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Assessment of toxic metals in groundwater and saliva in an arsenic affected area of West Bengal, India: A pilot scale study

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ABSTRACT

Communities in many parts of the world are unintentionally exposed to arsenic (As) and other toxic metals through ingestion of local drinking water and foods. The concentrations of individual toxic metals often exceed their guidelines in drinking water but the health risks associated with such multiple-metal exposures have yet to receive much attention. This study examines the co-occurrence of toxic metals in groundwater samples collected from As-rich areas of Nadia district, West Bengal, India. Arsenic in groundwater (range: 12–1064 μg L^{-1}; mean ± S.D: 329 ± 294 μg L^{-1}) was the most important contaminant with concentrations well above the WHO guideline of 10 μg L^{-1}. Another important toxic metal in the study area was manganese (Mn) with average concentration of 202 ± 153 μg L^{-1}, range of 18–604 μg L^{-1}. The average concentrations (μg L^{-1}) of other elements in groundwater were: Cr (5.6 ± 5.9), Mo (3.5 ± 2.1), Ni (8.3 ± 8.7), Pb (2.9 ± 1.3), Ba (119 ± 43), Zn (56 ± 40), Se (0.60 ± 0.33), U (0.50 ± 0.74). Saliva collected from the male participants of the area had mean concentrations of 6.3 ± 7.0 μg As L^{-1} (0.70–29 μg L^{-1}), 5.4 ± 5.5 μg Mn L^{-1} (0.69–22 μg L^{-1}), 2.6 ± 3.1 μg Ni L^{-1} (0.15–13 μg L^{-1}), 0.78 ± 1.0 μg Cr L^{-1} (< DL–5.9 μg L^{-1}), 0.94 ± 0.90 μg Pb L^{-1} (< DL–4.2 μg L^{-1}), 0.56 ± 0.37 μg Se L^{-1} (0.11–1.5 μg L^{-1}) and 194 ± 54 μg Zn L^{-1} (112–369 μg L^{-1}). The high concentrations of salivary As and Mn are believed to be indicative of intake from the groundwater. The clustering of salivary As and Mn in principal component analysis further indicated influence of the common exposure source. Zinc and selenium comprised a separate component presumably reflecting the local deficiencies in intakes of these essential elements from drinking water and foodstuff. Thus the study reveals that the concentration of other metals beside As must be monitored in drinking water before implementation of any policies to provide safe water to the affected communities.

1. Introduction

The groundwater of Bengal delta plain comprising of Bangladesh and West Bengal, India has drawn the attention of the scientific community all over the World due to the presence of arsenic (As) in groundwater at levels much above the WHO permissible limit of 10 μg L^{-1} (Bhattacharyya et al., 2003; Bhowmick et al., 2013b). The crisis in health of local communities stemming from the switch from surface water to groundwater for drinking, cooking and irrigation purposes has been well documented (Chowdhury et al., 2000; Guha Mazumder et al., 2010). The chronic intake of As is associated with a range of dermal, neural, cardiovascular, hematological, renal, endocrine and hepatic diseases along with increased risk of cancer of various organs (Rahman et al., 2001; Ahsan et al., 2006; Kapaj et al., 2006; Guha Mazumder et al., 2010). Among the health effects, skin lesions are the most common identifiable health outcomes in human (Haque

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et al., 2003; Ahsan et al., 2006). Under continued exposure, the skin-identifiable manifestations are generally characterized by changes in pigmentation (melanosis including diffuse and spotted) and/or keratosis (with thickening of skin) of varying severity (Ahsan et al., 2006; Rahman et al., 2006).

Groundwater in West Bengal is a complex mixture of various dissolved metals of different concentrations, and as a result, there is unintentional co-exposure of several contaminants to individuals (Frisbie et al., 2009; Bacquart et al., 2012). Friesbie et al. (2002) found elevated levels of manganese, lead, nickel and chromium along with arsenic in the groundwater of Bangladesh and stated that strategies should be implemented not only to provide As free water but also to eliminate other toxic elements that may accompany As poisoning. Thus the exposure from groundwater source is commonly in the form of multiple metals and considering the single-As exposure does not appropriately represent the health risk of a community (Berglund et al., 2011). Upon exposure, the combined effect of the metals may be different from the sum of the individual effects and can be synergistic or antagonistic of each other. As example, while dietary supplement of selenium has been shown to reduce the toxicity of arsenic (Wang et al., 2002; Christian et al., 2006), nickel acts synergistically with As to exaggerate lung cancer (Denkhaus and Salnikow, 2002). Thus while assessing the health risk of a community, apart from the individual contaminants, attention should also be paid to the combination of contaminants.

