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ORIGINAL PAPER

Availability of iodide and iodate to spinach (*Spinacia oleracea* L.) in relation to total iodine in soil solution

J. L. Dai · Y. G. Zhu · Y. Z. Huang · M. Zhang · J. L. Song

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Abstract A greenhouse pot experiment was carried out to investigate the availability of iodide and iodate to soil-grown spinach (*Spinacia oleracea* L.) in relation to total iodine concentration in soil solution. Four iodine concentrations (0, 0.5, 1, 2 mg kg^{-1}) for iodide (I^-) and iodate (IO_3^-) were used. Results showed that the biomass productions of spinach were not significantly affected by the addition of iodate and iodide to the soil, and that iodine concentrations in spinach plants on the basis of fresh weights increased with increasing addition of iodine. Iodine concentrations in tissues were much greater for plants grown with iodate than with iodide. In contrast to the iodide treatments, in iodate treatment leaves accounted for a larger fraction of the total plant iodine. The soil-to-leaf transfer factors (TF_{leaf}) for plants grown with iodate were about tenfold higher than those grown with iodide. Iodine concentrations in soil solution increased with increasing iodine additions to the soil irrespective of iodine species. However, total iodine in soil solution was generally higher for iodate treatments than iodide both in pots with and without spinach. According to these results, iodate can be considered as potential iodine fertilizer to increase iodine content in vegetables.

Keywords Biofortification · Iodide · Iodate · Spinach · Soil solution

J. L. Dai · Y. G. Zhu (✉) · Y. Z. Huang
Research Center for Eco-environmental Sciences,
Chinese Academy of Sciences,
100085 Beijing, China
e-mail: ygzhu@rcees.ac.cn

J. L. Dai · M. Zhang · J. L. Song
College of Environment and Resource,
Shandong Agricultural University,
271018 Tai'an, China

J. L. Dai
Environment Research Institute,
Shandong University,
Ji'nan 250100, China

Introduction

The linkage between iodine deficiency and the onset of health problems such as goitre, growth impairment, mental retardation and even cretinism has been known since 1895. These health problems are generally regarded as iodine deficiency disorders (IDD), and WHO currently estimates that about 1 billion people around the globe today are at risk from IDD. In particular, iodine deficiency among pregnant women and preschool children constitutes a severe public health problem that must affect the social and economic development of many countries. Despite the wide adoption of iodized salt in China, iodine deficiency, particularly in western China, can be severe. A survey in 1999 showed that in Xinjiang, about 23% of children

aged between 8 and 10 had goiter (Xinjiang Disease Control Centre 2000).

People at risk are often the poorest members of the population who are totally dependent on subsistence agriculture for their dietary needs. The inability of their local environment to provide the correct mineral balance can lead to serious health problems and diseases. Although many successful remediation methods involve dietary interventions such as the provision of iodized salt and iodized oil, they can be difficult to implement in regions where these approaches are not acceptable to the local population and culture (Zhang et al. 2000). Furthermore, these techniques do not address the underlying issue of environmental iodine deficiency and the iodine content of natural food intake. Animals and plants in the food chain ultimately depend upon soil, water and rock in the environment for the provision of essential minerals to meet the human need. Therefore supplementation of iodine in food chains through plant uptake is believed to be a cost-effective way to improve human nutrition and help to reduce the worldwide incidence of IDD (Jopke et al. 1996).

Although it is well known that iodine is an essential nutrient for human and animal health, its essentiality has not yet been established for plants, although many studies have shown that plants can accumulate iodine (Mackowiak and Grossl 1999; Zhu et al. 2003; Whitehead 1973; Yuita 1982; Dai et al. 2004a). Some previous results have shown that increasing iodine applications to the soil could enhance iodine accumulation in the edible parts of vegetables (Dai et al. 2004a; Whitehead 1973). However, excessive iodine in the soil can be toxic to plants, such as rice of 'reclamation akagare' disease, a physiological disorder caused by flooding paddy fields on iodine-rich soils. Iodine toxicity to the plant may be caused by the intracellular oxidation of I^- to I_2, resulting in the inhibition of photosynthetic processes (Mynet and Wain 1973). Furthermore, the uptake of iodine by plants is governed ultimately by many factors (e.g. genetic differences and environmental factors) and is dependent upon the amounts and forms of I present at the root surface (Mackowiak and Grossl 1999). Both forms of I, iodate (IO_3^-) and iodide (I^-) can coexist in soils (Yuita 1992). Thus, it is important that I availability studies consider both forms of soluble iodine in soil.

