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Study and estimation of arsenic in vegetables and groundwater of Buxar

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Abstract
The total concentration of arsenic in vegetables, fruits, grains and water samples was estimated by Graphite Furnace Atomic Absorption Spectrophotometer. Digestion was carried out with an HNO3-HClO4 mixture on hot plate at 60-120 degree Celsius respectively. Arsenic determination was performed by the standard addition method. The highest arsenic level was found in inner part of vegetables where moisture content is highest. This was found to be higher level than those recommended by WHO and Food Agricultural Organization limits. Arsenic is an element that raises much concern from both environmental and human health aspects. The incidence of arsenic contamination of vegetable samples and ground water samples has taken the dimension of epidemiological problem. Initially it enters into the human body through ingestion, inhalation or skin absorption. After entering into the body it is distributed into large number of organs including lungs, liver, kidney and skin. It is very difficult to diagnose early symptoms of arsenicosis because such non-specific symptoms may also be present in many other diseases. The correct diagnosis depends on awareness of the problem. Medicine used for remedy of arsenicosis has been found to be dissatisfactory. Melanosis may disappear but keratosis is not altered. The symptoms and signs may be reduced by improving the quality of drinking water.

Keywords: Arsenic, GF-AAS, arsenicosis, vegetables

Introduction
Contamination of groundwater through naturally occurring arsenic (As) has been reported in many countries around the world, particularly in Southeast Asia causing serious threat to humans as reported by Mandala and Suzuki in their paper. (Mandal and Suzuki, 2002; Mukherjee *et al*., 2006; Naidu *et al*., 2006). This has received significant attention in the last three decades due to its serious health effects on millions of people and has been termed "the biggest As calamity in the world" said by Smith (Smith *et al*., 2000). Arsenic contamination of tubewells in the middle Gangetic plain was first reported in 2002 in Semria Ojha Patti village (area 4 km2), Sahapur block in the Bhojpur district of Bihar, India reported by Chakraborti (Chakraborti *et al*., 2003) [6]. About 89% geographical area of Bihar (~94,000 km2), India is located in the middle Gangetic plain and is known for surplus food production and intensive groundwater e extraction for drinking and irrigation as said in the paper of Saha (Saha, 2009) [28]. According to Rahman and Naidu arsenic in low concentration can stimulate plant growth but in higher concentration it can be accumulated above the WHO threshold concentrations for safe ingestion of food crops (Rahman and Naidu, 2009) [25]. As a consequence, food crops accumulate elevated As which pose serious risks to the health of local residing population mentioned by Dudka (Dudka and Miller, 1999). Therefore it is crucial to assess As exposure via seasonal vegetables intake like potato, spinach, cabbage, chilli. High concentrations of As in tubewell water and sediments of the middle Gangetic plain have been documented by Chauhan, Saha (Chauhan *et al*., 2009; Kumar *et al*., 2010a,b; Saha, 2009; Saha *et al*., 2010a,b; Shah, 2013) [28].
Hughes said that arsenic is a toxic element, known as class (I) human carcinogen and widely distributed in the environment as both inorganic and organic forms (Hughes *et al*., 2011). In general, the inorganic forms (arsenite and arsenate) of As are much more toxic than the organic forms (monomethylarsonic acid, dimethylarsinic acid, arsenobetaine etc.) of As. Arsenite is generally more toxic than arsenate and humans are exposed to both forms of

inorganic As from water and food. There are many pathways by which As can enter the human body via food chain (ingestion by water and food sources) and occupational exposure are the most common (Rahman *et al*., 2009) [24]. Various inorganic species (arsenite and arsenate) and organic species (methylated anionic species, volatile As hydride and organo-As) in food materials have been reported as the main pathways to human exposure (Momplaisir *et al*., 2001). From a health perspective, it is equally important to study the trace elements other than As present in water and in dietary food components. Several attempts have been made to investigate the concentration of As in groundwater, health effect due to As toxicity and the mechanism of mobilization process of As in the middle Ganga plain of Bihar (Chakraborti *et al*., 2003; Chauhan *et al*., 2009; Kumar *et al*., 2010b; Kumar *et al*., 2015; Saha, 2009) [6, 17, 28]. Arsenic above 50 µg/L was detected in 47% of tubewells in Bhagalpur, Bihar (Kumar *et al*., 2010b), while it was 58.6% in Semaria Ojha Patti, Bihar, India (Chakraborti *et al*., 2003) [6]. A range of 6–389.4 µg/L of As was also observed in Samastipur, India (Saha and Shukla, 2013). Very limited efforts have been directed to determine the degree of As contamination in food components and the associated risk to human through consumption of food. It is therefore timely to determine the level of As and other toxic elements present in water, vegetables and other food components grown in this particular area. The total exposure to As and other elements in water, vegetables and other dietary components in the gangetic plains of Bihar, India.