Human biomonitoring involves the study of biological fluids, tissues or other accessible structures as biomarkers of exposure to and effects of contaminants (Manno et al., 2010). There are numerous reports in the literature, where As in blood, urine, hair and nail has been used as a biomarker so as to assess the exposure of a community to As (Rahman et al., 2001; Shraim et. al. 2003; Marchiset–Ferlay et al., 2012, references therein). Recently, saliva has been shown to be a competent biological matrix that can be used as a biomarker of As toxicity in local communities (Yuan et al., 2008; Bhowmick et al., 2013a). The non-invasive and easy sample collection procedure along with unsophisticated requirement for sample storage are among the advantages of saliva especially for screening large populations, involving children and patients with limited coping abilities. Therefore, although there is increasing evidence that support the use of saliva for biological monitoring, until now, we have limited understanding of the concentration of various metals in saliva of a population that are exposed to a range of toxic metals (Gil et al., 2011).

The primary aim of the present study was to monitor a suite of toxic metals in ground water with respect to the WHO standard and examine saliva as a representative biological matrix for assessing multiple metal exposures in a rural population of West Bengal, India. To achieve this, we measured the As concentration along with other metals (Mn, Cr, Mo, Ni, Pb, Ba, Zn, Se, U) in groundwater of the area and also analyzed the saliva samples for the metals that were important from the exposure point-of-view. The present study is designed to provide baseline value for future work, where saliva will be used as an easily available biological fluid to monitor the multi-metal exposure for a community.

2. Materials and methods

2.1. Study area and sample collection

Nadia is one of the districts of West Bengal, India where the arsenic concentration in groundwater is above the WHO guideline ($10\ \mu g\ L^{-1}$) (Guha Mazumder et al., 2010). The study areas, Debagram and Chhoto-Itna are neighboring villages which are located in the northern part of the district (Fig. 1). The details of the study

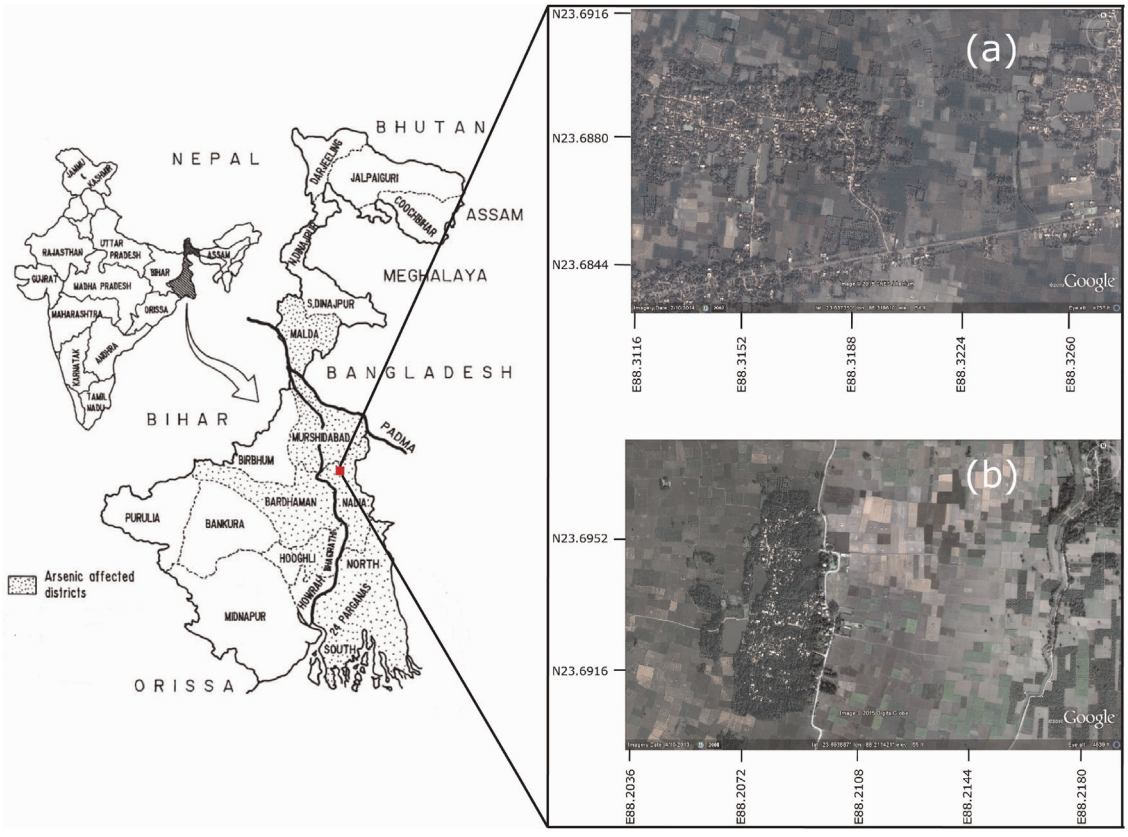

Fig. 1. Map of the study area. The sampling areas are marked with the red circle. The map also shows the Google Earth image of the study areas; (a) Debagram and (b) Chhoto-Itna. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

area and the characteristic geomorphic and geologic features have already been reported in our earlier study (Bhowmick et al., 2013b). Fifty groundwater samples from tubewells were collected randomly so as to have a representative groundwater dataset for the area. The tubewells were selected from the residential areas and are used by the inhabitants for household purposes, such as cooking, cleaning of foods items and vegetables, washing, bathing etc. Before sample collection, tubewells (hand operated) were manually pumped for several times to discharge the standing volume of groundwater and the fresh water was collected in pre-cleaned acid washed polyethylene (PE) bottles. All the water samples were acidified with conc. HNO_3 on-spot ($pH < 1$), so as to prevent the precipitation of dissolved iron and preserve the trace metals in dissolved state. The samples after return to laboratory were stored at 4 °C, until further analysis. For on-site As speciation, 50 mL of the groundwater samples were passed through an ion-exchange cartridge (Metal Soft Center, Highland Park, USA) where As(III) is eluted, while As (V) are adsorbed onto the resin. The eluent was acidified with HNO_3 and measured for As(III). As (V) was obtained from the differences in concentrations of total As and As(III).