Solution culture studies have shown that plants can tolerate higher levels of iodate than iodide (Borst and Pauwels 1961; Mackowiak and Grossl 1999; Zhu et al. 2003), and that very low concentrations of iodine (0.02–0.2 mg kg^{-1}), regardless of form, can be beneficial to several crop plants, particularly halophytes (Borst and Pauwels 1961). Our previous studies for selecting iodine-enriched vegetables showed that vegetables can take up iodine from soil fertilized with iodate and that spinach was considered as an efficient vegetable for iodine bio-fortification. Therefore, based on these results, the objectives of the present study were to investigate the effects of iodine forms in soil on accumulation by spinach (*Spinacia oleracea* L.), and to elucidate the linkage between iodine in soil solution and plant accumulation.

Materials and methods

Soil

Soil was obtained from Huai'rou district of Beijing in China. General properties of the soil used in this study are shown in Table 1. The soil sample was air-dried and ground to pass a 2-mm sieve before potting for planting vegetables, and were sieved through a 1-mm sieve for soil pH, analysis, ground to pass through a 0.25-mm sieve for the analyses of total iodine, cation exchange capacity (CEC) and free Fe/Al oxides, and through a 0.125-mm sieve for organic matter determination.

Fertilizers

Potassium iodate (KIO_3) and potassium iodide (KI) were used as iodine fertilizer. Basal fertilizers: NH_4NO_3 (150 mg N kg^{-1} soil), KH_2PO_4 (150 mg P kg^{-1} soil, 189 mg K kg^{-1} soil). All fertilizers were thoroughly mixed with the soil prior to potting.

Determination of soil properties

Soil pH was determined with a Thermo Orion pH meter (Model 828) in a 1:2.5 suspension in H_2O.

Table 1 Basic properties of soils used in this study

Soil type (Gong, 1999)	pH (H_2O)	OM (g kg^{-1})	CEC (cmol kg^{-1} soil)	Free Fe_2O_3 (g kg^{-1})	Free Al_2O_3 (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Total I (mg kg^{-1})
Udic Luvisols	7.85	13.9	9.16	10.8	1.07	65.39	14.78	59.67	1.55

Organic matter was determined by oxidation with of potassium dichromate-titration of $FeSO_4$. Free Fe/Al oxides were extracted with DCB reagent (dithionite citrate sodium-bicarbonate reagent), Fe was determined by Atomic Absorption Spectrometry (AAS, Z-6100, Hitachi Co., Japan) and Al was determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Optima 2000, PE Co., USA). Cation exchange capacity (CEC) was determined with the method of acetic ammonium saturation. Procedures for the determination of soil properties were standard methods recommended by the Chinese Society of Soil Science (Lu 2000). Total iodine in soil was determined by Neutron Activation Analysis (NAA).

Plant culture in soil

The pot experiment was carried out in a greenhouse with temperature of 25 ± 3°C at daytime and 20 ± 3°C at night. Seeds of spinach (*Spinacia oleracea* L.) were disinfected in 10% H_2O_2 solution for 10 min followed by thorough washing in deionized water. Seeds were then germinated on moist filter paper for 2–3 days. Three geminated seeds were sown in pots with 500 g soil per pot. The soil was supplied with the following amounts of iodine as KIO_3 and KI in soil: 0, 0.5, 1.0, 2.0 mg I kg^{-1} soil. Basal fertilizers were applied in the same time as iodine was applied with thorough mixing, and each treatment had four replicates for planting spinach and three replicates for the controls with no planting. Ten days after emergence, seedlings were thinned to one uniform plant. The water content of the soil was adjusted to 20%. Throughout the growth period, water losses were compensated by the addition of deionized water by weighing.

