CHAPTER-2

EPIDEMIOLOGICAL STUDY OF ARSENICAL EFFECTS AT NATURALLY CONTAMINATED SITES OF CHHATTISGARH
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NATURALLY CONTAMINATED SITES OF CHHATTISGARH

ABSTRACT
This chapter reports the results of epidemiological study on prevalence of arsenic and its effect in arsenic contamination in the District Kanker and Rajnandgaon. Arsenic concentrations in water and soil and food materials consumed by animals of both the districts and its adjoining area have been reported here. The region has been found to contain an elevated level of arsenic. Bioaccumulation of arsenic in animals and humans were also studied. All segments of environment are contaminated with arsenic of varying degree. The levels of arsenic keep on changing depending on the season and location. Epidemiological studies were performed that showed that ruminants like cattle, buffalo and goats have higher concentration of arsenic in their body which is responsible for the development of toxicity symptoms. As the animals have short life span and are more tolerant to arsenic so we do not found any lesions of arsenicosis but presence of toxic levels of arsenic in their body fluids like blood, urine, faeces and hairs have been detected. It was found that food consumed by animals and humans of the contaminated regions was responsible for the development of arsenicosis. A case study of episodic arsenical diarrhoea is presented.

INTRODUCTION
Arsenic is one of the most important global environmental toxicant, not only can contaminate water, but also intoxicate soil and crops and consequently endanger the human and animal health. Extensive use of arsenic containing pesticides and other agricultural products in the past leads to outbreaks of arsenic poisoning. In animals, several types of inorganic and organic arsenical compounds cause is responsible for arsenicosis. Toxicities vary with factors such as oxidation state of arsenic, solubility, and species of animal involved and duration of exposure. Poisoning is practically always due to human carelessness. While many arsenicals have been replaced by
less dangerous compounds for a number of applications, animals can still be poisoned by this persistent element.

The principal natural reservoirs of arsenic are rocks. Release and mobilization of arsenic from these sources constitute the availability of this element in soil, water and air in various forms. Arsenic is typically released to the environment in an inorganic form, and it tends to adsorb strongly to soils. Leaching into subsurface soils is generally not significant, except under reducing conditions (ATSDR, 2000).

India is basically an agricultural country, where about 75% of the total population depends upon agriculture. In the present scenario, animal husbandry plays an important role in country agriculture economy by producing milk, meat leather, manure and animal draught power for agricultural purpose. Therefore, any condition or circumstances, which influence the production of livestock, also seriously affect the economy of rural farming community.

Drinking water containing more than 0.25% arsenic is considered potentially toxic, especially to large animals. Herbivores are commonly poisoned because they eat contaminated forage. Pastures near smelters may be contaminated with arsenic. Empty containers may be licked by the animals. Fluids used for dipping and spraying of animals to control ectoparasite is the commonest source; animals may swallow the solution while in the dipping or spraying. Percutaneous poisoning can occur if animals are dipped in strong solutions of lead arsenate. Milk from arsenic poisoned cattle may be toxic for calves. In most instances, poisoning occurs when animals accidentally gain access to recently insecticidal sprayed areas, wood preservative specially arsenic-copper-chromium are used to treat pine in wooden calf pens (Booth & Mc Doland, 1988).
Fig 2.1 Arsenic Cycle in Environment (Adapted from Yan-Chu, 1994; Bhumbla and Keefer, 1994; Carter and Fairlamb, 1993)
Major reactions in the soil–water and sediment–rock systems to influence the environmental transport, distribution and availability of arsenic. Oxygen availability controls the arsenate–arsenite redox reactions. Adsorption and precipitation of arsenate and arsenite immobilize the soluble arsenic. Slow release of arsenic from rocks and sediments or oxidative dissolution of arsenopyrite (FeAsS) from sediments contribute flux of arsenic in the environment. Methylation of arsenite to monomethylarsonic acid (MMA) or dimethylarsinic acid (DMA) followed by other organoarsenic compounds, constitute the major biological reactions in the arsenic cycle.

The use of inorganic arsenicals (Sodium arsenite and arsenic trioxide) as herbicides has been reduced greatly in developed countries because of livestock losses and the environmental persistence of arsenic. Yet, this compound is still been used in our country and can be hazardous to animals.

Ruminants including wild animals are apparently attracted to and lick plants poisoned with arsenite. The highly soluble arsenicals can concentrate in pools in toxic quantities after a rain has washed them from recently treated plants (Susan, 1998).

Chronic arsenical poisoning has been encountered from time to time in areas near smelting works and mines which contaminate the surrounding pasture with arsenic. Sheep and cattle often develop a taste for arsenic and will selectively graze contaminated pastures. Pharmaceuticals and growth stimulants including arsenic acid and sodium arsenilate and phenylarsonic acid preparations such as roxarsone, nitarsone are used both as feed additives and in the control and treatment of vibrionic dysentery in animals and as antidote of selenium poisoning. Overdosing with them can occur accidentally by carrying on the administration for too long or when there is an error in mixing. Toxicosis results from an excess of arsenic containing additives (Clark et al., 1981)
Currently the high probability zone, moderate probability zone and low probability zone of arsenic contaminated area together cover almost about 60% of the total country area. High arsenic levels in the ground water of central east India has been reported by Pandey et al., (1999, 2002, 2004). It has been found that the area
between Dongargaon to Mohla (about 500 sq. km) and a wide area of Chowki block of Chhattisgarh state is affected by the presence of arsenic in varying concentration. The arsenic concentration was discernible in different reasons and show wide seasonal variability. They also reported that there are thousand of people facing elevated average arsenic levels which in some cases are much higher than 0.01 mg/As (total)/L, the provisional guideline value recommended by the World Health Organization is 0.01 mg/L and the Indian guide value is 0.05 mg/L. Human epidemiological study was also carried out by and Pandey et al., (2002) Yadav, (2003) that arsenicosis symptoms are manifested even at 2 years and there is a continuous increase in number of patients up to the age 20 years where maximum number of patients are noted. Yet, there had been almost no study on the epidemiological aspect in ruminants.