Fifty saliva samples were collected from the male residents of the area. For this study, male participants were only considered so as to minimize the gender differences. Written consent was obtained from the participants and the content was explained to each participant in the local language of Bengali. The recruited participants were between 18 and 65 years of age and have shared the same domicile for a minimum of 10 years prior to the interview. The participants washed their mouths with Milli-Q water and discarded the few mL of saliva that was formed immediately. After 2–3 min, the saliva sample was collected in 15 mL LDPE bottles. Each saliva sample collection took approximately 5 min so as to yield 5 mL of sample, for the minimum sample requirement of 3 mL. We asked the participants not to eat or drink for 1 h prior to saliva sample collection, so as to avoid food particulate contamination. Following saliva collection, the samples were stored and transported in salt-ice mixture and were later stored at −20 °C, until analysis. The details of the sample collection procedure have been described in our previous publication (Bhowmick et al., 2013a, 2014).

Individual ethical committees of University of Kalyani, University of Michigan and DNGM research foundation approved the study on the Ethics of Research on Human Beings.

2.2. Sample analysis

The cations and trace elements in water samples were analyzed using ICP-AES (Perkin Elmer 3300 DV optima). Hydride generation Atomic Absorption spectroscopy (HG-AAS; Varian, AA220) was used for the analysis of As concentration in water samples following the manufacturer's instruction. For quality control, standard reference material for drinking water (SRM 1643e) from National Institute of Standards and Technology (NIST) and laboratory blanks was included during the analysis.

Analysis of arsenic in saliva samples has already been validated in our previous study (Bhowmick et al., 2013a). We used the same method with slight modification to measure the elemental composition in saliva samples. In brief, saliva samples were brought to room temperature and were centrifuged. To 1 mL of the sample, appropriate amount of HNO_3 and Bruker "Internal Standard" solution was added so as to make a final concentration of 2% v/v and 1 $\mu g\ L^{-1}$ standard respectively. The solution was finally diluted to 3 mL with Milli-Q water and was measured for trace elements using ICP-MS (Bruker, Aurora Elite) in normal (Pb), helium (As, Cr, Mn, Ni, Zn) and hydrogen mode (Se). The use of saliva for measuring the exposure of a community is new and currently, there is no SRM for saliva. Thus in absence of SRM, percentage recovery of the element was used for the validation of our method. Six different saliva samples were spiked with various concentrations of As, Mn, Ni, Cr, Pb, Zn and Se so as to account for the sample matrix. Our results show good recoveries of the elements with percentage ranging from 98.26% to 105.2%.

The data was checked for reproducibility ($\pm$ 0.2–0.4%) through frequently run laboratory standards. Three times the standard deviation for reagent blank was attributed the detection limit for each element.

2.3. Statistical analysis

Software package SPSS (IBM, version 17) was used for all statistical analyses. The salivary elemental concentration was skewed and was Log transformed before statistical analysis. The inter-elemental relationship was determined by Pearson Correlation. Principal Component analysis (PCA) was used as a measure for the association among metals. A p-value < 0.05 was considered significant. 50% of detection limit was assigned an arbitrary value for the samples with concentration below the detection limit.

3. Results and discussion

3.1. Groundwater portability and health

All the groundwater samples were analyzed for toxic elements (As, Mn, Cr, Mo, Ni, Pb, Ba, Zn, Se, U) and the results are summarized in Table 1. The range of toxic elements is compared with

Table 1
The concentrations of toxic metals in groundwater samples and the respective WHO health-based drinking water guidelines.

Analyte	Detection limit (DL) ($\mu g\ L^{-1}$)	Max ($\mu g\ L^{-1}$)	Min ($\mu g\ L^{-1}$)	Mean ($\mu g\ L^{-1}$)	SD	WHO guideline ($\mu g\ L^{-1}$)	% of sample exceeding WHO guidelines
As(T)	0.33	1064	12	329	294	10	100
As(III)	0.33	862	7.0	266	256	–	NA
As(V)	–	234	5.0	64	45.2	–	NA
Mn	0.04	604	18	202	153	–	NA
Cr	0.31	38	BDL	5.6	5.9	50	0
Mo	0.73	11	BDL	3.5	2.1	–	NA
Ni	0.52	48	1.3	8.3	8.7	70	0
Pb	0.51	5.2	BDL	2.9	1.3	10	0
Ba	0.04	197	32	119	43	700	0
Zn	0.29	159	15	56	40	–	NA
Se	0.11	1.7	BDL	0.60	0.30	40	0
U	0.12	3.4	BDL	0.50	0.70	30	0

BDL: Below Detection Limit.
NA: Not Applicable.

