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ARSENIC ACCUMULATION IN PUMPKIN THROUGH CONTAMINATED GROUNDWATER AND VARIETAL EVALUATION THEREOF IN GANGETIC ALLUVIUM OF WEST BENGAL, INDIA

Rajib Kundu*, Sukanta Pal and Aparajita Majumder

ICAR Niche Area of Excellence, Arsenic Research Laboratory, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal - 741 235 (India)
*e-mail: *rajibagro2007@gmail.com*

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ABSTRACT

Arsenic (As) is the most hazardous toxic metalloid found in natural ecological units and is widely distributed in various parts of the world. Nearly about 50 million people suffer from its toxicity in Indo-Gangetic alluvium particularly in West Bengal. Present study was aimed to evaluate As accumulation in pumpkin (*Cucurbita moschata*), varietal tolerance, As intake and dietary risk in Nadia (West Bengal) during summer 2008-09 and 2009-10 where contaminated ground water is frequently used for irrigation. The experiment was laid out in a randomized block design using 5 pumpkin cultivars each replicated 4 times. As uptake and its translocation to the edible parts varied in the pumpkin cultivars grown. Provisional tolerable weekly intake (PTWI) of inorganic As was 15 µg body weight^{-1}. The locally grown cultivar showed maximum As accumulation but lower yield, whereas high yielding cultivars 'Kali Kumro' and 'Ambili' had less As load in fruits. As accumulation in different parts of pumpkin remained in an order of root > stem > leaf > fruit across the cultivars.

Keywords: Arsenic, cultivars, pumpkin, risk assessment, yield

INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch. ex Poir.) is one of the most popular summer vegetable grown all over India on commercial scale both for its immature and mature fruit. These are often used as fresh vegetable, processed food or stock feed, sometimes young tender tops of shoots and leaves are cooked as vegetable. Owing to its high carotene content and good keeping quality, pumpkin is considered as a vegetable of immense value (Thamburaj and Singh, 2005). Under intensive cropping system pumpkin is successfully grown as a remunerative vegetable in Gangetic alluvium of West Bengal due to its low production cost and longer keeping quality. However, arsenic contamination in ground water has been reported in these areas over past decade (Mitra *et al.,* 2002; Pandey *et al.,* 2002). The arsenic poses serious threat to human race because of its capability to cause terrible human health hazards (Srivastava *et al.,* 2001; Rahman, 2002). Out of 20 countries in the world where groundwater arsenic contaminations have been observed the magnitude is of human suffering reportedly high in Bangladesh followed by Indian state West Bengal (Sanyal, 2005). The serious problem in these areas arises because of groundwater contamination and its subsequent use for drinking purpose (WHO, 2001). However, the emerging areas of As hazards in agricultural systems through use of contaminated irrigation water and entry of toxin in crops has largely been overlooked. Increased As levels in ground water-irrigated soil and As uptake by plants grown in soils contaminated with high As concentration and irrigated with As contaminated groundwater have been well documented (Sanyal and Nasar, 2002; Dahal *et al.*, 2008; Bhattacharya *et al.*, 2009).

The As problem requires immediate attention as As finds its way into the food web, other than drinking water, accompanied with possible biomagnification at different levels in a food chain (Roychowdhury *et al.*, 2002). Recent studies have shown that the contribution of food-chain towards As pollution in human is manifold greater than that of drinking water (Roychowdhury *et al.*, 2003; Díaz *et al.*, 2004). Presence of As in vegetable crops and its translocation to the edible parts were observed to vary with crops (Alam *et al.*, 2003; Das *et al.*, 2004) and even within the cultivars (Rahman *et al.*, 2007; Kundu *et al.*, 2012). In this context, a thorough understanding of arsenic-plant-soil interaction is necessary to examine As uptake by plants grown in soils contaminated with high As content, and irrigated with As-contaminated groundwater as well as the entry of As in food chain. Rice-based cropping system is most predominant in West Bengal; however, rainy season rice is not a major problem due to its rainfed cultivation (Duxbury *et al.*, 2003). However, the winter and summer crops mainly cultivated through irrigated ground water create a great problem in the form of As uptake (Ghosh *et al.*, 2004; Huang *et al.*, 2006). Keeping this view, the present investigation was undertaken to study As-accumulation in pumpkin (known for high water requirement and possibilities to accumulating more toxin from the system), the varietal tolerance, relative pattern of As uptake by selected cultivars, As intake and dietary risk.