This study intends to fill this gap in the understanding. Studies on the epidemiological aspects of arsenicosis in ruminants are necessary for proper understanding of the arsenical effects on animals and particularly ruminants. While carrying out this study we came across many cases of human exposure to arsenic hence a study was further carried out to find out the reasons and manifestations of arsenicosis in human's vis-à-vis the ruminants. A case study on arsenic and its epidemiological manifestation in the form of mass diarrhoea is also reported.

MATERIALS AND METHODS

STUDIED LOCATIONS

Category I: Areas with higher level of arsenic in ground water.

This area comprised of parts of Rajnandgaon and Kanker district. The Rajnandgaon district is situated in the southeastern part of India and is in the state of Chhattisgarh. The district lies between 20° 70' to 22° 29'N Latitudes and 81° 29' to 88°. The total area of the district is 6396.28 square kilometres out of which 2987.19 square kilometre is forest area. The climate is tropical and the average rainfall is 1275mm.
FIG 2.3 LOCATION OF CHHATTISGARH IN INDIA
River Sheonath originates in Garhchiroli district of Maharashtra and its origin is very near to Ambagarh-Chowki block. This river flows towards northeast and enters the Durg district. The principal river of the district is Sheonath, a tributary of Mahanadi, the biggest river of central east India.

Demographically the total population of the district is 1,439,951 (1991 census) out of which the scheduled tribe population is 25.1% and the scheduled caste population is 20.28%. Out of eight tehsils of the district three tehsils i.e. Mohla, Manpur & Chowki are tribal (aboriginals). The main tribes of the district are Gond, Kanwar, halba & Baiga. The tribal population of the district is around 25.16% of the total population. Almost entire district depends on tube wells and dug wells for the drinking water.

The second set of contaminated locations was located in the district Kanker. This district is an important district of Bastar Commissionerate in the state of Chhattisgarh. The Kanker District is situated in the southern region of the state. Four districts namely Bastar, Dhamtari, Durg and Rajnandgaon surround it. Kanker is situated within the longitudes 20.6-20.24 and latitudes 80.48-81.48. The total area of the district is 5285.01 square kilometres. The district is situated on the National Highway number 43 between the two well-developed cities of Chhattisgarh named Raipur.
(Capital of Chhattisgarh) and Jagdalpur. The district is monsoon fed and the average rainfall is 1492 mm mainly during the months of June to October.

Kanker district is divided in six tehsils named Kanker, Charama, Narharpur, Bhanupratapur, Antagarh and Pakhanjoor and seven blocks named Kanker, Charama, Bhanupratapur, Narharpur Antagarh, Durgu-Kondal and Koyalibeda. The number of villages in the district is 1074. The literacy percentage in district is 74.7% and the total population of the district is 651,333. The ratio of male female is 10:6. The density of population is 100 per square kilometre.

Geographically the district is divided in five Groups namely Vindhyan hill Group, Archean hill Group, Dharwar hill Group, Mahanadi plains and Kotri plains. Vindhyan hill Group is situated in the Southeastern part of the district. Quartzite sand is a main feature of this Group. Achaean hill Group covers the 95% area of the district. Granite and gneiss rocks are the major component and are spread over almost entire district. Dharwar hill Group comprises of weathered hills in Sambalpur and Bhanupratappur blocks of the district.

Mahanadi plain and Kotri plains are two plain areas in otherwise hilly district. Height from the mean sea level is between 300-600 meters in this area. The Northeastern part of Kanker falls under Mahanadi plain. The height of this plane is less than 500 meters. The main rivers draining this area are Mahanadi, Hatkul, Chinar, Doodh, Sendoor, Nakti and Doori. The Kotri plains comprise mainly the Bhanupratappur area. Kotri, Handi and Valier are the main rivers of this area. The height of this plain is less than 400 meters.

**Category II: Control Areas (no report of ground water contamination) and experimental Group**

We selected the Durg area as control area where the As has not been detected. We took 10 cattle, 10 buffaloes and 12 goats irrespective of age, sex and breed. These were found free from parasitic infection on blood smear and faecal examination. These animals belonged to college dairy farm and goat farm of College of Veterinary Science and Animal Husbandry Anjora, Durg, Chhattisgarh. These were treated as healthy control.

Different samples like water, soil, vegetation, rock. Cattle, buffaloes and goats reared in the above mentioned rural localities were taken as study population and biological samples like blood, urine, hair, milk and faeces were collected.
Prevalence of arsenic poisoning in cattle buffaloes and goats were studied in different villages of Rajnandgaon districts and Kanker districts. To study the prevalence of chronic arsenicosis in Chhattisgarh state a total of 152 ruminants were screened during October 2003 to September 2004. The information was statistically analysed to study the prevalence of diseases in this area and its correlation with age, sex, breed, feeding habit and season.

**SAMPLING PROTOCOL**

All moist solid samples were dried at 40°C in uncontaminated petridishes till dryness. All solid samples were finally crushed and weighted accurately by Digital weighing machine (Denver, USA). Appropriate weight of sample was taken for digestion in clean uncontaminated Teflon beakers with few glass beads. Standard digestion procedure was followed (APHA 1992). Arsenic was analysed by atomic absorption spectrophotometer with hydride generation and background correction facility (Chemilo 201).

