Application of synchrotron radiation X-ray fluorescence to investigate the distribution of mineral elements in different organs of greenhouse spinach

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ABSTRACT: Consumption of vegetables is one of the most important ways of providing the body with mineral elements. However, it is not clear how mineral elements are distributed in different organs of vegetables, especially vegetables grown in greenhouses. The distribution of mineral elements in the root and leaves of greenhouse spinach was determined using synchrotron radiation XRF, the results indicated that the amount of various elements in different parts of the leaves, roots and stems were inequable. Generally, the content of the elements in the root base were slightly higher than in the other parts. The amounts in the root apices were much lower than those in the other parts while the amount of every element decreased gradually from the root base to the root apices. In stems, the amount of K, Ca, Fe, Ni, and Zn were higher in the base of the stem than in the top of stem while the amounts of Co and Cu were higher in the top of the stem than in the base of stem. From all the elements detected in the stem, Mn was at the lowest concentration. In leaves, Co and Zn were primarily accumulated around the main veins and the amount of Mn in the tip of the leaf was higher than in the other parts. In contrast, K, Ca, Ni, and Cu were higher in the center of the leaf.

Keywords: spinach; distribution of mineral elements; synchrotron radiation XRF; leaves; roots; stem

The importance of mineral elements in maintaining good health is well known. Some elements help to combat infection, but they are also associated with many chronic, epidemic, endemic, and even malignant diseases. Numerous epidemiological investigations have pointed out that a lack of essential mineral elements can precipitate an increase in sensitivity to illness, leading to suboptimal health or an enhancement of disease occurrence and development. If the human body is lacking zinc, copper and other trace elements, or if there is an imbalance in the body, it will lead to or aggravate some diseases, including coronary heart disease, diabetes, hyperlipidemia, hypertension, and childhood zinc deficiency among others. Iodine, selenium, zinc, iron, copper, manganese, and chromium have been recognized as the essential mineral elements indispensable to maintain normal life activities (Li 1998).

Although mineral elements cannot be synthesized by the human body, they can be obtained from consumption of certain foods. Through the continuous progression in techniques for the sensitive quantitative analysis of trace elements, it became recognized that green and natural foods are important sources (Han et al. 2005; Wang 2005; Rui et al. 2007, 2008).

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Vegetables are one of the major sources of mineral elements (Anonymous 1991). Vegetables contain a variety of trace and major elements, including copper, iron, zinc, calcium, magnesium, phosphorus, chromium, cobalt, nickel, lead, vanadium, manganese, and selenium. Therefore, a thorough study of mineral elements found in vegetables should help people to understand how to meet their required intake of trace elements while ensuring good health. At the same time, studies of mineral elements in vegetables will help to expand interest in consumption of vegetables which in turn, will promote vegetable industry.

Heavy metals are mobile and are easily taken up by plants in the environment (Khairiah et al. 2004; Chojnacka et al. 2005), so they are commonly found in trace amounts in the plant (Kou et al. 2007). Voutsa et al. (1996) found that the dominant pathway for most trace elements to vegetable roots was from the soil, while trace elements in vegetable leaves appeared to originate mostly from the atmosphere. High accumulation due to atmospheric deposition was found for Pb, Cr and Cd, especially in leafy vegetables. Root vegetables were found to accumulate soil Cd more efficiently than the other trace elements (Voutsa et al. 1996). And element concentrations differed from one vegetable to another as the result of differences in element selectivity and accumulation from soil solution. In the family Chenopodiaceae, salq had higher levels of Cd, Co, Mn, Ni, Pb, and Zn than spinach (Mohamed et al. 2003). Trace element determinations from Spanish tea were proposed by Fernandez et al. (2002). Micronutrients differ widely in their distribution within plants and their ability to be remobilized from source tissues to sink tissues (Grusak et al. 1999a,b). Guo et al. (2008) showed that the content of the same elements in different parts of the vegetable are different from each other; the contents of trace elements in leaves were higher than those in the stem of lettuce, so the lettuce leaf is more valuable than the stem for supplying trace elements to the human body (Guo et al. 2008). Another study showed that the Mn content of the tissue in the central region of the fruit was reduced by NH_4 ions, whereas the Cu content of this tissue increased, and the highest concentrations of K and NO_3^- were found in the neck and skin tissues, whereas the highest concentrations of Mn and Cu were observed only in the fruit skin (Kotsiras et al. 2002). Some studies also proved that time also affects the distribution of elements. For example, Paivi et al. (2007) reported that remarkable changes were found in the average of trace element contents of vegetables, most of which showed decreases during the past 30 years. The contents of Mn, Zn, Cu, Al, Pb, Cd, and Ni decreased, the contents of Fe and Co remained unchanged and Se was the only trace elements showing increased content (Paivi et al. 2007). In this report, we analyzed the distribution of trace elements in the leaves, roots and stems of greenhouse spinach.

