ADVERSE EFFECT OF ARSENIC EXPOSURE ON ANIMAL HEALTH AND NATURAL RESOURCES

A Thesis

Submitted to the

West Bengal University of Animal and Fishery Sciences

In partial fulfillment of the requirements

for the Degree of

Master of Veterinary Science

In

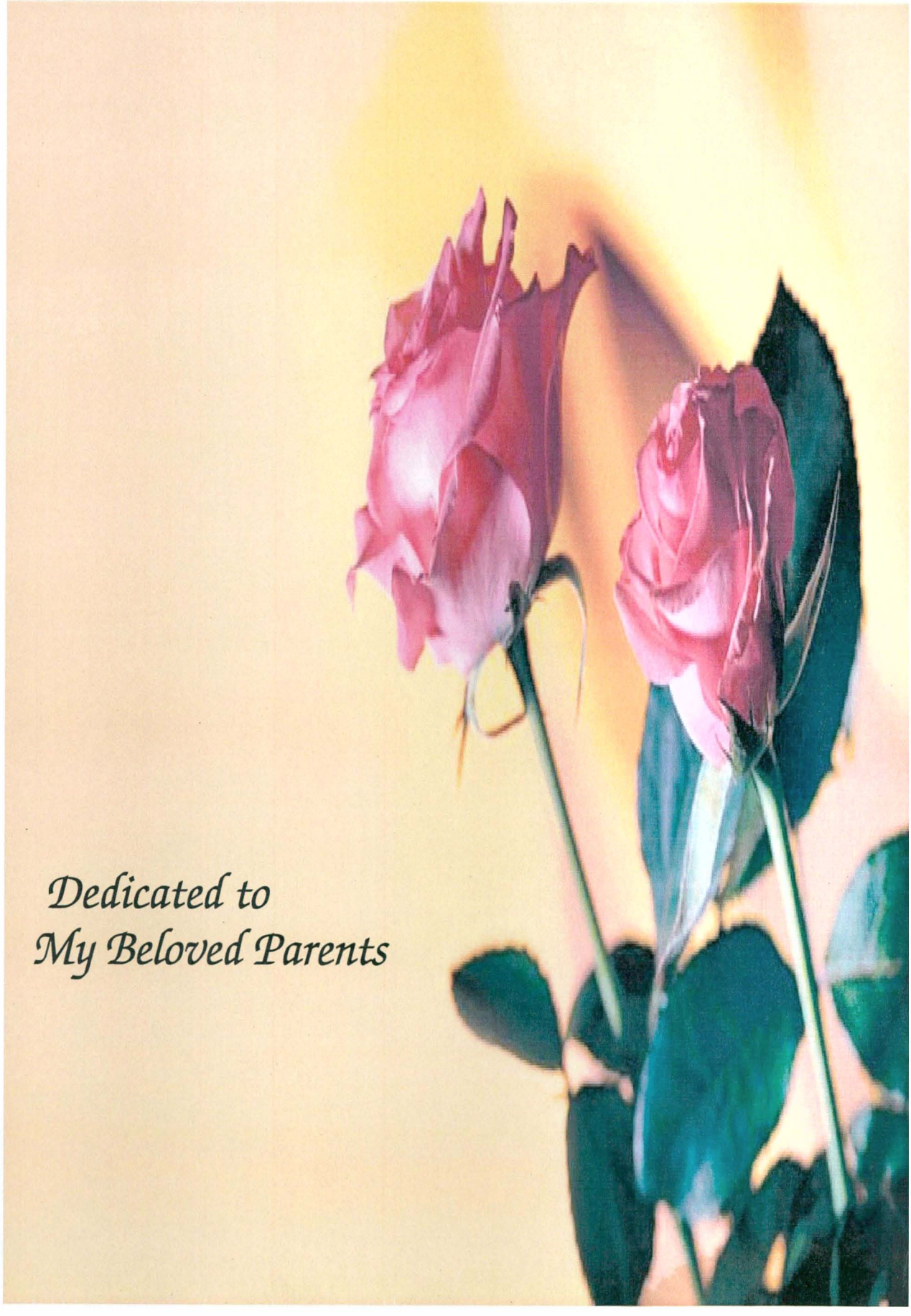
VETERINARY MEDICINE

By

PRADIP KUMAR MANDAL
B.V.Sc. & A.H.

Department of Veterinary Medicine, Ethics and Jurisprudence
Faculty of Veterinary and Animal Science
West Bengal University of Animal and Fishery Sciences
37 & 68, Kshudiram Bose Sarani,
Belgachia, Kolkata-700 037

2008


Dedicated to
My Beloved Parents

West Bengal University of Animal and Fishery Sciences
Department of Veterinary Medicine, Ethics & Jurisprudence
Faculty of Veterinary and Animal Sciences
37& 68 Kshudiram Bose Sarani, Belgachia, Kolkata – 700037

Prof. (Dr.) Samar Sarkar
Head, Department of V.M.E.J.

37 Kshudiram Bose Sarani
Belgachia, Kolkata-700037
Telex: VETUNIV
+91332557 1986

Ref: No. ..... ***Date .....***

CERTIFICATE

This is to certify that the work recorded in the thesis entitled ***"ADVERSE EFFECT OF ARSENIC EXPOSURE ON ANIMAL HEALTH AND NATURAL RESOURCES"*** *submitted by Pradip Kumar Mandal in partial fulfillment of the requirements for the degree of* ***Master Of Veterinary Science*** *in Veterinary Medicine under West Bengal University of Animal and Fishery Sciences, is the faithful and bonafide research work carried out by the candidate himself under my personal supervision and guidance. The research findings presented in the thesis have not so far been submitted for any other degree or diploma. The assistance and help received during the course of investigation have been duly acknowledged.*

Prof. (Dr.) Samar Sarkar
Chairman
Advisory Committee

Dated:
Place: Belgachia, Kolkata

APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF VETERINARY SCIENCE

(VETERINARY MEDICINE)

We, the undersigned, having been fully satisfied with the performance of Dr. Pradip Kumar Mandal, in the Viva-Voce Examination, conducted today, the 11th November, 2008, recommended that this thesis be accepted for the award of the Degree of 'Master of Veterinary Science' in Veterinary Medicine of the West Bengal University of Animal and Fishery Sciences, Kolkata.

Name	Signature
1 Prof. Samar Sarkar Chairman, Advisory Committee	.....
2. Prof. K. Roy. External Examiner	.....
3. Prof. A. Chakrabarti Member, Advisory Committee	.....
4. Prof. N. R. Pradhan Member, Advisory Committee	.....
5. Prof. T. K. Mandal Member, Advisory Committee	..... 11/11/08
6. Mr. R. Dasgupta Member, Advisory Committee	..... 11/11/08

ACKNOWLEDGEMENT

A formal statement of acknowledgement will hardly meet the ends of justice in the matter of expression of my deeply felt sincere and allegiant gratitude to all who encouraged and helped me during my study. I feel really sorry if I forget to mention any one.

There author express his deepest sense of gratitude and sincere indebtedness to his relevant guide. Prof. S. Sarkar, Head, Deptt. of Veterinary Medicine Ethics and Jurisprudence, Faculty of Veterinary and Animal Sciences. West Bengal University of Animal and Fishery Sciences, for his valuable guidance, constructive criticism, inexhaustible interest, scholarly advice, constant inspiration and whole hearted encouragement during the entire course of present investigation and in preparation of this thesis.

I feel immense pleasure to express the special gratitude to Prof. T. K .Mandal, Deptt. of Pharmacology & Toxicology, Faculty of Veterinary and Animal Science. West Bengal University of Animal and Fishery Sciences for his benevolent guidance, valuable suggestions, untiring supervision, sustained interest, and encouragement through out the period of the study.

I would like to express my respect and thankfulness to Prof. A. Chakarbarti, PhD., Deptt of Veterinary Medicine Ethics and Jurisprudence, Faculty of Veterinary and Animal Science, for his valuable suggestions.

I would also like to convey my sincerest regards and gratitude I wish to express my sincere thanks to Prof. N. R. Pradhan, PhD., Deptt. Of Veterinary Medicine Ethics and Jurisprudence, Faculty of Veterinary

and Animal Science, Controller of Examinations, West Bengal University of Animal and Fishery Sciences, for their intensive encouragement and suggestions during my research works.

The author also expresses his sense of gratitude and acknowledgement to Mr. Raju Dasgupta for constant help and cooperation during the present study and statistical analysis.

I would like to offer my appreciation to Prof. C. S. Chakraborty, Vice- chancellor, West Bengal University of Animal and Fishery Sciences, Kolkta-37, for kindly allowing me to prosecute higher studies in this university.

I am highly obliged and very much grateful to Dr. C. Lodh, Lecturer, Dept. of Veterinary Medicine Ethics and Jurisprudence, Faculty of Veterinary and Animal Sciences for providing me all necessary facilities and help during the period of my study.

I am delighted to convey my hearty thanks to my seniors specially Dr. Chhton Ghosh, Dr. Bakul Dutta, and Dr. Prasun Biswas, Dr. Samir Kr. Maiti, Dr. Stephen Soren for their kind cooperation & advice in this study.

I am highly obliged and thankful to my friends Dr. Nitya Gopal De, Dr. Sujoy Bisai, Dr. Lalon Kole, Dr. Devapriya Har, Dr. Pamela Halder, Dr. Debjani Das, Dr. Sunirmal Saren, Dr. Kaushik Pal , Dr. Subhasis Das for their help and encouragement.

I also convey my sincere thanks to my dear juniors Dr. Taraknath Karmakar, Dr. Parameswori Mandal, Dr, P. Thakur, Dr. Nirmalya Bar, Dr. Santu Dutta, Thuin Samanta, Suman Biswas, Abhijit Barui, Prasanta Roy, Supada Biswas, Mayuk Ghosh.