MATERIALS AND METHODS

An experiment was conducted during summer seasons of 2008-09 and 2009-10 at two different sites in village Nonaghata (latitude $22^{0}57$’N, longitude $88^{0}33$’E and altitude 7.8 m above sea level) in alluvial soil of district Nadia (West Bengal). In the area many wells contaminated with arsenic are used as irrigation source. At the experimental site, the climate is subtropical humid with an average rainfall between 1200 and 2500 mm and mean minimum and maximum temperature 12 and 40^{0}C, respectively. The experiment was laid out in a randomized block design using 5 pumpkin cultivars viz., ‘Pusa Vikas’, ‘Kali Kumro’, ‘Arka Chandan’, ‘Ambili’ and a locally grown cultivar. Each treatment was replicated 4 times. The experiment was conducted for two consecutive years on the same land without disturbing the experimental layout. The soil texture at experimental sites was silty clay loam having pH 7.21, EC 0.27 dsm^{-1}, organic carbon 0.53%, available nitrogen 167 kg ha^{-1}, available phosphorus 34 kg ha^{-1} and available potassium 139 kg ha^{-1}, respectively. However, the arsenic content in irrigation water and soil at site I and site II was in the range of 0.115 and 0.122 mg L^{-1} and 8.47 and 9.31 mg kg^{-1}, respectively.

Crop management

The pumpkin was planted in ending April at a plant spacing of 3x2 m^2. The recommended dose of fertilizers (75:80:80 kg NPK ha^{-1}, well decomposed farmyard manure was applied uniformly as basal along with ½ N and full dose of P and K at the time of final land preparation. Remaining nitrogen was applied in two equal split doses at veining and full blooming stages) were applied in the form of urea, SSP and MOP. Irrigation was given immediately after seed germination and subsequent irrigations applied once at 5-7 days interval and As contaminated shallow tube-well (STW) water used as the source of irrigation.

Sampling

Irrigation water samples were collected from the shallow tube well pumps, used for soil irrigation in the area. Prior to sample collection, the pumps were kept running for about 10-15 min. in order to have uniform rate of discharging water. The water samples were collected in plastic containers and preserved with concentrated HNO_3. The plant samples were taken from the experimental sites at vegetative and mature stages. The plant samples were washed with pure water to remove soil debris attached to the plant body and then with ultra-pure de-ionized water. The root, stem, leaves and fruits were collected separately and cut into small pieces. The samples were dried, ground and kept in a sample container.

Arsenic concentration determination

The plant samples (1 g each) were digested with tri-acid mixture (HNO_3: H_2SO_4: $HClO_4$: 10:1:4, v/v) until a clear solution was obtained. These digested materials were adequately filtered through Whatman No. 42 filter paper. The filtrate (10 ml) was taken in 50 ml volumetric flask and 5 ml conc. HCl and 1 ml mixed reagent [5% KI (w/v) + 5% ascorbic acid (w/v)] added to it. The flasks were kept as such for 45 min. to ensure complete reaction and volume was made up to 50 ml. The total As content in solution was determined by using atomic absorption spectrophotometer (AAS), Perkin Elmer Analyst 200 coupled with Flow Injection Analysis System (FIAS 400) where the carrier solution was 10% HCl (v/v) following the Olsen method (McLaren *et al.*, 1998). A set of standard solutions of 2.5, 5.0, 10.0, and 20.0 mg L^{-1} As were used for calibration.

Arsenic intake through food and risk assessment

A dietary exposure assessment is the process of estimating how much of a food chemical a population or population sub-group consumes. Dietary exposure (or intake of) to food chemicals was estimated by combining food consumption data with food chemical concentration data. The process of doing this is called ‘dietary modeling’.

Dietary exposure = Food chemical concentration x Food consumption

The dietary intake and the risks associated with As contamination of food materials were assessed by determining Provisional Tolerable Weekly Intake (PTWI) of the contaminant [PTWI of As is 15 μg body weight^{-1} (i.e. 900 μg for an adult of 60 kg body weight)] as described by WHO (2000).

Statistical analysis

Analysis of variance was used for statistical analysis (Gomez and Gomez, 1976). The significance of different sources of variation was tested by error mean square with the help of Fisher’s (F) test at probability level of 0.05. For comparison of F-value and computation of critical different at 5% level of significance, Fisher and Yates tables were consulted.

