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Determination of Microelements in Sprouts Grown on Metal-Enriched Solutions by Ion Chromatography

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Summary. The paper discusses a possibility to grow seeds on solutions of microelements and application of sprouts enriched in such a way as an alternative to commercial dietary supplements. It contains a short review of the approaches reported till now and a systematic experimental study, carried on the most frequently used seeds (*Lens culinaris*, *Helianthus annuus*, *Vigna radiata*, *Glycine max*, and *Lepidium sativum*).

Seven metals (Fe, Cu, Zn, Ni, Co, Cd, and Mn) were studied. Seeds were grown on cellulose in 20°C temperature using deionized water enriched with metals in concentrations: 100, 50, 25, 12.5, 6.25, and 3.125 mg/L in a period of 4 days. The reference samples were the seeds grown on pure deionized water. Sprouts were mineralized by microwave radiation, and the metal content was quantified by ion chromatography with on-line post-column derivatization and spectrophotometric detection.

The conclusions can be treated as general recommendations, which seeds should be grown and what concentrations of metals in solutions should be applied to provide good enrichment and to avoid risk of microelement overdose.

Key Words: sprouts, microelements, metals, dietary supplements

Introduction

One of the recent trends in nutrition is to point the attention to advantages of germinating seeds called sprouts. They have been proven to be a rich source of many basic food components: amino acids, vitamins, minerals, unsaturated fatty acids, and dietary fiber [1]. They may be grown regardless of the season from appropriately stored seeds and therefore can be a valuable part of the human diet. They are also the kind of food that interacts weakly with drugs [2].

The total content of the metal ions in sprouts is the sum of the metals being inside the seeds and taken from the soil or growing solution. In the case of toxic heavy metals, this is treated as a big disadvantage; but in the case of microelements, there is a possibility to enrich the sprouts with them, making such sprouts an alternative to dietary supplements.

There are several papers regarding the metal contents in sprouts in the literature, and they will be mentioned chronologically. Popov and Mashev investigated the effect of zinc content to growing process of maize seeds [3]. Freeland-Graves et al. checked the zinc and copper contents in several food products including sprouts [4]. Bansal et al. pointed out the attention that high rates of zinc reduced the content of Cu, Fe, and Mn in sprouts grown on cultivated sod-podzolic soil and chernozem [5].

Wills et al. [6] reported the levels of sodium, potassium, calcium, iron, magnesium, and zinc in several Chinese foods, including bean sprouts. Kadam and Salunkhe [7] undertook a study of composition of horse gram and moth bean sprouts including microelements. Goodrich et al. [8] checked cadmium level in sprouts of cabbage. Leh [9] checked the content of lead, cadmium, and zinc in growing vegetables.

Lujan et al. [10] reported the ability to uptake metal ions for freeze dried roots, stems of cattail plants, the leaves of tumble weeds and alfalfa sprouts (*Medicago sativa*). Metz et al. [11] checked the interactions of several organic pollutants with a metal ion uptake in growing plants.

Laric et al. [12] published an interesting study on a level of several metals (Na, K, Mg, Ca, Fe, Cu, Zn, and Mn) in wheat sprouts grown on distilled water. The sprouts were separated into three fractions (root, culm, and grain) manually, and the differences in the metal levels were comprehensively examined and discussed. Bibak et al. [13] checked the level of 63 elements in cabbage sprouts during a long comprehensive study. Next, Elik et al. [14] developed a new procedure of analyzing metals in sprouts (Pb, Cd, Cr, Ni, Cu, Fe, and Al), called Bio Collector-Ultrasonic Leaching Method (BC-ULM). This study was followed by a next one [15] where levels of metals (Pb, Cu, Ni, Zn, and Cd) were checked in sprouts grown in different regions of Turkey.

En et al. [16] discussed the connection of metal contents in growing plants and a soil contamination. They concluded that the metal uptakes from the soil to plants are high enough to assess environmental contamination by toxic elements. Next, Saiki et al. [17] studied the iron uptake by sprouts of Japanese radish.

Inaba and Takenaka [18] investigated the effect of dissolved organic components on bioavailability of copper for lettuce sprouts. Zieliński et al. [19] checked a mineral composition of sprouts of Cruciferae family and reported higher amounts of calcium, magnesium, copper, and zinc comparing to seeds (a significant uptake of these bioelements). Urbano et al. [20] checked a content of zinc and magnesium in sprouts of pea. Sulieman et al. [21] checked the contents of several minerals (P, Ca, Fe, Mg, Cu, and Zn) in lentil cultivars during germination. There is also a study of Zeng et al. [22] regarding the effect of cadmium content on germination of many rice varieties.

