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Arsenic exposure on bovine health and environmental pollution with special emphasis on groundwater system in Manipur

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ABSTRACT

There was high arsenic content in soil, drinking water and grass in Manipur suggestive of environmental pollution by the arsenic contaminant, which was due to groundwater containing arsenic more than the permissible limit. The cattle of 2 zones in Manipur were selected for the present study, i.e. Kakching block in Thoubal district as exposed zone and Kamjong block in Ukhrul district as control zone. The arsenic content in faeces, urine, milk, hair and serum in cattle of exposed zone were higher compared to control zone. High concentration of arsenic in milk indicated the presence of arsenic in food-chain. It suggests the testing of milk and hair for arsenic concentration that could be considered as biomarker to assess the arsenic exposure.

Key words: Arsenic, Biomarker, Cattle, Environment, Food-chain, Groundwater contamination

Contamination of groundwater with arsenic is one of the serious problems encountered all over the world. The World Health Organization set a provisional level of 0.01 ppm for arsenic (WHO 1993) in drinking water, however in India, 0.05 ppm is commonly adopted as the guideline values (Morales *et al.* 2000). At present, 21 countries in different parts of the world like USA, Afghanistan, Bangladesh, India, Cambodia, Canada, Hungary, China, Chile, Argentina, Japan, Mexico, Mongolia, Myanmar, Nepal, Pakistan, Poland, Taiwan, Thailand and Viet Nam have reported high levels of arsenic in groundwater resources (Biswas *et al.* 1998). In India, excess level of arsenic in drinking water was found in samples from 51 villages in 3 arsenic affected districts of Uttar Pradesh, 202 villages in 6 districts of Bihar, 11 villages in 1 district of Jharkhand, 3500 villages in 9 districts (of a total 19) of West Bengal, 17 villages in 2 districts of Asom and 2000 villages in 50 districts (of a total of 64) of Bangladesh also (Sengupta *et al*. 2003).

Arsenic is eliminated by many routes (e.g. faeces, urine, sweat, milk, skin and lung), although most of it is excreted through urine in humans (Klaassen 2006). Human health can be affected through intake of milk of dairy animals reared in arsenic prone zone where the arsenic concentration in water and cattle feed is much higher than the permissible limit of 0.05 ppm (USEPA 1973). This paper reports arsenic concentration in water and substrates like soil and grass, excretory products of cattle like faeces and urine; secretory product like milk; serum and hair as well as seasonal influence on the concentration of arsenic in Manipur, India.

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MATERIALS AND METHODS

Two zones in Manipur were selected for the present study, i.e. Kakching block in Thoubal district as exposed zone, and Kamjong block in Ukhrul district as control zone. This was also supported by the report of Chakraborti *et al.* (2008) who recorded arsenic contents in groundwater more than the permissible limit in 4 districts, out of 9 districts in Manipur. The study was conducted from October 2007 to September 2008. Samples, 20 each, of soil, drinking water and grass were collected in 3 batches, viz. winter (October–January), summer (February–May) and monsoon (June–September) both from exposed and control zone. At the same seasons, 20 samples of each were collected from excretory materials of cattle staying in the same environment such as faeces, urine; and serum and hair.

Selection of animals: Milch cows (20) from the exposed zone were selected randomly and considered for the study throughout the year. All the animals were in between 6 and 10 years of age and the duration of exposure was between 4

and 6 years. The animals were fed with straw and grass and were provided with tubewell water. Apparently healthy milch cows (20) were selected from the Kamjong block in Ukhrul district, which is non-arsenic prone zone (Chakraborti *et al.* 2008). All the selected animals subjected to the study were thoroughly examined clinically for the detection of any disease condition or any characteristic sign of arsenic exposure. Faecal and blood samples were also examined for any abnormalities and the presence of parasitic ova/oocyst or haemoprotozoan parasite in blood. If the samples were found positive for oocyst or ova, deworming was administered to reduce the parasitic load. The apparently healthy animals of control group were also screened similarly to detect abnormality if any.