I am extremely delighted in extending my thanks to the staff of dog ward and Department of Veterinary Medicine , Ethics, and Jurisprudence, West Bengal University of Animal & Fishery Sciences, for helping to undertake research work.

A word of thanks is due to the library, West Bengal University of Animal and Fishery Sciences for the help extended for collection of all relevant literature through CD ROM, books and journals.

The author feels very happy to acknowledge M/S Dhar Brothers for excellent printing and binding work.

I wish to record my eternal sense of gratitude and indebtness to my parents and brother for their constant inspiration, blessings, sacrifice during the period of study.

Lastly but not the least, the author seeks the kind grace and blessing of the omnipotent, omniscient Almighty.

Pradip Kr. Mandal

Pradip Kumar Mandal

Date: 23.09.08.

Place: Kolkata.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS USED

%	: Percent.
µl	: Microlitre.
µg	: Microgram (10^{-6}g)
@	: At the rate
etc.	: Etcetera.
µg/L.	: Microgram (10^{-6}g)/liter.
&	: And
gm/dl.	: Gram per deciliter.
Hb.	: Haemoglobin.
AAS	: Atomic Absorption Spectrophotometer
As	: Arsenic
ASA	: Arsanilic Acid
AST	: Aspertate transaminase
ALT	: Alanine Aminotransaminase
BAL	: British Antilewisite
DMSA	: Meso-2-3 dimercapto succinic acid
DSMA	: Disodium Methane Arsenate
MMA	: Monomethyl 1 arsonic acid
mmol	: Milimole
MSMA	: Monosodium Methane arsenate
/	: Per
ppb	: Parts per billion
ppm	: Parts per million
SPSS	: Statistical package for social science
EDTA	: Ethylene diamine tetra acetate
nm.	: Nanometer.
mg %	: Miligram percentage
^{0}C	: Degree centigrade

ml.	: Mililitre
IU/L.	: International Unit per litre.
GIT	: Gastrointestinal tract
b. wt	: Body weight
Gr.	: Group.
III	: Trivalent
PTH	: Parathyroid hormone.
TEC	: Total erythrocytic count.
TLC	: Total leucocytic count.
B.U.N.	: Blood urea nitrogen
BGL	: Blood Glucose Level
TSP	: Total Serum Protein
Conc.	: Concentration
lb	: Pound
<	: Less than
>	: More than
OD	: Optical density
et al.	: And other
Fig	: Figure
gm	: Gram
hrs	: Hours
i.e	: That is
L	: Liter
mg	: Milligram(10^{-3}g)
mm	: Millimeter
ng	: Nanogram (10^{-9}g)
No.	: Number
V/V	: Volume by volume
viz.	: Namely
V or v	: Pentavalent
Kg.	Kilogram.

CHAPTER-I

INTRODUCTION

INTRODUCTION

Arsenic is one of the toxic environmental pollutants which have recently attracted mass attention because of its chronic and epidemic effects on human health. The widespread water and crop contamination through natural release of this toxic element from aquifer rocks has been reported in Bangladesh and West Bengal, India (Fazal *et al.*, 2001; Smith *et al.*, 2000; Hopenhayn, 2006).

Arsenic (As) is in Group V of the periodic chart of elements. It is metalloid with some properties similar to phosphorus, antimony and bismuth. Arsenic can exist in number of valance states from -3, 0, +3 to +5, although latter two oxidation states are the most common (Eisler, 1994).

Arsenic concentration at dangerous level in natural water is a worldwide problem. At present, among 21 countries in different parts of the world like the USA, Afghanistan, Bangladesh, India, Cambodia, Canada, Hungary, China, Chile, Argentina, India, Japan, Mexico, Mongolia, Myanmar, Nepal, Pakistan, Poland, Taiwan, Thailand, Viet Nam have reported high levels of As in part of their groundwater resources (Biswas *et al.*, 1998).

The high levels of arsenic in groundwater in the affected countries are predominantly of geogenic origin. Reductive dissolution of iron hydroxides stimulated by microbial activity and organic materials is regarded the most important mechanism of release of As into the aquifer (McArthur *et al.*, 2004; Zheng *et al.*, 2004).

The World Health Organization (2005) set a provisional level for arsenic in drinking water i.e. 10µg/L.

In Australia, the guideline value for drinking water set by the National Health and Medical Research Council and the Agricultural and Resource Management Council of Australia and New Zealand is 7µg/L (NHMRC and ARMCANZ, 1996).

However, in many developing countries, including Bangladesh, 50µg/L is commonly adopted as the guideline value, often for economic reasons, thus exposing the population to long-term risks (Morales *et al.*, 2000).

Environmental arsenic exposure mainly occurred from arsenic contaminated drinking water. Arsenic in drinking water is often from natural sources (Garland, 2007). Environmental exposure to arsenic also occurs from burning of coal containing naturally high level of arsenic (Liu *et al.*, 2002). In some coal, mined in Czechoslovakia, the concentration of arsenic has been shown to be as high as 1500 mg/kg (Cmarko, 1963).

Arsenic is found as different ores and rocks being mined, then smelted resulting in elemental arsenic and arsenic trioxide. In the environment, arsenic usually exists as pentavalent form and soil micro-organism may methylate it. Since it is ubiquitous in many forms, it is not likely that complete avoidance is possible (Garland, 2007).

Global natural emission of arsenic have been estimated to be 7,900 tones per year, while anthropogenic emission is about three times higher i.e. 23,600 tones per year (Woolson, 1983).

Arsenic in air is present mainly in particulate form as inorganic arsenic. It is assumed that methylated arsenic is a minor component in the air of suburban, urban and industrial areas and that major

inorganic portion is a variable mixture of the trivalent and pentavalent forms and the latter being predominant (Environmental Protection Agency, 1984).

In arsenic contaminated areas of the Ganga - Meghna – Brahmaputra (GMB) plain (area 5,69,749 km^2 ; population over 500 million) where traditionally cow dung cake is used as a fuel in unventilated oven for cooking purpose, people are simply exposed to1859.2 ng (nano gram) arsenic per day through inhalation (Pal *et al.*, 2007).

The order of toxicity from greatest to least follows this schematic: inorganic As^{+3} (arsenite) > inorganic As^{+5} (arsenate) > trivalent organics > pentavalent organics (Garland, 2007).

The amount of arsenic requires causing adverse health effects depends on the chemical and physical forms of the arsenic that is ingested. Inorganic forms are generally more acutely toxic than organic forms and more water-soluble forms tend to be more toxic than those that dissolve poorly in water. Also, the oxidation state of arsenic affects its toxicity, with As III being more toxic than As V. Recent evidence indicates that trivalent methylated metabolites of inorganic arsenic can be more toxic than arsenite in both *in vitro* and *in vivo* tests (Styblo *et al.*, 2002).

Human and animal data indicate that over 90% of the ingested dose of dissolved inorganic trivalent or pentavalent arsenic is absorbed from the gastrointestinal tract. Organic arsenic compounds in seafood are also readily absorbed (75–85%). Absorption of less soluble forms, e.g. arsenic trioxide, is much lower (Freeman *et al.*, 1993).

Arsenate (As^{5+}) is rapidly reduced to arsenite (As^{3+}) by arsenate reductase (presumably purine nucleoside phosphorylase). Arsenite is then sequentially methylated to form methyl arsonate (MMA^{5+}) and dimethylarsonic acid (DMA^{5+}) by arsenic methyl transferase or arsenite methyl tranferase using δ-adenosylmethionine (SAM) as methyl group donor. The intermediate metabolites, methylarsonous acid (MMA^{3+}) and dimethylarsonous acid (DMA^{3+}), are generated during this process, and these trivalent methylated arsenicals are now thought to be more toxic than even the inorganic arsenic species (Aposhian and Aposhian, 2006).

Arsenic is eliminated by many routes (e.g. faeces, urine, sweat, milk, skin and lung), although most is excreted in urine in humans (Klaassen *et al.*, 2006).

