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Lead and Other Elemental Content of Normal and *Erwinia carotovora* Infected *Amorphallus konjac* Corms

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Abstract *Amorphallus konjac* corms are important agriculture products in Yichang, Hubei Province, China. The *Erwinia carotovora* infected *Amorphallus konjac* corms are processed to food as normal corms. The contents of elements and L-Proline in the normal and infected *Amorphallus konjac* corms are analyzed for food safety. Even growing in the almost same soil condition, the contents of Pb, Cd, Mn and L-Proline in infected corms are significantly higher than those of normal corms (show data as suggestion by peers). Our study suggested that the infected corms are not suitable for food purpose.

Keywords Element · Lead · Food · *Amorphallus konjac* · *Erwinia carotovora*

Heavy metals can be gradually enriched by plants and be further transferred into human body through the food chain. The accumulation of heavy metals in human body is a potential threat to human health, especially to children (Ebenso and Ologhobo 2009; Sizer and Whitney 2000). Lead (Pb) is phytotoxic and reduces the growth of plants significantly at high concentration (Buchanan et al. 2000).

The main component of *Amorphallus konjac* corm is konjac glucomannan (i.e. KGM), a high molecular weight polysaccharide consisting primarily of mannose and glucose sugars. As an important source of soluble fiber and its extensive applications in food, medicine, chemical engineering, agriculture, oil and other fields, KGM has received a lot of attention recently (Liu et al. 2004; Alvarez-Mancenido et al. 2008).

In Yichang, Hubei Province, China, *Amorphallus konjac* Cultivation is one of the main local agricultural products. *Amorphallus konjac* farms are frequently bothered by *Erwinia carotovora* infection. *Erwinia carotovora*, which is reported to be ubiquitous in soil, is a kind of soft rot bacterium that softens and rots storage tissues of many plants. This bacterium typically enters plant tissues through injuries caused by insects, wind, tools, and the like. When temperature and moisture conditions are allowed, bacteria grow rapidly and then macerate the tissue (Liu et al. 2004). However, the infected *Amorphallus konjac* corms are used in food industrials as the same as the normal corms. While the size of infected *Amorphallus konjac* is usually smaller, we proposed that lead and other metals may play roles here.

On the other hand, L-proline works as radical scavenger, electron sink, stabilizer of macromolecules, and a cell wall component (Buchanan et al. 2000). Increased levels of L-Proline correlate with enhanced salinity tolerance, toxic impacts of lead and protect plant tissues against stress by acting as an N-storage compound, osmotic-solute and hydrophobic protectant for enzymes and cellular structures. L-Proline accumulation is also a defense against metal-induced stress in plant cells. L-Proline accumulation in plant may be used as an indicator of heavy metal accumulation (Andrade et al. 2009; Choudhary et al. 2007; Li et al. 2007).

Here we report the results of lead concentration and L-Proline accumulation in the infected *Amorphallus konjac* corms in Yichang, Hubei Province, China. The infection causes significant increase of lead and Mn accumulation, thus the infected *Amorphallus konjac* corms may not be suitable for food purpose.

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Materials and Methods

The Chang-yang Lang-ping *Amorphallus konjac* farm is located in Yichang, to the south of the Chang-Jiang river, Hubei Province, southwestern China. There is abundant plant biodiversity in the healthy vegetation next to these plantations. The study sites have a subtropical climate. The elevation is 850 m above sea level, where the frost-free period is 270 days in a year. The annual average temperature there is 13°C and the annual rainfall is 1,400–1,500 mm. It is far away from industrial area. The sample plantation site occupies 150 m^2, where the soil is yellow soil and the bedrock is limestone (Yichang Institute of Geology and Mineral Resources, Chinese Academy of Geological Sciences 1987). The upper 60 cm of soil was turned over and mixed thoroughly in November 2003 and in March 2004.