MATERIAL AND METHODS

Plant sampling

We selected a spinach named Big leaf spinach, and the spinach growing conditions were as follows: the treatments consisted of following fertilizer application: 65 kg N/ha (as $Co(NH_2)_2$), 24 kg P/ha (as calcium super-phosphate, 12% as P_2O_5), 110 kg K/ha (as K_2SO_4), 10.4 kg Zn/ha (as $ZnSO_4$).

The spinach was planted on October 26, 2008, and harvested on December 20, 2008 leaving them growing as usual in order to measure the intensity. The temperature of greenhouse during the living stage of spinach ranged from 12°C to 18°C. The air humidity was 60–80% relative humidity, and irrigation for sufficient water supply was done when the soil water potential was below –30 Pa.

The fresh plant was taken from the soil in the afternoon of December 27 and was divided into three parts, including leaves, stems and roots on December 28 to measure the intensity, separately. Within a leaf, a number of representative points were selected to determine the intensity of the trace elements. Within the root and stem, we selected points every 0.5 cm to measure the intensity.

Analysis theory

Synchrotron radiation (SR) as one of X-ray sources has superior properties of high-intensity (10^3 to 10^6 stronger than the conventional X-ray sources), highly collimated and linearly polarized in the electron orbital plane. Its advantages greatly improve the analytical sensitivity and space resolution of XRF. An absolute detection limit of 10^{-12} to 10^{-15}g, and a relative detection limit at several µg/g, even as low as 10 ng/g, can be achieved with only micrograms of samples required. Therefore, synchrotron radiation X-ray fluorescence analysis (SRXRF) is characterized by multi-elements, high sensitivity microanalysis and positional analysis (Feng et al. 2005). It is thus possible to measure the element distribution in different organs of plants.