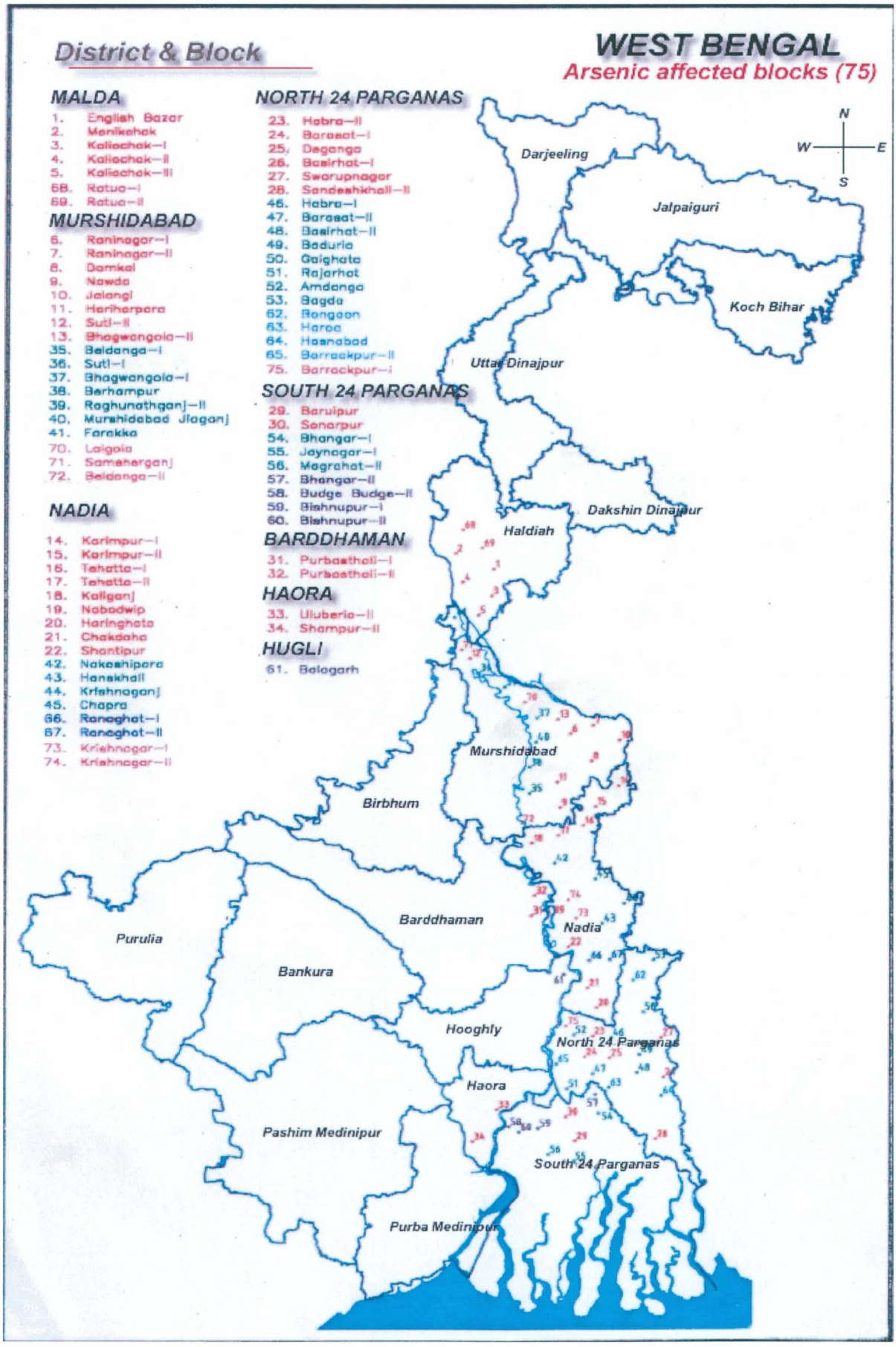
Human health can be affected through intake of milk of the cattle reared in the arsenic prone zone where the arsenic concentration in water to feed cattle is much higher than the permissible limit i.e. 0.05mg/L (USEPA, 1973). Concentrations of 3.0μg/l00ml of arsenic in milk have been recorded in normal condition (Kirchgessner *et al.*, 1967).

Uncontaminated soils were found to contain arsenic levels between 0.2 to 40 mg/kg, while arsenic-treated soils contained up to 550 mg/kg (Walsh and Keeney, 1975). Studies also reported that the arsenic concentration in straw of rice (*Oryza sativa*) is directly proportional to soil arsenic concentrations (Rahman *et al.*, 2007a).

The use of groundwater for irrigation has increased abruptly over the last couple of decades. About 86% of total groundwater withdrawn is utilized in agricultural sector (WRI, 2000).

West Bengal is one of the 29 states in India. The area of West Bengal is 89,193 sq. km having a population of about 80.1 million. Its administrative structure consists of several districts. Each district has several blocks/police stations; each block has several Gram Panchayets (GPs), which are cluster of villages. There are 19 districts, 341 blocks and 37910 villages in West Bengal. Based on the arsenic concentrations found in the 19 districts of West Bengal, it is classified into three categories: Severely affected, mildly affected and arsenic safe. Nine districts (Malda, Murshidabad, Nadia, North-24-Parganas, South-24-Parganas, Bardhaman, Howrah, Hoogly and Kolkata), where more than 300 μg/L arsenic concentrations were found in tube wells are categorized as severely affected. Out of 1,35,555 samples analyzed from these districts, 67,306 (49.7%) had arsenic concentrations above 10μg/L and 33,470 (24.7%) above 50μg/L. The five districts (Koch Bihar, Jalpaiguri, Darjiling, North Dinajpur and South Dinajpur) where the contaminated tube wells showed arsenic concentrations mostly below 50μg/L (only a few above 50μg/L but none above 100 μg/L), termed as mildly affected. A number of 2,923 water samples from these districts were analyzed of which 285 (9.8%) had arsenic concentration between 4 to 10μg/L, 163 (5.7%) had above 10μg/L and 6(0.2%) showed above 50 μg/L. The rest five districts (Bankura, Birbhum, Purulia, Medinipur East and Medinipur West), where all the recorded concentrations were below 10 μg/L termed as unaffected or arsenic safe. All the samples (n=1,672) analyzed from these five districts had arsenic concentrations below 3 μg/L. (SOES, 2006).

The Planners, Politicians, Bureaucrats, Administrators may give a thought upon and feel the necessity of all-round development of animal husbandry sectors and the role of the veterinarians for the socio-economic cause of the country.


District & Block
WEST BENGAL
Arsenic affected blocks (75)
MALDA
1. English Bazar
2. Manikchak
3. Kaliachak—I
4. Kaliachak—II
5. Kaliachak—III
68. Ratua—I
69. Ratua—II
MURSHIDABAD
6. Raninagar—I
7. Raninagar—II
8. Domkal
9. Nawda
10. Jalangi
11. Hariharpara
12. Sutl—II
13. Bhagwangola—II
35. Beldanga—I
36. Sutl—I
37. Bhagwangola—I
38. Berhampur
39. Raghunathganj—II
40. Murshidabad Jiaganj
41. Farakka
70. Lalgola
71. Samsherganj
72. Beldanga—II
NADIA
14. Karimpur—I
15. Karimpur—II
16. Tehatta—I
17. Tehatta—II
18. Kaliganj
19. Nabadwip
20. Haringhata
21. Chakdaha
22. Shantipur
42. Nakashipara
43. Hanskhali
44. Krishnaganj
45. Chapra
66. Ranaghat—I
67. Ranaghat—II
73. Krishnagar—I
74. Krishnagar—II
NORTH 24 PARGANAS
23. Habra—II
24. Barasat—I
25. Deganga
26. Basirhat—I
27. Swarupnagar
28. Sandeshkhali—II
46. Habra—I
47. Barasat—II
48. Basirhat—II
49. Baduria
50. Gaighata
51. Rajarhat
52. Amdanga
53. Bagda
62. Bongaon
63. Haroa
64. Hasnabad
65. Barrackpur—II
75. Barrackpur—I
SOUTH 24 PARGANAS
29. Baruipur
30. Sonarpur
54. Bhangar—I
55. Jaynagar—I
56. Magrahat—II
57. Bhangar—II
58. Budge Budge—II
59. Bishnupur—I
60. Bishnupur—II
BARDDHAMAN
31. Purbasthali—I
32. Purbasthali—II
HAORA
33. Uluberia—II
34. Shampur—II
HUGLI
61. Balagarh
N
W
E
S
Darjeeling
Jalpaiguri
Koch Bihar
Uttar Dinajpur
Dakshin Dinajpur
Haldiah
Murshidabad
Birbhum
Barddhaman
Nadia
Purulia
Bankura
Hooghly
North 24 Parganas
Haora
Pashim Medinipur
South 24 Parganas
Purba Medinipur

In view of the increasing arsenic related health hazards for million of peoples in India, ICAR (Indian Council of Agricultural Research) has implemented a project in West Bengal under National Agricultural Innovation Project (NAIP) from 2007 with an integrated approach to form a new organizational vehicle by the inclusion of scientist and teachers from the discipline of Agricultural, Veterinary, Fishery and Medical sciences.

In West Bengal though literatures on the status of arsenic in water is available but still there is no such report of the effect of arsenic on the substrates of cattle in the ambit of arsenic prone zone.

The present study therefore envisages with the following objectives.

1. Evaluation of the status of arsenic in feed stuffs and drinking water of animals.

2. To estimate the amount of arsenic excreted through urine and faeces of cattle.

3. To measure the arsenic concentration in milk of cattle belonging to arsenic prone zone.

4. Estimation of amount of arsenic deposited in hair.

5. Estimation of different biochemical and haematological changes due to arsenic toxicity in animal.

CHAPTER-II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 STATUS OF ARSENIC IN WATER, SOIL AND PLANTS:-

Chakravarty and Saha (1987) recorded arsenic content ranging between 0.2 to 0.64 mg/L in drinking water from shallow tube wells in 5 districts in West Bengal.

Chatterjee *et al.*(1995)analysed thousands of tubewell water samples from six districts for four arsenic species namely, arsenite, arsenate, monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) in which MMAA or DMAA in any of those samples were nil and more toxic arsenite is present in ground water at about 50% of the total arsenic level.

Pandey *et al.* (2002) stated that surface water of the rivers is being contaminated because of the probable dislocation of contaminated groundwater due to the heavy rains in monsoon season, which indicates that the river water could be a major carrier of arsenic in dissolved or absorbed forms that may be a cause of contamination of delta plains.

Ghosh *et al.* (2004) reported that the ground water As concentration (50-1600 mg/L) from the affected areas of West Bengal are several orders of magnitude higher than the stipulated Indian Standard or the permissible limit in drinking water(50mg/L)which is also the maximum acceptable concentration, (MAC), for drinking water in Bangladesh, India. They also reported that the loading of the groundwater which is used as irrigation source varied from 0.06 to 0.53 mg/L in Nonaghata mouza of the Haringhata block of Nadia district in West Bengal.

Rahaman *et al.* (2005) analyzed 29,612 hand tubewell water samples for arsenic from both contaminated and non-contaminated areas, and 26% of the tubewells were found to have arsenic above 50 µg/ L in Murshidabad, West Bengal.

In Haringhata block of Nadia district, 20.6% of ground water samples contained As more than the permissible limit (0.05 mg/L) and 58.4% contain arsenic more than 0.01 mg/L (SOES, 2006).

Sanyal and Dhillon (2005) opined that the total arsenic concentration of soil at Nonaghata Dakhinpara and Nonaghata Uttarpara of Haringhata Block in Nadia district, West Bengal, India were 11.3 mg/kg and 14.7 mg/kg respectively.

