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Risk assessment of vegetables irrigated with arsenic-contaminated water†

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Arsenic (As) contaminated water is used in South Asian countries to irrigate food crops, but the subsequent uptake of As by vegetables and associated human health risk is poorly understood. We used a pot trial to determine the As uptake of four vegetable species (carrot, radish, spinach and tomato) with As irrigation levels ranging from 50 to 1000 μg L^{-1} and two irrigation techniques, non-flooded (70% field capacity for all studied vegetables), and flooded (110% field capacity initially followed by aerobic till next irrigation) for carrot and spinach only. Only the 1000 μg As L^{-1} treatment showed a significant increase of As concentration in the vegetables over all other treatments ($P < 0.05$). The distribution of As in vegetable tissues was species dependent; As was mainly found in the roots of tomato and spinach, but accumulated in the leaves and skin of root crops. There was a higher concentration of As in the vegetables grown under flood irrigation relative to non-flood irrigation. The trend of As bioaccumulation was spinach > tomato > radish > carrot. The As concentration in spinach leaves exceeded the Chinese maximum permissible concentration for inorganic As (0.05 μg g^{-1} fresh weight) by a factor of 1.6 to 6.4 times. No other vegetables recorded an As concentration that exceeded this threshold. The USEPA parameters hazard quotient and cancer risk were calculated for adults and adolescents. A hazard quotient value greater than 1 and a cancer risk value above the highest target value of 10^{-4} confirms potential risk to humans from ingestion of spinach leaves. In our study, spinach presents a direct risk to human health where flood irrigated with water containing an arsenic concentration greater than 50 μg As L^{-1}.

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Environmental impact

The use of As contaminated irrigation water to cultivate food crops is a potential threat to humans. We investigated the uptake response of four common vegetable crops to As contaminated irrigation water using two irrigation techniques. Our research highlights that spinach leaves present a potential carcinogenic and non-carcinogenic risk to humans when grown under As-contaminated flood irrigation. Crop selection and irrigation strategies to mitigate As exposure to humans are discussed in this work.

1 Introduction

Arsenic (As) is toxic to all forms of life. Although As contamination is a global concern, some parts of the world are more severely affected. For example, in Asia, serious As toxicity symptoms in humans have been linked to the consumption of As-contaminated drinking water and food.[1–3] The situation is worst in South and Southeast Asian countries, where around 110 million people are under the threat of As poisoning.[4] Arsenic exposure can cause several carcinogenic (tumors of the skin, lung, urinary bladder and other organs) and non-carcinogenic (gastrointestinal disturbances, skin lesions, peripheral vascular diseases, reproductive toxicity and neurological disorders) effects in humans.[5] Arsenic contamination also has economic consequences through increased healthcare costs, loss of agricultural productivity, a deterioration of natural resources (water and soil) leading to increased cost for their rehabilitation, and trade barriers that may prevent the export of food products.[6]

There has been an increased concern in recent years over the presence of As in food crops. Rice and vegetables contribute a substantial share to overall As ingestion.[1,7,8] Vegetables may accumulate this element through growing in contaminated soils and/or in soils that are irrigated with contaminated water.[9–12] Under conditions of high As exposure, vegetables can continue to grow and accumulate arsenic to a concentration that can exceed critical levels.[13–15]

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Despite the apparent risk, As uptake by vegetables irrigated with contaminated water is poorly understood. Most reports on As in vegetables are monitoring surveys where various crops, soil and water samples are collected from affected areas, analyzed and the As concentration and estimated daily intake is reported.[7,9,16–18] Some studies have been conducted using hydroponic or soil media with various levels of As to determine uptake potential and associated risk.[19–21]

We propose that As-irrigated soil studies are different to hydroponic studies and to those where As-contaminated soil is used as a medium for plant growth. This is because (i) As in irrigation water is initially available to plants before it is adsorbed onto soil particles, (ii) As can be directly absorbed by plant leaves during the event of irrigation, and (iii) an excessive use of irrigation water (flood irrigation) will develop an anaerobic condition in soil which will result in increased solubility and release of As from As-binding minerals.[22,23] Differences in As uptake between irrigation studies and the more widely reported contaminated soil or hydroponic studies highlight a need for research on contamination of vegetables and soil when As-contaminated water is used for irrigation.

Understanding the As uptake response of vegetable crops to irrigation with As-contaminated water has practical significance for South and Southeast Asian countries where As-contaminated water is used to irrigate food crops. A common irrigation technique used in South Asia is flood irrigation (also known as surface irrigation) where a field is divided into small plots surrounded by earth banks. Water is applied to plots by an adjacent channel and flows over the soil surface by gravity. When the plot is saturated/flooded (defined as about 2–3 cm water head), the water inflow is diverted to irrigate an adjacent plot. The surface water moves downward slowly in the field over a period of 2–3 days, during which time the soil remains in a flooded state. Aerobic conditions are re-instated once the surface water has drained. The effect of flood irrigation on As chemistry in soil where irrigation water is contaminated with this metalloid has not been previously considered and may affect both As solubility and uptake by plants. Research is required to investigate the potential for As accumulation in different vegetables irrigated with water containing variable levels of As under variable water management practices, and the risk to humans that is associated with the ingestion of these vegetables.

Only one study appears to have been conducted where As-contaminated water (as a treatment) was used for radish cultivation under soil conditions.[24] In this work an increase in the As concentration of irrigation water corresponded to an increase in the As concentration of radish tuber and caused several changes to the tubers' internal structure (black spots over the hypocotyl, and changes in the thickness and structure of the outermost cell layer). Marconi's study[24] on radish was limited to low levels of As in irrigation water (maximum 104 μg L^{-1}) and did not calculate the potential risk to humans through ingestion. The arsenic concentration in irrigation water used around the world for crop cultivation ranges from <0.001 to 1.014 mg L^{-1} for groundwater[7,25,26] and from 5.92 to nearly 100 mg L^{-1} for industrial effluent.[16,27] Therefore, the potential for As uptake in some scenarios may be many orders of magnitude higher.