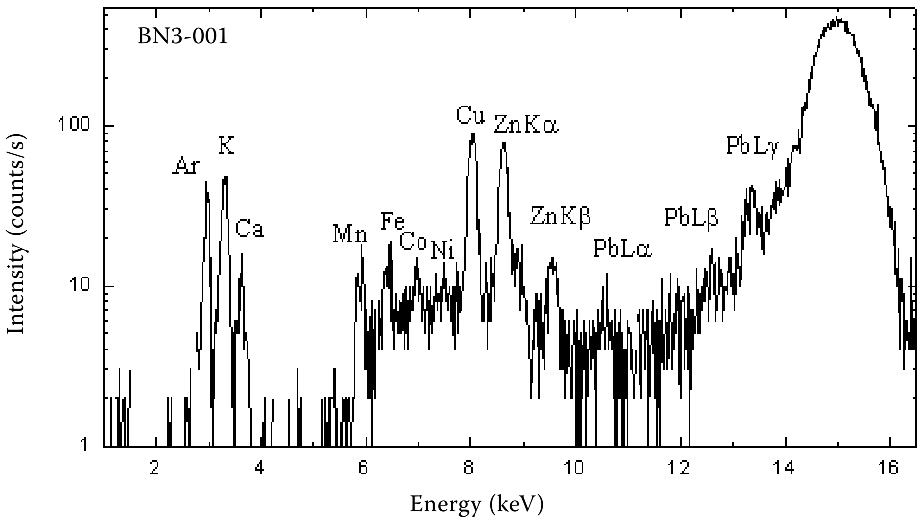

Fig. 1. Typical spectral lines of the spinach leaf

The samples were analyzed by synchrotron radiation XRF. The process is like as follows: the application of the electron-position collider results in the acceleration of particles (such as protons and electrons) to high energy, followed by focusing the particles to bombard a fixed target. During this process, the K-α line and K-β line of elements above P (15) will be measurable when the X-rays irradiate the samples. There are two α, β lines in the spectrum, but generally, the α-ray spectrum line was chosen for analysis. At the same time, the intensity of the spectrum lines is not the same because of the different intensity of incident light and variations in measurement times. For the following comparison treatment, we adopted the counts of the ionization chamber (recording the number of incident photons) to a single count, that is, the spectrum areas were divided by the count respectively. After this, we obtained the line intensity of each element. Since there is a direct relationship between the element concentration and spectral line intensity, the concentration ratio for each element can be determined. Thus, the distribution trends of different elements throughout the plant can be identified. Fig. 1 showed the typical spectral lines of the spinach leaf.

Data analysis

The relative intensity values from different leaves cannot be analyzed statistically, because matrix of one leaf is different from other leaves and different positions of one leaf have the same and good matrix. So the distribution of elements in the same organ detected by synchrotron radiation X-ray fluorescence is usually not analyzed statistically when the value is the relative intensity.

Table 1. Relative intensity of the mineral elements in the spinach leaf (counts/s)

Points	K	Ca	Mn	Fe	Co	Ni	Cu	Zn
1	0.0712	0.0056	0.0205	0.0245	0.0209	0.0215	0.1866	0.1738
2	0.0891	0.0044	0.0048	0.0261	0.0171	0.0189	0.0299	0.0323
3	0.1531	0.0113	0.0121	0.0566	0.0216	0.0272	0.0385	0.0381
4	0.1350	0.0110	0.0057	0.0285	0.0120	0.0198	0.0234	0.0378
5	0.0963	0.0041	0.0049	0.0305	0.0045	0.0207	0.0267	0.0350
6	0.1055	0.0092	0.0031	0.0273	0.0081	0.0143	0.0092	0.0160
7	0.1172	0.0055	0.0148	0.0254	0.0077	0.0119	0.0225	0.0267

The locations of points were referred to in Fig. 2.

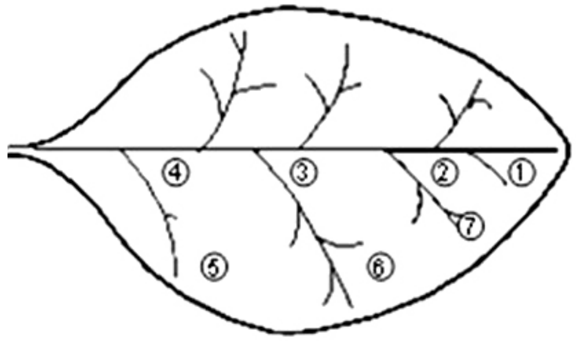

Fig. 2. Parts of spinach leaf used to determine the elements

RESULTS AND DISCUSSION

Mineral elements distribution in spinach leaves

Seven locations in half of leaf were selected to detect the relative content of mineral elements (Fig. 2), the results are shown in Table 1. The results showed the relative intensity of every element in the spinach leaf. According to the change rules of relative concentration, which are similar to the change rules of relative intensity, it was determined that the relative contents of potassium, calcium, manganese, iron, cobalt, nickel, copper, and zinc were higher than the upper range of mineral elements that can be measured.

The content of K was higher in the leaf center while the content of Ca was higher in the leaf center than in the other parts. The highest content of Mn was in the leaf-tip. The distribution of Fe in the leaf was generally uniform, with a slight decrease from leaf base to tip. Co and Zn were mainly accumulated around the primary veins. The relative content of Ni was higher in the center of the leaf than in the other parts, with a gradual decrease in the amount from the leaf base to the tip. Cu was also primarily accumulated in the leaf center, with a similar decrease in amount from the leaf base to tip.