Singh (2006) reported that the surface reactivity of iron (Fe) and aluminium (Al) plays an important role in adsorbing the bulk of As in the sedimentary aquifers in the Ganges-Brahmaputra.

Adak *et al.* (2002) stated that the arsenic content of the leaves, stem and potato tuber at harvest were 5.51, 9.34 and 10.2 mg/kg respectively, when potato was grown with irrigation water with arsenic loading of 0.22 mg/L.

Sanyal and Dhillon (2005) opined that the arsenic content in roots, stems, leaf and economic products of rice plants of Nonaghata Mouza of Haringhata Block were 5.40 mg/kg, 3.54 mg/kg, 2.58 mg/kg and 1.44 mg/kg respectively.

Abedin *et al.* (2002) reported significant increase of arsenic in rice (*Oryza sativa*) straw with the increase of arsenate concentrations in irrigation water & they also found 3.9 mg of As kg^{-1} dry straw at the lowest arsenate treatment (0.05 mg L^{-1}), which increased progressively with increasing the concentration

of the element in irrigation water and reached to 91.8 mg kg^{-1} dry weight at the highest arsenate treatment (8.0 mgl^{-1}).

Alsono *et al.* (2004) determined the concentration of arsenic, copper, and zinc in the liver, kidney, muscle, and blood of calves from across Galicia in Spain related them to the metal conc. in the soil from the areas in which the animals were reared and for each element, liver (but not usually kidney, muscle, or blood) concentration was significantly elevated in animals from areas with higher soil conc. They also told that liver arsenic conc. were only markedly greater in animals from areas with soil arsenic levels more than 20mg/kg

2.2 STATUS OF ARSENIC IN URINE AND FAECES:-

Peoples (1964) claimed that increasing arsenic intake results to elevation of As content in urine.

Wang *et al.* (2002) revealed that the most common arsenic biomarker of exposure is the analysis of total arsenic in urine. Urinary porphyrins have also been proposed as a biomarker of arsenic exposure.

Biswas *et al.* (1998) revealed that chronic arsenic in experimentally produced animals causes reduced body weight, coffee coloured urine, congested mucous membrane and polyuria.

Browning (1969) reported that urinary As excretion rises with increasing As intake so that total urinary As excretion provides a useful index of exposure.

Satoskar and Bhandarkar (1993) stated that urinary excretion occurs within 2 to 8 hrs after oral administration and continuous for 8-10 days.

Buck *et al.* (1976) reported that the kidneys are a primary route of excretion with approximately 36% of the initial dose cleared by 2 hr post injection. Only about one-fifth of absorbed arsenic is promptly excreted through urine and faeces. The remaining four fifths is stored widely in the body. A single dose may require 10 days for complete elimination.

Biswas *et al.* (1998) revealed that the level of arsenic in urine and faeces of experimentally induced arsenic toxicity in goat varies from 45.8 ± 0.77 to 215.6 ± 1.73mg%.

2.3 STATUS OF ARSENIC IN MILK:-

Peoples (1964) observed that daily doses of 0.03-0.66 mg/kg body weight given to cows in the form of arsenic acid for up to 8 weeks did not cause the arsenic levels in milk to rise

Mahreu *et al.* (1977) stated that high levels of arsenic (117 µg/L) were found in the cow's milk in France.

Sahli (1982) opined that milk from healthy cows contained 0.0005-0.81ppm of As in U.K where contaminated grazing pastures contained 0.07-1.5 ppm As.

2.4 STATUS OF ARSENIC IN HAIR:-

Biswas (1993) measured arsenic concentration in hair in the ranges from 4.77 ± 0.06 to 18.19 ± 0.27mg/kg.

It is also well known that inorganic arsenic binds to sulfhydryl groups and it has been suggested that arsenic concentrates in tissues with a high content of cysteine-containing proteins, including hair, nails, skin and the lungs (NRC, 1999).

Radostits *et al.* (2000) reported that arsenic content in hair of cattle went as much as 5-10 mg/kg where as animals not exposed to arsenic (normal) should contain less than 0.5mg/kg.

Hindmarsh (2002) observed that major issue in the use of hair and nails as biomarkers of exposure is their adsorption of arsenic from external sources. For someone who consumes and bathes in water or is in contact with soil with elevated levels of inorganic As, arsenic from internal and external exposure would most likely be detected in hair and nails. This would complicate the exposure analysis. Although washing procedures have been developed, the possibility exists that this procedure may remove arsenic in the specimens that originated from internal sources. It is also not presently possible to distinguish between externally and internally derived arsenic in hair

Chandra Sekhar *et al.* (2003) reported that the arsenic contents in hair in eight industrial contaminated sites of Hyderabad were 0.9 mg/kg, 0.8 mg/kg, 0.9mg/kg, 0.5mg/kg, 0.7mg/kg, 0.06mg/kg, 0.5mg/kg, 0.8mg/kg, and 0.4 mg/kg repetitively.

Mitranescu *et al.* (2003) cited that the highest values of arsenic were observed in young and dark-coloured bulls and it came into the animal body through water and fodder and accumulate in the skin and bones.

2.5 HAEMATOLOGICAL CHANGES :-

Goodman and Gilman (1990) stated that haematological examination shows anaemia with the effect of inorganic arsenicals. There is slight to moderate leukopenia and eosinophilia.

Biswas *et al.* (1998) observed that chronic arsenic toxicity of experimentally induced goats reveals lowered Hb, PCV, TEC and TLC.

Kent (1998) revealed that arsenic suppress cellular formation in the bone marrow resulting in leukopenia, granulocytopenia and anaemia.

Fusari and Ubaldi (2000) noted that in chronic arsenic poisoning in dairy cows, there is decrease in erythrocytic count and increase in serum potassium levels.

Ianchev (2001) reported low haemoglobin percentage in ruminants suffering from arsenic toxicosis.

Ng *et al.* (2005) revealed that the haematological consequences of chronic exposure to arsenic may include interference with haeme synthesis, with an increase in urinary porphyrin excretion, which has been proposed as a biomarker of arsenic exposure.

2.6 BIOCHEMICAL CHANGES:-

Biswas (1993) observed that acute and subacute exposure of arsenic toxicity in goat show reduced Total Serum Protein, albumin, globulin, sodium, potassium, chloride but increased

blood glucose level, ,alkaline phosphatase, acid phosphatase, AST and ALT.

Biswas *et al.* (2000) opined that chronic exposure of arsenic toxicity in goat leads to significant decrease in total serum protein and albumin globulin ratio, and increase in blood glucose and various enzymatic activities.

Santra *et al.* (2000) reported the hepatic damaged caused by chronic arsenic toxicity in experimentally animals and there was elevated level of serum ALT & AST.

Ianchev (2001) analysed 21 blood samples of arsenic affected sheep (3 years old) that contain high concentration of AST and ALT and cholesterol in blood.

2.7 METABOLISM OF ARSENIC:-

Buck *et al.* (1976) reported that the animals dying of acute or subacute poisoning might have contained from 2 to 100 ppm of As on a wet weight basis in two vital organs such as liver and kidney. He also opined that the levels above 10 ppm on a wet weight basis would be the considered confirmatory of arsenic poisoning.

Satyanarayana (2002) reported that arsenic binds with -SH groups of several enzymes and inhibits biochemical reactions, e.g. pyruvate dehydrogenase. It causes coagulation of proteins and blockage of ATP generation (function as an uncoupler).

Styblo *et al.* (1996) noticed that arsenic methylation activity is localized in the cytosol and appears to occur sequentially and mainly in the liver.

Radostits *et al.* (2000) reported that the liver is the best organ for assay and levels of over 10-15 mg/kg wet matter of arsenic trioxide in the kidney or liver. The maximum concentration of arsenic in tissue reaches about 8 hrs after ingestion and the animals that survive for 2-3 days may have levels as low as 3mg/kg. The toxic dose of inorganic arsenic poisoning in cattle varies with an average of 36mg/kg.

Suzuki *et al.* (2002) opined that arsenic metabolites in the body fluids and organs/ tissues remained in the form of inorganic (arsenite and arsenate) and methylated arsenics (MMA and DMA), although pentavalent arsenics can be present mostly in the form of free ions where as trivalent ones may be present more in forms conjugated with thiol groups of glutathione (GSH) or proteins and arsenic in the body fluid present in soluble forms can be speciated on Ion exchange columns by HPLC.

Ratnaike (2003) stated that the absorbed arsenic undergoes hepatic biomethylation to form monomethylarsonic acid and dimethylarsinic acid that are less toxic and about 50% of the ingested dose may be eliminated in the urine in 3-4 days. He also revealed that dimethylarsinic acid is the dominant urinary metabolite (60-70%) compared to monomethylarsonic acid and a small amount of inorganic arsenic is also excreted unchanged. He told that in acute poisoning and in chronic arsenic ingestion, the highest concentration of arsenic accumulates in the kidneys, liver and liver, kidneys, heart, lungs, muscles, nervous system, gastrointestinal tract, and spleen(small amount) respectively. The residual amount remains in the keratin-rich tissues, hair and skin.

Sharma (2007) stated that arsenic inhibits enzyme system containing sulfhydryl group even in low concentration and interfere with cellular metabolism. It also binds to various macromolecules in membranes and cytosol & accumulates in the body as a complex that are not metabolised.

2.8 HISTOPATHOLOGICAL CHANGES:-

Nielberle and Cohrs (1966) stated that liver is enlarged & heavier than normal, doughy in consistency, uniformly light yellow in colour & reddish yellow congestion that lead to saffron yellow coloured liver due to arsenic toxicity.