The above publications indicate that the level of microelements of the sprouts depends significantly not only on the level in the seeds but also on the respective level in soil or growing solution. This makes some interesting idea—instead of taking tablets made of inorganic salts as dietary supplements, there is a possibility to grow the sprouts in metal-enriched solutions and treat the sprouts obtained in this way as a dietary supplement rich in one or several particular microelements. So far, in the literature, there is not any comprehensive study on microelement uptakes in several species of sprouts and in different concentrations of enriched growing solutions. This paper is intended to fill this gap and give some recommendations, which sprouts are especially recommended to this task, based on ion chromatography and AAS assays.

Experimental

Five common sprouting seed species were studied (manufactured by Diet-Food, Warsaw, Poland): A. lentil (*Lens culinaris*), B. sunflower (*Helianthus annuus*), C. mung bean (*Vigna radiata*), D. soybean (*Glycine max*), and E. garden cress (*Lepidium Sativum*). Deionized water was obtained using an EASY PURETM RF Barnstead deionizer.

The seeds were dipped in different solutions of respective metals (3.125, 6.25, 12.5, 25, 50, and 100 mg/L of Fe, Cu, Zn, Ni, Co, Cd, and Mn) and grown for 4 days in temperature of 20°C in standard day–night cycle, in daylight conditions (window). As reference samples, the seeds grown on pure deionized water were used. All experiments were performed without any substrate (seeds were dipped in solutions). After germination, the sprouts were washed five times with 500 mL of deionized water.

The mineralization of samples was done in a teflon cuvette inside a microwave mineralizer (UniClever BM-1z, Plazmatronika, Poland). The weighed 0.5 g samples were mineralized with 1 mL of concentrated nitric acid (*V*) (Suprapur grade, Merck, Germany) and 9 mL of deionized water. The mineralization process lasted 20 min with increasing pressure up to 45 atm and 100% of microwave power.

A Dionex DX-500 IC ion chromatograph was used to quantify the metal content in mineralized samples (except for Ca and Mg). The dedicated precolumn IONPAC CGP 5A 4 × 50 mm (Thermo Scientific, USA) and column IONPAC CS 5A 4 × 250 mm (Thermo Scientific, USA) were used. The aqueous mobile phase containing 35 mol/L pyridinecarboxylic acid, 0.37 mol/L formic acid, 0.33 mol/L KOH, and 28 mmol/L potassium sulfate was used. The derivatization was carried out using the aqueous solution containing 2.8 mmol/L of 4-(2-pyrridazo)resorcinol, 0.3 mol/L of calcium carbonate, 1 mol/L dimethylaminoethanol, and 0.5 mol/L of ammonia. The detection was done spectrophotometrically at 530 nm.

The calcium and magnesium contents were determined by atomic absorption spectrometry on a Pye Unicam SP-192 spectrophotometer (Cambridge, UK), using the standard conditions recommended by a manufacturer (422.7 nm for Ca and 285.2 nm for Mg).

Results and Discussion

The chromatographic conditions described above allowed to separate all analyzed metals *(Fig. 1)*. The mean retention times were (in minutes): 4.61 (Fe), 6.41 (Cu), 6.86 (Ni), 7.26 (Zn), 7.89 (Co), 8.52 (Cd), and 8.87 (Mn). Preliminary visual examination of the results (presented in a logarithmic scale in *Fig. 2*) indicated the positive dependence between the respective solution concentration and content of metals in the sprouts. The results were then concatenated into the series and fitted to the following linear dependence:

$$\mathrm{Cf} = s^{*}\mathrm{Cs} + i$$

where Cf is a concentration of metal found in grown sprouts, Cs is a concentration of metal solution where the sprouts grew in, s is a slope, and i is an intercept.

The correlation coefficient in such an equation can be interpreted as the linearity of the metal uptake dependence. If the dependence is linear enough, the intercept can be interpreted as an extrapolated concentration for sprouts grown in deionized water. The more important parameter is the slope which characterizes the ability of the seeds to accumulate the metals from the solution. If the slope is less than unity, there is a situation when some barrier exists and seeds take only some part of metal solutions. The slope equal to unity indicates the situation when metal ions migrate freely to sprouts without any barrier. The slope larger than unity would indicate some active uptake of microelements, whereas negative value means that metals go from seeds to water.