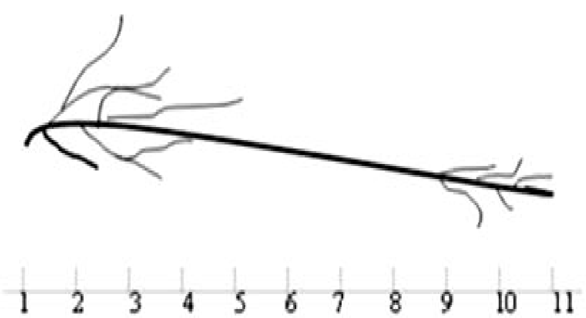

Fig. 3. Positions in root used to determine the amount of elements

Using the elements spectral line of point 1 in spinach leaf as an example (Fig. 1), every element is represented by an absorption peak area and it also indicates a high content of trace elements at this location. Using the α-line instead of the β-line as the experimental criterion, it was concluded that the leaf contains higher amounts of K, Ca, Mn, Fe, Co, Ni, Cu, and Zn.

Mineral elements distribution in spinach root

Eleven locations in root were selected to detect the relative content of trace elements (Fig. 3), the distance of the neighboring location is 0.5 cm, results are shown in Table 2. The spinach root mainly contained K, Ca, Cu, Mn, Co, Ni, Fe, and Zn, similar to the leaf, but the content of K in the root was slightly lower. The amount of every element gradually decreased from the root base to the root apices.

Mineral elements distribution in spinach stem

Three locations in stem were selected to detect the relative content of mineral elements (Fig. 4); the distance of the neighboring location is 1.0 cm, the results are shown in Table 3. The varieties and amounts of mineral elements in the spinach stem were similar to the leaf and root, and thus spinach stem mainly contains K, Ca, Cu, Mn, Co, Ni, Fe, and Zn. All of the mineral elements were quantified at a high level, with a lower amount in the middle of the stem and a higher amount in two ends except for Mn. The amounts of K, Ca, Fe, Ni, and Zn were higher in the stem base than in the top while the contents of Co and Cu showed the opposite trend. Similar as in root and leaf, the amount of Mn was lower but it was slightly higher in the middle of the stem than at the two ends.

Mineral elements distribution in whole spinach plant

From Tables 1–3, it is evident that the elements found in the root base were slightly higher than in the other parts. Mineral elements in the leaf and stem were uniform. The further the portion of the

Table 2. Relative intensity of the mineral elements in the spinach root (counts/s)

Points	K	Ca	Mn	Fe	Co	Ni	Cu	Zn
1	0.2699	0.0420	0.0214	0.1041	0.0174	0.0297	0.0441	0.1143
2	0.2835	0.0204	0.0166	0.0586	0.0197	0.0328	0.0415	0.1078
3	0.2805	0.0283	0.0073	0.0716	0.0205	0.0233	0.0379	0.0681
4	0.1794	0.0504	0.0292	0.2149	0.0195	0.0214	0.0362	0.0803
5	0.2241	0.0345	0.0043	0.0974	0.0046	0.0114	0.0105	0.0903
6	0.1282	0.0209	0.0102	0.0911	0.0097	0.0247	0.0244	0.0856
7	0.0008	0.0010	0.0038	0.0144	0.0077	0.0098	0.0074	0.0053
8	0.0006	0.0012	0.0010	0.0054	0.0080	0.0088	0.0076	0.0039
9	0.0006	0.0005	0.0037	0.0055	0.0097	0.0087	0.0107	0.0070
10	0.0016	0.0024	0.0040	0.0082	0.0047	0.0061	0.0092	0.0032
11	0.0002	0.0034	0.0013	0.0115	0.0060	0.0084	0.0092	0.0047

The locations of points were referred to in Fig. 3.

plant is from the root base, the lower is the amount of mineral element.

Fusun (2005) found that increasing the nitrogen amounts of the fertilizers resulted in significant decreases in the K, Zn and Mn content in spinach. Similarly, the Ca content of the fruit and other plant tissues of cucumber was reduced when the NH_4^+ levels were increased in the nutrient medium (Alan 1989). Assimakopoulou (2006) found that Fe and Mn concentrations in shoots were reduced when N was added as 100% NO_3^-. Therefore, the method of fertilization can have definite effects on the nutrient content of vegetables.