**Water samples**

Sampling of the contaminated area was carried randomly. The sampling bottles were cleaned with chromic acid, detergent, tap water and finally with double distilled water. Duplicate samples were collected for each sampling site. Arsenic preservation was done with EDTA (Pandey et al. 2004) and HNO₃ (till pH 2). Soil, rocks, and vegetation sample were kept in clean, uncontaminated polythene pack.

**Feed and Fodder**

Feed and fodder samples were collected freshly from the field in polyethylene bags in required amounts at marketable stage of development. To assess the dietary intake of arsenic by human and animals all the samples were washed with tap water followed by double distilled water. 0.5 gram of hot air oven dried (80°C) sample was digested in Teflon bomb at 100°C for six hours in hot air oven to avoid the losses of arsenic. Dilution to known concentration was made with triple distilled water and samples were kept in deep freeze until further analysis.

**Soil Samples**

Composite soil samples were collected at a depth of 15-45 cm below the ground surface. The samples were immediately stored in a sealed in a zip-lock polyethylene bag.
Blood samples

Blood sample from animals was collected from jugular vein by taking all aseptic precautions. The instruments and glassware used to store and process the blood were soaked overnight in 10% concentrated Nitric acid than washed properly with detergent and rinsed in double distilled water. Heparinised blood was collected in sterilized vials. Blood and urine samples were digested immediately after collection by adding concentrated Nitric acid and 30% Hydrogen peroxide. Digestion was carried out in bomb at 100°C for 6hrs in hot air oven. Dilution to known concentration was finally made with triple distilled water. Samples were finally stored at -20°C till analysis.

Urine samples

Urine samples are collected for the estimation of arsenic in acid washed sterilized bottles after discarding the first stream of urine and are kept in ice. Samples are digested immediately on the same day of collection and stored at -20°C till further analysis.

Milk samples

After discarding few streams, milk samples were collected directly from the teats of cattle fed on forage grass grown on above-mentioned contaminated sites. Milk samples are collected in sterile polyethylene sampling bottles with KmnO4 as preservative.

Faeces samples

Fresh samples were collected in polyethylene bottles and are kept in deep freeze until further analysis. For the estimation of arsenic in the samples, they were dried in hot air oven at 80°C. Dried samples were digested and final volume of known dilution was made by triple distilled water.

Hair samples

Hair samples were collected from tail region of cattle, buffalo and goat with the help of a stainless steel scissor and were kept individually in polythene packets at room temperature to determine arsenic level in hair. Samples were washed with absolute alcohol and then with soap and water followed by rinsing with double distilled water.
TABLE 2.1 BRIEF DETAIL OF THE ANALYTICAL METHODS ADOPTED IN THE PRESENT WORK.

<table>
<thead>
<tr>
<th>Sample matrix</th>
<th>Preparation method</th>
<th>Analytical method</th>
<th>Sample detection limit</th>
<th>Percent recovery</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Reduction to arsine in acid solution; reaction with SDDC</td>
<td>EPA Method</td>
<td>10 μg/L</td>
<td>100</td>
<td>US EPA, 1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>206.4; SDDC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>colorimetric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>spectrophotometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>at 535 nm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water/soil/solid waste</td>
<td>Digestion with nitric/sulphuric acid; reduction to As +3 with tin chloride; reduction to arsine with zinc in acid solution</td>
<td>HGAAS, EPA Methods 206.3 and 7061;</td>
<td>2 μg/L</td>
<td>85–94</td>
<td>US EPA, 1983, 1986</td>
</tr>
</tbody>
</table>

ANALYSIS PROCEDURE

Arsenic was analysed by hydride generation atomic absorption spectrophotometer with background correction facility (HG-AAS, Chemito-2001) and occasionally by Silver diethyldithiocarbamate method using scanning UV-visible spectrophotometer (Chenito-UV 2100) following the APHA, 1992 (American Public Health association) standard methods. Merck certified standard solutions and chemicals were used in the analysis.

SUMMARY OF METHOD

Samples were prepared according to the nitric/sulphuric acid digestion procedure described in this method. The arsenic in the digestate was then reduced to the trivalent form with tin chloride or potassium iodide. The trivalent arsenic was then converted to a volatile hydride using hydrogen produced from a zinc/hydrochloride acid reaction or sodium borohydride. The volatile hydride was swept into an oxy-acetylene flame located in the optical path of an atomic absorption spectrophotometer. The resulting absorption of the lamp radiation is proportional to the arsenic concentration. The typical detection limit for this method is 0.002mg/L.
APPARATUS AND MATERIALS

Atomic absorption spectrophotometer – Used Chemito - AAS 201) is a single channel, double-beam instrument having a grating monochromator, photo-multiplier detector, adjustable slits, a wavelength range of 190 to 800 nm. The burner used was special corrosion resistant metal as recommended by the particular instrument manufacturer. The arsenic hollow cathode lamp used was manufactured by Photron, Australia.

Reagents required

Analytical reagent grade chemicals (Merck, Germany/India) were used in all tests. The reagent water used was deionised and double distilled, interference free water. All references to water in the method refer to reagent water unless otherwise specified. Acid viz. HNO₃, H₂SO₄, and HCl etc. were analysed to determine levels of impurities. The acid was used only when the method blank was less than the detection limit (< MDL).

- Potassium iodide solution – 15/10 g KI was dissolved in 100 mL water.
- Stannous chloride solution/sodium borohydride solution – 40 g SnCl₂ was dissolved in 100 mL concentrated HCl/2.0 g sodium borohydride in 0.05 N sodium hydroxide solution.
- Silver diethyldithiocarbamate (SDDC reagent) (0.5%) – 0.5 gm of silver diethyldithiocarbamate was dissolved in 100 ml pyridine with thorough mixing.