Our research was designed to directly address the identified lack of information on As uptake by vegetables as a function of the As concentration of irrigation water and water management technique used, and the risk to humans that would be associated with the ingestion of potentially contaminated vegetables. The specific objectives of the current study were to determine: (i) the extent of arsenic accumulation and distribution in tissues of four vegetable species cultivated using a range of As concentration levels in irrigation water; (ii) the critical As concentration in water that may be acceptable for irrigation; (iii) the effect of irrigation techniques (flood *versus* non-flood irrigation) on As accumulation by vegetables; and (iv) the human risk associated with the ingestion of the vegetable specie(s) which accumulate the most arsenic.

2 Materials and methods

Four commonly grown vegetables, four concentrations of As in irrigation water, and two irrigation management techniques were used in the current study. The two irrigation techniques were (i) non-flooded water management where soil moisture was maintained at 70% field capacity (Fc) of the soil throughout the plant growth period and (ii) flooded water management where an alternating regime of saturation to 110% Fc of soil for three days followed by draining to attain aerobic condition until the next irrigation event was used.

2.1 Crops

Four commonly grown vegetable species, carrot (*Daucus carota* cv. All Year Round), radish (*Raphanus sativus* cv. Champion), spinach (*Spinacia oleracea* cv. Perpetual), and tomato (*Solanum esculentum* cv. Italian Dwarf Romandore F1 hybrid) were selected for the current research. These crops are commonly grown around the world and show a high potential for As uptake when grown under hydroponic and soil conditions.[12,13,28,29] Carrot and spinach were grown under both non-flooded and flooded water management, while radish and tomato were only cultivated under non-flooded water management. The experiment was conducted in a glasshouse at the Plant Growth Unit of Massey University Palmerston North, New Zealand with one healthy plant per pot. The experiment was laid down in a complete randomized design with three replications per treatment. The glasshouse temperature was maintained at 12 ± 2 °C minimum (night) and 22 ± 2 °C maximum (day).

2.2 Pot preparation and soil

Plastic pots (16.5 × 16.5 × 19 cm) were prepared to allow for both irrigation techniques. For the non-flood irrigation, the pots were drilled at the bottom (5 holes per pot) to provide aeration to plants. For the flood irrigation, a hole was drilled at one side of the pot (2 cm from the base) and a silicone rubber pipe (0.5 cm × 4 cm) was inserted. The portion of the silicone rubber which was inside the pot was attached to a PVC pipe (0.8 cm × 14 cm). This internal PVC pipe was cut through the upper side and wrapped with Coolaroo non-woven mulch mat (made of polypropylene material) to drain water. The portion of

the silicone rubber which was outside the pot was attached to a PVC pipe (0.3 cm × 28 cm), and clamped to the top edge of the pot (Fig. 1). Both types of pots were filled with a basal layer (500 grams) of gravel (>2 mm) to facilitate water drainage and aeration. Each pot was filled with 4 kg of Rangitikei silt loam soil collected from a quarry adjacent to the Manawatu River near Palmerston North, New Zealand. The soil was irrigated with distilled water (to 50% field capacity) and fertilized with P and K prior to seeding. Nitrogen was applied to plants in two splits, prior to seeding and with the second irrigation. The rate of NPK used for the experiment was 50 mg N kg^{-1} (equivalent to 100 kg N ha^{-1} applied as DAP and urea), 22.5 mg P kg^{-1} (equivalent to 45 kg P ha^{-1} applied as DAP), and 67.5 mg K kg^{-1} (equivalent to 135 kg K ha^{-1} applied as K_2SO_4) according to the fertilizer guidelines of Wallace.[30]

2.3 Irrigation

Four arsenic concentrations in water (50, 100, 200, and 1000 μg L^{-1}) and a control (distilled water) were used as treatments. Arsenic irrigation waters were prepared from a stock solution of 1000 mg L^{-1} of sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$). The irrigation was initiated once all the plants were germinated (10 days after sowing). At the day of irrigation, each pot was weighed and adjusted to 70% and/or 110% Fc with treatment water for non-flooded and flooded water management respectively. Flooded conditions were maintained for three days by clamping the external/exhaust pipe. On day four, the clamp was removed and the pots were drained. The irrigation frequency was every ten days for the non-flooded plants and fortnightly for the flooded plants. Immersion of the basal leaves was observed in the flooded plants, and we assume that some surface absorption of As would have been apparent for these plants while water remained standing on the soil surface (~2–3 cm head).

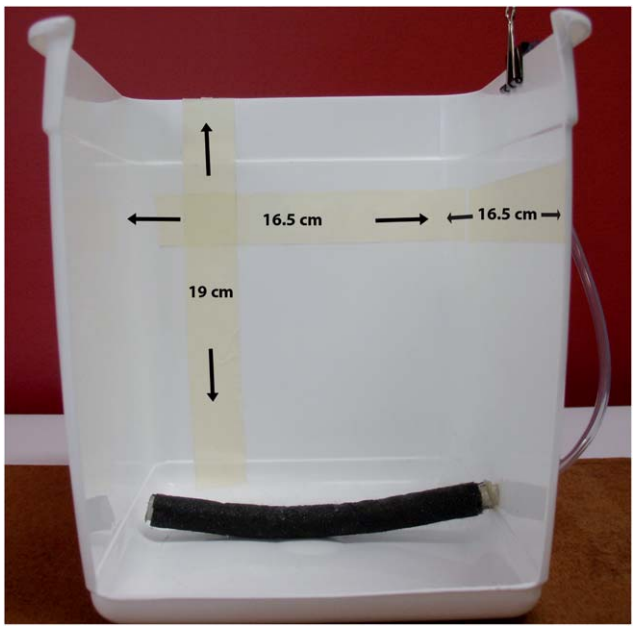

Fig. 1 Image of the pot designed to model flood irrigation.