Spinach was found to contain a certain amount of trace elements (Mn, Fe, Co, Ni, Cu, and Zn) and the macro-elements (K, Ca). These elements are essential for maintaining proper acid-alkali balance and normal osmotic pressure in the body. Once the body starts to lack these essential elements, illness will likely result, which may even be life-threatening. However, excessive intake of essential elements can also result in abnormal physiological function and illness. Therefore, taking these results into consideration, in order to optimize the intake of trace elements from spinach, a vegetable well known for its rich content of trace elements, portions of the root base should be included for consumption.

CONCLUSIONS

In spinach in greenhouse conditions, the content of mineral elements in the root base was slightly higher than in the other parts. The amounts in the root apices were much lower than those in the other parts while the amount of every element de-

Table 3. Relative intensity of the mineral elements in the stem (counts/s)

Points	K	Ca	Mn	Fe	Co	Ni	Cu	Zn
1	0.1350	0.0094	0.0026	0.0250	0.0138	0.0234	0.0320	0.0360
2	0.0002	0.0011	0.0030	0.0061	0.0051	0.0089	0.0091	0.0034
3	0.1679	0.0250	0.0019	0.0385	0.0131	0.0244	0.0235	0.0657

The locations of points were referred to in Fig. 4

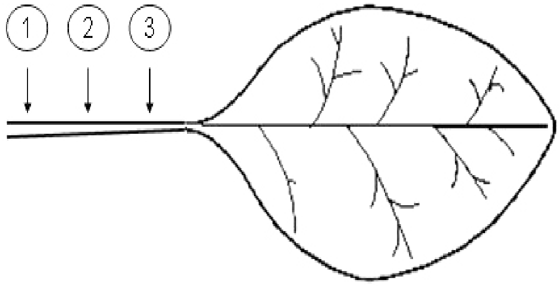

Fig. 4. Positions in the stem used to determine the amount of elements

creased gradually from the root base to the root apices. In stems, the amount of K, Ca, Fe, Ni, and Zn were higher in the base of the stem than in the top of stem while the amounts of Co and Cu were higher in the top of the stem than in the base of stem. In leaves, Co and Zn were primarily accumulated around the main veins and the amount of Mn in the tip of the leaf was higher than in the other parts. In contrast, K, Ca, Ni, and Cu were higher in the center of the leaf.

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Využití rentgenové fluorescence (XRF) synchrotronového záření při studiu zastoupení minerálních prvků v různých orgánech špenátu ze skleníkové výsadby

ABSTRAKT: Konzumace zeleniny je jednou z nejdůležitějších cest, jak zásobit organismus minerálními prvky. Zastoupení minerálních prvků v různých částech zeleniny, pěstované ve skleníku však není zcela známo. Rozložení minerálních prvků v kořenech a listech špenátu bylo stanoveno pomocí XRF synchrotronového záření; výsledky ukazují, že množství různých prvků v jednotlivých částech listů, kořenů a stonků není stejné. Obsah prvků v bázi kořene byl zpravidla mírně vyšší než v ostatních částech, zatímco obsah prvků v kořenových špičkách byl mnohem nižší. Množství všech prvků postupně klesalo od báze kořene směrem ke kořenovým špičkám. Ve stonku byl zjištěn vyšší obsah K, Ca, Fe, Ni, a Zn v bázi stonku v porovnání s jeho vrcholem, zatímco obsah Co a Cu byl vyšší ve vrcholu než v bázi. Obsah Mn ze všech sledovaných prvků byl ve stonku nejnižší. V listech byly Co a Zn akumulovány okolo hlavních žil a obsah Mn byl vyšší ve špičce listu než v ostatních částech. Naproti tomu obsahy K, Ca, Ni, a Cu byly vyšší ve střední části listu.

Klíčová slova: špenát; rozložení minerálních prvků; rentgenová fluorescence (XRF) synchrotronového záření; listy; kořeny; stonek

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