Collection of samples: The soil samples for analysis were collected from the grazing field in Kakching block of Thoubal district in Manipur. Each soil sample thus collected was placed in a double walled cloth or paper bag. The water samples were collected in plastic bottles previously rinsed with 20% nitric acid and deionised water and were preserved with 4 ml of concentrated HCl per liter (Das and Dutta 1991). The grass samples were collected from the feeding troughs. Milk samples were obtained during milking and preserved at –20°C. Urine samples were collected and preserved with HCl (1: 100) and stored at –20°C. Faecal and hair samples were collected in clean polythene bag separately (Sarder 2004). Blood samples were collected from each animal for arsenic estimation in serum.

Sample preparation for arsenic estimation: The bulk soil samples were digested in double acid mixture for analysis of arsenic. Samples of 5 ml of drinking water were digested with 3 ml of HNO_3 at 180–200ºC. An amount of 0.5 g of dried grass samples and faecal samples were digested with 8 ml tri-acid mixture at 180–200ºC for 1–2 h. Samples of 1 ml milk and urine were processed with 5 ml of tri-acid mixture. Samples of 2 ml of serum were digested with 8 ml of tri-acid mixture (180–200ºC for 2 h). The hair samples were cleaned with acetone and distilled water before digestion and dried at 60ºC for 1 h in hot air oven. Samples of 0.2 g were heated (180–200ºC) with 5 ml of tri-acid mixture (Sarder 2004). All the digested samples were transferred to 10 ml volumetric flask and the total volume was made up to 10 ml with triple distilled water which then filtered. All the filtrates were used for the estimation of arsenic by commercial kit. An arsenic cathode lamp with slit 0.5 nm and wave length 193.7 nm was used as a light source. Aqueous solution of 0.6% sodium borohydrate in 0.4% NaOH and 66% hydrochloric acid as a carrier solution were used to reduce the analyte to its hydride form. The arsenic stock standard was used for preparation of these series of standard solutions and the concentration was 1000 µg/ml. Rigorous quality control procedures were followed throughout the analysis and the recovery of arsenic in fortified samples ranged from 86.6 to 90.4% in soil, 87.8 to 92.5% in water, 85.2 to 87.5% in grass, 85.1 to 88.3% in faeces, 87.4 to 93.2% in urine, 86.2 to 89.5% in milk, 85.6 to 92.2% in serum, and 88.3 to 91.4% in hair.

All the data obtained were analysed in SPSS (version 10.0) by one-way ANOVA for more than 2 groups of observations. Multiple comparisons were made by Duncan's multiple range tests (Duncan 1955). For 2 groups of observations, Fisher's 't' as test statistics was used (Fisher 1950). Probability of $P < 0.05$ and $P < 0.01$ was described as significant and highly significant respectively.

RESULTS AND DISCUSSION

The concentration of arsenic in control zone varied from season to season and the highest being recorded in monsoon season (except water). Like wise, the arsenic content in the soil of exposed zone varied from season to season, and the highest value was observed in monsoon. The concentration of arsenic in the soil of exposed zone was significantly ($P<0.01$) higher than that of corresponding control zone (Table 1). The arsenic concentration in the water of both the control and exposed zone also varied from season to season and the highest being recorded in the water of summer. Comparatively, arsenic concentration in the water of exposed zone were significantly ($P<0.01$) higher from corresponding season of control zone (Table 1). Arsenic content of grass in different seasons of control zone varied and the highest

Table 1. Seasonal variation of arsenic content (ppm) in soil, water and grass in between control and arsenic exposed zone (n-20; mean with SE)

Substrates (ppm)		Season		
		Winter (Oct – Jan)	Summer (Feb – May)	Monsoon (June – Sep)
Soil	Control	$0.441^{b} \pm 0.021$	$0.434^{b} \pm 0.021$	$0.55^{a} \pm 0.02$
	Exposed	$4.187^{**} \pm 0.026$	$4.484^{**} \pm 0.012$	$5.01^{**} \pm 0.068$
Water	Control	$0.014^{a} \pm 0.002$	$0.043^{b} \pm 0.002$	$0.028^{c} \pm 0.003$
	Exposed	$0.245^{**} \pm 0.038$	$0.391^{**} \pm 0.096$	$0.13^{**} \pm 0.01$
Grass	Control	$0.041^{a} \pm 0.002$	$0.034^{b} \pm 0.001$	$0.43^{a} \pm 0.002$
	Exposed	$0.438^{**} \pm 0.08$	$0.448^{**} \pm 0.022$	$0.481^{**} \pm 0.037$

Control, Kamjong block in Ukhrul district, exposed zone, Kakching block in Thoubal district; P, <0.01 between control and exposed values in each season; value of dissimilar superscript in row vary ($P<0.05$) from each other.