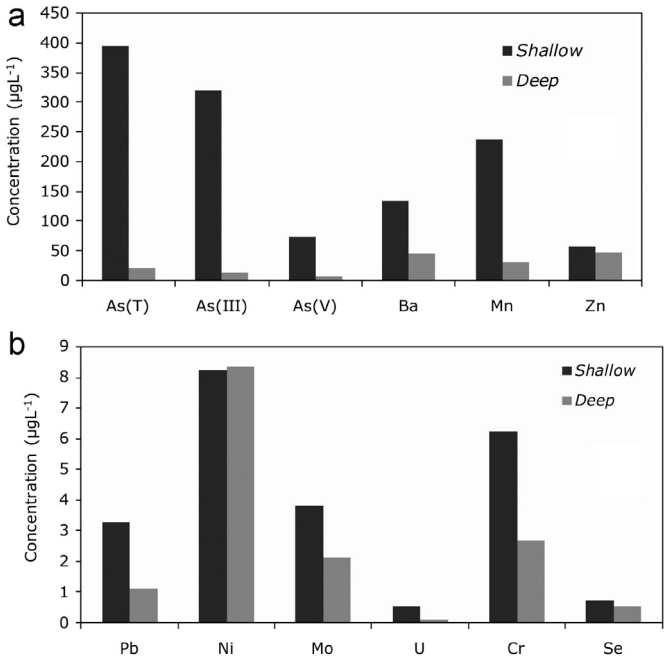

Fig. 2. Concentration of (a) trace and (b) ultra-trace metals in shallow and deeper groundwater.

the WHO drinking water guidelines (WHO, 2004a, 2011a), thereby giving an indication of the safe/unsafe levels of the metal composition which may potentially be of health concern.

The results indicate that the presence of As in groundwater of our study area are of prime concern with all the sampled groundwater exceeding the WHO drinking water guideline value ($< 10\ \mu g\ L^{-1}$), both in the shallow wells (< 100 m) as well as in the deeper wells (> 100 m). Chronic As poisoning from drinking As rich groundwater is the most significant health problems of these communities (Haque et al., 2003; Guha Mazumder et al., 2010). A common practice among the villagers is the use of water from deeper tubewells for their source of domestic water supply (drinking, cooking, bathing, washing etc.) as it is considered to be safe (Nath et al., 2007). However this study showed that the water from the deeper tubewells can have As concentration greater than the WHO guideline value of $10\ \mu g\ L^{-1}$ (Fig. 2a) and therefore needs to be tested, before declaring it as a safe source of water supply with regards to As. It should be noted that there remains considerable uncertainties over the actual risk from drinking water with As concentration within the WHO drinking water guideline ($< 10\ \mu g\ L^{-1}$). In fact, the current guideline for As is based on male lifetime skin cancer risk in the order of 6×10^{-4}, which is obviously much higher value (1×10^{-5}) than that generally used to protect public health (WHO, 1996). Although $0.17\ \mu g\ L^{-1}$ was suggested as a guideline for As concentration in drinking water by WHO, a less rigorous guideline of $10\ \mu g\ L^{-1}$ was set owing to such range of detection limits for most routine laboratories (WHO, 1993). WHO experienced that the available data on mode of action do not provide a biological basis for using extrapolation; yet, a choice overestimation has been set due to practical quantification limit and therefore there exists significant uncertainties surrounding the risk assessment for As carcinogenicity (WHO, 2004a). Until now, the drinking water guidelines for As set up by WHO and followed by various environmental and health research studies is based on the quantification of the total As concentration without giving due consideration to the arsenic species. It is well known that the health effect due to arsenic is dependent upon its species and each species exerts different orders of toxicity when compared with each other. For instance, As(III) is labile in environment and are more cytotoxic compared to their pentavalent analogue (Cullen and Reimer, 1989; Styblo et al., 2000). Thus if we are to proportionate the risk of arsenic toxicity from drinking contaminated groundwater with respect to the arsenic species, the WHO guideline value for As ($< 10\ \mu g\ L^{-1}$) may not be sufficiently low enough in this part of the globe as the groundwater often contains more As(III) than As(V) (Nath et al., 2007; Bhowmick et al., 2013b) (Fig. 2a).

The WHO drinking water guideline value for As ($< 10\ \mu g\ L^{-1}$) is provisional and purported to prevent or at least decrease the occurrence of adverse health effects after consumption of water is now debatable and the role of the other toxic metals present in the groundwater needs further attention. In our study area, there has been an increase in As awareness among the people due to the widespread visibly detected arsenic induced skin lesion and also of the extensive health based research that has taken place over the past decades involving the residents (Guha Mazumder et al., 2010). As a result, the communities are now mostly relying on the safe municipal water supply for drinking purpose. Nevertheless, the skin lesions are still prevalent and have been attributed to additional As exposure routes, mainly through rice consumption (Chatterjee et al., 2010; Halder et al., 2013). However our field experience shows that the water from the tubewells are still being used for daily activities (cooking, washing) and also, people occasionally consume water from tubewells, specially the farmers, who work for long hours in the agricultural fields. Thus the role of few other toxic metals is worthy to investigate so as to minimize the health risk of the residents from drinking As-contaminated (or even safe) groundwater.