Arsenic solutions

Arsenic stock solution (1,000 mg/L) – A certified aqueous standard was obtained from Merck Germany and was verified by comparison with a second standard. Subsequently 1.734 g of sodium arsenite (NaAsO₂) was dissolved in 1000 mL of deionised water.

Intermediate arsenic solution – 1 ml stock arsenic solution was pipetted into a 100-mL volumetric flask and make up to mark with deionised water. (1 ml = 10 µg As).

Standard arsenic solution – 10 ml intermediate arsenic solution was pipetted into a 100-mL volumetric flask and brought to volume with deionised water. (1 ml =1.0 µg As).
Procedure adopted in AAS

Transfer 0.00, 0.1, 0.2, 0.3, 0.4 ml standard solutions of As(III) was pipetted uncontaminated beaker and brig to volume up to 1.00-ml with deionised water. This yields blank and standard solutions of 0.1, 0.4, 0.6, 0.8, 1.00 µg /ml. (APHA, 1992).

A standard volume of sample was transferred to the reaction vessel and 20 ml of 20% HCl and 1 mL KI solution were added to it and the contents were allowed at least 10 minutes for the metal to be reduced to its lowest oxidation state. The metal hydride was produced by adding 2 ml Sodium Borohydride solution, which produced a peak almost immediately. The wavelength of 193.7-nm and background correction was used for the analysis of arsenic. The calibration curve obtained by the AAS and UV-VIS is shown in fig 2.5.

![Fig 2.5 A Typical Arsenic Analysis Calibration Graph Obtained by HG-AAS](image)

![Fig 2.6 A Typical Arsenic Analysis Calibration Graph Obtained by UV-VIS](image)
TABLE 2.2 GENERAL INSTRUMENTAL PARAMETERS EMPLOYED IN ARSENIC ANALYSIS BY HG-AAS.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>193.7</td>
</tr>
<tr>
<td>Current (mA)</td>
<td>7-10</td>
</tr>
<tr>
<td>Flame</td>
<td>Air-Acetylene (A-A)</td>
</tr>
<tr>
<td>Normal working range (mg/L)</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>Spectral band width (nm)</td>
<td>0.5</td>
</tr>
<tr>
<td>N2 flow rate (litre/minute)</td>
<td>0.4-1</td>
</tr>
</tbody>
</table>

Procedure adopted in UV-Visible spectrophotometer

In a series of arsenic generator, pipette 0.0, 1.0, 2.0, 10.0 ml of arsenic standard solution. Diluted to 50 ml with deionised water. Added 5 ml concentrated HCl, 2ml of 15% KI (freshly prepared), and 8 drops of 40% SnCl₂ subsequently in each arsenic generator with thorough mixing. Allowed 15 minutes for complete reduction of As(V) to As(III). Taken 4.0 ml of SDDC reagent in absorber tube and connected to arsenic generator assembly. Added 3 gm of Zn metal powder in arsenic generator assembly and allowed 30 minutes for the complete evolution of arsine. Poured the solution from the absorber tube into 1 cm cell and measured the absorbance of solution at 535 nm on UV-Visible spectrophotometer. Prepared the calibration curve and same procedure was followed for sample with taken appropriate volume of sample.

INTERNAL QA/QC PROCEDURE

The following instruments were employed in the course of analysis:

- Hydride generating Atomic Absorption Spectrophotometer (HG-AAS), Chemito (India) AAS 201 equipped with Background correction facility.
- Scanning UV-VIS Visible Spectrophotometer, Chemito (India)
**TABLE 2.3 QUALITY ASSURANCE AND QUALITY CONTROL STEPS ADOPTED IN THIS WORK.**

<table>
<thead>
<tr>
<th>QA/QC Steps</th>
<th>Description</th>
<th>Adopted procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory reagent blank</td>
<td>At least one determination of a blank to establish the contribution to the analytical signal by reagents, glassware, etc. The blank should be subtracted from the gross analytical signal for each analysis before calculation of sample analyte concentration.</td>
<td>Measurement of sample blanks and laboratory blanks was carried out to control the contamination arising out of the sample preparation, respectively. Blank analysis was carried out with every determination and the signals (OD) were deducted from the analytical signal (OD) of the analyte (As)</td>
</tr>
<tr>
<td>Replicate analysis</td>
<td>Duplicate analysis of at least one sample from the batch. The variation between replicate analysis should be recorded for each batch to provide an estimate of the precision of the method.</td>
<td>Carried out in every batch of 10 samples and the variation (nil or otherwise) were recorded.</td>
</tr>
<tr>
<td>Quality control sample</td>
<td>Analysis of at least one control sample, a standard reference material, a laboratory reference material or a control matrix fortified with analytes representative of the analyte class. Recovery check portions should be fortified at concentrations, which are easily quantified but within the</td>
<td>*Elemental standards used were prepared by dilution of the stock certified single element solution for AAS supplied by Merck, Germany.</td>
</tr>
<tr>
<td></td>
<td>*Measurements of calibration check with known analyte concentration but of different origin than the calibration standards, maximum allowed</td>
<td></td>
</tr>
</tbody>
</table>
RESULT AND DISCUSSION

ANIMAL EPIDEMIOLOGY AND PREVALENCE OF ARSENICOSIS

Prevalence of arsenicosis in ruminants in Chhattisgarh was monitored based on blood, urine, faeces, and hair arsenic level in areas with ground water reported to have higher level of arsenic. A total of 152 ruminants from different villages of some areas were examined and altogether 12 (24%) out of 50 cattle, 3 (10%) out of 30 buffalos and 21 goat (29%) out of 72 goats were found affected by arsenicosis. The overall prevalence of arsenicosis in ruminants in the studied locations of Chhattisgarh was 23.6% (Fig 2.4).

