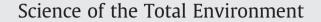
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# Chronic arsenicosis in cattle with special reference to its metabolism in arsenic endemic village of Nadia district West Bengal India

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# ABSTRACT

Thirty Milch cattle were selected randomly from a village of Nadia district of West Bengal, India containing high arsenic in water and soil samples. Milk, feces and hair samples were collected to analyze arsenic status in animals. Water and straw samples were also estimated for arsenic. Milk products prepared from milk of cattle rearing in arsenic prone village were also collected to quantify total arsenic and speciation of arsenic in milk and feces samples were also carried out. It was observed that high amount of arsenic was present in milk, feces, hair of cattle and water and straw samples in arsenic prone village. Milk product also contained significant amount of arsenic than that of milk product of control village. Speciation study revealed arsenite fraction was mainly eliminated through milk, whereas organoarsenic species were mainly excreted through feces.

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# 1. Introduction

It has been reported that ground water of South and East Asia are contaminated with arsenic. Approximately 60 million people are at risk of arsenic exposure of Asia alone, of which two lakh people are exposed to arsenic endemic region in West Bengal, India. 1 lakh people exhibited the arsenic toxicosis by maintaining skin lesion, remaining one lakh people are at risk due to consumption of water (10-12 times of minimum permissible limit of arsenic) having maximum permissible limit of 0.05 µg/ml. The source of arsenic resulting in pollution in aquifer in this vast region of West Bengal is thought to be geogenic (Roy and Saha, 2002). It has been reported that the people of arsenic endemic region suffer from arsenicosis though they are supplied with arsenic free drinking water for more than 12 years. (News letter of Indian training network, 1996). Therefore it is expected that arsenic may be consumed by people through their foods like agricultural produces, fishes and animal products like meat, milk, egg, etc. A large number of cattle population maintained by those people take their drinking water from arsenic affected tube well and they ingest large amount of arsenic contaminated straw (Bhattacharya et al., 2009) The ingested high amount of arsenic in cattle may come out by milk of respective animals which is consumed by human beings directly and indirectly through arsenic contaminated milk products.

From the view of animal health, it is observed that most of the animals rearing in arsenic prone area are not showing any specific clinical symptoms. The possible reasons of such occurrence in animals are not properly evaluated. An attempt was made in this study to find out:

- Residue of arsenic in feces, hair and milk of cattle as well as their drinking water and straw feed by respective cattle.
- Residue of arsenic in different milk products produced from milk of arsenic prone area.
- Speciation of arsenic in milk and feces of arsenic endemic village.

# 2. Material and methods

### 2.1. Selection of study area

People of Ghetugachi village under Chakda block of district Nadia, West Bengal, India are badly affected with ground water arsenic poisoning and was selected as experimental village, and Akna village of district Hooghly, West Bengal, India was considered as control arsenic unaffected village.

### 2.2. Design of experiment

Thirty milch cattle of each of both control and experimental villages were selected randomly for the study and arsenic

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Table 1

Validation of total arsenic by fortification of known quantity of ICPMS grade arsenite standard.

Substrate	Concentration of total arsenic in substrates (natural) (X) ( $\mu$ g/L)	Fortification with As <sup>+3</sup>		Recovery (%)
		Fortified concentration (Y) ( $\mu$ g/L)	Concentration observed (Z) ( $\mu$ g/L)	$(Z - X) \neq - \times 100$
Water	11	1, 2, 4, 8, 16	11.96, 12.90, 14.75, 18.60, 26.20	94-96%
Milk	15	1, 2, 4, 8, 16	15.89, 16.80, 18.65, 22.40, 29.90	89-93%
Milk product	40	1, 2, 4, 8, 16	40.90, 41.84, 43.60, 47.29, 55.20	90-95%
Hair	459	1, 2, 4, 8, 16	459.93, 460.85, 462.75, 466.44, 474.40	92-96%
Feces	155	1, 2, 4, 8, 16	155.95, 156.80, 158.80, 162.60, 169.85	90-95%
Straw	259	1, 2, 4, 8, 16	259.94, 260.90, 262.8, 266.42, 274.40	93-96%

concentration of different substrates like hair, feces and milk were collected from individual cattle and drinking water, and straw samples were also collected from the respected farmer house. Besides, heat and acid coagulated indigenous Indian milk product (Chana) were also collected from different sweet shop, where milk of respective village was used for production of such sweet. Arsenic speciation in milk and feces of experimental village were also carried out.

# 2.3. Reagent

All chemicals of analytical grade were purchased from Rankem Pvt. Ltd., E-Merck (India), and Sigma Aldrich (USA).