RESULTS AND DISCUSSION

Arsenic accumulation pattern

The arsenic concentration in different parts of pumpkin plants revealed maximum As accumulation in roots as compared to other plant parts (Fig. 1). Arsenic accumulation in different parts of pumpkin remained in an order of root > stem > leaf > fruit across the tested cultivars and across the sites, irrespective of the time of sampling (vegetative or mature stage). Comparison of As accumulation in different parts of pumpkin plants showed that the translocation of As in edible part was relatively lower than any other plant part. In a similar study, significantly lower amount of uptake and translocation of this toxic metalloid have been reported in rice grain as compared to the uptake in rice straw and root parts (Liu *et al.*, 2007). Such findings are quite consistent with general observations wherein the distribution of As in plant parts is in the order of below-ground parts > areal parts (Sanyal, 2005). In present study As concentration varied in different parts of the plants. Similar high As accumulation in root as compared to other parts has been reported by other workers (Norra *et al.*, 2005; Rahman *et al.*, 2007; Bhattacharya *et al.*, 2009). Again, As concentration in different plant parts of pumpkin varied over sites. The site-II recorded maximum As values across the plant parts and cultivars. It may possibly be attributed to the source of irrigation water and soil. Similar findings have been reported by Bhattacharya *et al.* (2010), who found that As-contaminated irrigation water and soil considerably influence the As-accumulation in different crops especially vegetables.

Arsenic accumulation at vegetative stage

At the vegetative stage of pumpkin (60 days after sowing), the As accumulation by different plant parts varied significantly across the tested cultivars (Table 1). Maximum As accumulation in roots was noticed in pumpkin cv. 'Arka Chandan' (2.14 and 2.45 mg kg^{-1} at site-I and site-II, respectively) which was, however, at par with cv. 'Pusa Vikas' and locally grown cultivar. Least As load was noticed in cv. 'Kali Kumro' (1.37 and 1.61 mg kg^{-1} at site-I and site-II, respectively) which was at par with cv. 'Ambili'. In case of pumpkin stem, locally grown cultivar accumulated maximum amount of this toxic metalloid (1.69 and 1.91 mg kg^{-1} at site-I and site-II, respectively). Across the tested cultivars, 'Pusa Vikas' and 'Arka Chandan' contained least amount of As at site-I and site-II, respectively. In leaves the locally grown cultivar accumulated significantly high amount of As (1.21 and 1.35 mg kg^{-1} at site-I and site-II, respectively), whereas least As load was observed in cv. 'Kali Kumro' (0.73 and 0.97 mg kg^{-1} at site-I and site-II, respectively) followed by cv. 'Ambili'.

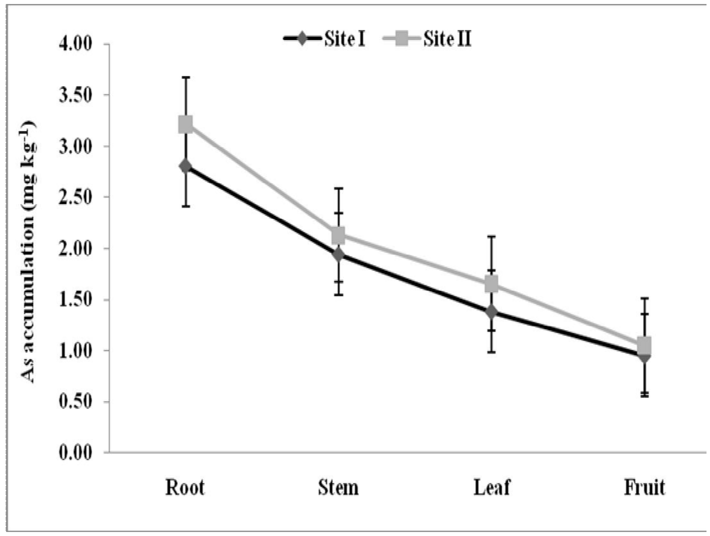

Fig. 1: Arsenic accumulation pattern of pumpkin (average) at maturity

Table 1: Arsenic accumulation (mg kg^{-1}) in different plant parts of pumpkin cultivars at vegetative stages (pooled data)

Cultivars	Root		Stem		Leaf	
	Site I	Site II	Site I	Site II	Site I	Site II
Pusa Vikas	1.91	2.22	1.30	1.72	0.86	1.15
Kali Kumro	1.37	1.61	1.34	1.57	0.73	0.97
Arka Chandan	2.14	2.45	1.48	1.55	0.93	1.17
Ambili	1.43	1.72	1.40	1.62	0.85	1.03
Local cultivar	1.89	2.13	1.69	1.91	1.21	1.35
S.Em (±)	0.09	0.11	0.05	0.07	0.03	0.04
$CD_{0.05}$	0.27	0.33	0.15	0.22	0.09	0.11

Arsenic content in irrigation water and soil of the experimental sites was 0.115 and 0.122 mg L^{-1}, and 9.31 and 9.43 mg kg^{-1} at site I and site II, respectively.