Hatch (1969) reported gross lesions characteristic of inorganic arsenic poisoning include reddened abomasal or duodenal mucosa and submucosal oedema and haemorrhages in the abomasum and duodenum leading to sloughing of the duodenal mucosa or perforation of the gut wall. The intestinal content are fluid filled, bloody, sheds of intestinal mucosa with foul smelling. The liver may be soft and yellow and lungs may be oedematous and congested. Haemorrhages on the heart, pericardium, kidneys, and liver may be observed.

Hatch (1982) reported that capillary degeneration is observed in gut, skin, lungs and other organs. Degenerative changes occur ranging from cloudy swelling to necrosis in the gut mucosa, liver, kidney and heart

Anderson (1992) stated that gross lesions in the arsenic toxicity includes ulceration of mucous membrane, abomasal & intestinal erosions & ulceration, fatty liver, and pale, swollen kidneys.

Andrews *et al.* (1992) revealed that accidental contamination of feed with inorganic arsenicals or organic (herbicides, pesticides) arsenic leads to sudden death, severe colic, salivation, teeth grinding, weakness, in coordination, rapid collapse and death in acute case and in sub-acute case there is ruminal stasis, diarrhoea, severe thirst, dehydration, collapse and death. Arsenic toxicosis produces a dry, dull, rough, easily epilated hair coat, progressive to alopecia and severe seborrhoic skin diseases, occasionally focal areas of the skin necrosis and slow healing ulcers are seen.

Biswas *et al.* (1993) studied that gross lesions in the arsenic toxicity in goat include haemorrhages, swelling, sloughing of entire gastrointestinal tract, pulpy kidney, pale & small haemorrhagic spots in liver, lungs, heart and spleen. He also noticed that arsenic toxicity in goat reveals vascular congestion, cloudy swelling, fatty degeneration and massive haemorrhages of liver, kidney and heart and spleen.

Howard and Smith (1999) cited that arsenic toxicity in bovine causes hypermia of abomasum and sometimes duodenum, petechiae of Gastro Intestinal serosa, congestion of liver, kidney and lungs, oedema and necrosis in mucosa and submucosal of abomasum and duodenum, hepatic and proximal tubular degeneration and necrosis

Radostis *et al.* (2000) stated that pronounced hypermia and patchy submucosal haemorrhage in the stomach is observed in acute and subacute cases of inorganic arsenic poisoning. Ulceration of gall bladder and severe intravascular haemolysis are common in sheep.

Sastry and Rao (2002) observed that arsenic being an active irritant causes severe haemorrhagic gastroenteritis and lesions of the walls of capillaries. In chronic poisoning, lesions appear on the skin, gastrointestinal tract and the nervous system and stomach & intestine show congestion, oedema and small ulcer.

Chen *et al.* (2005) reported that after chronic ingestion of inorganic As, dermatologic lesions may develop. These lesions have been used as a long-term biomarker of cumulative arsenic exposure.

Sastry and Rao (2002) stated that in arsenic toxicity, myelin degeneration in the nervous system and pigmentation and severe keratinisation of the skin are found. Fatty degeneration of the liver, kidney and myocardium may be observed.

2.9 ARSENIC TOXICITY IN ANIMALS AND THERAPY:-

Smith *et al.* (1972) stated that chronic poisoning occurs in animals grazing on the land subjected to precipitated fumes from smelters and blast furnaces using arsenic containing ores.

Buck *et al.* (1976) reported that the lethal oral dose of sodium arsenite for most species of animals was found to be from 1 to 25 mg/kg body weight.

Sodium arsenite caused a slight increase in chromosomal aberrations in the bone marrow cells of mice treated in vivo (IARC, 1980)

Robertson *et al.* (1981) reported that one hundred and one herd of cattle died due to percutanious absorption of arsenic trioxide following the application of a medicant for killing lice.

Biswas *et al.* (1998) reported that chronic arsenic toxicity was induced in goats by oral administration of sodium arsenite @ 25.0 mg/kg body weight daily for 12 weeks and clinically there were development of gastrointestinal and nephritis signs with 100% mortality. Intoxicated goats had leukopenia, anaemia and increased erythrocyte fragility rate.

Mukherjee *et al.* (2004) stated that on chronic oral exposure to arsenic, rabbit shows an oxidation stress to cause deleterious effects on the endocrine pancreas.

Faires (2004) opined that over a 44day period, 4 0f 5 affected calves in a 170 herd of beef cattle died after exhibiting clinical signs of lethargy, ataxia, anorexia, and diarrhoea. Histopathological examination of tissues and toxicological analysis of a suspicious powder discovered in the pasture confirmed arsenic trioxide toxicosis.

Bahri *et al.* (1991) and Fletcher (1966) stated that Chronic arsenic poisoning is rarely seen in domestic animals because arsenic is rapidly excreted in the urine. Arsenic may be absorbed percutaneously, causing blistering, edema, and necrosis of the skin due to capillary dilatation and degeneration.

Anderson (1953) stated that the clinical characteristic of acute arsenic poisoning includes intense abdominal discomfort, vomiting and diarrhoea followed by rapid circulatory collapse in

animals. Death may occur within a few days and sometimes in less than one hour.

Smith *et al.* (1972) reported that the chronic form of arsenic toxicity in cattle includes particular fibrosis producing stiffness and asymmetrical enlargement of hocks or other joints of the limbs.

Despite repeated tests in multiple species at very high doses, animal testing has generally failed to detect carcinogenic effects of inorganic arsenic (IARC, 1987).

Hungerford (1975) reported that ascending degeneration of the peripheral nerve occurs in arsanilic acid poisoning in pigs. He also reported that chronic arsenic poisoning will result on the long haired coat which is scurfy and the horse is emaciated.

Winek *et al.* (1977) stated that toxicity results if feed levels greater than 250 ppm are fed for several weeks. They also reported that toxicity leads to incoordination with ataxia and posterior paresis but alertness and appetite is normal.

Kent (1998) cited that acute arsenic toxicity in human being causes diarrhoea characterised by Rice water stool, often garlicky breath, abdominal pain, laryngitis, dehydration and shock.

Howard and Smith (1999) stated that As is a nephrotoxic agent in bovine showing complication of weakness, trembling, ataxia, depression, colic, rumen atony, diarrhoea, prostration. Course of the disease is hours to several days and case mortality is high.

Radostits *et al.* (2000) stated that arsenic poisoning is characterised by dysentery, toxaemia, normal temperature and nervous signs in animals.

Sharma and Sharma (2007) reported that inorganic arsenic is absorbed from GIT and skin and arsine gas is absorbed from lungs. They also reported that chronic arsenic poisoning leads to skin irritation and colour changes, hair loss, nausea, GIT disturbance, bone marrow depression. Arsine causes massive haemaglobinuria and acute renal failure and it is also established as a carcinogen which can cause cancer in lungs and skin.

Acute arsenic poisoning causes gastro-enteritis dehydration, laryngitis and shock.

A sweet garlicky odour in breath and of stool is indicative of arsenic poisoning in human.

Clarke and Clarke (1975) recommended that BAL (Dimercaprol), a sulfhydryl containing specific antidote should be given at a rate of 3 mg/kg body weight intra muscularly every 4 hours.

Buck *et al.*(1976) made an recommendation of use of sodium thiosulfate against arsenic poisoning in a large group of animals at a dose rate of 20-30 gm orally in approximately 300 ml of water and 8-10 gm in the form of 10-20% solution intravenously.

The treatment regime for livestock includes supportive and decontamination procedures, administration of sodium thiosulfate, IV and PO, and antidotal therapy. Chelating sulfate, antidotes contain sulfhydryl groups that compete with

sulfhydryl-containing enzymes for available arsenic (Radostits et al, 2000). The classic chelating antidote for arsenic toxicosis is dimercaprol (BAL). Thioctic acid, mesodimercaptosuccinic acid, and dimercaptosuccinic acid are alternative cinic chelating agents (Osweiler, 1996).

CHAPTER-III

MATERIALS AND METHODS

3.1.1.2 SELECTION OF CONTROL ANIMALS:

Ten apparently healthy cattle were selected from the Akna village which was not affected with arsenic exposure and were kept as control group (Group I) and the eighteen experimental animals of Dakshin Panchpota village were kept as exposed group (Group II).

3.2 PREPARATION OF THE ANIMALS AND DESIGN OF EXPERIMENT:

All the animals subjected to the study were thoroughly examined both physically and clinically for the detection of any disease condition. Fecal and blood samples were also examined for any abnormalities to identify the presence of parasitic ova/oocyst and haemoprotozoan parasite(s). Rational treatment was implemented in every positive case. All the animals of different groups were subjected to the following experimental scrutiny:

1. Determination of arsenic concentration in drinking water of the cattle.
2. Determination of arsenic concentration in the feed, usually supplied to the cattle as per socio-economic condition.
3. Determination of arsenic concentration in milk.
4. Determination of arsenic concentration in urine and feces of cattle.
5. Determination of arsenic concentration in hair of cattle.
6. The selected animals were thoroughly examined for detection of any characteristic sign relating to arsenic exposure.

7. The laboratory examinations of blood for haemoglobin, TEC, TLC and serum biochemical profiles like serum glucose, total protein, albumin, AST, ALT, BUN and creatinine were carried out for assessment of their body condition and also for detection of the abnormalities.

During sampling, data were collected on the basic information of arsenic contamination using questionnaire according to specific objectives.

3.3 COLLECTION OF SAMPLES:

3.3.1 COLLECTION OF WATER SAMPLES:

The water offered for drinking to the animals was collected in plastic bottles previously rinsed with 20% nitric acid and deionized water. Water samples were preserved with 4ml of concentrated HCl per liter and analyzed within 7 days of collection.

3.3.2 COLLECTION OF FEED SAMPLES:

The paddy straw and grasses usually consumed by the animals were collected from the feed container of animals and transported to the laboratory in the plastic bag envelope containing information like sample no., place and date of collection etc.

3.3.3 COLLECTION OF FAECAL SAMPLES:

The faecal samples were collected (10 gm) in polythene zipper bags and stored at -20° C until further analysis (Sarder, 2004).