Naturally occurring levels of arsenic in vegetables, grains, meats and Fish present a significant source of arsenic exposure worldwide (Gibb *et al*., 1994) [10]. The arsenic comes from uptake by food crops from the soil and irrigation water. In addition, arsenic in water can contaminate food during processing and cooking. According to a recent World Health Organization (WHO) background document on global arsenic exposure (WHO 2011), arsenic in contaminated water is completely bioavailable and provides the majority of daily arsenic dose (Abernathy, Thomas, *et al*., 2003) [1]. However, as water arsenic concentration decrease, the relative contribution of dietary sources becomes more significant to human arsenic exposures.

As indicated by its IARC classification, arsenic exposure increase the risk for a number of important cancers. Numerous epidemiological studies indicate an association between arsenic exposure and an increased risk of lung cancer mortality (Schoof, Yost, *et al*., 1999) [29] and lung cancer may be the leading cause of arsenic associated cancer deaths. Meta –analysis of available epidemiological studies performed in Bangladesh, Chile, Argentina, Taiwan and the United Sates (Kile, Houseman, *et al*., 2007) [16] estimated about 4.51 additional lung cancer cases per 100,000 people for maximum contamination level of 10µg/l of arsenic in drinking water. An association between arsenic exposure and bladder cancer has been substantiated by multiple ecological, as well as case-control and cohort studies (Begam, Horowitz, *et al*., 2012, Christoforidou, Riza, *et al*., 2013) [4, 8]. In addition, the ingestion of arsenic to increased incidence of non-melanoma skin cancer i.e. basal cell and squamous cell carcinoma. Cohort studies from IARC, 2012 reported risks of skin cancer to be significantly related to average concentration of arsenic in drinking water and index for cumulative exposure to arsenic.

Materials and Methods

1. Sample collection

The water and food samples were collected. Food samples were collected in sealable plastic pouches. While the water sample is collected in the polypropylene plastic bottles from hand pumps. Earlier the bottles were acidified with nitric acid with laboratory grade (1:1) and finally washed with demineralized water. Immediately after collection, one drop of dilute nitric acid (1:1) was added as preservative. Food samples were immediately stored in 4^0 C at Mahavir Cancer Institute and Research Centre, Patna. Total arsenic in water and food was determined by Graphite Furnace atomic absorption spectrometry (GF-AAS).

2. Food sample preparation

Initially 0.5g food sample is weighed and digested with 5ml of Conc. HNO_3 in overnight condition. After completing of the overnight digestion the food samples were redigested in water bath at 60^0 C for 2 hours. After the water bath digestion the samples were allowed to cool at room temperature and 2ml of $HClO_4$ is added and then again heated at heating block at 160^0 C for 4-5 minutes until the white dense fumes of $HClO_4$ is not emitted. Now cool the sample and make final volume of 25ml with demineralized water. Filter the sample with Whatmann no 41. Now the sample is ready for the GF-AAS analysis.

3. Water sample preparation

50ml of water sample is taken into griffin beaker with 5ml of conc. HNO_3. Then the samples were covered with the watch glass and placed on the hot plate at 90-120^0 C and allowed it to continuously evaporate until it comes to 5ml, while evaporation sample should not boil. Now cool the sample and add another 5ml of conc. HNO_3 and cover the sample with watch glass and return into the hot plate until digestion is complete (3ml portion should be left). Allow the sample to cool at room temperature. Filter the sample with watt man no 41 to remove the silicate and other insoluble impurity. Now finally adjust the final volume to 50ml with 1% HNO_3. Now the sample is ready for the GF-AAS analysis.