Based on feeding habit in ruminants, it was observed that out of 36 ruminants 32 (88.88%) grazing ruminants were affected while 4 (11.11%) stall fed ruminants were containing higher arsenic level in hair (Table 2.4).

Literature regarding the prevalence of chronic arsenic poisoning in ruminants is lacking, though acute arsenic poisoning in cattle have been reported by some scientists. Monies (1997) diagnosed arsenic poisoning by post-mortem in a heifer. He also reported that soil from the site contained arsenic 36125 mg/kg. Lergerguist (2000) reported arsenic poisoning in cattle and 8 of 11 young cattle were found dead and liver samples contained very high levels (15.6 mg/kg) of arsenic.

In the studied location, Pandey et al. (1999) reported the total arsenic contamination between 0.01 to 1.01mg/L with a mean concentration of 0.35mg/L of the affected localities. The arsenic contamination was discernible in different seasons and it has been observed that cattle, buffaloes and goats reared in the localities where ground
water is used for drinking purpose contain higher level of arsenic. This might be due to the reason that most of the ruminants are grazing around the localities where the contaminated ground water is used. However, in control areas arsenic in drinking water was below the detection limit and animals of such areas do not have arsenic burden.

![Graph showing species of animal most susceptible to arsenic poisoning in the areas polluted with arsenic.](image)

**Fig 2.7 Showing species of animal most susceptible to arsenic poisoning in the areas polluted with arsenic.**

**Table 2.4 Prevalence of arsenic toxicity on the basis of feeding habit in affected areas.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Animals</th>
<th>Number of animals infected</th>
<th>Animals on Grazing</th>
<th>Percentage of animals affected</th>
<th>Stall fed animals</th>
<th>Percentage of animal affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>12</td>
<td>9</td>
<td>75</td>
<td>3</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Buffalo</td>
<td>3</td>
<td>2</td>
<td>66</td>
<td>1</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>Goat</td>
<td>21</td>
<td>21</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>32</td>
<td>88.88</td>
<td>4</td>
<td>11.11</td>
<td></td>
</tr>
</tbody>
</table>
**TABLE 2.5 SHOWING AGE WISE PREVALENCE OF ARSENICOSIS IN AFFECTED LOCATION.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Age Group</th>
<th>Number Examined</th>
<th>Number Affected</th>
<th>Percentage Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>0-3 Years</td>
<td>18</td>
<td>4</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>3-6 Years</td>
<td>16</td>
<td>7</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>6-9 Years</td>
<td>16</td>
<td>1</td>
<td>6.2</td>
</tr>
<tr>
<td>Buffalo</td>
<td>0-3</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>3-6 Years</td>
<td>10</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>6-9 Years</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Goat</td>
<td>0-2 Years</td>
<td>30</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
<td>28</td>
<td>11</td>
<td>40.2</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>16</td>
<td>1</td>
<td>5.5</td>
</tr>
</tbody>
</table>

**Age wise prevalence**

It was observed during the course of study that 22.2%, 42.5% and 6.2% of cattle in the age Groups of 0-3, 3-6 and 6-9 age were affected respectively. In case of buffaloes 10% and 20% in the age Group of 0-3 and 3-6 years, were affected, while none of the buffaloes out of 10 between 6-9 years of age were affected. In case of goat, it was observed that 30%, 40.2% and 5.5% of goats in the age Group of 0-2 year, 2-4 years and 4-6 years of age were affected respectively (Table 2.5). The present findings indicate that the prevalence was being highest in the age Group of 3-6 years in cattle and buffaloes and 2-4 years in goats. The prevalence was found to higher in younger (<3 year and 3-6 years) age Groups in cattle and buffaloes and (<2 year and 2-4 years) of age Groups in goats (Table 2.4), which corroborates the findings of Hinderwood et al., (2003). They reported that moderately higher arsenic residue in young animals could be attributable to the higher methylating capacity of older individuals than younger ones.
**TABLE 2.6 SEX WISE PREVALENCE OF ARSENIC TOXICITY IN AFFECTED AREAS.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Total Number of animals examined</th>
<th>Number of Animals affected</th>
<th>Percentage of animal affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Male</td>
<td>28</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22</td>
<td>5</td>
<td>22.7</td>
</tr>
<tr>
<td>Buffalo</td>
<td>Male</td>
<td>18</td>
<td>2</td>
<td>11.11</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>12</td>
<td>1</td>
<td>8.33</td>
</tr>
<tr>
<td>Goat</td>
<td>Male</td>
<td>53</td>
<td>16</td>
<td>30.1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>19</td>
<td>5</td>
<td>26.2</td>
</tr>
</tbody>
</table>

**Sex wise prevalence**

The prevalence of arsenicosis was studied sex wise also to see if there is any difference in the prevalence. The results showed that the prevalence of arsenicosis in the male and female was 25% and 22.7% in cattle, 11.11% and 8.33% in buffaloes and 30.1% and 26.2% in goats respectively (Table 2.6). Comparatively higher percentages of males were found to be positive for arsenicosis. The exact cause of these findings is not explainable, but it might be due to the reason that unlike milking females most of the male animals are kept on open grazing, thereby increasing the chances of their exposure to arsenic poisoning. Miranda et al., (2000) failed to record any sex wise discrepancies in blood arsenic accumulation in calves.