3.3.4 COLLECTION OF URINE SAMPLES:

About 10 ml of urine of each animal were collected in pre-washed and dried plastic bottles. Immediately after collection, Hydrochloric acid (1 ml in 100 ml) was added to prevent bacterial growth.

3.3.5 COLLECTION OF MILK SAMPLES:

Cow's milk was obtained during milking and was collected in a clean plastic container.

3.3.6 COLLECTION OF HAIR SAMPLES:

Hair samples were collected from tail tip and were kept in a clean dried polythene pack.

All the collected samples after transporting to the laboratory were preserved at -20°C, until the samples were analyzed.

3.3.7 COLLECTION OF BLOOD FOR HEMATOBIOCHEMICAL EXAMINATION:

The blood samples from each animal were collected from the jugular vein aseptically by using 10 ml disposable syringe. 6 ml of blood was taken from each animal, of which around 2 ml was collected in the sterile blood vials containing EDTA (1 drop of 10% EDTA as dried form) and the rest was left in the syringes undisturbed for collection of serum. The formed serum was transferred to the serum vials with proper identification (case no., sex, age etc.) and stored at -20°C for biochemical investigations. However for estimation of enzymes, fresh serum was used.

3.4 SAMPLE PREPARATION FOR ARSENIC ESTIMATION:

3.4.1 DIGESTION OF WATER SAMPLES:

Samples of 5 ml of drinking water were taken in a conical flask with 3 ml of HNO_3. Then it was heated in sand bath till the volume reduced to 1-2 ml. After cooling, the samples were transferred to 10 ml volumetric flask and deionized water was added to make a volume of 10 ml. These samples were used for estimation of arsenic in water samples in atomic absorption spectrometer (AAS) and the values were expressed in terms of mg/L.

3.4.2 DIGESTION OF FEED SAMPLES:

All the feed samples (straw & grasses) collected was washed with deionized water and dried at 60° C. (Rosas *et al.*, 1999)

Oxidation of the organic matter of the plant tissue and release of the mineral elements were effected through wet oxidation by means of oxidizing acid such as HNO_3, H_2SO_4 and $HClO_4$ acid mixture in the ratio of 10:1:4 (V/v) respectively. 0.5 gm of the dried feed sample was taken in 100 ml conical flask. 10 ml of tri-acid mixture was added to it. It was kept for overnight. Then digestion was completed on hot plate at 180-200°C until dense white fumes of H_2SO_4 and $HClO_4$ were evolved. The content in the flask, after digestion, was transformed to mineral crystal of each sample. After cooling it was transferred to 50 ml volumetric flask by several washings through Whatman filter paper (No. 1). Washing of each sample was done by triple distilled water and made up the final volume to 50 ml (Jackson, 1967).

3.4.5 DIGESTION OF MILK SAMPLES:

Samples of 1 ml raw milk were digested with 5 ml tri-acid mixture (HNO_3: H_2SO_4:$HClO_4$= 10:1:4 V/v) on hot plate for 1-2 hours. After that 2 ml concentrated nitric acid was added in each sample and again heated to reduce the volume to 1-2 ml. After cooling, the samples were transferred to 10 ml volumetric flask and volume made up to 10 ml. Then the samples were filtered through Whatman filter paper (No. 1).

The above extractant was used for the estimation of arsenic in milk in atomic absorption spectrometer (AAS) and values were expressed in terms of mg/L.

3.4.6 DIGESTION OF HAIR SAMPLES:

The hair samples were thoroughly cleaned before digestion, with acetone and distilled water. The cleaned samples were dried at 60°C for one hour in hot air oven.

Samples of 0.2 gm of hair were digested with 5 ml of tri-acid mixture (HNO_3: H_2SO_4:$HClO_4$= 10:1:4 V/v) on hot plate for one to two hours at 150-200°C. After cooling the samples were transferred to 10ml volumetric flask, and volume made up to 10ml with deionised water. Then these samples were filtered through Whatman filter paper (No. 1).

The above extractant was used for the estimation of arsenic in hair in atomic absorption spectrometer (AAS) and values were expressed in terms of mg/kg.

3.5 ARSENIC ANALYSIS:

A Varian atomic absorption spectrometer AA240, coupled with vapour generation accessory VGA77 was used for arsenic estimation. A Varian arsenic cathode lamp with slit 0.5 nm and wave length 193.7nm was used as a light source. Aqueous solution of 0.6% sodium borohydrate in 0.4% NaOH and hydrochloric acid as a carrier solution used to reduce the analyte to its hydride form.

To determine total arsenic in different type of samples 10 ml aliquots were taken in a 50 ml volumetric flask. Then 5 ml concentrated HCl and 1 ml freshly prepared 5% solution of KI and ascorbic acid were added and kept for 45 min. After 45 min. the volume of the samples were made up to 50ml and analysed within 1 hour by hydride evolution method.

3.6 CALIBRATION CURVE:

Calibration of machine was performed with fresh sets of standard solutions of 5, 10, 20 & 40 ppb arsenic. The arsenic stock standard used for preparation of these series of standard solutions was procured from AccuStandard^{R}, New Haven, USA and the concentration was 1000μg/ml.

3.7 STANDARDIZATION OF PROCEDURE AND RECOVERY TEST:

Rigorous quality control procedures were followed throughout the analysis. For evaluation the percentage of recovery of arsenic from different samples collected from the unaffected zone, a recovery test was done for each category of sample separately. This was done after

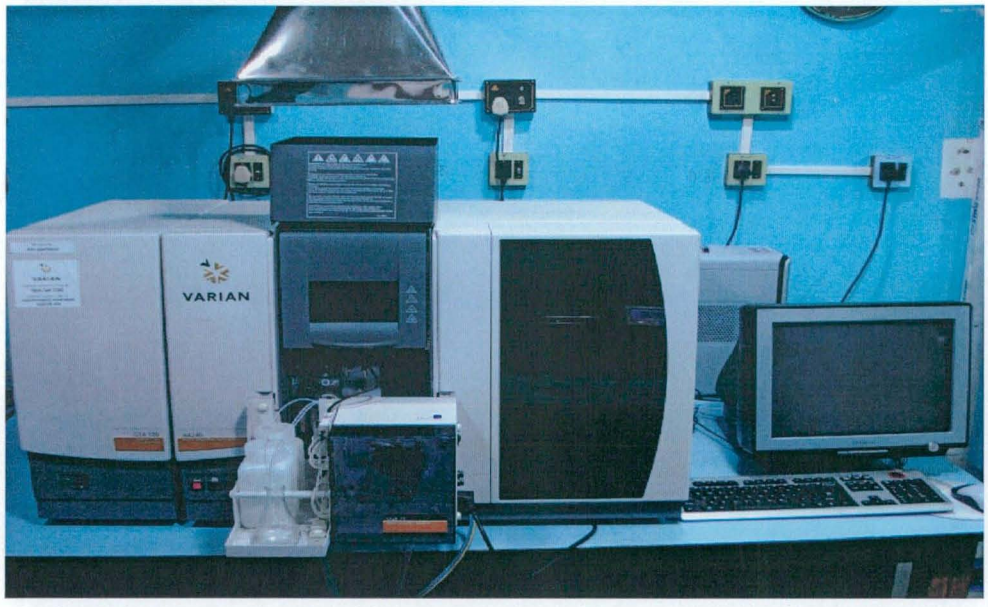


Figure: 1 shows Atomic absorption spectrometer (Varian AA240).

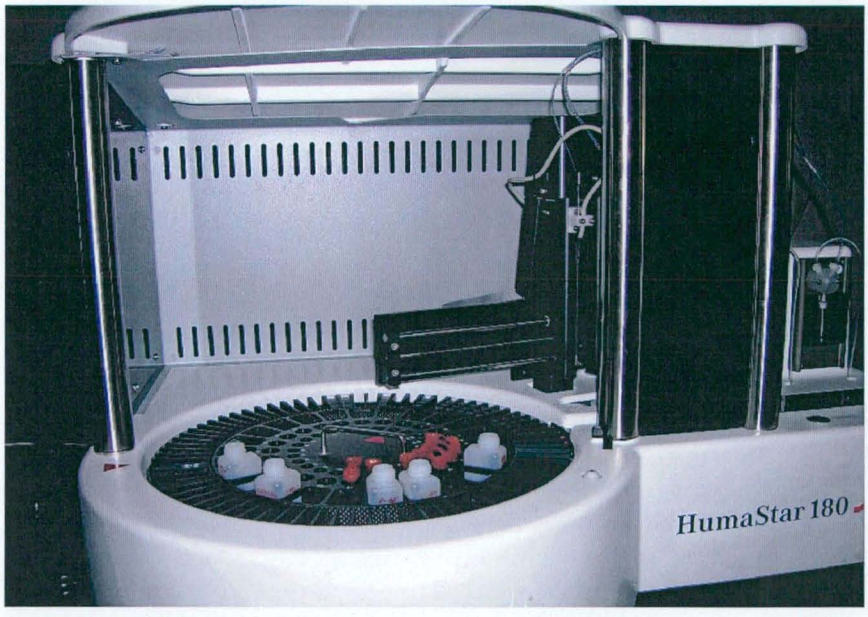


Figure: 2 shows serum auto analyzer for biochemical estimation.

fortification of different samples (homogenous) with 3 different concentration of arsenic solution, i.e. 1, 2 and 3 ppm. All the samples were digested and analysed by the same procedure as described in each samples. Recovery of arsenic in fortified samples was ranged from 85.2 to 87.5% in straw, 87.8 to 92.5% in water, 86.2 to 89.5% in milk, 88.3 to 91.4% in hair, 87.4 to 93.2% in urine and 85.1 to 88.3% in faeces. For each group of processed samples, blanks (deionized water & reagents) were included throughout the entire sample preparation and analytical process.