Similar to As, Mn concentrations were high in the groundwater (Table 1), mostly in the shallow aquifers (Fig. 2a), where the aquifers are under reducing condition (Nath et al., 2007; Chatterjee et al., 2010; Bhowmick et al., 2013b). The WHO health based guideline value for Mn is currently being discontinued from the former value of $400\ \mu g\ L^{-1}$ (WHO, 2011a). In the present study, Mn concentration in the groundwater often exceeds the previous WHO standard value and a high value up to $604\ \mu g\ L^{-1}$ has been found (Table 1). Mn is known to cause neurological effects, mainly hallucinations, tremors like Parkinson's disease, balance problem in adults (Aschner et al., 2007) and also effect the intellectual function of children (Wasserman et al., 2006; Bouchard et al., 2011). The infants are more prone to manifest the clinical symptoms of Mn poisoning as children tend to accumulate Mn in their brain (Howe et al., 2004). For a median Mn concentration of $34\ \mu g\ L^{-1}$ in groundwater (range 1–2700 $\mu g\ L^{-1}$), Bouchard et al. (2011) found a decrease of 2.4 IQ point in school-age children for a10-fold increase in manganese concentration in water. In another such population based study, Wasserman et al. (2006) also found decreased intellectual function in 10 year old Bangladeshi children that were exposed to high concentration of Mn in drinking water, even when the As co-exposure was controlled. Similar environmental condition also persists in our study area where there is co-occurrence of high levels of Mn along with As in groundwater that is often being used for drinking and other domestic purposes. The earlier WHO drinking water guideline value of $400\ \mu g$ Mn L^{-1} established in 2004 did not deliberate at the recent findings showing adverse neurological effects in infants and thus, warrant that the guideline should be re-introduced and needs to be revised downwards.

The WHO guidelines for Cr, Mo, Ni and Pb in drinking water are listed in Table 1. The mean values for all the elements analyzed in groundwater of our study area were below the guideline values except Mo, where there is no guideline value as per latest WHO edition (WHO, 2011a). It has been found that the shallow aquifers are mostly more prone to contamination from these toxic trace elements than deeper aquifers (Fig. 2b). The WHO revised the

guideline value for Pb to 10 μg L^{-1} in drinking water based on the toxicological evidence of human infants (WHO, 1996). No samples exceeded this guideline value for both shallow and deeper aquifers. Similarly, Ba was also found well below the WHO recommended value (Table 1). Although U has often been found in concentration greater than the WHO limit of 30 μg L^{-1} in neighboring country of Bangladesh with similar groundwater hydrological features (Frisbie et al., 2002, 2009), this toxic element has not been detected in the groundwater of West Bengal (Bacquart et al., 2012). Our results also showed a low concentration of U in the groundwater (mean: 0.50 ± 0.74 μg L^{-1}) with a maximum of 3.4 μg L^{-1}, and thereby are in agreement with the previous finding.

The Zn and Se are essential trace elements and often found in food and portable water. The Zn concentration in groundwater of our studied area ranged from 15–159 μg L^{-1}, with a mean concentration of 56 μg L^{-1} (Table 1). In adult humans, the daily requirement for Zn is 15–22 mg day^{-1} while the provisional maximum tolerable daily intake is 1.0 mg kg^{-1} of body weight. On this basis, WHO concluded, that for Zn, "the derivation of a health-based guideline value is not required at this time" (WHO, 2004a, 2011a). On the contrary, WHO has recently suggested an increase in the guideline values for Se in drinking water from its former 10 μg L^{-1} to 40 μg L^{-1} (WHO, 2011a, 2011b). Se also had a low concentration in our groundwater with a mean Se concentration < 1 μg L^{-1} (Table 1). The use of such characteristic groundwater for irrigation purposes has resulted in the low concentration of Se and Zn in the soils and locally grown crops and eventually, small amount of these metals are being ingested through diet (Roychowdhury et al., 2002; Roychowdhury et al., 2003; Ortega et al., 2003). Zn, an essential element for DNA repair has been shown to reduce the arsenic-induced oxidative stress and liver damage in rats when co-administered with As (Kumar et al., 2010). Thus the exposure of Zn can act in favor for the residents in any As *"hot-spot"* areas and can ameliorate the As poisoning to a certain extent. Selenium is also known to antagonize the effect of As and the As detoxification process takes place through formation of biologically inert conjugates [seleno-bis(*S*–glutathionyl)arsinium ion] along with selenium-dependent antioxidant enzymes (Chen et al., 2007; Zwolak and Zaporowska, 2012). Higher concentration of Se in the blood samples were related to the reduced risk of As-related skin lesion in a case-cohort study in Bangladesh (Chen et al., 2007). Thus the deficit of Zn and Se in groundwater and finally diet for our studied community may potentially magnify the toxicity of As.