Five hundred *Amorphallus konjac* basic seed corms, the weight of which was 0.210 ± 0.02 g, were planted in April 2004. Fertilizer was used in April 2004 and in August 2004. The corms were harvested on November 2, 2004. Normal and *Erwinia carotovora* infected corms were sorted according to the literature (Liu et al. 2004). The weight of normal corms ranged from 9.90 to 12.00 g with the mean values of 11.00 g. The weight of infected corms ranged between 3.50 and 6.00 g with the mean values of 4.50 g. The weight of normal corms used for this study was 11.00 ± 0.10 g and 4.50 ± 0.10 g for the infected corms.

The soil samples were taken from the plantation according to the literature (Liu et al.2004), at a depth of 0–20 cm below the surface. The experiment, laid out in a randomized block design, had eleven replications.

Reagents of higher purity grade were used. High-purity deionized water with a resistivity of 18.0 MΩ cm was used to prepare all standard and sample solutions. The experiment was completed at room temperature (Liu et al.2004; Li et al. 2006). The standard reference materials (SRM) GBW07605 were obtained from the National Research Centre for Certified Reference Materials, China and used as control samples.

Soil samples were air-dried and passed through a 2 mm sieve to determine the pH (pH meter, soil:deionized water = 1:1) (Liu et al. 2004; Li et al. 2006). The corm samples were cleaned with deionized water, oven-dried at 60°C to constant weight, and ground to pass through a 2 mm sieve, then oven-dried at 110°C for 48 h.

The closed PTFE reaction vessels used in all digestion procedures were cleaned by using 2 × 3 mL of concentrated nitric acid before each digestion. Generally, 2.0000 g dried samples and 10.00 mL concentrated nitric acid, 2.50 mL concentrated $HClO_4$ and 2.50 mL concentrated HF were mixed in a PTFE vessels. The vessel was tightly capped and heated for 5 min in the microwave digestion system, and then the vessel was taken out and allowed to cool down for 3 min in a fume hood. This step was repeated for three times. After the vessel cooled to room temperature, 4.00 mL of 30% H_2O_2 was added drop by drop to the vessel. After the sample was dissolved completely, resulting sample solution was heated to near dryness, then dissolved in 5.00 mL concentrated HNO_3, and lastly diluted to 50.00 mL in a calibrated flask with 1.00% HNO_3 (Li et al. 2006, 2007). After digestion, the total concentrations of Ca, Mg, Zn, Cu, Fe, Mn, K, Na, Cd, Pb in the samples were determined by an ICP-emission spectroscopy system (Thermo, USA) (Dean 1995; Li et al. 2006). The organic C content was corrected for carbonate, which was determined by subjecting the samples to 10% hydrochloric acid (HCl) and measuring the CO_2 evolution. The organic Carbon and Nitrogen contents of all samples were measured with a VarioEL III analyzer (Rosenberg et al. 2003).

L-Proline was extracted and its concentration was determined by the method of Bates et al. (1973). The samples were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged. The supernatant was treated with acetic acid and acid ninhydrin and the whole boiled for 1 h. The absorbance at 520 nm was determined. Levels of L-proline were expressed as mg kg^{-1} dry weight.

Eleven replicate measurements were made and the mean values and the standard deviations were calculated. All samples were used for experiments within 1 week.

The *T*-test was used in this study, in which a *p*-value smaller than 0.05 is obtained at 95% confidence level. The results are given as mean ±95% confidence interval for eleven separate analyses (Dean 1995; Li et al. 2006). Statistical analyses were performed by SPSS (SPSS 10.0, SPSS Inc. H, Chicago, USA).

Results and Discussion

The data obtained from the analyses of the standards show a strong agreement with the certified reference values provided by the standard reference materials (SRM, China) GBW07605. The recovery is 98.3–101.9% and the relative standard deviation (RSD) is 0.78–2.36% (Li et al. 2006).

Some chemical properties of the surface soil at the sample sites are presented in Tables 1 and 2. The *T*-test was used in this study, in which a *p*-value smaller than 0.05 is obtained at 95% confidence level (Dean 1995; Li et al. 2006). The results showed no statistically significant difference on soil moisture contents, pH, organic C, total N and mineral element (Ca, Mg, Zn, Cu, Fe, Mn, K, Na, Cd, Pb) between these two soil samples collected near the normal and infected corms.