2.4 Plant harvest and analysis

Plants were harvested at maturity (defined as the point of human consumption) and divided into various parts; spinach (leaves and roots), carrot and radish (leaves and taproot), and tomato (shoot, fruit and roots). Carrot and radish taproots were further divided into peel (skin) and the edible root. The removal of radish and carrot skin before eating or cooking is a usual practice in South Asia. These plant parts were washed with distilled water; surface dried using paper towels, and weighed to determine the fresh biomass yield. Plants were then oven dried at 70 °C for four days and re-weighed for dry biomass. The dried plant parts were homogenized to a powder using a Cyclotec herbage mill (Model 1093, Salmond Smith Biolab Ltd.).

Subsamples (0.5 g) were digested in 5 mL of concentrated nitric acid (HNO_3). The samples were kept overnight under a fume hood to provide sufficient time for the acid to pre-digest the biomass. The following day the samples were heated to 120 °C on a digestion block for 2 to 3 hours until fumes were no longer emitted and the digest volume was reduced to 1–2 mL. The samples were subsequently cooled to room temperature and diluted with 10 mL deionized water, filtered with Whatman filter paper (42), and then further diluted to a final volume of 25 mL with deionized water. An aliquot of each digest solution was analyzed for its total As concentration using Hydride Generation Atomic Absorption Spectrometer (HGAAS, Perkin Elmer, FIAS 400). As a pre-requisite for As analysis on HGAAS, the samples were reduced to As^{III} by taking 1 mL of sample and adding 1 mL of concentrated HCl, and 1 mL of 5% (w/v) potassium iodide plus 5% (w/v) ascorbic acid. The treated samples were allowed to stand for 1 hour at room temperature and finally diluted to 10 mL with 10% HCl.

A standard reference material (1573a, Tomato leaves, NIST) and sample blanks were included in the procedure to evaluate the reliability of analytical method. The limit of detection (LOD) for As in HGASS was 0.145 μg L^{-1} and the sample blanks were below this LOD value. The analyzed As concentration of the standard reference material (0.112 ± 0.007 μg g^{-1}, $n = 13$) was in good agreement with certified reference value (0.112 ± 0.004 μg g^{-1}). The precision was maintained at 10% relative standard deviation (RSD) and any sample value higher than described value was repeated.

2.5 Soil analysis

The experimental soil was analyzed for the fertility parameters pH, P, SO_4, and CEC according to the methods of Blakemore *et al.*,[31] total C and N using a Leco furnace, and soil texture according to pipette method.[32] Total Fe and Al were analyzed as described by Gartler *et al.*[33] using inductively coupled plasma optical emission spectrometry (ICP-OES Varian 720 ES-USA). These analytical determinations were performed by commercial laboratories of the Fertilizer and Lime Research Centre Massey University, Lincoln University, and Landcare Research in New Zealand (Table S1, ESI†).

The soil was an acidic silt loam with low levels of available P and CEC (Table 1). For total As, accurately weighed subsamples (1 g) of soil were predigested overnight in 10 mL *aqua regia*

Table 1 Physico-chemical properties of the Rangitikei silt loam soil used for the experiment

pH (H_2O)	6.1
Olsen P (μg P g^{-1})	7.1
SO_4 (μg S g^{-1})	4.8
Total C (%)	0.8
Total N (%)	<0.005
Total Fe (%)	2.3
Total Al (%)	2.8
CEC (meq. per 100 g)	12
Texture	Silt loam (sand 14%, silt 65%, and clay 21%)
Total As (μg g^{-1})	4.9 ± 0.02 ($n = 3$)[a]

[a] The As concentration in soil was determined by the lead author during the experimental work. All other soil determinations were performed by commercial laboratories.

(HCl : HNO_3, 3 : 1). The next day the samples were placed on a digestion block at 120 °C for 2 to 3 hours. The samples were then cooled to room temperature and diluted with 20 mL of deionized water, filtered with Whatman filter paper (42) and made to 50 mL with deionized water. The samples were pre-reduced to As^{III} as described for plant samples, prior to analysis by HGASS. A reference soil material (CRM-GBW 07403, National Research Center for CRMs of China, Beijing), was used to check the accuracy of analytical technique. Replicate sample blanks were analyzed in parallel with the soil samples and were found below the instrument detection limit. The analyzed As concentration of the reference soil material (4.2 ± 0.05 μg g^{-1}, $n = 7$) was within the range of reported values (4.4 ± 0.6 μg g^{-1}). The As concentration in the experimental soil was below the New Zealand environmental quality standard for total As (20 mg kg^{-1}, ANZECC/NHMRC, Table 1).[34]

2.6 Statistical analysis

The data for As concentration in plant tissues was tested for normality and then analyzed by ANOVA using SAS 9.2 (SAS Institute Inc., USA). The Tukey's test was used to determine significant differences among treatment means using a probability value of 0.05.

2.7 Human health risk assessment

To evaluate the risk presented to humans through ingestion of edible parts of the vegetables in the current study, the As concentration in each vegetable was re-expressed on fresh weight, and assumed to be present in an inorganic form. The fresh weight concentrations were then compared to the Chinese food safety standard for inorganic As in food (0.05 mg kg^{-1} fresh weight, Heikens[35]). Vegetables with an As concentration below this guideline value present an acceptable risk to humans while vegetables with an As concentration above the guideline value represent a potentially unacceptable risk to consumers.