Arsenic accumulation at maturity

At the maturity of pumpkin (120-150 days after sowing, depending upon cultivars), the degree of As load differed significantly across the cultivars (Table 2). At this stage As accumulation in below-ground parts of pumpkin i.e. roots recorded more or less similar trend as observed in its vegetative stage. The cultivar 'Pusa Vikas' had significantly high amount of As in its stem and leaves (2.11 and 2.56 mg kg^{-1} in stem and 1.72 and 2.03 mg kg^{-1} in leaves at site-I and site-II, respectively). Among the cultivars least As load was seen in cv. 'Kali Kumro' (1.77 and 1.90 mg kg^{-1} in stem and 1.07 and 1.41 mg kg^{-1} in leaves at site-I and site-II, respectively) which closely followed by cv. 'Ambili'. In fruits, the As concentration was significantly high in cv. 'Arka Chandan' (1.39 and 1.47 mg kg^{-1} at site-I and site-II, respectively), whereas cv. 'Kali Kumro' contained significantly least amount of this toxic metalloid (0.42 and 0.55 mg kg^{-1} at site-I and site-II, respectively) which was followed by cv. 'Ambili'. The As accumulation have been observed to increase with the advancement of crop growth and at the same time such increase in As load is maximally manifested in pumpkin root.

Table 2: Arsenic accumulation by different plant parts and yield of pumpkin cultivars at maturity (pooled data)

Cultivar	Arsenic accumulation (mg kg^{-1})								Yield (kg ha^{-1})	
	Root		Stem		Leaf		Fruit			
	Site I	Site II	Site I	Site II	Site I	Site II	Site I	Site II	Site I	Site II
Pusa Vikas	2.96	3.07	2.11	2.56	1.72	2.03	1.06	1.13	3036	2977
Kali Kumro	2.53	2.77	1.77	1.90	1.07	1.41	0.42	0.55	3353	3471
Arka Chandan	3.12	4.31	2.01	2.05	1.50	1.82	1.39	1.47	2879	2785
Ambili	2.67	2.91	1.85	2.01	1.21	1.43	0.71	0.85	3517	3633
Local cultivar	2.75	3.02	1.97	2.12	1.42	1.57	1.18	1.23	2451	2513
S Em (±)	0.12	0.14	0.03	0.09	0.04	0.06	0.03	0.05	69.75	66.11
$CD_{0.05}$	0.38	0.42	0.09	0.27	0.11	0.18	0.10	0.15	214.9	203.7

Arsenic content in irrigation water and soil of the experimental sites was 0.115 and 0.122 mg L^{-1}, and 9.31 and 9.43 mg kg^{-1} at site I and site II, respectively.

Yield and arsenic uptake

Fruit yield of pumpkin was recorded at harvest which differed significantly within the cultivars (Table 2) and did not remarkably vary over experimental sites. Across the tested cultivars, 'Ambili' gave maximum fruit yield (3517 and 3633 kg ha^{-1} at site-I and site-II, respectively) which was at par with cv. 'Kali Kumro' (3353 and 3471 kg ha^{-1} at site-I and site-II, respectively). On the other hand, significantly less fruit yield was obtained in locally grown cultivar (2451 and 2513 kg ha^{-1} at site-I and site-II, respectively), followed by 'Arka Chandan' (2879 and 2785 kg ha^{-1} at site-I and site-II, respectively).

The As uptake by fruits increased at site-II across the tested cultivars (Fig. 2). The maximum As uptake was noticed in cv. 'Arka Chandan' across the sites (4.00 and 4.09 g ha^{-1} at site-I and site-II, respectively) which was followed by 'Pusa Vikas' and locally grown cultivar. The cultivar 'Kali Kumro' showed least As uptake across the experimental sites (1.41 and 1.91 g ha^{-1} at site-I and site-II, respectively), followed by cv. 'Ambili'. The high As uptake by pumpkin fruits cv. 'Arka Chandan' may be responsible for lower fruit yields, although there may be several other factors controlling yields.

