42±0.13a | ND     |
| ST   | 66.4±7.7a | 16.1±4.1a | ND     | ND     | 0.63±0.49a | 0.08±0.06a |
| LD   | 104.6±7.1a | 11.5±2.0a | ND     | ND     | 0.20±0.11a | 0.76±0.26a |
| BH   | 68.0±2.6a | 19.6±10.8a | ND     | ND     | 0.23±0.07a | 0.41±0.17a |

Information on sampling site is shown in Table 1. Values are means±SD. Means followed by the different superscript letters significantly differ from each other using the Student’s t-test (P<0.05). ND: not detected.
farms was facing a high risk of heavy metal pollution. Contamination of Cr and Pb was not clearly revealed in soil, seawater and freshwater runoff in the present study, probably due to the sampling time. We only collected seawater and freshwater for one time, which might miss pollution of Cr and Pb. Secondly, S. europaea and P. oleracea have been reported to accumulate Pb, Cr and other heavy metals when growing in saline soils [20-22]. Thus, as novel vegetables, seawater-irrigated plants might raise concerns of food safety. Besides, high concentrations of Cu were detected in soil and seawater samples, but the level of Cu in plant samples was low. Previous reports claimed that treatment with salinity reduced Cu bioaccumulation in halophytes [23], which might explain the observed phenomenon in the present study.

P. oleracea was considered as a candidate of phytoremediation species for Cu, Cr, Cu, Fe, Ni, Mn, Pb and Zn [22]. In the present study, levels of Cu, Cr, Pb and Ni in P. oleracea were higher than those in stems of the other two species, suggesting that P. oleracea had higher ability of accumulating heavy metals than S. glauca and S. europaea. However, levels of tested metals were lower in almost all stem, root and leaf samples of P. oleracea than surrounding soil, demonstrating no significant biomagnification effects. Thus, the present results suggested that P. oleracea might be unsuitable for phytoremediation. Nevertheless, the performance of P. oleracea in a highly polluted area required further elucidation.

Conclusion

In the present study, heavy metal contents in P. oleracea, S. glauca and S. europaea collected from five farms in Jiangsu Province (China) were investigated. The results suggest that these seawater-cultivated vegetables can accumulate an excess of heavy metals when planting on saline soils and might subsequently endanger health of human beings. Irrigation using contaminated seawater and runoff might be the reason. Moreover, P. oleracea has a higher ability to accumulate heavy metals than S. glauca and S. europaea.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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