To further quantify this potential risk, the USEPA defined hazard quotient (HQ) and cancer risk (CR) was derived.[36,37] The HQ was calculated according to eqn (1), where EDI is estimated daily intake (mg kg^{-1} day^{-1}) and RfD is reference dose for As (0.0003 mg As kg^{-1} body weight day^{-1}). An HQ value greater than 1 defines a non-carcinogenic toxic risk to human health.

$$HQ = EDI/RfD \quad (1)$$

The parameter EDI was calculated according to eqn (2), where C is concentration of arsenic in edible part of the plant (mg kg^{-1} fresh weight); Fi is food intake (kg per person per day); Ef is exposure frequency (days per year); Ed is exposure duration (70 years); W is average body weight (60 kg for adults 18 years and older,[38] and 50 kg for adolescents between 12 and 18 years age); Te is average exposure time (=Ed × 365 days)

$$EDI = (C \times Fi \times Ef \times Ed)/(W \times Te). \quad (2)$$

The CR was calculated according to eqn (3), where the parameter CSF is a cancer slope factor for As (1.5 mg kg^{-1} day^{-1}). The USEPA has proposed a range of 1 in 10 000 (10^{-4}) to 1 in 1 000 000 (10^{-6}) as acceptable As cancer risk in humans.[37]

$$CR = EDI \times CSF \quad (3)$$

3 Results and discussion

3.1 Arsenic concentration in vegetables

Arsenic accumulation and distribution within plant tissues and among plant species varied as a function of the As treatment and the irrigation technique used. The results for each species are reported separately, followed by comparison of As bio-accumulation among the studied vegetables and possible factors affecting the accumulation of As by the plants.

3.1.1 Radish. The As concentration in radish tissues (leaves, radish skin and edible root) was significantly higher in the plants irrigated with 1000 μg L^{-1} when compared to all other treatments ($P < 0.05$, Fig. 2). An increase in As concentration in radish as a function of As exposure has been reported by other researchers.[24,39] Among the various plant tissues, leaves accumulated the highest concentration of As, followed by radish skin and the edible root. A relatively higher concentration of As in leaves in current study is in agreement with previous hydroponic and soil studies where a higher concentration of As has been observed in leaves.[39–41] However, our finding of a higher concentration of As in leaves also contradicts previous hydroponic and soil studies where As was mostly retained in the roots.[13,19,20] Among the components of the taproot, skin accumulated a higher concentration of As by a factor of 2.7 to 5.3 relative to the edible root. A higher concentration of As in radish skin observed in this study is consistent with previous hydroponic and soil studies.[13,42]

3.1.2 Tomato. The As concentration in shoots and roots of tomato was significantly higher at an irrigation water concentration of 1000 μg As L^{-1} relative to the other treatments ($P < 0.05$, Fig. 2). In contrast, there was no significant difference of As concentration in tomato fruit among the various treatments. An increase in the As concentration of tomato roots and shoots as a function of As concentration in various growth media (soil, mixtures of soil and As-contaminated mine tailings and

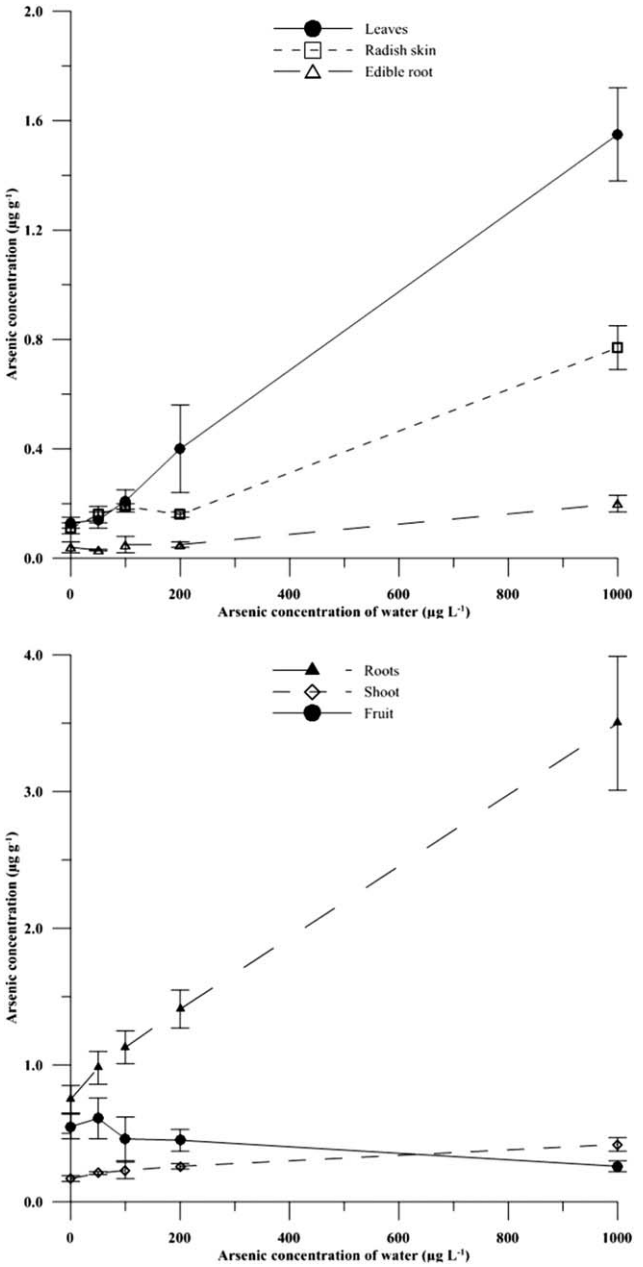

Fig. 2 Total arsenic concentration (μg g^{-1} dry weight) in various parts of radish (top) and tomato (bottom) grown under non-flooded irrigation. Data are means ± SE ($n = 3$).