Arsenic intake and dietary risk assessment

For risk assessment, the exposure estimates are compared with 'reference health standards', if available, to assess the potential risk to health associated with changes to the food supply. The dietary intake and the risks associated with As contamination of food materials were assessed in terms of provisional tolerable weekly intake (PTWI) of the contaminant. PTWI of inorganic As is 15 μg body weight^{-1} (900 μg for an adult of 60 kg body weight) as described by FAO and WHO (2000). It has been reported that in fruits and vegetables the inorganic As accounts for approximately one-half of the total As (Schoof *et al.*, 1999). Ingestion of

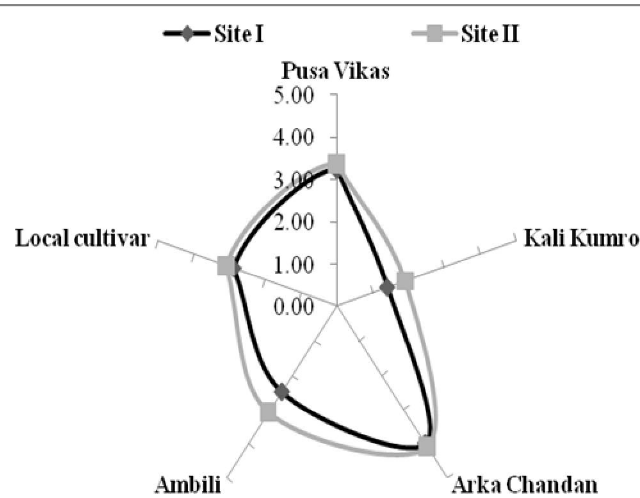

Fig. 2: Arsenic uptake (g ha^{-1}) by different cultivars of pumpkin at maturity

significant proportion of inorganic As intake (1596 and 1688 μg $week^{-1}$ $adult^{-1}$ at site-I and site-II, respectively) through consumption of pumpkin has been observed in the present study. It poses a risk of 177 and 188% (at site-I and site-II, respectively) of PTWI through dietary intake of inorganic As which is much beyond the WHO permissible limit but still pretty low as compared to such risk of exposures to contaminated rice (Table 3). The field based experiment revealed that continuous use of As contaminated irrigation water increases with crop age which possibly increases As load in pumpkin plants with time. Again As intake and dietary risk assessment through pumpkin varied over the source of irrigation water and cultivated soil. As contaminated irrigation water and soil considerably influenced the accumulation of As in different crops and vegetables (Bhattacharya *et al.*, 2010). The cultivars 'Kali Kumro' and 'Ambili' accumulated least amount of As in their fruits with maximum yielding capacity across the cultivars, whereas the locally grown cultivar loaded moderate amount of As in its fruit and gave less fruit yield. On the other hand, cv. 'Arka Chandan' showed highest recovery of this toxic metalloid with lower yielding capacity. So, yield of pumpkin may not be affected by the level of As concentration in its environment whereas the quality of fruits and aerial parts

Table 3: Arsenic intake and dietary risk assessment through pumpkin

Food item	Weekly consumption (g)	Maximum total As concentration (μg kg^{-1})	Maximum weekly total As intake (μg)	Maximum weekly inorganic As intake (μg)	PTWI (%)
Pumpkin (Site-I)	2296	1390	3191	1596	177
Pumpkin (Site-II)	2296	1470	3375	1688	188
Rice**	10965**	357**	3911**	1956**	217**

***Final Report of "Total diet study in West Bengal" (2010) http://whoindia.org/LinkFiles/Food_Safety_Total _Diet _Study_in_West_Bengal.pdf*

(use of leafy vegetable) is highly affected. Preference should, therefore, be given to those cultivars which have higher production ability with least As accumulation in its edible parts. Chaturvedi (2006), Rahman *et al.* (2007) and Kundu *et al.* (2012) reported that As toxicity varied widely within plant genotypes probably due to varietal differences in As translocation and phyto-extraction or phyto-morphological potential of the varieties. Our observations support the fact that cultivar 'Kali Kumro' accumulated least As and cultivar 'Arka Chandan' accumulated higher As under the same growth conditions.

Conclusions: In Eastern India, particularly West Bengal, pumpkin is commonly consumed cucurbitaceous vegetable. Though As accumulation in pumpkin fruit is relatively low than the other plant parts, but the leaves and top of shoots are often used as cooked vegetable. So, there is need for appropriate agricultural management practices towards minimizing the As load in pumpkin. The accumulation of toxic heavy metals like As remained significantly high in pumpkin and other vegetables than cereals and other crops; and also varied across the cultivars. Selection of appropriate cultivars of pumpkin that accumulate less amount of As without hampering its production rate in As-affected belt have immense ecological importance, as it might have a beneficial role towards mankind. Pumpkins accumulate much high As in edible portion than rice, still the risk associated with dietary exposure to As contaminated pumpkin is less than rice which is due to far less weekly consumption of pumpkins. Though the level of As ingestion through pumpkin exceeded the WHO permissible limit for clinically safe food item, such food items are largely ignored in toxicological investigation and demand due attention of researchers.

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