Table 2. Seasonal variation of arsenic content (ppm) between the substrates from cattle in control and arsenic exposed zone (n=20; mean ± SE)

Substrates (ppm)		Season		
		Winter (Oct–Jan)	Summer (Feb–May)	Monsoon (June–Sep)
Faeces	Control	$0.098^{a} \pm 0.008$	$0.107^{ab} \pm 0.001$	$0.125^{b} \pm 0.009$
	Exposed	$0.221^{*} \pm 0.005$	$0.246^{*} \pm 0.024$	$0.278^{*} \pm 0.015$
Urine	Control	$0.01^{a} \pm 0.002$	$0.017^{ab} \pm 0.001$	$0.011^{b} \pm 0.007$
	Exposed	$0.078^{*} \pm 0.031$	$0.138^{*} \pm 0.027$	$0.086^{*} \pm 0.020$
Milk	Control	$0.028^{b} \pm 0.002$	$0.032^{a} \pm 0.002$	$0.031^{a} \pm 0.003$
	Exposed	$0.288^{*} \pm 0.027$	$0.304^{*} \pm 0.035$	$0.331^{*} \pm 0.096$
Serum	Control	0.038 ± 0.002	0.024 ± 0.001	0.125 ± 0.02
	Exposed	$0.111^{*} \pm 0.027$	$0.074^{*} \pm 0.003$	$0.29^{*} \pm 0.027$
Hair	Control	$0.063^{*} \pm 0.002$	$0.079^{ab} \pm 0.003$	$0.11^{b} \pm 0.009$
	Exposed	$0.682^{*} \pm 0.011$	$0.724^{*} \pm 0.014$	$0.758^{*} \pm 0.008$

Control, Kanjong block in Ukhrul district; exposed zone, Kakching block in Thoubal district; P<0.01 between control and exposed values in each season: value with dissimilar superscript in row vary (P<0.05) from each other.

content was in the grass of monsoon season. Arsenic content in the grass of all the seasons of exposed zone was significantly (P<0.01) higher from the corresponding season of control zone (Table 1).

Arsenic concentration in the milk of cattle in summer of the control zone was higher followed by monsoon season and winter in sequence. Arsenic concentration of all the seasons in the milk of cattle of exposed zone was significantly (P<0.01) higher than the corresponding seasons of control zone. The maximum increase was noticed on monsoon season followed by summer and winter (Table 2). Arsenic in the faeces of cattle of control zone was lowest in winter followed by summer and monsoon season in sequence. The values of arsenic content in faeces of cattle of exposed zone were significantly (P<0.01) higher in all the corresponding seasons of control zone (Table 2). Arsenic in the urine of cattle of control zone was almost same in all the 3 seasons. However, the level of arsenic in the urine of cattle of exposed zone were significantly (P<0.01) higher from the corresponding seasons of control zone. Arsenic concentration in serum of cattle of control zone was highest in winter followed by summer and monsoon seasons. Arsenic concentration in the serum of cattle of all the seasons of exposed zone were significantly (P<0.01) higher from the corresponding seasons of control zone. The maximum concentration was noticed in serum of cattle in monsoon season followed by winter and summer in sequence (Table 2). Arsenic concentration in hair of cattle of control zone varied from season to season. The highest concentration was recorded in the hair of cattle at monsoon season followed by summer and winter in sequence. The arsenic concentration in the hair of cattle of exposed zone was significantly higher from all the corresponding seasons of control zone. The increase of arsenic in winter, summer and monsoon season was 10.82, 9.1 and 6.89 times, respectively (Table 2).