![Comparison of the Data Shows that Indigenous Animals are Most Susceptible for Arsenic Toxicity.](image-url)
We also carried out a study on the effect of genotypic difference on the arsenic susceptibility. For this we targeted the crossbred and local or non-descript breed. The prevalence of arsenicosis was found to be 27.02% and 15.36% respectively in local or non-descript breed and cross breed. In case of indigenous or non-descript buffaloes and goats the prevalence was found to be 11.11% and 29% respectively. (Fig 2.8). Moreover it was interesting to note that the prevalence of arsenicosis was more in local or non-descript breed of cattle, buffaloes and goat than in cross bred. The exact cause of these findings is not explainable as literature in this regard is scarce. But it might be due to the reason that most of the cross bred animals were maintained as stall fed, so they used to get more protein in there diet as compared to grazing animals and protein increases the ability of an animal to methylate arsenic, resulting in decreased toxic effects.

BIOLOGICAL MONITORING OF ARSENIC POISONING IN ANIMALS AT THE AFFECTED LOCATIONS

The accumulation of arsenic in polluted soil; water and its subsequent uptake by plants represent a direct pathway of arsenic into animal and human food chain, which is of major concern. It was reported in the literature (Hananota, 1955; US PEA (United states Environmental protection Agency), (1984) that skin abnormalities appear in the infants who are fed on arsenic contaminated milk and it was calculated that daily arsenic intake was about 3 mg (US EPA, 1984). To understand the potential pathways of arsenic into samples of animal body fluids viz. blood, urine and milk, solid excretion and the hairs were analysed for arsenic levels in various animals of the affected area. The reported normal levels of arsenic in blood, urine, faeces and hair are 0.03 ±0.12 mg/L, 0.10 ± 0.02 mg/L, 0.03 ±0.10 mg/L, 0.16 ± 0.08 mg/L respectively and we do not found arsenic in milk samples. Compared to this the animals studied in the region have shown 0.06 ± 0.11 mg/L in blood, 0.18 ± 0.21 mg/L in urine, 0.04 ±0.13 mg/L in faeces, 0.43 ±0.26 mg/L in hair samples and the milk samples of affected locations were free from arsenic. (Table 2.7).

When arsenic is absorbed in body, the major portion is excreted in urine (approximately 50%), and a small portion via the faeces and through the skin, hair and nail and possibly a trace through the lungs. Storage in body is the result of increased
intake and slow elimination rate from the body. This corresponds to the slight enrichment of arsenic in animal body

**TABLE 2.7 SHOWING THE CONCENTRATION OF ARSENIC (ppm.) IN BIOLOGICAL SAMPLES OF ANIMALS OF POLLUTED AREAS.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Healthy Animals</th>
<th>Animals of affected area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.03 ±0.12</td>
<td>0.06 ± 0.11</td>
</tr>
<tr>
<td>Urine</td>
<td>0.10 ± 0.02</td>
<td>0.18 ± 0.21</td>
</tr>
<tr>
<td>Faeces</td>
<td>0.03 ±0.10</td>
<td>0.04 ±0.13</td>
</tr>
<tr>
<td>Hair</td>
<td>0.16 ± 0.08</td>
<td>0.43 ±0.26</td>
</tr>
<tr>
<td>Milk</td>
<td>BDL</td>
<td>BDL (Below detection limit)</td>
</tr>
</tbody>
</table>

**EVALUATION OF ARSENIC LEVELS IN CONTAMINATED AREA**

Epidemiological studies in areas with raised arsenic content in drinking water have suggested a relatively high incidence of skin cancer, which increased with increases in the arsenic concentration in the drinking water, and the age of the individual. The arsenic levels of two districts Rajnandgaon and Kanker districts of Chhattisgarh were studied. Increased concentration of arsenic was present in water samples of affected areas. It was observed that in Rajnandgaon district maximum concentration of arsenic in water is present in Chowki block having the mean value of 229 µg/L followed by Dongargaon (150 µg/L). Analysis of ground water sources from certain locations in Kanker district has shown high values of arsenic. Most of the contaminated sites are located in the Kotri plains. Maximum concentration of arsenic is present in Durgukundal and Koraki block that is 400 µg/L.

Soil contamination is a complex issue, which is greatly affected by local geology and source of the contamination. It was found that the Chowki block of the Rajnandgaon district is the most contaminated block having mean arsenic concentration 216 mg/kg arsenic followed by the Dongargaon block (88.12 mg/kg) than in Mohla, the arsenic concentration is 25.93 mg/kg and the lowest arsenic level was observed in Manpur block. The soil analysis of the area presents another remarkable picture. Soil arsenic levels are high but not as high as could be expected in the soil. The soil analysis
presents a picture of metal enrichment with a mean arsenic concentration of $34 \pm 13$ ppm.

Therefore, sufficient amount of arsenic is present in soil and water of the contaminated sites and this is responsible for the development of arsenicosis in animals in humans.

**HUMAN EPIDEMIOLOGICAL STUDY AND THE FOOD BASKET APPROACH FOR ESTIMATING THE ARSENIC LOAD AT A CONTAMINATED LOCATION**

In arsenic contaminated soil, the uptake of arsenic by the plant tissue is significantly elevated, particularly in vegetables and edible crops (Larsen, 1992). According to the Total dietary Study of the Food and Drug Administration (FDA), arsenic ($> 0.03$ mg/kg) was found in 63 (24%) of foods/dishes analysed in the United States (Tao and Bogler, 1998). The highest concentration was found in seafood, followed by rice/rice cereal, mushrooms and poultry. Grains and produce are expected to be significant contributors to dietary inorganic arsenic intake (Schoof et al., 1999).