After 7 weeks of growth period, harvest plants were divided into roots and shoots, then washed with deionized water and dried with soft paper, and fresh weights were determined immediately. Plant materials were then oven-dried at 70°C for 72 h, and dry weights were determined for all tissues. Tissues were all ground for iodine analysis by Neutron Activation Analysis (NAA).

Soil solution

Soil solution was sampled directly from soil using rhizon soil moisture samplers (Rhizon SMS: Rhizosphere Research Products. Doderstraat 62, NL 6706 JG Wageningen, The Netherlands). The rhizon soil moisture sampler is known to be a non-destructive, simultaneous, sequential, convenient and rapid sampling tool for soil pore-water extraction and provides an in situ monitoring technique. The rhizon soil moisture sampler in this experiment is a standard set with porous polyester tubes, complete with PVC/PE tubing connectors and protective caps. The sampler is connected to a standard syringe using luer-lock fittings and PVC tubing. Evacuating the syringe by drawing the piston is sufficient to withdraw filtered soil solution.

One soil moisture sampler was inserted to soil per pot prior to planting, and the stoppers were sealed with protective caps. Vacuum is kept by keeping plunger in place with wooden spacer. Plants were grown for 24 days after seedling, and began to extract soil solution by soil moisture samplers for the first time, soil solution yielded (approx. 7–10 ml) after 24 h. Soil solution was obtained and total iodine was determined immediately. At later times, solution was sampled once a week, four times altogether before harvest.

Iodine analysis: soil solution

Iodine in soil solutions was determined by Ion chromatography (Dionex 600, USA), and the conditions of determination were as follows: the

ion chromatography (Sunnyvale, CA, USA) was equipped with a GP50 gradient pump and ED50A electrochemical detector in the pulsed amperometric detection mode, an IonPac AS11-HC analytical column (250 × 4 mm) and IonPac AG11-HC guard column (50 × 4 mm), a silver working electrode with pulsed amperometric method and an Ag/AgCl reference, with a 25 µl and 125 µl sample loop. Both instrument control and data collection were performed by a Dionex PeakNet 6.4 chromatography workstation. All samples were filtered with 0.22 µm filters (Tianjin, China) prior to analysis. For the determination of iodate, the soil solution was reduced to iodide by 1% Vc (50 µl in 10 ml solution, Fang et al. 1994), and was determined by Ion Chromatography (Dai et al. 2004b).

Iodine analysis: plant tissues

Neutron activation analysis (NAA) was used to analyze total iodine in plant tissues (Shinonaga et al. 2000; Hou et al. 1997). In brief, the procedure was as follows. Dried plant samples and reference materials (150–200 mg per sample) were double-sealed in a polyethylene tube for irradiation. Each sample was irradiated for 15–20 min in a miniature Neutron Source Reactor (MNSR) of the Chinese Institute of Atomic Energy Science in Beijing. The thermal neutron flux used was 7.0×10^{11} n cm^{-2} s^{-1}. In the irradiation facility, samples were shielded in a BN (boron nitride) box.

The radioactivity of ^{128}I was measured by high purity Ge detectors (Ortec, USA) for 10 min at 442.09 keV. Analysis accuracy was measured on the reference material and was between 2% and 3.6%.

Data analysis

The iodine concentrations (IC) in leaves and roots were calculated both on fresh weight basis.

Soil-to-leaf transfer factors (TF_{leaf}) were calculated as follows:

$$TF_{leaf} = [IC_{leaf}]^{fresh}/IC_{soil}$$

where $[IC_{leaf}]^{fresh}$ is iodine concentration in leaves on a fresh weight basis and IC_{soil} is iodine concentration in the corresponding soil.

All data are subjected to analysis of correlation (ANOVA, two-way) performed using Windows-based Genstat (6th edition, NAG, England).

