



Quantification of inorganic arsenic exposure and cancer risk via consumption of vegetables in southern selected districts of Pakistan



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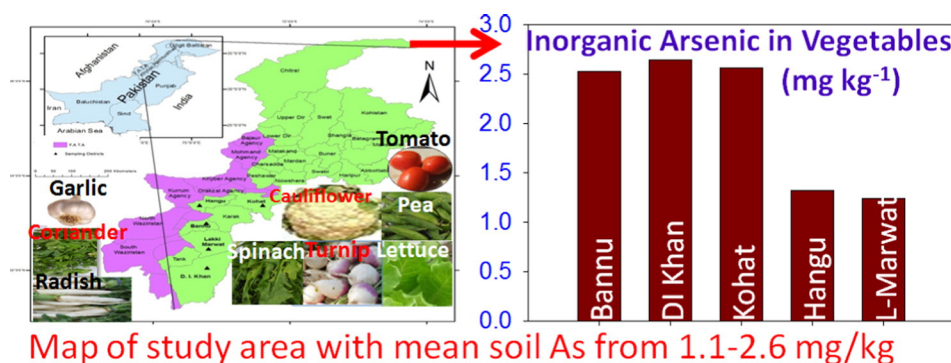
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HIGHLIGHTS

- Cancer risk due to inorganic As exposure via vegetable consumption was determined.
- Total As in soil ($3.0\text{--}3.9\text{ mg kg}^{-1}$) and vegetable ($0.03\text{--}1.4\text{ mg kg}^{-1}$) were quantified.
- InAs in soil and vegetable were $1.2\text{--}2.6\text{ mg kg}^{-1}$ and $\text{BDL}\text{--}1.3\text{ mg kg}^{-1}$, respectively.
- As contents in 75% of vegetable samples exceeded limit set by WHO/FAO.
- As intake, health quotient (<1) and cancer risk ($0.6\text{--}13 \times 10^{-5}$) were quantified.

GRAPHICAL ABSTRACT



Map of study area with mean soil As from 1.1–2.6 mg/kg

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ABSTRACT

Human exposures to arsenic (As) through different pathways (dietary and non-dietary) are considered to be one of the primary worldwide environmental health risks to humans. This study was conducted to investigate the presence of As in soil and vegetable samples collected from agricultural lands located in selected southern districts of Khyber Pakhtunkhwa (KPK) Province, Pakistan. We examined the concentrations of total arsenic (TAs), organic species of As such as monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA), and inorganic species including arsenite (AsIII) and arsenate (AsV) in both soil and vegetables. The data were used to determine several parameters to evaluate human health risk, including bioconcentration factor (BCF) from soil to plant, average daily intake (ADI), health risk index (HRI), incremental lifetime cancer risk (ILTCR), and hazard quotient (HQ). The total As concentration in soil samples of the five districts ranged from $3.0\text{--}3.9\text{ mg kg}^{-1}$, exhibiting minimal variations from site to site. The mean As concentration in edible portions of vegetable samples ranged from $0.03\text{--}1.38\text{ mg kg}^{-1}$. It was observed that As concentrations in 75% of the vegetable samples exceeded the safe maximum allowable limit (0.1 mg kg^{-1}) set by WHO/FAO. The highest value of ADI for As was measured for *Momordica charantia*, while the lowest was for *Allium chinense*. The results of this study revealed minimal health risk ($\text{HI} < 1$) associated with consumption of vegetables for the local inhabitants. The ILTCR values for inorganic As indicated a minimal potential cancer risk through ingestion of vegetables. In addition, the HQ values for total As were <1 , indicating minimal non-cancer risk.

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1. Introduction

The contamination of food crops by toxic elements is a universal health concern (e.g., Ng et al., 2003; Waqas et al., 2014). Arsenic (As) is considered to be the most widely distributed contaminant currently in the food chain (Joseph et al., 2015a, 2015b; Llobet et al., 2003). As is released from both natural sources (parent rocks) and also from various anthropogenic activities such as agricultural practices, irrigation with As-contaminated water (Waqas et al., 2015), the improper applications of arsenical fertilizers, insecticides, herbicides, use of poultry litter with As-based intestinal palliatives (Jia et al., 2012; Zhu et al., 2008), mining activities, and petroleum refineries (Martínez-Sánchez et al., 2011; Khan et al., 2014; Kabata-Pendias, 2011). Soil is a major sink of As, which can lead to contamination of vegetables because of its high mobility and uptake rate (Sridhar et al., 2011). The bioavailability of As and its subsequent bioaccumulation in vegetables (tomato, cucumber, cauliflower, pea, lettuce, spinach, cabbage, onion, radish, turnip, carrot, potato, etc.) depend on soil texture, pH, organic matter content and composition, redox condition, water regime, mineral composition, and microbial activity (e.g., Bergqvist et al., 2014; Khan et al., 2015a; Smith et al., 2009).

As is listed as a class one carcinogen (National Research Council, NRC, 2001; IARC, 2004), and is considered to be one of the most important toxicants of human health concern because of its ongoing potential threat to the health of hundreds of millions of individuals worldwide (Baig et al., 2009; Christen, 2001; Zavala and John, 2008). There is no evidence that As is essential for human health and its exposure has been linked to severe health complications such as hyperkeratosis, gangrene, hypertension, peripheral vascular disease, melanosis, keratosis, bladder, and internal cancers, and also its carcinogenic consequences on skin and lungs (Fatmi et al., 2009; IARC, 2004; Ramadan and Al-Ashkar, 2007).