The contents of mineral element in three different *Amorphallus konjac* corms (normal, infected and seed) are

Table 1 Chemical properties of surface soils at Chang-yang Lang-ping *Amorphallus konjac* plantation in Yichang, Hubei Province, China

No	Organic C (g kg^{-1})	Total N (g kg^{-1})	pH	Soil water content (%)
NS	13.67 ± 0.30	1.25 ± 0.25	6.46 ± 0.11	18–26
I S	13.38 ± 0.39	1.23 ± 0.22	6.40 ± 0.15	18–26

NS is the soil of normal *Amorphallus konjac* plantation

IS is the soil of infected *Amorphallus konjac* plantation

The results are mean values, n = 11, $p < 0.05$

Table 2 The contents of lead and other mental (as mg g^{-1} dry weight for Ca, Mg, K, Fe, as mg kg^{-1} dry weight for Mn, Zn, Cu, Na, Cd and Pb) in surface soils

Element	NS (n = 11)	IS (n = 11)
Ca	23.12 ± 0.96	23.01 ± 0.95
Mg	8.408 ± 0.361	8.399 ± 0.355
K	2.165 ± 0.100	2.159 ± 0.102
Fe	21.90 ± 0.086	21.92 ± 0.087
Zn	69.2 ± 3.7	71.5 ± 3.8
Cu	32.2 ± 2.1	32.7 ± 2.3
Mn	529 ± 25	513 ± 26
Na	59.0 ± 2.4	58.1 ± 2.2
Cd	0.079 ± 0.03	0.079 ± 0.03
Pb	17.00 ± 0.19	16.98 ± 0.20

NS is the soil of normal *Amorphallus konjac*

IS is the soil of infected *Amorphallus konjac*

The results are mean values, n = 11, $p < 0.05$

presented in Table 3. The contents of L-proline in the basic seed corms, the corms of normal and infected *Amorphallus konjac* are 282 ± 7, 279 ± 7, 658 ± 10 mg kg^{-1} dry weight, respectively.

L-Proline accumulation in plant cells exposed to such stress as salt or water is a widespread phenomenon. Metal-induced stress also causes L-proline accumulation in some algae exposed to copper and cadmium (Buchanan et al. 2000). As expected, the normal and the basic seed corms show no statistically significant difference on the contents of Ca, Mg, Zn, Cu, Fe, Na, K, Mn, Cd, Pb and L-proline. However, the contents of Ca, Zn, Cu and Fe in the normal corms are significantly higher than those in the infected corms. The contents of Mg and K show no statistically significant difference. The contents of Pb, Mn and Cd in the normal corms are significantly lower than those in the infected corms. It is very interesting that all the ratios of the contents of Pb, Mn and L-proline in the infected corms to those in the normal and the basic seed corms are almost the same around 2.3.

Different species often have different sorption characteristics (Liu et al. 2004; Dahiya et al. 2005; Cui et al. 2004). The soil texture, pH, organic matter, the metal concentration, cation exchange capacity (CEC), the organism living in contact with the soil and even presence of competing ions like Na and Ca are the major factors in assessing the impact of plant acquisition and transport of mineral element on terrestrial ecosystems (Buchanan et al. 2000). The availability of metals depends on retention capacity of the soil and particular solid phases with which metals associate. Soils properties such as pH and high contents in clays and crystalline iron oxides greatly influence metal such as Pb

Table 3 The range and means values of lead and other mental (as mg g^{-1} dry weight for Ca, Mg, K, as mg kg^{-1} dry weight for Fe, Mn, Zn, Cu, Na, Cd, Pb) in the basic seed corms and the corms of normal or infected *Amorphallus konjac* in Yichang, Hubei Province, China