4. GF-AAS analysis

A PerkinElmer Pin AAcle 900T flame and longitudinally Zeeman atomic absorption spectrometer was used for all measurement. Samples were automatically pipette into standard transversely heated graphite atomizer (THGA) tubes using AS900 auto sampler while single element electrode less discharge lamps (EDLs) were used for arsenic detection. For the detection of arsenic the required wavelength is 193.7 nm, slit 0.7 nm, Lamp type EDL, Lamp current 380mA. Argon is the normal gas which is used and the special gas type was 95% Ar/5% H_2 and was used in steps 1-3. Step 4 removed the H_2 gas from the tube before atomization and the read step was step 5 for all elements. All samples and standards used an injection volume of 20µl sample plus 5µl of matrix modifier. Each analyte was run twice.

Table 1: Furnace parameters for arsenic (AS)

Step	Temp (°C)	Ramp Time	Hold Time	Internal Flow	Gas Type
1	110	1	30	250	Special
2	130	15	30	250	Special
3	900	10	20	250	Special
4	900	1	15	250	Normal
5*	2300	0	5	0	Normal
6	2450	1	3	250	Normal

**Read step*

5. Chemicals used

- Nitric acid (concentrated) - 5 ml
- Perchloric acid – 2 ml

6. Calculation

Amount of samples taken (a) = 0.5g
Final makeup volume = 25ml
Dilution factor = b/a
= 25/.05
= 50
AAS reading = X
So, final arsenic concentration in food sample = X*50

7. Quality control

For the analytical quality control, the standards, the calibration and the correlation coefficient (at 0.999) were maintained during the analysis in the Atomic Absorption Spectrophotometer. The known standard concentration of arsenic was prepared from the standard arsenic stock solution (1000µg/l) procured from Perkin Elmer (CAS#:As 7440-38-2; Lot#; 20-85ASX1; PE #; N9300102), Singapore before the start of the assay of the field samples. The food arsenic detection limit was at 0.010µg/kg, while for water the detection limit was 0.04µg/l. hence all the standard norms were maintained during the analysis.

8. Statistical analysis

Data were analaysed with statistical software (Graphpad Prism 5) and values expressed as Mean ± SEM.

Results and Discussion

In the study vegetables were collected from different locations of Bihar. These vegetable samples were analysed by Graphite-Furnace Atomic Absorption Spectroscopy. The concentration of arsenic in different vegetables were found to be higher. Arsenic concentration is found to be higher where moisture content is higher. Arsenic ranging from 0.02µg/kg of vegetable samples to 586µg/kg. This exceeds the permissible limits of the BIS and WHO. Inner part and outer part of some vegetables like potato peas, brinjal, chilli, beans etc were analysed. According to analytical report inner part of vegetables have elevated graph were observed.

Ground water arsenic concentration in different regions of Bihar situated near Ganga river bank

A total of 100 water samples were collected from (from the rural areas of Buxar) from the hand tube wells. Samples were representative of the households use. Depth of the installed hand pumps was noted on the basis of information given by the majority of the communities. Depth ranged from 80 feet to 155 feet with mean value of 108.5 feet. (n=20). The ground water was founded highly contaminated with arsenic ranging from 4µg/l of as to 225µg/l. This exceeds the permissible limits of the BIS and WHO guidelines of 50µg/l and 10µg/l as respectively.

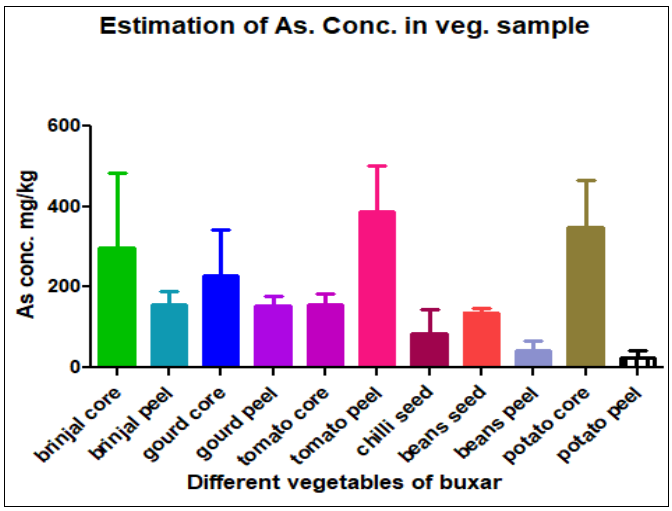

Fig 1: Estimation of different vegetable samples of Buxar.