Previous studies have revealed that human exposure to As contaminated water and soil is of great concern, while food ingestion is one of the chief pathways of human exposure to toxic metals (Dummer et al., 2015; Joseph et al., 2015a; Khan et al., 2015b). Consequently, it is of practical significance to assess the extent of As accumulation from soil into plants. This research area has gained increasing attention throughout the world (e.g., Chang et al., 2014). Food crops such as vegetables and cereals are a potential entry source of As into the food chain and can reflect the levels of As that exist in the soil in which they are cultivated (Arain et al., 2009; Joseph et al., 2015b; Rahman et al., 2008; Ramirez-Andreotta et al., 2013a). Millions of individuals in south and south-east Asia have been affected by As poisoning from exposure to As-contaminated food-crops from the last several years (Jia et al., 2012; Neumann et al., 2011; Zhu et al., 2008).

Numerous studies have been conducted in order to assess the amount and toxicity of chemical forms of As such as organic species (monomethylarsonic acid (MMA), dimethylarsonic acid (DMA)) and inorganic species (arsenite (AsIII), arsenate (AsV)) present in soil and food plants (Ruiz-Chancho et al., 2007). Inorganic As (iAs) is considered to be more toxic and mobile than organic As (Egbenda et al., 2015; Van-Herreweghe et al., 2003).

The speciation of As in soil depends on numerous factors including pH, soil organic carbon (SOC), electron donor/acceptor availability, oxidation potential, and bacterial community structure and function. AsIII is the dominant species in reducing environments (~0.0–0.8 VpE and pH 2–10), while AsV is more prevalent under aerobic conditions (Acosta et al., 2015; Álvarez-Ayuso et al., 2016; Wilson et al., 2010). As methylation into MMA and DMA occurs in soil mediated by both bacteria and plants (Huang et al., 2012). The literature supports both the ability and inability of plants to methylate iAs inside the tissues (Lomax et al., 2012; Raab et al., 2007). However, As distribution and translocation (root to shoot) occur through both xylem and phloem depending on plant species (Bergqvist et al., 2014).

Previously, several studies have been carried out to investigate As accumulation in vegetables grown in various areas (Garcia et al., 2014;

Kronbauer et al., 2013; Martinello et al., 2014a,b; Oliveira et al., 2013; Oliveira et al., 2012; Ramirez-Andreotta et al., 2013a; Ribeiro et al., 2013a; Sanchis et al., 2015; Silva et al., 2009; Silva et al., 2012). However, this is the first study of its nature conducted in the southern section of Khyber Pakhtunkhwa Province, Pakistan to investigate total As (TAs) and its organic (MMA and DMA) and inorganic species (AsIII and AsV) in agricultural soil and vegetables. Furthermore, we evaluate the daily As intake and cancer risk posed by the consumption of vegetables by local inhabitants.

2. Materials and methods

2.1. Site description

The study was conducted in the southern selected districts Hangu, Kohat, Bannu, Lakki Marwat and Dera Ismail Khan (DI Khan) of Khyber Pakhtunkhwa, Pakistan (Fig. 1). The study area encompassed 15,359 km² (see Table S1) and has very fertile land, cultivated with a variety of crops and vegetables. In most of the areas, these food crops and vegetables are irrigated with water may contain high As concentration. Cucumber, bitter melon, ridge gourd, onion, garlic, mint, lady finger, squash-melon, lettuce, spinach, pea, pumpkin, cabbage, cauliflower, potato, bringal, turnips, carrot, radish, tomato, yam, perslane, oriental onion and coriander were selected as the most important food crops in this study (DCR, 1998; Waqas et al., 2014). The English and botanical names of selected food stuffs are provided in Table S2.

2.2. Soil sampling and processing

The soil samples (n = 175) were collected with a stainless steel auger from a depth of 0–20 cm. Multiple soil samples were collected from randomly selected locations for each site, followed by homogenization to form a composite sample (1 kg), using the quartile method (Wu et al., 2010). The soil samples were transported to the laboratory and then air dried. The dried samples were mechanically ground into fine powder and sieved to 2 mm, removing unwanted granulated substances and placed in clean plastic bags for further analyses.

Soil samples (1 g each) were subjected to acid digestion in Teflon vessels, following the protocol from US EPA SW-846 (1986) in the Arizona Laboratory for Emerging Contaminants (ALEC), University of Arizona, Tucson, USA. The samples were treated with 2.5 ml HNO₃ (analytical grade) in microwave assisted reaction system (CEM MARs 6, NC, USA). After filtration through a membrane (0.22 µm), the filtrates were diluted up to 15 ml with milli-Q water and analyzed for TAs using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700x, Agilent Technologies, Santa Clara, USA). The As species such as AsIII, AsV, DMA, and MMA were determined using ICP-MS connected with high performance liquid chromatography (HPLC-ICP-MS) (Hamilton company, USA) and separated with HPLC system comprising a Agilent 1100 HPLC with Hamilton column (PRP-X100) and guard cartridge. The mobile phase was ammonium carbonate (pH 8.75) with 2% methanol in a gradient. The sum of AsIII and AsV was considered as the total iAs. To ensure accuracy and precision, soil standard certified reference material (CRMs, 2702) purchased from National Institute of Standards and Technology (NIST), USA and reagent blanks (without sample), were used with each batch of samples. The recovery rates were satisfactory and ranged from 92–113%.

2.3. Vegetable samplings and acid digestion

Vegetable samples (n = 175) were collected from the same fields used for soil sampling. Further detail is given in SI (Table S2). The edible portions were separated, and all visible soil particles were removed using a soft bristled brush. The samples were then rinsed with tap water and finally washed with distilled water several times. The edible parts of vegetable were dried at 60 ± 5 °C for 72 h and then powdered

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