3.8 GLASS WARES AND CHEMICALS:

For different estimations, glass wares were used and they were procured from M/S Borosil, Mumbai. The acids were procured from Rankem, RFCL Ltd., New Delhi. Sodium borohydrate was procured from E. Merck (India) Ltd., Mumbai. Ascorbic acid & KI were procured from SRL, Mumbai. The kits for biochemical estimations were procured from Span diagnostics Ltd., Sachin 394 230 (Surat) India, Medsource Ozone Biomedicals Pvt. Ltd., 14/3 Milestone, Delhi Mathura Road, Faridabad 121003 (Haryana) India.

3.9 HAEMATOLOGICAL CHANGES:

3.9.1 ESTIMATION OF HAEMOGLOBIN (Hb):

Haemoglobin was determined by Sahli's method as described by Schalm *et al.* (1986) and the volume was expressed in gm/dl.

3.9.2 TOTAL ERYTHROCYTE COUNT (TEC):-

TEC was estimated by the haemocytometers as described by Schalm *et al.* (1986). Anticoagulated blood was drawn up to 0.1 mark

of the RBC pipette followed by RBC diluting fluid up to 101 marks. Then the pipette was rotated between the fingers for few seconds in order to facilitate proper mixing of the contents. Following few minutes, the counting chamber was charged after discarding the first few drops of the diluted sample. Once the cell settled down, the erythrocytes were counted in the exact position of the Neubaur's chamber. The values were then expressed as millions per cubic millimetres (10^6/ cmm).

3.9.3 TOTAL LEUCOCYTE COUNT (TLC):

Anticoagulated blood was drawn upto the 0.5 mark of the WBC pipettes followed by WBC diluting fluid up to 11 marks. The pipettes were then rotated between the fingers for few seconds in order to facilitate proper mixing of the contents. Following few minutes, the counting chambers were charged after discarding the first few drops of the diluting samples. Once the cell settled down, the leucocytes were counted in five large squares. The values were then expressed in terms of thousand per cubic millimetres (10^3 / cmm).

This was also estimated by haemocytometers as described by Schalm *et al.* (1986).

3.10 BIOCHEMICAL CHANGES:

3.10.1 ESTIMATION OF TOTAL SERUM PROTEIN (TSP):

Estimation of Serum total protein was done by Biuret method as described by Webster (1977) mentioned in the diagnostic kit (Medsource Ozone Biomedicals, Ref. No. TPA 200) and was expressed in gm/dl.

3.10.2 ESTIMATION OF SERUM ALBUMIN:

Estimation of serum albumin was done by Bromocresol Green (BCG) method as described by Doumas *et al.* (1971) mentioned in the diagnostic kit (Medsource Ozone Biomedicals, Ref. No. TPA 200) and was expressed in gm/dl.

3.10.3 ESTIMATION OF SERUM UREA AND BLOOD UREA NITROGEN:

Estimation of Serum Urea was done by the 'UREASE-GLDH', enzymatic UV method (Hellen *et al.*, 1971) as described in the diagnostic kit (Medsource Ozone Biomedicals, Ref. No. URG090) and was expressed in mg/dl.

BUN (mg/dl) = concentration of serum urea X 0.467

3.10.4 ESTIMATION OF SERUM CREATININE:

Estimation of Serum creatinine was done by modified Jaffe's kinetic method that was described by Browers (1980) mentioned in the diagnostic kit (Medsource Ozone Biomedicals, Ref. No. CRS 100) and was expressed in mg/dl.

All the serum analysis was done using fully automated serum analyzer (HUMASTAR 180).

3.10.5 ESTIMATION OF SERUM GLUCOSE LEVEL:

The level of serum glucose was estimated by the method GOD-POD of Trinder (1969) mentioned in the diagnostic reagent kit (Medsource Ozone Biomedicals, Ref. No. GLU300) and was expressed in mg/dl.

3.10.6 ESTIMATION OF SERUM ASPARTATE AMINO TRANSFERASE (AST):

The level of AST in serum was estimated by the method of Reitman and Frankel (1957) mentioned in the diagnostic reagent kit (COGENT, Code No. 25913) and was expressed in IU/L.

3.10.7 ESTIMATION OF SERUM ALANINE AMINO TRANSFERASE (ALT):

The level of ALT in serum was estimated by the method of Reitman and Frankel (1957) mentioned in the diagnostic reagent kit (COGENT, Code No. 25912) and was expressed in IU/L.

3.11 STATISTICAL ANALYSIS:

All the data obtained were analysed in SPSS (version 10.0) with Fisher's independent't' as a test statistics. Comparison had been made between control groups and test groups. The results were expressed as mean ± SE. Significance level <0.05 was described as significant (significant at 5% level) and <0.01 was described as highly significant (significant at 1% level).

CHAPTER-IV

RESULT AND DISCUSSION

RESULTS AND DISCUSSION

Under the scheme sponsored by World Bank through ICAR in the name of National Agricultural Innovation Project (NAIP), a detailed investigation was carried out to observe the impact of Arsenic (As) exposure on cattle. For this purpose, the village Dakshin Panchpota, under Chakadha block of Nadia district was selected which is considered as arsenic prone zone (affected) as per the report of Belon *et al.* (2006).

For statistical comparison of the data generated from this affected area, the Akna village of Polba block of Hoogly district was selected as the non arsenic prone zone (control).

The samples of water and feed (mixture of straw & grass) and the substrate like, excretory materials such as faeces and urine and the secretary products such as milk along with blood and hair collected from the affected zone would be treated as Group-II and the materials of same profiles collected from non arsenic prone (Control) would be treated as Group-I.

The detail clinical observations of animals (cattle) have been recorded as well as hematological and biochemical changes have been recorded in this present study. A format in connection with questionnaire was made to have detailed record about the socio-economic condition of the farmers, breeds of animal, nature of feeding etc.

The results of arsenic estimation in different samples like water and feed, excretory and secretary product and hair of cattle of arsenic

Sample I.D. No.	Name of households	Feed (mg/kg)	Water (mg/L)	Urine (mg/L)	Feces (mg/kg)	Milk (mg/L)	Hair (mg/kg)
P1	Ananta Mandal	2.29	0.03	0.34	-	0.02	-
P7	Bidhan Biswas	3.56	0.03	-	0.66	0.03	2.96
P14	Palash Paik	0.96	0.03	0.25	0.61	0.08	5.64
P15	Kiran Chandra paik	1.12	0.03	0.18	0.67	0.08	1.69
P28	Subol Biswas	2	0.03	-	0.24	0.04	4.64
P29	Nilratan Roy	1.6	0.05	0.22	0.46	0.04	4.59
P33a	Haripada naskar	0.68	0.03	0.29	0.98	0.04	5.24
P33b	Haripada naskar	1.59	0.03	0.07	0.55	0.04	4.42
P37	Ramsundar Biswas	0.8	0.02	-	0.52	0.04	4.49
1-DP	Krishnakanta Mondal	1.61	0.03	-	0.57	0.1	5
5-DP	Rameshwar Mahanta	1.77	0.03	-	0.64	-	5.84
18-DP	Bimal Gharami	0.88	0.03	-	0.76	0.07	3.4
19-DP	Nirangan Gharami	1.3	0.03	-	1.26	0.06	4.99
20-DP	Ramchandra Das	0.7	0.03	-	0.48	0.08	2.55
27-DP	Billo Biswas	1.68	0.05	-	1.06	0.1	6.42
38-DP	Tarak Gharami	1.49	0.02	0.16	0.52	0.06	1.07
52-DP	Manarama Baroi	3.39	0.03	-	0.62	0.05	4.24
60-DP	Sunil Ray	1.64	0.03	0.19	1.08	-	2.15

Table-1: Concentration of arsenic in different samples of Dakshin Panchpota village, Nadia, West Bengal (In mg/kg or ppm).

prone zone i.e. Dakshin Panchpota village have been presented in Table-1.

4.1 STATUS OF ARSENIC IN DRINKING WATER:

Tube well water as a major source of drinking water for cattle was collected. However, the pond water as a source of drinking water in a few instances was also collected. The results of arsenic content of water have been shown in Table-2 and Fig.-3. The mean arsenic concentration in water of control group (Gr. I) was 0.012 ± 0.002mg/L and of exposed group (Gr. II) was 0.031 ± 0.001mg/L, ranging from 0.02 to 0.05 mg/L.

Statistical analyses showed significant difference ($P<0.01$) existed between Gr. I and Gr. II. The arsenic content of water of exposed area was higher than control area.

The mean arsenic concentration of exposed area was higher than the WHO (1993) standard (10μg/L or 0.01 mg/L) but was lesser than the National drinking water standard (50μg/L or 0.05 mg/L). The allowable limit for water used to feed cattle is 0.05 mg/L (USEPA, 1973).

The present findings in respect with the status of arsenic in the drinking water were in close similarity with Rahaman *et al.* (2005).

Out of 18 samples 2 samples contained arsenic more than national standard of 0.05 mg/L and 16 samples contained arsenic more than 0.01 mg/L, less than national standard. This warrants further study to know the mystery of this difference of arsenic content within the same ambit of arsenic exposure, considering the facts like

comparison of the depth of tube well, duration of setup and consumption limit etc.

The present findings of ground water arsenic concentration are closely similar with SOES (2006). According to SOES (2006), in Haringhata block of Nadia district, 20.6% of ground water samples contained arsenic more than the permissible limit (0.05 mg/L) and 58.4% contained arsenic more than 0.01 mg/L.

4.2 STATUS OF ARSENIC IN FEED OF CATTLE:

The animals are mostly maintained marginal farmers and landless labourers and therefore the animals are not supplied with concentrates and feed additives. They are given mostly straw and grass. To asses the amount of arsenic consumed by the animals through feed, the feed samples comprising of straw and grass were collected from feeding turf and analyzed for arsenic content.