3.2. Elemental composition in saliva

Saliva, secreted in the salivary glands including parotid, submandibular, and sublingual glands, represents a simple matrix and typically mirrors the constituent of the extracellular fluid. Water, chemicals and their metabolites from plasma are transported to the salivary glands via several mechanism including active transport, passive diffusion and ultrafiltration and thus, provide additional means for assessment of the exposure of toxic substances in humans (Esteban and Castaño, 2009). Saliva being physiologically and biochemically heterogeneous in nature (Wang et al., 2008), there is actually a scarcity of report for the elemental concentrations in saliva for a general population. Nevertheless, the whole saliva collected in our study overcomes the variation in composition from the influence of various glands that secretes saliva (Esteban and Castaño, 2009). The concentration of various elements detected in saliva for our studied population is shown in Table 2. Our results are compared with those reported in the scientific literature that have detected metals in the same matrix (saliva) and are shown in Table 3. Although the concentration of the elements in such biological samples may vary due to differences in environmental, dietary and occupational factors, such assessment helps us to understand the nature of elemental excretion compared with other differentially exposed communities. For example, Wang et al. (2012) showed an increase in salivary As with increase in arsenic concentration of drinking water. Similar findings were also reported by Yuan et al. (2008) where a mean salivary As concentration of 11.9 μg L^{-1} was found for the population of Inner Mongolia that was exposed to high concentration of As while a concentration of 0.79 μg L^{-1} As was determined in saliva of the population of Edmonton, Canada consuming drinking water with As content below 5 μg L^{-1}. Our measured salivary As concentration was in-between these ranges as the participants have shifted their drinking water sources from tubewells to municipal water supply; although they are occasionally exposed to high As concentration in groundwater (Guha Mazumder et al., 2010). Moreover there are numerous reports which show increasing risk of As consumption from rice and other foods locally irrigated with As contaminated groundwater (Roychowdhury et al., 2002, 2003; Chatterjee et al., 2010; Halder et al., 2013). Thus the exposure of As is complex and there are wide number of components that actually contributes to such exposure, resulting in the reflection on the concentration of salivary As (Bhowmick et al., 2013a, 2014). Similar situation also exists for Mn, with the groundwater of our study area containing elevated levels of this element along with As (Table 1). The recommended daily intake of Mn for adult is about 2 mg, with dietary supplement being the major source for the element while water contributes approximately 20% (WHO, 2004b). Considering an average daily water consumption of 2 L for the participants in our study area, the amount of Mn ingested solely through water ranges from a small fraction to more than half of the adequate daily intake. The Mn intake from water may further be elevated as the villagers tend to drink more than 2 L water per day due to the hot and humid climate. Moreover, rice has significantly high concentration of Mn (Pennington and Young 1991) and thus for a population with rice based diet, similar to our study area, the diet is susceptible to have high amount of Mn. Additionally, due to the use of Mn-rich groundwater for irrigation and high Mn content in the soil (Sankar et al., 2014), the locally grown crops tends to have a high concentration of Mn, thereby further increasing the Mn exposure of the community. In one such study, Roychowdhury et al. (2003) investigated the concentration of As and other heavy metals in food and drinking water in an As affected community close to that of our study area. The report estimated a daily dietary intake of more than 8000 μg Mn per day for adults and more than 4500 μg Mn per day for children with 10 years of age. Thus the manifestation of such high Mn exposure was seen in the salivary Mn and our results are in agreement with the other communities occupationally exposed to Mn (Table 3). Presently there is lack of suitable biomarker for biomonitoring Mn exposure (Zheng et al., 2011). Wang et al. (2008) studied the Mn

Table 2
Metal levels in 50 saliva samples from the study population.

Element	Detection limit (DL) (μg L^{-1})	Mean ± SD (μg L^{-1})	Range (μg L^{-1})	Median (μg L^{-1})	Geometric mean (μg L^{-1})
As	0.018	6.3 ± 7.0	29–0.70	4.1	4.1
Mn	0.022	5.4 ± 5.5	22–0.69	2.6	3.1
Ni	0.073	2.6 ± 3.1	13–0.15	1.12	1.4
Cr	0.012	0.78 ± 1.0	5.9 – BDL	0.47	0.48
Pb	0.031	0.94 ± 0.90	4.2 – BDL	0.60	0.60
Se	0.091	0.56 ± 0.37	1.5–0.11	0.52	0.44
Zn	2.1	194 ± 54	369–112	182	188

BDL: Below Detection Limit.