nutrient solution) has been reported.[21,29,39] Among the various plant tissues, the As concentration of roots was higher than in fruit and shoots under all treatments. Roots accumulated an As concentration that was 1.4 to 5.1 times higher than that recorded for the fruit and shoot. This higher concentration in roots relative to other tissues of tomato is consistent with previous studies that have investigated the response of tomato to arsenic.[21,39,43] Burlo *et al.*[29] reported that As in tomato plants was mainly accumulated in roots (85% of total As), followed by shoots (14%) and fruit (1%) when grown in nutrient solution. Carbonell-Barrachina *et al.*[43] suggested that tomato plants tolerate As by exclusion, limiting its transport to shoots by increasing the As concentration in the roots. Among the upper plant parts, the As concentration in tomato fruit was significantly higher than in shoots for all the treatments, with the exception of the 1000 μg L^{-1} treatment ($P < 0.05$). This indicates that shoots may transport a large quantity of As to fruit at low As levels (<1000 μg As L^{-1}), but transfer is inhibited or restricted above this level. Carbonell-Barrachina *et al.*[43] suggested that when As is above a threshold level, the growth and transport function of a plant is affected, resulting in limited As translocation.

3.1.3 Spinach. Spinach was grown under both non-flooded and flooded irrigation. The response of spinach to As in water under both irrigation techniques is presented in Fig. 3. The

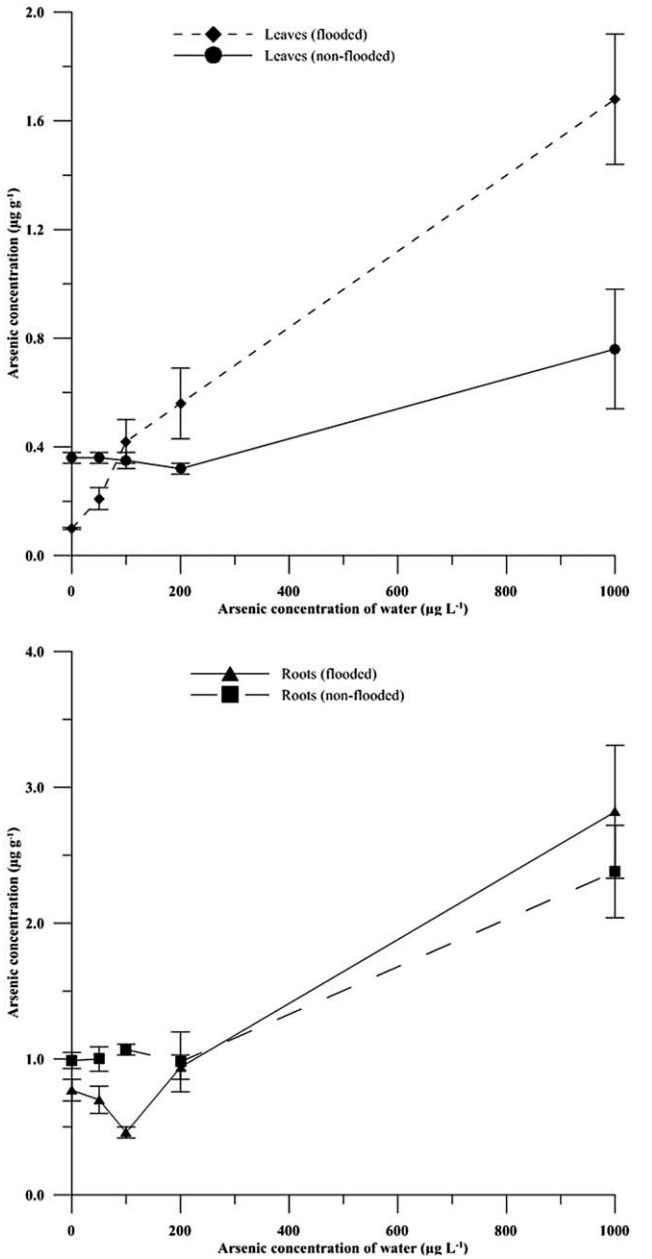

Fig. 3 Total arsenic concentration (μg g^{-1} dry weight) in various parts of spinach grown under variable As treatments and irrigation techniques. Data are means ± SE ($n = 3$).

effect of the 1000 μg As L^{-1} irrigation treatment was significant under both irrigation techniques and resulted in a higher concentration of As in the leaves and roots of plants relative to the other treatments ($P < 0.05$). An increased As concentration in leaves of spinach with increasing As concentration in soil has been documented previously.[44] Among the plant tissues, roots had a higher As concentration than in leaves under both irrigation techniques. The As concentration in roots was 2.7 to 3.1 times higher than in leaves for plants under non-flooded water management, and 1.1 to 7.7 times higher under flooded water management. Tlustos *et al.*[45] also demonstrated a higher concentration of As in spinach roots relative to the aerial biomass. Nonetheless, a comparison of the As concentration in roots shows no significant difference for the same concentration of irrigation water under the two irrigation techniques. For leaves, a relatively higher As concentration was observed in flood irrigated plants for the 100, 200 and 1000 μg As L^{-1} treatments relative to plants subject to non-flood irrigation. There could be three possible explanations for such an increase, (i) a higher amount of As was introduced in the flooded pots due to an increase in the total volume of irrigation water (110% Fc *vs.* 70% Fc), (ii) direct absorption of As by spinach leaves from standing water during the initial phase of the flood irrigation event, and (iii) more translocation of As to aerial parts. An increased translocation of As in the current study is in agreement with the findings of Talukder *et al.*,[46] who reported that As is more easily translocated to the above-ground biomass of rice plants under anaerobic conditions than aerobic conditions.