In view of the arsenic concentration in foods is an important consideration. One of the most comprehensive studies of arsenic in food was published in 1993 (Dabeka et al., 1993). The average daily dietary ingestion of total arsenic was estimated to be $14.9$ to $59.2 \mu g$ (Dabeka et al., 1987) for adults.

To analyse the cause of arsenicosis we carried out detailed analyses of the constituents of the food basket of the animals. Forage grass and feed samples were collected in triplicates and results were presented in Fig 2.9. Plants accumulate arsenic extensively in leaves from areas with high levels of arsenic in soils (Gonzalez et al., 1996). It is suggested that arsenic uptake is passive and that it is translocated to most part of plant, most being found in the roots and leaves. Natural arsenic levels in plants seldom exceed $11$ mg/g. Fig 2.9 shows the concentration of arsenic in feed and forages. It was observed that maximum concentration of arsenic was present in rice bran ($7.5$ mg/kg) and lakhadi husk ($7.2$ mg /kg), which are the main crops of the affected area. Rice plants are known to be very susceptible to arsenic toxicity, since arsenic would be more prevalent under reducing conditions, which are favourable for the growth of arsenic plants. The high arsenic concentration in straw may have the potential for adverse health effects on the ruminants and an increase of arsenic exposure in humans via the plant-animal-human pathway (Abendin et al., 2002).
In this study, it is experimentally proved that tube well water in the studied area is contaminated with arsenic and acts as the primary source of arsenic poisoning among the inhabitants. The results indicate that human population is affected with arsenic locally using the contaminated water for a long time.

Although food chain contamination is not firmly established in this study, yet some trend of arsenic contamination through localized groundwater source found in plants could be considered. It was observed that maximum concentration of arsenic was found in rice and lakhdi, which are main food consumed by the people of affected locations. Result showed that Para and doob grass consumed by grazing animals was responsible to increase their body burden of arsenic. From this study, it can be concluded that shallow groundwater is contaminated with arsenic. Plants accumulated arsenic through the localized contaminated groundwater sources. Population in those areas is being affected through drinking and cooking of the contaminated tube well water for a long time. The continued use of contaminated groundwater for drinking and cooking may give rise to a build-up of the toxin in human food chain.

Plants accumulated arsenic through the localized contaminated groundwater sources. Population in those areas is being affected through drinking and cooking of the contaminated tube well water for a long time. The continued use of contaminated groundwater for drinking and cooking may give rise to a build-up of the toxin in human food chain. The elevated levels of arsenic in the above compartments reflect the translocation of biodiversity equilibrium. Further research is necessary for conclusive remarks.
A CASE STUDY ON THE ARSENICAL HEALTH EFFECTS IN HUMANS AT THE STUDIED LOCATION

The leaching and mineral dissolution process-taking place in the region appears to be sporadic and it appears that some kind of intermittent injection process is going on in the region. We have collected a proof of this process in case of a mass-diarrhoea at a village named as Jatadah in the Durg District, which adjoins the Kanker district. In the location during the months of September-October of 2003, a case of mass diarrhoea was reported and a large number of people had to be admitted in the hospital. The whole village was dependent on boreholes. In the beginning, it was presumed that a bacterial infection is spreading by contaminated food or by water.

We carried out an epidemiological investigation where any case of gastroenteritis was defined as diarrhoea characterised by three or more loose stools in a 24-hour period or vomiting. A standardized questionnaire was filled in collecting the details of the foods the villagers ate before the onset of epidemic. The questionnaire was also used to characterize the demographics, timing of illness, attack rates, symptomatology, and risk factors for illness. Twenty-five patients were studied in detail and the manifestations included abdominal cramps (25 {100%}), diarrhoea (20 {80%}), bloody stools (6 {24%}), and vomiting (10 {40%}). The median age was 28 years (range: 5-54 years), and 15 (60%) patients were female. The signs and symptoms of the patients included the following:

- Gastrointestinal: abdominal pain, nausea and vomiting, and rice-water diarrhoea.
- Haematological: anaemia, leucopenia, thrombocytopoenia, and disseminated intravascular coagulation (studied after 15 days of the diarrhoeal epidemic).
- Other: difficulty in swallowing, abdominal pain.

Stool cultures were prepared for Salmonella, Shigella, Campylobacter, and Escherichia coli and none of the samples were positive. Then we analysed the drinking water and urine samples for the presence of arsenic. Both the drinking water and urine samples collected after about four days of outbreak, showed the presence of arsenic. The mean As level in groundwater was 112 µg/L with a standard deviation of 83.9 and maximum As level of 330 µg/L compared to the National Drinking Standard of 50 µg/L of As. To confirm the diagnosis, urinary As level were measured and the analysis of 14 patients showed mean As level of 177.9 µg/L standard
deviation of 101.4 and maximum of 390 µg/L. These results confirm the diagnosis as sub-acute arsenic poisoning. The Agency for Toxic Substance and Disease Registry (ATSDR 2003) proclaims that in sub acute poisoning the onset of milder gastrointestinal symptoms may be so insidious that the possibility of arsenic intoxication is overlooked. As a result of inorganic arsenic's direct toxicity to the epithelial cells of the gastrointestinal tract and its systemic enzyme inhibition, profound gastroenteritis, sometimes with haemorrhage, can occur within minutes to hours after acute ingestion. Symptoms may last for several days. Difficulty in swallowing, abdominal pain, vomiting, diarrhoea, and dehydration may result. Clinical diagnosis of arsenic intoxication is often difficult because both acute and chronic poisoning present a wide spectrum of signs and symptoms, which are largely dependent upon route of exposure, chemical form, dose, and time elapsed since exposure. In many cases, the patient or person providing the history might not have all of the information, or the source of exposure might not be apparent. By integrating laboratory results with history and clinical findings, it is often possible to confirm a diagnosis.