Table 3
Concentration of metal ions in saliva reported in literature (the concentration are in $\mu g\ L^{-1}$, unless specified).

n	As	Mn	Pb	Ni	Cr	Zn	Se	Reference	Study population
32	0.91							Yuan et al. (2008)	Volunteers from Canada with drinking water As concentration $< 5\ \mu g\ L^{-1}$
301	12.5								People from Inner Mongolia exposed to varying concentrations of As
969			2.40 ± 0.13					Nriagu et al. (2006)	Low-income population in Detroit, Michigan
153–178		3.68	0.42	1.72	0.64			Gil et al. (2011)	Individuals working in the iron and steel Industry (median value)
242							4.62–0.05	Benson (2013)	Primary school children's
100				4.45 ± 2.87	0.76 ± 1.43			Agaoglu et al. (2001)	Unexposed group
26							3.1 ± 0.2	Hadjimarkos and Shearer, (1971)	Children attending school
17				18 ± 11	20 ± 11			Eliades et al. (2003)	Unexposed group
21	5.54 ± 1.06							Wang et al. (2012)	Individuals with As $< 50\ \mu g\ L^{-1}$ in drinking water
22	13.23 ± 1.98								Individuals with 50–200 $\mu g\ L^{-1}$ concentration in drinking water
27	27.93 ± 2.69								Individuals with As $> 200\ \mu g\ L^{-1}$ in drinking water
33		3.04 ± 1.40	25.5 ± 14.4			260 ± 132		Wang et al. (2008)	Unexposed group
49		4.45 ± 2.17	24.9 ± 11.8			191 ± 107			Welders
78	1.1 ± 2.1							Lew et al. (2010)	Children playing in CCA playground
47	1.4 ± 1.1								Children playing in non-CCA playground
88			0.05–4.40					Barbosa et al. (2006)	Population living near contaminated area
10		2.94 ± 2.82	0.08 ± 0.037			13.5 ± 12.2		Kim et al. (2010)	Non smoker
30				0.5–2.0				Burguera et al. (1998)	Unexposed group
40	3.29 ± 0.69							Hinhumpatch et al. (2013)	Children's that has been exposed to arsenic in utero
20	0.84 ± 0.11								Unexposed children's
444			0.10–17.2					Costa de Almeida et al. (2010)	Children attending school
18				13–96				Petoumenou et al. (2009)	Patients before orthodontic therapy
45				1.16 ± 0.28 μg/mL	2.2 ± 1.3 μg/mL			Kocadereli et al. (2000)	Unexposed group
249		0.11 nmol/L				0.64 ± 0.39 mg/L		Benson (2011)	School children
20							2.3 ± 1.2 ng/mL	Hojo (1987)	Male
10						411.30 ± 152.24		Ayinampudi and Narsimhan (2012)	Patients with oral cancer
15						284.26 ± 124.64			Patients with premalignant oral lesions
50	4.11	2.63	0.60	1.12	0.47	182	0.52	Present study	Resident living in As-rich area (median value)

concentration in saliva of Mn-exposed carrier welders and found that salivary Mn concentration has the potential of reflecting the airborne exposure of Mn from work sites. Our result also hints to such notion that saliva can be used for such biomonitoring purpose, however more focused research should be carried out.

Nriagu et al. (2006) found an average concentration of $2.4 \pm 0.13\ \mu g\ L^{-1}$ of Pb in saliva in non-occupationally exposed African-American living in Detroit. In another study, Costa de Almeida et al. (2010) assessed the Pb exposure of 444 school children in Brazil and reported a salivary Pb concentration range of 0.1–17 $\mu g\ L^{-1}$, with a median of 1.7 $\mu g\ L^{-1}$. Studies have usually reported low concentrations of Pb in saliva for unexposed community and our results are consistent to that previously published data (Table 3). Ni and Cr are probably the most investigated elements in saliva and studies have reported large variations in the Cr and Ni contents of saliva, particularly in patients with orthodontic application (Table 3). While some studies have shown an increase in the metal concentration in saliva of orthodontic patients (Agaoglu et al., 2001), others have reported no significant differences in the Ni and Cr concentration of saliva between subjects with and without fixed orthodontic applications (Kocadereli et al., 2000). However since such type of patients were not recruited in our study, the interferences were possibly eliminated. Our measured concentrations for these metals fall on the lower end of these ranges for both metals and are close to values reported by Gil et al. (2011). The low concentration of Pb, Cr and Ni in saliva may therefore be related with the safe levels of these metals in groundwater of our study area (Table 1). As expected, due to low levels of Se in water and foods in this part of the World (Frisbie et al., 2009; Roychowdhury et al., 2003), the concentration of salivary Se of our studied population was low compared to others (Table 3). Zn, on the other hand, is one of the primary elements that is associated with enzymes and thus are generally present in higher ranges in biological tissues and fluids compared to the other trace elements reported here. Our measured concentration of Zn in saliva ranged from 112 to 369 $\mu g\ L^{-1}$; with a median value of 182 $\mu g\ L^{-1}$. A comparison with other studies revealed that the Zn concentration was lower (Table 3) and can possibly be attributed to deficiencies in exposure of this metal via food and drinking water (Frisbie et al., 2002; Ortega et al., 2003; Frisbie et al., 2009).

Table 4 shows the inter-elemental correlations in saliva using bivariate model. The most significant correlation was obtained for As and Mn ($r=0.77$; $p<0.01$), while there are a number of element pair with small but significant correlation including As and Se ($r=-0.28$; $p<0.05$); Se and Zn ($r=0.35$; $r<0.01$); Zn and Pb ($r=-0.24$; $r<0.05$); Pb and Cr ($r=0.28$; $r<0.05$) (Table 4). The

Table 4
Pearson correlation coefficient between elements in saliva samples.