3.1.4 Carrot. Carrot was also grown under both non-flooded and flooded irrigation. Among the various As treatments, plants irrigated with 1000 μg As L^{-1} had a significantly ($P < 0.05$) higher As concentration in their leaves than for other treatments under both irrigation techniques (Fig. 4). A similar effect at this treatment level was also observed for edible roots subject to flood irrigation. Among the plant tissues, the trend of As concentration as a function of As in irrigation water differed between the two irrigation techniques. Under non-flood irrigation, carrot skin accumulated more As than leaves, while under flood irrigation, leaves accumulated more As than carrot skin. The reported higher concentration of As in leaves subject to flood irrigation may be attributed to enhanced translocation from roots under this water management, and surface absorption by leaves. Liu *et al.*[40] found a higher concentration of As in carrot leaves than in edible parts and suggested that carrot leaves are efficient bio-accumulators of heavy metals/metalloids. Considering the components of the taproot, the skin accumulated more As than the edible root under both irrigation techniques. This is consistent with other studies where a higher concentration of As in carrot peel (approximately 3 times) than the edible root has been reported.[28,47] Zandstra & De Kryger[48] reported a higher concentration of As in the root shoulder and peel than the peeled root and attributed this to direct contact with soil particles. Overall, the As concentration in plant tissues (leaves, carrot skin and edible root) was higher in plants grown under flood irrigation relative to those grown under non-flood irrigation.

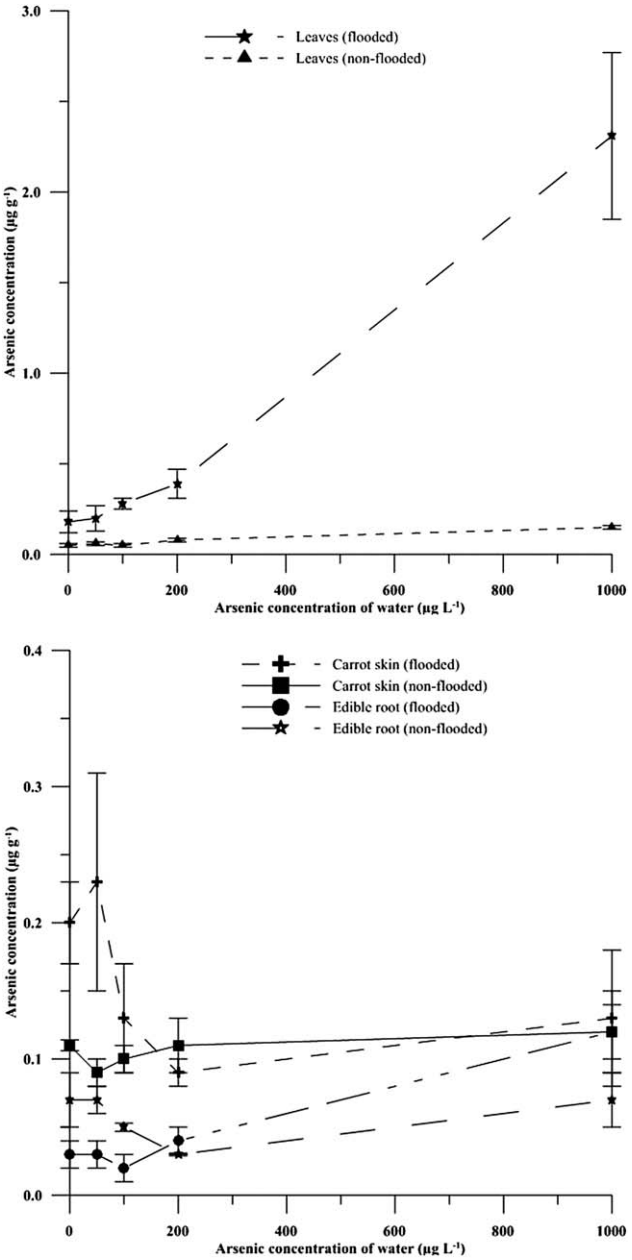

Fig. 4 Total arsenic concentration (μg g^{-1} dry weight) in various parts of carrot grown under variable As treatments and irrigation techniques. Data are means ± SE ($n = 3$).

3.1.5 Arsenic content in vegetables and possible factors affecting accumulation in plants. Arsenic is a non-essential element for plants. The uptake of As by plants is a complex phenomenon and depends on various As–plant–soil factors, including As concentration and species, crop species, and soil redox conditions.[19,49–52] Our results show that As accumulation by plants was dependent on the As concentration of irrigation water, the irrigation technique used, and the vegetable species tested. Fig. 5 depicts the As content of the studied vegetables, where content is defined as the As concentration (μg g^{-1}) × dry matter yield (g). The As content in plants varied among species, and can be ranked from high to low as spinach (flooded) > tomato > spinach (non-flooded) > radish > carrot (flooded) >

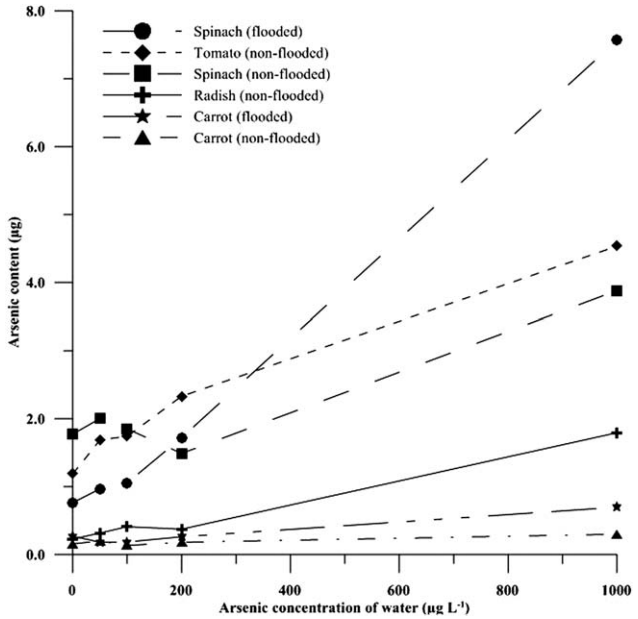

Fig. 5 Arsenic content (μg) in four vegetables as a function of As treatments and irrigation techniques. Data points represent the mean value for each species calculated as a weighted function of the relative mass of each plant organ. *n* was therefore variable among crops. For radish, carrot and tomato $n = 9$ (for example in radish, triplicate values of each of leaves, radish skin and edible root). For spinach $n = 6$ (triplicate values of each of leaves and roots).