#### 2.4. Collection of samples

Milk samples were taken in a dry plastic container pre-washed with nitric acid (20%) with cap after milching manually. Pooled milk samples were obtained from all 4 teats. Feces samples were taken from rectum manually and kept in plastic zipper bag. Hair samples were collected from tail. Straw and water samples were collected directly from manger of respective cow. All the above mentioned samples were collected from both control and experimental cows. Samples of milk product were collected from sweet shop of both control and experimental zone and kept in plastic zipper. All samples except straw and hair were stored in  $-20^{\circ}$  C until processed.

# 2.5. Analysis of arsenic

### 2.5.1. Total arsenic

Total arsenic was analyzed in all samples by wet ashing procedure in hot plate: Milk, milk products and water samples were digested with 70% of nitric acid as per the method of Carbrey et al (2009), while feces, straw, hair samples were digested using Tri acid mixture of nitric acid, perchloric acid and sulphuric acid at 10:4:1 ratio following some modification of the method of Welsch et al (1990) and properly digested samples were diluted with Millipore water, passed through filter paper, and made the volume 10 ml. Concentrated hydrochloric acid (5 ml) was added to it and shaken well. Then 1 ml of potassium iodide (5%) and ascorbic acid (5%) mixture was added and kept the aliquot for 45 min. for transformation of arsenate to arsenite (Haring et al., 1982). Then final volume was made up to 50 ml with Millipore water for reading in Atomic Absorption Spectrometer (AAS) equipped with vapor generation accessories.

# 2.5.2. Instrument

A Varion AA240 model AAS equipped with vapor generation accessories (model no VGA77) was used for total arsenic estimation. Reducing agent (Aquas solution of 0.6% sodium borohydride in 0.5% sodium hydroxide) and Acid (40% Hydrochloric acid) were prepared freshly before use.

#### 2.5.3. Instrumental condition

Operating parameters for Varion AA240: lamp, arsenic hollow cathode lamp; wavelength, 193.7; slit width; 0.5 nm; lamp current, 10.0 mA; vapor type, air/acetylene; air flow, 10.00 L/min; inert gas for hydride generation, Argon.

# 2.5.4. Working standard solution

Working standard solutions were prepared by dilution of stock (1000  $\mu$ g/ml) and intermediate (10  $\mu$ g/ml) standards. The working standards were as follows: 2.5, 5, 10, 15 and 20  $\mu$ g/L and prepared it by same procedure as test sample.

# 2.5.5. Instrument calibration

The calibration curve for determination of arsenic was prepared using a blank and working standard solution  $(2.5-20 \,\mu\text{g/L})$  of arsenic. The calibration was periodically verified by analyzing a standard at the frequency of 20 readings. If the recovery was outside the limits, the analysis was stopped. The problem was corrected and the system was recalibrated.

#### 2.5.6. Validation of total arsenic analysis methods

For validation of total arsenic analysis, substrates like milk, feces, hair were collected from animals of control zone. Likewise water, straw and milk products were collected from specified zone respectively. Arsenic content of each substrate was then assayed. Arsenic content of each substrate was found to be below the permissible limit and considered for validation. The natural arsenic content of each substrate was deducted from the fortified result and expressed in percentage. Known quantity of arsenite and arsenate ICPMS grade standard solution were spiked in a single and combined manner in different concentration in milk, milk product, feces, hair, straw and water so that final total arsenic concentration would be 1, 2,

Table 2

Validation of total arsenic by fortification of known quantity of arsenite and arsenate ICPMS grade standards.

Substrate	Concentration of total arsenic in substrates (natural) (X) (µg/L)	Fortification with $(As^{+3} + As^{+5})$ (1:1)		Recovery (%)
		Fortified total arsenic (Y) ( $\mu$ g/L)	Concentration observed (Z) ( $\mu$ g/L)	$(Z - X) \neq Y \times 100$
Water	13	1, 2, 4, 8, 16	13.95, 14.90, 16.78, 20.70, 28.25	93-96%
Milk	17	1, 2, 4, 8, 16	17.92, 18.78, 20.70, 24.50, 31.95	89-94%
Milk product	33	1, 2, 4, 8, 16	33.94, 34.90, 36.78, 40.55, 48.25	90-95%
Hair	440	1, 2, 4, 8, 16	440.91, 441.9, 443.80, 447.44, 455.25	90-95%
Feces	138	1, 2, 4, 8, 16	138.96, 139.80, 141.85, 145.70, 153.10	90-96%
Straw	285	1, 2, 4, 8, 16	285.94, 286.88, 288.78, 292.44, 300.38	93-96%

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