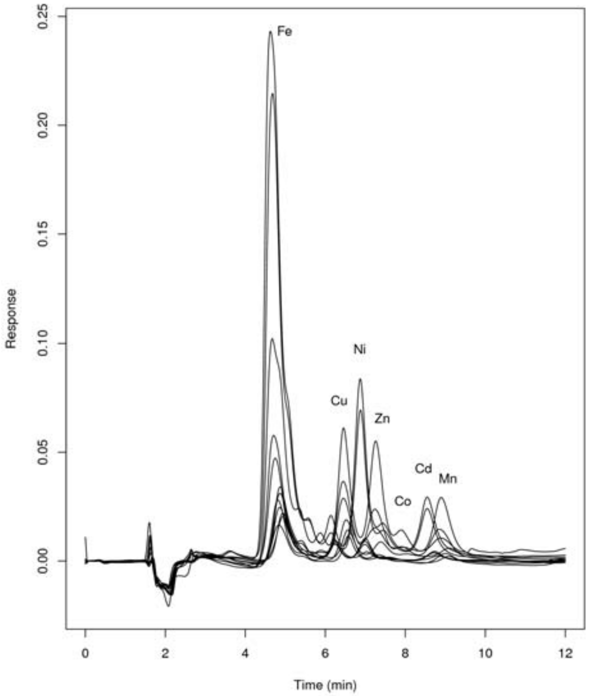

Fig. 1. The representative set of annotated ion chromatograms, showing separation of analyzed metals

The results are summarized in *Table I*, whereas slopes and intercepts are also depicted in *Fig. 3* in logarithmic scale for better readability. The correlation coefficient is, in most cases (except calcium) larger than 0.9, which indicates a linear uptake dependence over the investigated range. The following conclusions can be drawn:

1. The most intensive uptake of iron can be observed in the case of lentil. Whereas the seeds themselves are not rich in this element, sprouts grown on large concentrations (for example 100 mg/L) are the richest ones.
2. Copper is taken by the seeds with a linear dependence but with very weak slope. The largest uptake is observed in the case of lentil, too.
3. The largest uptake of nickel is observed for mung bean—the sprouts contain almost half the amount of the solution concentration overall to investigated range.
4. The lentil seeds cannot be recommended to be enriched by zinc. The other seeds accumulate zinc better, whereas sunflower and soybean are the best recommendations.

5. In the case of cobalt, the most intensive uptake is observed in the case of lentil and soybean.
6. Although cadmium cannot be discussed as a nutrient, we have investigated its uptake in the same conditions. It is similar among all the species investigated.

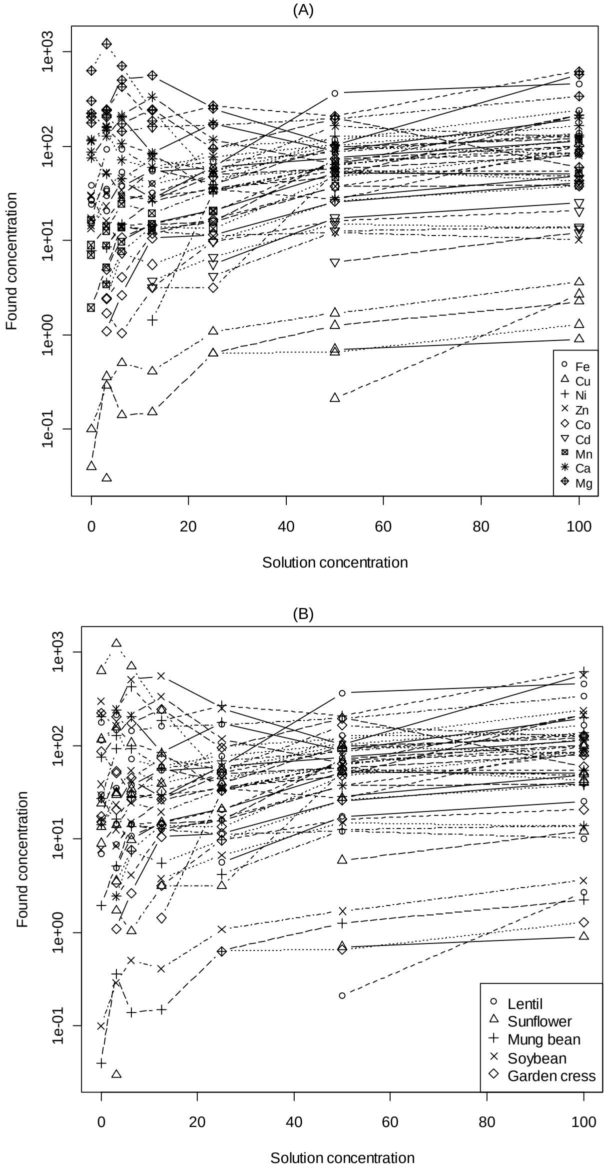

Fig. 2. The dependence of concentration of the solution (mg/L) and concentration found in germinated sprouts (mg/kg) depicted as a function of metals (A) and species of the seeds (B)

7. Lentil, soybean, and garden cress sprouts have the largest slopes in the case of manganium.
8. Due to a high level of calcium in the seeds (comparing to the other metals), there is no easy way to obtain further enrichment of this microelement.
9. The lentil sprouts can accumulate the largest amount of magnesium.