Element	NC (n = 11)	IC (n = 11)	BSC (n = 11)
Ca	1.985–2.433 (2.132)	1.064–1.638 (1.327)	1.879–2.511 (2.129)
Mg	4.789–5.128 (4.986)	4.431–4.786 (4.681)	4.653–5.123 (4.938)
K	31.44–39.67 (34.54)	30.74–38.81 (34.10)	30.99–40.14 (34.55)
Fe	45.06–54.78 (49.53)	35.23–44.13 (41.96)	42.34–52.69 (49.77)
Zn	65.2–70.8 (68.7)	39.8–57.0 (49.2)	65.0–71.1 (68.6)
Cu	7.64–8.71 (8.38)	5.22–6.17 (5.72)	7.69–8.73 (8.39)
Mn	51.6–71.4 (58.4)	90.8–173.4 (137.6)	50.8–72.1 (58.5)
Na	210.5–231.8 (224.0)	246.2–280.4 (258.4)	211.3–230.8 (220.1)
Cd	0.036–0.057 (0.047)	0.060–0.079 (0.069)	0.037–0.057 (0.047)
Pb	0.54–0.88 (0.69)	1.48–1.97 (1.62)	0.55–0.91 (0.70)

NC is the corms of normal *Amorphallus konjac*

IC is the corms of infected *Amorphallus konjac*

BSC is the basic seed corms

The results are mean values, n = 11, $p < 0.05$

sorption (Liu et al. 2004). The small difference of soil properties between the normal and infected *Amorphallus konjac* plantation is perhaps not the main reason for the significant difference on contents of Pb, Mn, Cd in the corms.

Plant calcium has a decisive role in disease development. The *PehN* gene is specifically required in the early stages of the *Erwinia carotovora* infection observed in high-calcium plants. Increment in extracellular calcium can repress *pehN* gene expression and ectopic expression of *pehN* allows *E. carotovora* to overcome calcium-induced resistance (Flego et al. 1997). The lower Ca content in infected corms thus is very likely the result of plant pathogen interaction.

The significant increment of Mn in the infected corms may have a good reason correlated with the infected corms. The researches found both bacterial *Nramp* horologes and members of *LraI* family of proteins are Mn transporters. Mutations of these gene associate with decreased virulence of various bacterial species. Mn appears to be essential for bacterial virulence (Papp-Wallace and Maguire 2006). In our case, *Erwinia carotovora* might also need to uptake enough Mn for its survival.

In the brown seaweed *Lobophora variegate*, the biosorption capacity of Cd(II) and Pb(II) was significantly altered by pH of the solution. Kinetic data were satisfactorily manifested by a pseudo-second order chemical sorption process. The process mechanism consisting of both surface adsorption and pore diffusion was found. The biosorption system is possibly monolayer coverage of the *L. variegata* surface by the cadmium and lead ions (Jha et al. 2009). The Cd and Pb contents in the infected and normal corms showed statistically significant difference, although the pH, the Cd and Pb contents in the soils have no statistically significant difference. The Cd and Pb are not essential and beneficial element in plants (Buchanan et al. 2000). The Cd and Pb could easily surface adsorb and pore diffuse in the infected corms because the infected corms had no perfect cellular structures. Since both Mn and Pb contents increased 2.3 times in the infected corms, it was a reasonable implication of existing interaction of Pb and Mn in the infected corms.

The much higher L-proline content of infected corms than that of normal corms clearly appeared to be a defense against infection toxin stress, for example, Pb here.

Concentration of lead in the infected corms is close to the former maximum permissible concentration (MPC) in China for Pb of 2 mg/kg dry weigh. Since lead cannot be removed by simple heating process in food industrials, the using of infected corms for food purpose should be prohibited in the future.

By analysis of elemental contents of normal and the infected *Amorphallus konjac* corms, we found that infected corms contain 2.3 times more Mn, Pb and L-proline and less Ca, Mg, Zn, Cu and Fe than normal corms although the contents of mineral element and other properties of soils are not statistically significantly different. The infected *Amorphallus konjac* corms are not suitable for food purpose.

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