		As	Mn	Ni	Zn	Se	Pb	Cr
As	*r*	1	.77**	.11	−.13	−.28*	.22	.01
	p – value		.00	.22	.19	.02	.06	.48
Mn	*r*		1	.13	−.12	−.22	.20	.07
	p – value			.18	.21	.07	.09	.33
Ni	*r*			1	−.02	−.12	.15	.05
	p – value				.44	.21	.16	.38
Zn	*r*				1	.35**	−.25*	−.14
	p – value					.01	.04	.17
Se	*r*					1	−.11	−.09
	p – value						.22	.26
Pb	*r*						1	.28*
	p – value							.02
Cr	*r*							1
	p – value							

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

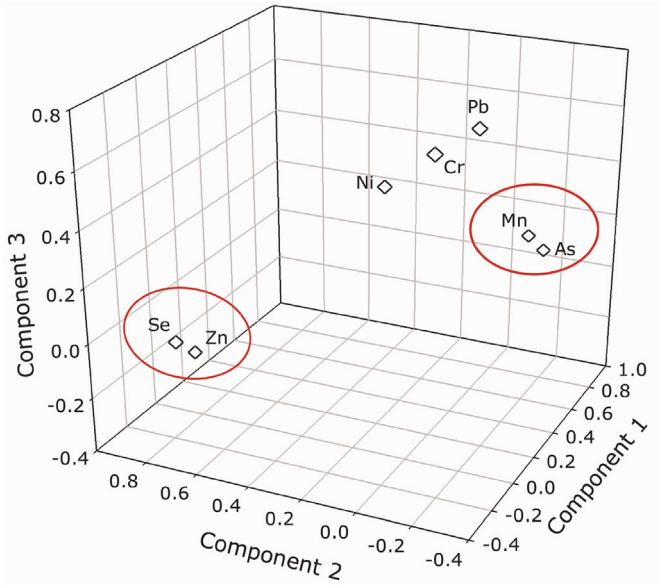

Fig. 3. Principal component analyses of the salivary elements.

extensive interaction of the elements in the human metabolic cycle is evident from the wide interdependency of element in saliva (Table 4) thereby, resulting as an important determinant of their concentration in saliva. However the bivariate model only considers the relationship among the pairs of metals and does not consider the presence of other elements. Thus to investigate the interaction among all the elements in saliva, the data was subjected to Principal Component Analysis (PCA) so as to examine the existence of cluster or groups within the data. The analysis shows that the first Eigen value estimated 32% of the variance from the matrix, while 65% of the variability was explained from the three Eigen value. An evaluation of the Principal Component (PC) space shows two distinct grouping: (i) As and Mn, and (ii) Se and Zn (Fig. 3). The clustering of As and Mn was in concordance with the high concentration of these elements in the groundwater and inevitable exposure from drinking water and locally irrigated foodstuff (Roychowdhury et al., 2002, 2003; Chatterjee et al., 2010; Halder et al., 2013). Food is the main source of dietary Zn and Se supplement. Unfortunately, the drinking water, crops and ultimately diet in this part of the globe (West Bengal, India and Bangladesh) are deficit in Se and Zn (Frisbie et al., 2002; Roychowdhury et al., 2002, 2003). Thus the scarcity of Se and Zn exposure in the residents of our study area is presumably being reflected in the clustering of these elements (Fig. 3). On the other hand, Pb, Ni and Cr are widely scattered in PC space which thereby indicates that the source-exposure route for these elements maybe different from the rest of the elements investigated in this study (As, Mn, Se and Zn).

4. Conclusion

Human exposure to As from drinking water and locally irrigated food of West Bengal, India is a major public health problem. During the last few decades, extensive research has been carried out on high As concentration in groundwater and the associated health effects. Recently, there has been an increasing concern across the globe about the co-occurrence of other metals with arsenic that can change the overall health risk. The present study that was carried out in one of the arsenic-affected areas of Nadia district, West Bengal, India addresses this issue. Analyses of the groundwater metal concentration showed elevated levels of As

with concentrations much greater than the WHO guideline of 10 μg L^{-1}. Although currently, there is no WHO guideline for Mn in drinking water and the earlier value of 400 μg L^{-1} has been withdrawn, our results show that the rather high concentration of Mn in groundwater can potentially cause adverse health effect. The high concentrations of these elements have also been reflected in the collected saliva samples where the salivary content of As and Mn are relatively high when compared to what have been reported in other communities. The significant negative associations between As and Se are interesting in suggesting that deficiencies in Zn and Se may be mediating the effects of arsenic exposure in a positive way. Thus our results implicates saliva as a biological fluid has the potential of reflecting the exposure of toxic metals and can potentially be used for biomonitoring purpose. The study further calls attention to the importance of multiple metal measurements in drinking water in an arsenic affected areas and a more careful evaluation on the interactions of several elements with As for the better understanding of arsenic toxicity.

Competing interests declaration

The authors do not have any competing interests to declare.

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