Results

Biomass production and iodine uptake

Although there were some fluctuations at different levels of iodine application, the fresh weights of plant biomass were not significantly affected by iodine application (Table 2). With increasing application of iodide, iodine concentrations in spinach increased greatly from 0.06 ± 0.01 mg kg^{-1} to 0.41 ± 0.05 mg kg^{-1} for leaves and from 0.43 ± 0.03 mg kg^{-1} to 2.28 ± 0.12 mg kg^{-1} for roots (Fig. 1); However, in the iodate treatments, iodine concentrations increased from 0.06 ± 0.01 mg kg^{-1} to 8.24 ± 0.65 mg kg^{-1} for leaves and from 0.43 ± 0.03 mg kg^{-1} to 5.21 ± 0.57 mg kg^{-1} for roots with increasing iodine application ($P < 0.001$, Fig. 1). Thus, with the iodate treatments, iodine concentrations in root or leaf tissues were much greater than with the iodide treatments, about twofold higher for roots and tenfold higher for leaves

Table 2 Biomass of spinach as fresh weights grown in soil supplied with different levels and forms of iodine

Treatments (mg I kg^{-1} soil)	Leaf (g)		Root (g)	
	Iodide	Iodate	Iodide	Iodate
0	13.48 ± 1.17[a]		3.44 ± 0.94	
0.5	13.23 ± 0.58	13.90 ± 1.02	3.26 ± 0.80	3.69 ± 0.57
1.0	14.70 ± 0.52	14.09 ± 0.72	3.69 ± 0.20	3.81 ± 0.20
2.0	13.62 ± 0.51	15.11 ± 0.22	2.79 ± 0.52	4.69 ± 0.43
	NS	NS	NS	NS

NS: Not significant

[a] Means ± standard error ($n = 4$)

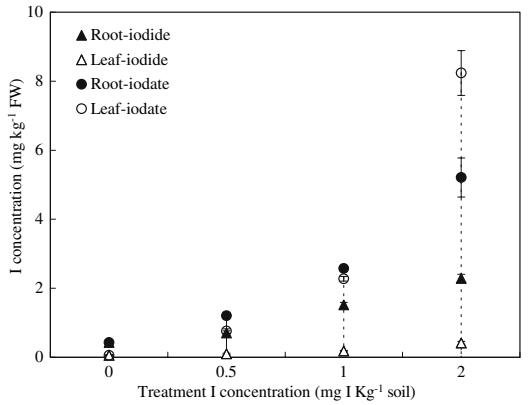

Fig. 1 Iodine concentration in plant tissues of spinach on fresh weight basis with iodide and iodate treatments Bars: standard errors. ($P < 0.001$, $n = 4$)

at the iodine application rate. In general, apart from the treatment with 2 mg I kg^{-1} iodate, iodine concentrations in roots were much higher than those of leaves at iodide.

Using the data of iodine concentration and biomass production, a partitioning budget was made for total plant iodine distribution between roots and shoots in spinach (Fig. 2). The total iodine content in spinach tissues increased with increasing iodine concentrations in soil ($P < 0.001$). It was much higher for iodate than

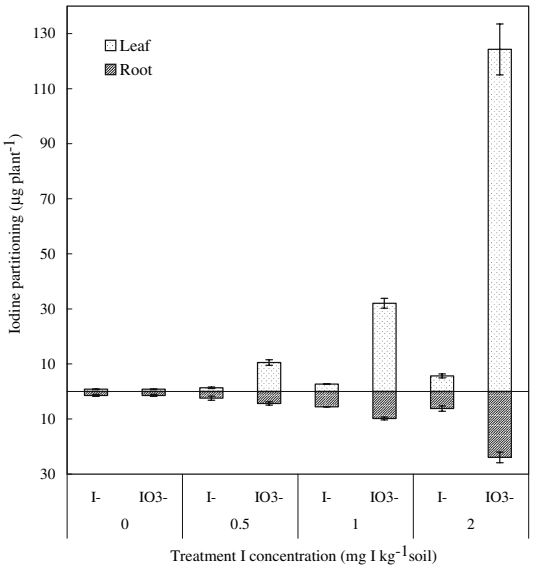

Fig. 2 Iodine partitioning of spinach plants between shoots and roots of spinach treated with different concentrations of iodide and iodate. Bars: standard errors. ($P < 0.001$, $n = 4$)

iodide treatments, up to tenfold for leaves with the same application rate. The iodate treatments had more iodine partitioning to the leaves (77%) on average than did the iodide treatments (39%).