carrot (non-flooded). This variation in As uptake among the vegetables may be attributed to genetic differences among these plants together with the irrigation technique used. Husaini *et al.*[17] reported As accumulation in the order spinach > tomato > carrot > radish when these plants were irrigated with untreated industrial effluent. Similarly, Arain *et al.*[18] reported higher As levels in leafy vegetables compared to root vegetables and grain crops collected from an agricultural field irrigated with As-contaminated lake water.

A second factor controlling As accumulation in plant species is the concentration of As present in irrigation water. In general, each species showed an increase in As content as a function of the As concentration in irrigation water (Fig. 5). Such an increase in plant tissues with As concentration in growth media is well documented in literature (for example ref. 14, 29 and 44). Among the various As irrigation water levels, the 1000 μg As L^{-1} treatment promoted the highest As concentration in these plant species. This is consistent with the findings of Dahal *et al.*,[26] who found a positive correlation between the As concentration in plants and the As concentration of irrigation water. These researchers also reported the highest As concentration in all parts of the studied vegetables in samples collected from plots irrigated with the highest As concentration of this study (1.014 mg As L^{-1} in groundwater).

A third possible factor affecting the As concentration in plants of the current study is the irrigation technique used. The flooded and non-flooded irrigation techniques could differentially affect soil redox potential which subsequently affect As solubility, speciation and uptake by plants.[46,49,53] The soil redox potential was not measured in the current work because the system was in a state of flux and measuring redox would have disturbed the system. However, we assume that the soil subject to flood irrigation will have had a periodically lower redox potential than the soil subject to non-flood irrigation. Li *et al.*[53] reported that flooded water management significantly reduced soil redox potential compared with aerobic treatment for an acidic silty clay loam soil. Similarly, Talukder *et al.*[46] reported a highly reduced redox potential (−41 to −76 mV) under flooded water management relative to aerobic water management (+135 to +138 mV) for an acidic sandy loam soil.

In our study there was a lower As content in the vegetables grown under non-flood irrigation than the plants under flood irrigation. A similar finding for rice was reported by Xu *et al.*[49] who found a 10–15 fold higher concentration of As in the grain of paddy rice relative to dry land rice. Lower As content under non-flooded conditions may be due to sorption of As with hydrous oxides minerals effecting a reduction in As solubility and uptake by plants.[4,23,49] In contrast, for soils subject to flood irrigation management, As solubility may have been higher due to (i) reduction of arsenate (As^{V}) to arsenite (As^{III}), and (ii) dissolution of Fe hydrous oxides which releases the adsorbed As, leading to increased uptake by plants.[23,49,54]

3.2 Risk assessment

The arsenic concentration in both the edible portion and taproot of carrot (μg g^{-1} fresh weight, Table 2) was less than

Table 2 Total arsenic concentration (μg g^{-1} fresh weight) in the edible parts of four common vegetables[a]

	Radish		Tomato	Spinach		Carrot			
	Non-flooded		Non-flooded	Non-flooded	Flooded	Non-flooded		Flooded	
As in water (μg L^{-1})	Edible root	Taproot	Fruit	Leaves	Leaves	Edible root	Taproot	Edible root	Taproot
0	0.003	0.011	0.055	0.058	0.019	0.008	0.022	0.004	0.032
50	0.002	0.014	0.061	0.058	0.040	0.008	0.019	0.004	0.036
100	0.004	0.018	0.046	0.056	0.080	0.006	0.019	0.003	0.021
200	0.004	0.015	0.045	0.051	0.106	0.004	0.018	0.006	0.017
1000	0.014	0.068	0.026	0.122	0.319	0.008	0.022	0.017	0.034

[a] Mean fresh weight was calculated as a product of mean As concentration (μg g^{-1} dry weight) and mean water content of each vegetable species according to the formula: fresh weight μg g^{-1} = (dry weight μg g^{-1}) × (1 − % moisture/100).

the Chinese maximum permissible concentration (MPC) for all irrigation treatments. Ingestion of carrot cultivated under the conditions of our study therefore poses a minimal and acceptable risk to human health. The edible portion of radish was also safe for consumption; however for the 1000 μg As L^{-1} irrigation treatment, the As concentration in taproot was above the MPC. In tomato fruit, the As concentration was equivalent to the MPC level for As concentrations in irrigation water less than 1000 μg L^{-1}. In contrast, for spinach leaves, the As concentration was above the MPC level for most treatments under both irrigation techniques. The As concentration in spinach leaves for the 100, 200, and 1000 μg L^{-1} treatments under flood irrigation and for the 1000 μg L^{-1} treatment under non-flood irrigation was of concern. For these treatments, As was 1.6 to 6.4 times higher than the MPC level. These values were further explored to determine potential risk to humans using the USEPA hazard quotient (HQ) and cancer risk (CR) calculations.