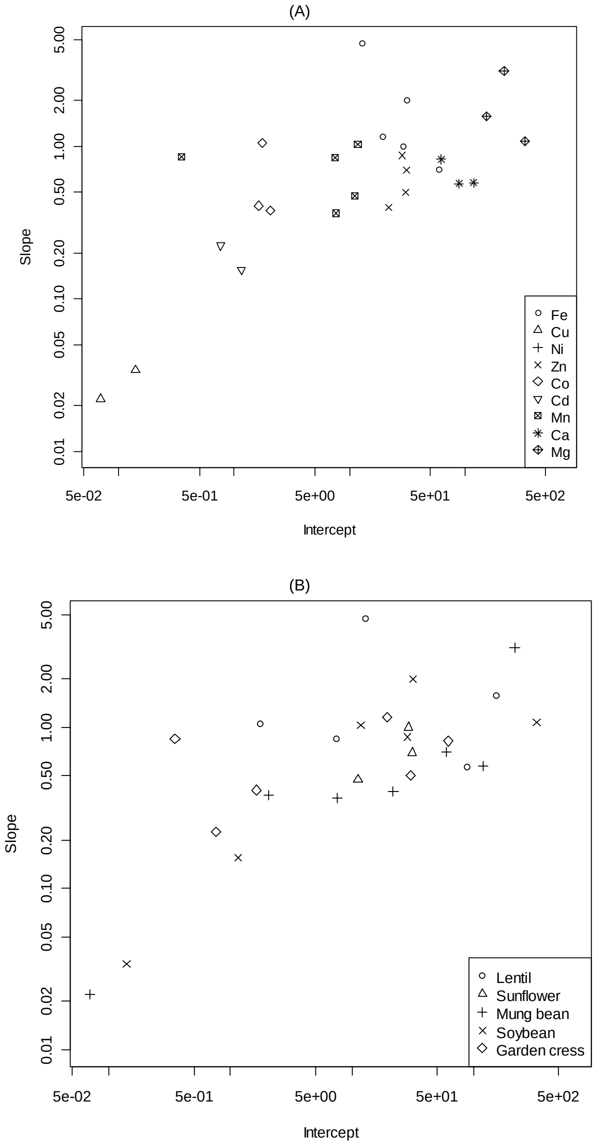

Fig. 3. The dependence between slope (dimensionless value) and intercept (mg/kg) of respective combinations metal species divided by metals (A) and species (B)

Table I. The slopes (s), intercepts (i, mg/kg) and correlation coefficients (r) for linear dependence of uptake of nine metals in investigated sprouts

	Lentil			Sunflower			Mung bean			Soybean			Garden cress		
	i	s	r	i	s	r	i	s	r	i	s	r	i	s	r
Fe	12.93	4.76	0.9489	29.08	1.00	0.9425	59.90	0.70	0.7436	31.63	2.00	0.9860	19.37	1.16	0.9879
Cu	-0.30	0.03	0.9095	-0.05	0.01	0.9335	0.07	0.02	0.9871	0.14	0.03	0.9954	-0.01	0.01	0.9574
Ni	-0.98	1.54	0.9164	0.83	-0.01	-0.3059	-3.79	0.52	0.9663	-3.96	1.93	0.9536	-2.19	1.08	0.9800
Zn	17.70	-0.10	-0.5157	31.21	0.70	0.8188	21.79	0.40	0.8830	28.43	0.87	0.9678	30.52	0.50	0.8490
Co	1.75	1.06	0.9705	-0.56	0.58	0.8746	2.05	0.38	0.9794	-0.97	0.83	0.9971	1.63	0.41	0.9850
Cd	-0.97	0.28	0.9760	-1.03	0.13	0.9739	-0.17	0.16	0.9334	1.16	0.16	0.8744	0.76	0.22	0.9493
Mn	7.48	0.85	0.9593	11.14	0.47	0.9088	7.61	0.36	0.9048	11.80	1.03	0.9932	0.35	0.85	0.9918
Ca	88.96	0.57	0.5223	100.39	-0.41	-0.3270	119.23	0.58	0.3795	201.52	-0.48	-0.2007	62.13	0.82	0.6781
Mg	154.40	1.58	0.8597	646.03	-8.08	-0.6507	217.49	3.14	0.7096	329.87	1.08	0.2125	208.35	-1.36	-0.7232

Conclusions

The above results suggest that the lentil and soybean seeds have the highest ability to accumulate microelements during germination process. This suggests that they could be primarily used for production of microelement-enriched sprouts.

From our correlation study, it could be concluded that the slope of fitted dependence does not exceed unity, so the concentration larger than the sum of solution content and seeds content cannot be expected during germination process, and the risk of overdose is very small. However, producing enriched sprouts would always require the metal levels control before passing a particular product to the market.

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