The soil-to-leaf transfer factor (TF_{leaf}) is broadly used as one of the parameters to estimate the intake of element through the food chain. As shown in Fig. 3, the TF_{leaf} of spinach increased significantly from 0.04 to 2.32 with increasing iodine concentrations in soil treated with iodate ($P < 0.001$), and the same trend was found for the treatment with iodide. The TF_{leaf} of spinach at different levels of iodate were about tenfold higher than those of iodide treatments.

Iodine concentration in soil solution

The total iodine concentrations in soil solution were determined to show the availability of iodine in soil (Table 3). Iodine concentrations in soil solution increased with increasing iodine concentrations applied to the soil as iodide or iodate. However, iodine concentrations in soil solution were generally higher for iodate treatments than iodide treatments both in the presence and absence of spinach, and iodine concentrations at the second sampling time (about 31 days after seedling) were higher than those of other times, then decreased sharply in pots with spinach and decreased slightly in pots without spinach. At the initial stage of growth (at the first and second

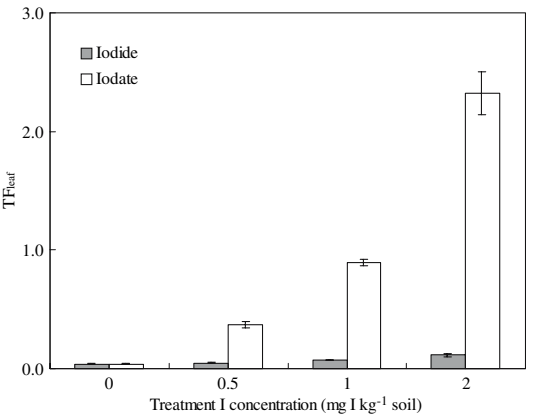

Fig. 3 Soil to leaf transfer factors for spinach plants treated with different concentrations of iodide and iodate. Bars: standard errors. ($P < 0.001$ for iodate treatment and $P = 0.003$ for iodide treatment, $n = 4$)

Table 3 Effect of different iodide and iodate treatments on the concentrations of iodine in soil solution

Treatments (mg I kg^{-1} soil)			Total I			
			First time (μg I l^{-1})	Second time (μg I l^{-1})	Third time (μg I l^{-1})	Fourth time (μg I l^{-1})
Pots with spinach	Iodide	0	22.5 ± 6.3[a]	47.4 ± 13.7	12.2 ± 6.5	2.2 ± 1.7
		0.5	54.1 ± 4	190 ± 35.9	34.3 ± 8.2	5.9 ± 1.2
		1.0	73.1 ± 12.9	297.2 ± 36	39.4 ± 4.4	10 ± 3.9
		2.0	90.2 ± 23.8	457.4 ± 99.1	96.7 ± 31.8	36.1 ± 16.5
			$P = 0.015$	$P = 0.001$	$P = 0.019$	$P = 0.026$
	Iodate	0	22.5 ± 6.3	47.4 ± 13.7	12.2 ± 6.5	2.2 ± 1.7
		0.5	63.5 ± 6.9	305.6 ± 111.3	28.2 ± 11.6	26.4 ± 15
		1.0	94.6 ± 6	251.6 ± 72.8	78.4 ± 7.2	23 ± 6.8
		2.0	845 ± 170.6	1000 ± 283.9	113.8 ± 15.1	24 ± 8
			$P < 0.001$*	$P = 0.003$	$P = 0.001$	NS
Pots without spinach	Iodide	0	16.1 ± 0.8	28.2 ± 2.3	8.9 ± 1.1	16.8 ± 2.4
		0.5	49.1 ± 8.9	87.5 ± 4.5	30.4 ± 5.3	10.6 ± 1.3
		1.0	68.7 ± 24.6	150.1 ± 6.6	73.0 ± 18.3	64.2 ± 21.2
		2.0	68.8 ± 13.7	234.1 ± 35.1	154.1 ± 48.6	130.7 ± 19.1
			NS	$P = 0.001$	$P = 0.019$	$P = 0.001$
	Iodate	0	16.1 ± 0.8	28.2 ± 2.3	8.9 ± 1.1	16.8 ± 2.4
		0.5	91.8 ± 6.1	121.9 ± 12	94.7 ± 10	57.5 ± 9.4
		1.0	221.9 ± 20	186.7 ± 49.9	175.8 ± 13.6	105.4 ± 18.5
		2.0	367.6 ± 29.1	818.9 ± 143.2	782.9 ± 43.3	383.8 ± 79
			$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.001$