Out of 9 districts of Manipur, 4 are arsenic exposed on the basis of the testing report in groundwater only (Chakraborti *et al*. 2008). But the consequent report of arsenic exposure either in environment or its effect in living system of both animals and human beings are lacking. The arsenic level in soil of control zone was below the permissible limit of 5 ppm (Mandal and Suzuki 2002). But the permissible limit of arsenic level in grass, was not available from literature. In control zone, arsenic content in water sample in all the seasons was less than the National Standard (0.05 ppm). Arsenic level in water during summer was observed more than those of winter and monsoon, which might be due to decline water level during summer. Arsenic content in soil and grass (control zone) during monsoon was maximum compared to winter and summer, which might be due to leaching of soil during monsoon leading to accumulation of arsenic in soil of Kakching block, a low lying area in comparison to surrounding. High arsenic content in soil as well as in grass during monsoon can be correlated. The range of arsenic in soil is highly variable compared to West Bengal state, where contamination of soil due to irrigation with arsenic laden water has been occurring since last 2–3 decades (Sanyal *et al*. 1993) and arsenic level varied from 8.4 ppm to 24.3 ppm in soil collected from exposed zone and its impact on crop (particularly paddy) and subsequent exposure to human being exhibited catastropic symptoms in victimized human being.

Arsenic in water samples of exposed zone of Manipur was 5 times higher than the recommended permissible limit of National Standard (0.05 ppm) and beyond which may produce a life time risk factor for skin cancer (WHO 1993). Concentration of arsenic in grass samples usually consumed by the animals during grazing was many folds higher than the permissible limit of arsenic content in water suggesting significant exposure of arsenic in the environment and possible subsequent effect in animal system. Studies also reported that the arsenic concentration in straw of rice (*Oryza*

sativa) is directly proportional to soil arsenic concentration (Sanyal and Dhillon 2005).

High arsenic content in faeces in monsoon of exposed area could be correlated with the presence of high arsenic content in samples of soil and grass in monsoon compared to other seasons. More than 7 folds higher arsenic content in exposed group than the control group was the cause of justification of arsenic exposure and concern about the impact on environment. High arsenic content in urine of cattle in summer was possibly due to concentrated urine due to decreased volume. However the value was below the permissible limit, i.e. 16 ppm (Radostits *et al*. 2003). The arsenic content in milk of cattle of exposed group was more than the permissible limit of 0.01 ppm (IDF 1986). Presence of arsenic in cow's milk corroborated with the report of Mahreu *et al.* (1977) who found high level of arsenic, i.e. 0.117 ppm in normal healthy cow's milk in France. During winter the increase in the value of arsenic in serum of exposed animals and also in summer and increased after monsoon than those of control values in spite of higher values. In all the substrates of cattle of exposed zone the cattle did not exhibit the characteristic symptoms of arsenic toxicity. This phenomenon is mysterious and warrants detailed study about arsenic metabolism in cattle which might explain the mechanism of non-exhibition of toxic symptoms. Arsenic in hair in exposed zone was 10 folds higher than the control zone, which resembles the findings of Riviere *et al.* (1981) who found 0.80 to 3.40 ppm arsenic in hair of cattle. On the contrary, Radostits *et al.* (2003) reported that normal non exposed animal should contain less than 0.5 ppm arsenic in hair. Arsenic has an affinity towards the sulphahydryl group of amino acid and therefore, arsenic in hair and nail is usually higher where maximum level of sulphahydryl group is required for keratin synthesis (Klaassen 2006). The animals reared in the same environment, i.e. at exposed and non-exposed region (control) were critically examined for the identification of classical symptoms as expected in human beings such as depigmentation, melanosis, leucomelanosis, keratosis, hyperkeratosis, non pitting oedema, ulceration and corn. Detailed examination indicated no characteristic clinical signs in animals as diagnostic point. However, occurrence of non specific symptoms like stunted growth, unthriftiness, loss of hair, inappetance to anorexia, loss of milk production, delayed oestrous, anoestrous, post-parturient anoestrous etc. can not be ruled out since these parameters have not been studied in this experiment. However, the quantification of arsenic in faeces, urine, milk, serum and hair indicated higher level of this metalloid to justify its presence in food-chain without showing clinical signs in animals does not agree with the possible chance of inducing symptoms during chronic exposure.

The attempt to record the significant content of arsenic in milk more than the permissible limit at Manipur was first time in India. The result of arsenic content more than the permissible limit in milk of exposed group of cattle indicated that the metalloid arsenic could be translocated from blood to the process of galactogenesis and eventually to be present in milk having tremendous risk factor for human consumption. Even, alarming call can be taken into consideration immediately to anticipate the ill-fate of babies. The progress of this integrated research project on arsenic hitherto significantly suggests the implementation of this kind of brainchild planning in other affected countries for covering all aspects of arsenic exposure.

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