It is further interesting to note that there was continuous decrease in the major water quality parameters (Fig 2.10 and 2.11). The water quality analysis of the water of affected village, collected after about four days of onset of epidemic showed elevated levels of As, Fe and Mn (188, 456, 556 µg/L). During the next week the levels of dissolved constituents showed a marked decrease (147.4, 394, 504 µg/L of As, Fe and Mn respectively) and eventually the arsenic was totally absent after 14 days and none of the sample during the said period showed any indication of microbial contamination of water as characterised by maximum probable number (MPN) tests.
Fig 2.10 Changes in the water quality parameters in the groundwater of diarrhoeal epidemic affected village.

Similarly, the laboratory tests to obtain the baseline urinary values showed a negligible concentration of arsenic. This follows the fact that the urinary levels of arsenic drop rapidly in the first 24 to 48 hours after acute exposure.

Fig 2.11 Changes in the water quality parameters in the surface water of diarrhoeal epidemic affected village

Hence, it appears that a regional arsenic contamination is in taking place in the central-east India. The arsenic is geological in origin and is related to the volcanic orogeny taken place during Middle Proterozoic age. Presence of arsenopyrite in certain geological formations has also been noted. This has also caused an elevated soil arsenic level. Yet, these levels are not as high as had been noted in some of the adjoining blocks of Rajnandgaon district. The arsenopyrite-oxidation could to be a
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cause of arsenic mobilisation in the Kanker district of Chhattisgarh state. Arsenic levels are high and show a seasonal variation, which indicates the involvement of leaching/mineral dissolution process.

Appearance of arsenic appears to be controlled by some complex geochemical phenomena and there are instances of sudden release of arsenic. The case studies of diarrhoeal epidemic prove that such a release could be a major health hazard also which may be wrongly attributed to the microbial contamination.

**Epidemiology of Human Arsenicosis**

In this study, it is experimentally proved that tube well water in the studied area is contaminated with arsenic and acts as the primary source of arsenic poisoning among the inhabitants. The results indicate that human population is affected with arsenic locally using the contaminated water for a long time.

Chronic ingestion of inorganic arsenic causes characteristic skin lesions including pigmentation changes, mainly on the trunk and extremities, and keratosis of the palms of the hands and soles of the feet. Hyper pigmentation has been described as raindrop-shaped discoloration spots, diffuse dark brown spots, or diffuse darkening of the skin on the limbs and trunk. Simple keratosis usually appears as bilateral thickening of the palms and soles; nodular keratosis appears as small protrusions usually confined to the palms and soles (Guha Mazumdar et al., 1998). The types of skin lesions occurring most frequently in arsenic-exposed humans are hyperpigmentation, hyperkeratosis, and skin cancer (Fig 2.12). Patchy hyperpigmentation, a pathologic hallmark of chronic exposure, may be found anywhere on the body, but occurs particularly on the eyelids, temples, axillae, neck, nipples, and groin. The classic appearance of the dark brown patches with scattered pale spots is sometimes described as "raindrops on a dusty road." In severe cases, the pigmentation extends broadly over the chest, back, and abdomen (Fig 2.13, 2.16). Pigment changes have been observed in populations chronically consuming drinking water containing 400 ppb or more arsenic (Yadav, 2003). Arsenical hyperkeratosis occurs most frequently on the palms and soles. Keratoses usually appear as small corn-like elevations, 0.4 to 1 cm in diameter (Fig 2.14, 2.15). In most cases, arsenical keratoses show little cellular atypia and may remain morphologically benign for decades. Arsenic induced neuropathy was observed in individual of affected areas. (Chakraborti et al., 1999b; Chowdhury et al., 2000a, 2000b, 2003). Arsenic toxicity
also causes anaemia, burning sensation of eyes, solid edema of legs, liver fibrosis and gangrene of toes (Guha Mazumder et al., 2001).

The studied locations present a vivid picture of arsenicosis at various stages in the humans, which are clinically manifest. Some of the pictures of such patients are also presented here. However, the clinical manifestation was not so apparent in the case of animals.
Fig 2.12 Hyperpigmentation in chronic arsenic toxicity.

Fig 2.13 (1) Leucomelanosis on the ventral side of trunk in chronic arsenic toxicity. (2) Ulcerative lesions on the finger of affected man Leucomelanosis.
CONCLUSION

The epidemiological study was carried on the arsenic levels in the caprines reared in the arsenic contaminated locations of Chhattisgarh. The study included all epidemiological, clinical, biochemical parameters in the target animals. An attempt was also made to find out the quantity of arsenic entering into the subjects at those locations by analysis the arsenic content of their food basket. Such studies provided vital clues on the arsenic burden and their probable manifestations/impact on ruminants and humans in the studied locations.

It appears that a regional arsenic contamination is in taking place in the central-east India. Arsenic levels are high in soil, water and vegetation of affected location. The elevated levels of arsenic in the above compartments reflect the translocation of equilibrium. Both animal and humans of affected area shows the signs of arsenicosis but clinical signs were observed in humans only as they are less tolerant to arsenic. Significantly, elevated levels of arsenic were found in biological samples and maximum concentration was in hair, the main accumulators of arsenic. Effect of sex, breed, age, feeding habits and species on arsenicosis was observed and it was concluded that there is impact of time and level of arsenic exposure as well as developmental changes takes place in body. Case studies of affected area prove that the diarrhoea may be caused by arsenic toxicity.
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