NS: Not significant

[a] Means ± standard error ($n = 4$ for pots with spinach and $n = 3$ for pots without spinach)

* Significant at the 0.05 level

sampling), iodine concentrations in soil solution inclined to much higher in pots with spinach than in pots with no spinach. However, they were significantly lower in pots with spinach than in pots without spinach at third and fourth sampling, irrespective of iodine species applied.

Discussion

Although a few studies have shown that there are differences in uptake or translocation by plants between iodine species (Zhu et al. 2003; Mackowiak and Grossl 1999), direct evidence of the relationship between soil solution iodine and plant uptake is lacking. Results from the present study demonstrated that iodine uptake by spinach was well related to iodine concentrations in soil solution obtained by an in situ technique. This pot experiment indicated that the potential for iodine enrichment in spinach plants was much greater with iodate than with iodide. In contrast, in solution culture it has been shown that the accumulation of iodate by spinach (Zhu et al. 2003) and rice (Mackowiak and Grossl 1999) was much lower than that of iodide. The discrepancy of species-specific enrichment of iodine by plants between soil and solution culture systems could be due to several reasons. Uptake of iodine by plants grown in soils is dependent on the availability of iodine in the soils, which is essentially governed by adsorption–desorption processes in soils. According to the iodine adsorption–desorption in different soils, the affinity of iodate to the soil used in this experiment was much higher than that of iodide (Dai et al. 2004c), therefore the availability of iodate in soils should be lower than that of iodide. However, results obtained from here showed that iodine concentrations in soil solution treated with iodide was generally lower than those treated with iodate. Therefore, the difference in iodine uptake by spinach plants supplied with iodide and iodate could be explained by the difference in iodine concentrations in soil solution, which is immediately available for root uptake.

The reason for low iodine concentrations in soil solutions in treatments with iodide could be due to substantial iodine volatilization. Fuge (1996) suggests that volatilization of iodine from soils plays an important role in the global iodine cycle and its transfer to the biosphere. Muramatsu et al. (1995) and Johnson et al. (2002) also found that iodine in the soil was volatilized from the soil–plant system into the atmosphere as organic iodine. Volatile iodine compounds (as organoiodides) can be released from terrestrial environments, such as rice fields (Redeker et al. 2000), peat bogs (Dimmer et al. 2001). The production of volatile organoiodides from soil is thought to be ultimately dependent on the amounts of iodide in soils (Keppler et al. 2000, 2003).

Since the adoption of application of iodine fertilizer to soil as a complementary strategy to supplement dietary iodine intake is relatively new, the fate and behavior of different species of iodine in soil–plant systems is largely unknown. Our studies (the present and earlier ones) demonstrated that although plants could more easily accumulate iodide, iodide in soils may be lost through volatilization, which may contribute to the discrepancy in iodine accumulation by spinach plants between solution and soil pot cultures. Taking all this information together, we may be able to conclude that the application of fertilizers containing iodate should be recommended. In order to have a better prediction of the fate of soil-applied iodine, further studies are needed to elucidate the processes of volatilization of iodide and iodate from various soil–plant systems, and the key influencing factors.

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