In the (fig. 1) estimation of As. Concentration in different vegetable samples of Buxar graph has shown in which brinjal core shows As concentration 450µg/kg whereas brinjal peel is about 200µg/kg. Gourd core shows more than 350µg/kg while gourd peel shows 180µg/kg. Tomato core has As concentration 200µg/kg while in tomato peel 465µg/kg is estimated. Chili seed has about 160µg/kg. Beans seeds has about 200µg/kg while beans peel has As concentration 50µg/kg. Potato core contains As concentration 500µg/kg whereas potato peel has 10-20µg/kg. This results shows that there is much concentration of As is present in core part of vegetables where moisture content is higher.

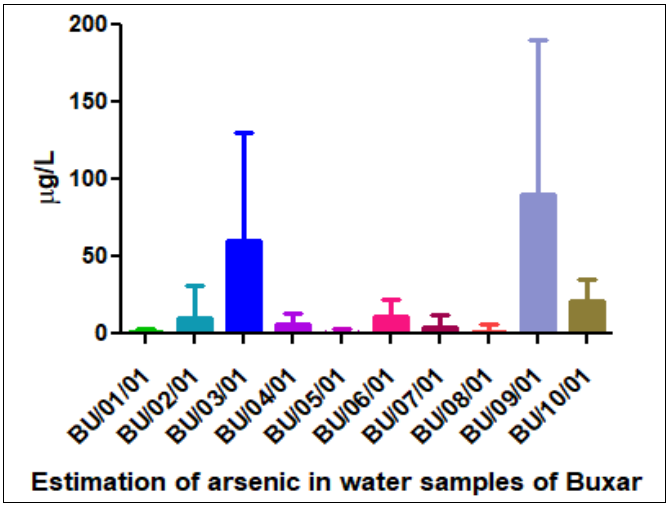

Fig 2: Graph showing arsenic concentration in water samples of Buxar.

In fig: 2, 10 water samples were taken in study from different panchayat of Buxar district. After statistically analysed the results came that samples taken from Bal Batra panchayat has 65µg/l As concentration while samples of Tilak rai kahatta has 80-85µg/kg of As concentration that is above the permissible limits of WHO which is 10µg/kg.

Effects of arsenic on human health

Arsenic toxicity and resulting health effects have bee associated with drinking and consuming of As- contaminated water and food (vegetables) containing As > 10µg/l and As > 10µg/kg respectively. (Base *et al*. 2014). Long term exposure to As in drinking water or chronic toxicity cause lung, skin and kidney cancers, pigmentation changes, neurological disorders, skin thickening (hyperkeratosis), loss of appetite, muscular weakness, nausea (Rahman *et al*; 2009) [25]. However acute poisoning causes Oesophageal and abdominal pain, vomiting and diarrhoea. Elevated levels of arsenic in drinking water can also cause an increase in miscarriages and spontaneous abortions (Smedley and Kinniburgh, 2002) [33].

Conclusion

Therefore, from the entire study, we conclude that arsenic contamination in drinking has become serious public health problem affecting more than 200 million people affected by it worldwide. More than a decade of exposure has led to the serious health related problems in the population like cancer. The entire study indicates that arsenic contamination in drinking water has exerted various deleterious health problems in the exposed population leading to skin manifestations like hyperkeratosis, melanosis, Bowen's disease. Furthermore, the other cancer incidences like lung cancer, bladder cancer, kidney cancer etc. has increased many folds in the population in the recent times which needs proper attention to the health department of the states. In the states like West Bengal and country like Bangladesh the incidences of cancer due to arsenic has decreased many folds. But, states like Bihar and Uttar Pradesh where huge population is presently exposed with arsenic poisoning needs the government support. Finally, awareness among the affected population related to safe drinking water sources should also be the prime vision of the stakeholders working in these regions.

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