Use of the HQ and CR risk assessment model requires quantification of the average daily vegetable consumption for the target population. The average daily consumption of vegetables per person varies among countries (Table 3). Considering the eating habit of people of South Asian countries, where vegetables are eaten with each of three meals a day,[1,55] the intake of 500 grams per day was used for further calculation. Only adults (>18 years old) and adolescents (12–18 years old) are considered in this discussion to provide a conservative picture of exposure. The vegetable intake data for children (<12 years) is insufficient for South Asian countries and therefore not considered in this discussion.

Assuming a scenario where 500 grams of spinach (Fi) is consumed on 52 days in a year (Ef), the HQ value ranged from 0.32 to 1.26 for adults and 0.38 to 1.51 for adolescents (Tables 4 and S2, ESI†). A higher HQ value for adolescents compared to adults infers greater risk for this demographic. The hazard quotient exceeded 1 and defines potential risk for both adults and adolescents consuming spinach cultivated under flood irrigation with water containing an As concentration of 1000 μg L^{-1}. Calculation of the parameter CR shows that there is an increased probability of cancer through ingestion of spinach leaves cultivated under flood irrigation with water containing more than 50 μg As L^{-1} (probability in excess of 1 in 10 000). The cancer risk also exceeded 1 in 10 000 for spinach cultivated using non-flood irrigation with water containing 1000 μg As L^{-1}. This risk from spinach consumption may be higher in areas where vegetables consumption is higher, and where As contaminated water is also used for drinking and cooking.

Table 3 Daily average vegetable intakes per capita around the world

Region/country	Vegetables consumption (g fresh weight day^{-1})
Bangladesh	130–205 (ref. 8, 56 and 57)
USA	161.5 (ref. 58)
Republic of Croatia	275 (ref. 59)
Santiago, Chile	327.3 (ref. 60)
Denmark	376 (ref. 47)
West Bengal, India	450 (ref. 1) to 500 (ref. 55)

3.3 Management strategies for irrigation with As contaminated water

Our results suggest that the choice of crop and irrigation management technique should be made with caution in scenarios where vegetable crops are irrigated with As-contaminated water. Where practicable, the practice of flood irrigation with As-contaminated water should be avoided as it increases As solubility and uptake in plants. Instead, non-flood irrigation should be practiced as this will minimize As transformation, solubility and uptake by plants. At locations where flood irrigation is practiced, crop species which actively accumulate As in their edible parts (*e.g.* spinach) should be replaced by species that show less propensity to accumulate As (*e.g.* carrot). Our data suggest that the skin of root vegetables (*e.g.* carrot, radish) should be removed before ingestion as a significant portion of the As burden of vegetables is found in the skin. Our research allows us to propose an acceptable level of As in irrigation water defined by the corresponding As concentration in the edible parts of vegetables which do not pose carcinogenic risk to humans upon their consumption. We propose that irrigation water with an As concentration higher than 50 μg L^{-1} should not be used for spinach cultivation where flood irrigation is practiced. However, for carrot, radish and tomato cultivation, an As concentration in irrigation water up to 1000 μg L^{-1} is acceptable.

Table 4 Hazard quotient (HQ) and cancer risk (CR) for the ingestion of spinach leaves as a function of the concentration of As in irrigation water and the irrigation technique used

			Hazard quotient (HQ)		Cancer risk (CR)	
As in water (μg L^{-1})	Irrigation technique	C^a (μg g^{-1} fresh weight)	Adults (>18 years)	Adolescent (12–18 years)	Adults (>18 years)	Adolescent (12–18 years)
100	Flooded	0.080	0.32	0.38	1.4×10^{-4}	1.7×10^{-4}
200	Flooded	0.106	0.42	0.50	1.9×10^{-4}	2.3×10^{-4}
1000	Non-flooded	0.122	0.48	0.58	2.2×10^{-4}	2.6×10^{-4}
	Flooded	0.319	1.26	1.51	5.7×10^{-4}	6.8×10^{-4}

[a] Concentration of arsenic in spinach leaves; parameters have been calculated where the As concentration in spinach exceeds the MPL of 0.05 μg g^{-1} fresh weight.

4 Conclusions

The arsenic concentration in carrot, radish, spinach and tomato increased as a function of the As concentration in irrigation water. The effect of irrigation with 1000 μg As L^{-1} was significant in all vegetable species relative to the other treatments and enhanced the As concentration of each plant. The distribution of As among vegetables tissues varied for the species used. Tomato and spinach accumulated a higher As concentration in roots relative to aerial biomass, while radish and carrot accumulated a higher As concentration in leaves and skin relative to edible root. Among the studied vegetables, As uptake increased in the order carrot < radish < tomato < spinach. The effect of irrigation technique was significant on the As concentration in the studied vegetables. Spinach and carrot grown under flood irrigation had a higher As concentration in aerial biomass relative to non-flood irrigation, possibly due to an increased solubility and bioavailability of As. In terms of risk to human health from consuming the edible parts of these vegetables, our findings indicate that spinach leaves accumulate a significant level of As under the treatments used, ranging from 1.6 to 6.4 times higher than the Chinese maximum permissible level of As in food (0.05 μg g^{-1} fresh weight). Spinach leaves also pose a carcinogenic and non-carcinogenic risk to humans upon their consumption. This is quantified by calculated HQ and CR values (USEPA), where an HQ value greater than 1 represents an unacceptable non-carcinogenic risk and a CR value greater than 10^{-4} represents an unacceptable carcinogenic risk. The HQ value for spinach ranged from 0.32 to 1.26 for adults and 0.38 to 1.51 for adolescents while the CR value ranged from 1.4×10^{-4} to 5.7×10^{-4} for adults and 1.7×10^{-4} to 6.8×10^{-4} for adolescents. Irrigation water with an As concentration greater than 50 μg L^{-1} should be avoided for spinach cultivation where flood irrigation is practiced.

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