The mean arsenic concentration in feed was 1.161 ± 0.191 mg/kg which varied from 0.70 to 3.56 mg/kg (Table-2 & Fig.-3) that was higher than the detection limit of 0.005 mg/kg (Korenovska, 2001).

The arsenic content in grass was 0.403 ±0.057 mg/kg in non arsenic prone zone (control) in comparison to 1.161 ± 0.191 mg/kg of arsenic zone. Statistical analysis showed that the difference was highly significant ($P<0.001$).

The mean concentration of arsenic in feed samples of control group (Gr. I) and exposed group (Gr. II) have been presented in Table-

Table-2: Mean ± S.E. of values of arsenic concentration in water and feed of cattle of control (Gr. I) and exposed (Gr. II) groups.

Sample / Groups	Water (mg/L)	Feed (mg/kg)
Gr. I	0.012 ± 0.002	0.403 ± 0.057
Gr. II	0.031 ± 0.001	1.161 ± 0.191
t value	6.37	4.37
Significance Level	<0.001	< 0.001

P<0.01 indicate significant at 1% level.
P<0.05 indicate significant at 5% level.

Table-3: Mean ± S.E. of values of arsenic concentration in faeces, urine and hair of cattle of control (Gr. I) and exposed (Gr. II) groups.

Sample / Groups	Faeces (mg/kg)	Urine (mg/ L)	Hair(mg/kg)
Gr. I	0.189 ± 0.033	0.057 ± 0.004	0.871 ± 0.124
Gr. II	0.687 ± 0.06	0.213 ± 0.029	4.078 ± 0.371
t value	5.43	5.2	5.57
Significance Level	< 0.001	< 0.001	< 0.001

P<0.01 indicate significant at 1% level.
P<0.05 indicate significant at 5% level.

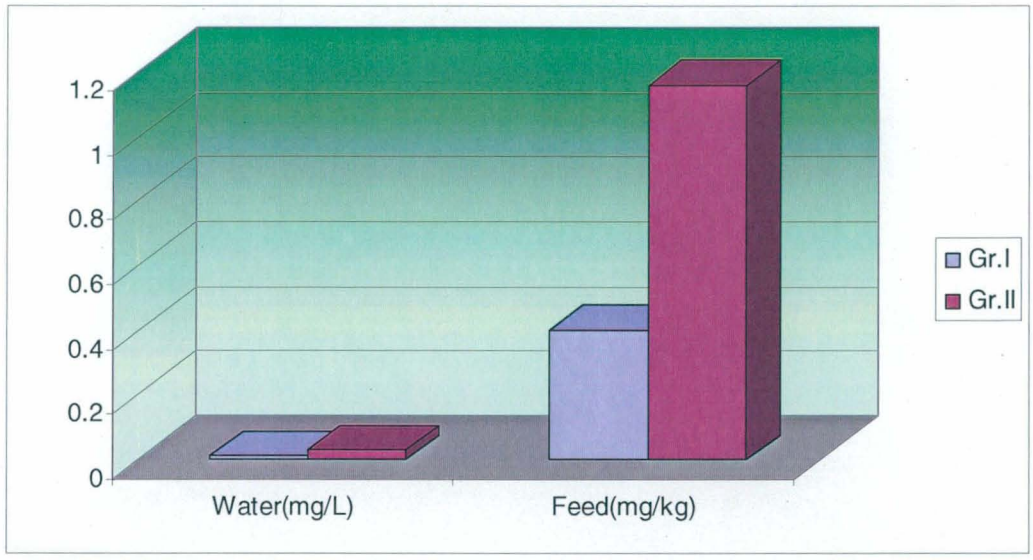


Figure: 3 Shows Comparison of arsenic content in water & feed of cattle between control (Gr. I) and exposed (Gr. II) group.

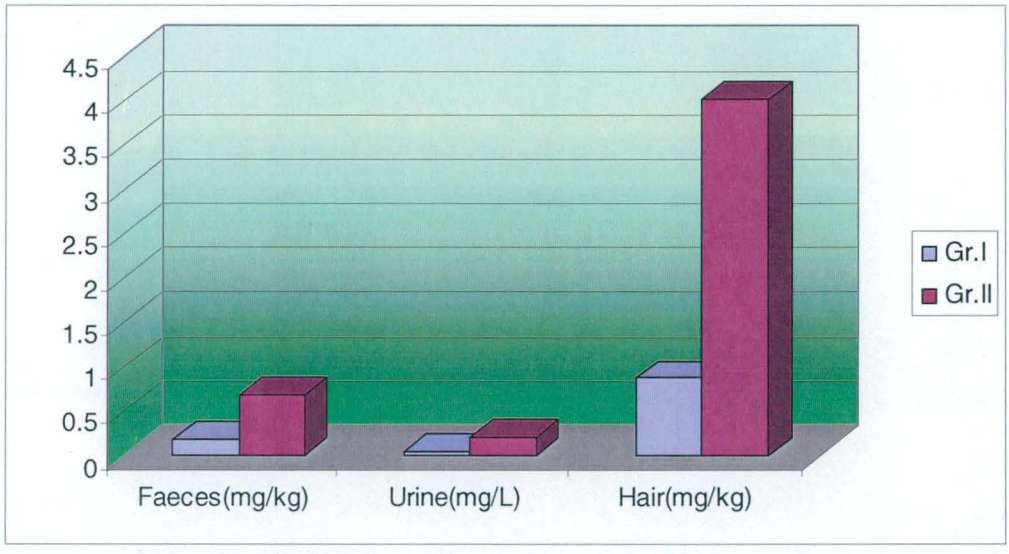


Figure: 4 Shows Comparison of arsenic content in faeces, urine and hair of cattle between control (Gr. I) and exposed (Gr. II) group.

2 and Fig. 3. The mean arsenic concentration in feed of Gr. 1 was 0.403 ±0.057 mg/kg and of Gr. II was 1.161 ± 0.191mg/kg.

The concentration of arsenic in feed samples of Gr. II was significantly higher ($P<0.001$) in comparison to Gr. 1.

The present finding in respect with arsenic content of feeds (paddy straw and grass) was closely similar with the findings of Abedin *et al.* (2002) and Sanyal and Dhillon (2005).

While browsing the literature from various available sources, it was pointed out that the As content in feed of cattle exposed under the environment of As contamination was nearer to the level as per US Public Health Service (Jones and Hatch, 1945) where recommendation of permissible limit of arsenic in edible crops is 2.6 μg/gm.

4.3 STATUS OF ARSENIC IN FAECES OF ANIMALS:-

The mean concentration of arsenic in faeces of cattle has been shown in the Table-3 and Fig.-4. The concentration of arsenic in the faeces of Gr. II (0.687 ± 0.06mg/kg) was significantly higher ($P<0.01$) than that of Gr. I (0.189 ± 0.033mg/kg).

The result of preset study was corroborated with the report of Rana (2007). The increased concentration of arsenic in feces of Gr. II might be due to higher concentration of arsenic in feed and water. Concentration of arsenic in faeces was higher than the concentration of arsenic in urine might be due to less absorption of arsenic from feed. The higher concentration of arsenic in urine and faeces confirms

the arsenic exposure of cattle and continuously spreading the contaminant to contaminate the environment.

Selby *et al.* (1974) reported that the major route of excretion of arsenic is through urine and faeces which supported the present findings.

4.4 STATUS OF ARSENIC IN URINE:-

From the Table-3 & Fig.-4 statistical analysis showed that the arsenic concentration in urine of experimental group (0.213 ± 0.029) was higher than the control animal (0.057 ± 0.004). The concentration of arsenic in urine significantly increased ($P<0.001$).

The increased concentration of arsenic in urine of Gr. II might be due to higher concentration of arsenic in feed and water as recorded earlier in the present study.

The report of present study was in accordance with the report of Browning (1969) who observed that urinary excretion of arsenic usually rises with increasing arsenic intake so that the total urinary arsenic excretion provides a useful index of exposure.

The findings are corroborated with the report of Lakso *et al.* (1975) who remarked that the permissible limit of arsenic in urine sample of cattle is 0.05-0.17 mg/L.

4.5 STATUS OF ARSENIC IN HAIR (TAIL) OF ANIMALS:-

The mean concentration of arsenic in hair of cattle has been shown in Table-3 and Fig.-4. The concentration of arsenic in hair of

Gr. I (control) was 0.871 ± 0.124mg/L and of Gr. II (exposed) was 4.078 ± 0.371mg/L.

The concentration of arsenic in hair of exposed group was significantly higher ($P<0.001$) than the control group.

The observation in relation with the arsenic content in hair of cattle was closely similar with the findings of Riviere *et al.* (1981), who estimated the arsenic content of hair of cattle and the value was found to be 0.80 to 3.40 mg/kg.

Radostits *et al.* (2000) reported that arsenic content in hair of cattle went as much as 5-10 mg/kg where as in animals not exposed to arsenic (normal) should contain less than 0.5mg/kg. The findings of present study were corroborated with the report of Radostits *et al.* (2000). Arsenic is a poisonous metalloid and exerts its toxic effect by combining with enzymes having sulfhydryl group, Sharma (2007).

Because of the high sulfhydryl content of keratin, the highest concentrations of arsenic are found in hair and nails. Deposition in hair starts within 2 weeks of exposure, and arsenic stays fixed at this site for years (Klaassen, 2006). Arsenic in nails and hair has been used as a biomarker for arsenic exposure, including both current and past exposure, while urinary arsenic is a good indicator for current exposure (Liu *et al.*, 2008).

Therefore, the significant presence of arsenic in hair in the present study may be considered as one of the very important diagnostic point for conclusion.

4.6 STATUS OF ARSENIC IN MILK:-

From the Table-4, statistical analyses showed that the arsenic concentration in milk (Fig.-5) of Gr. II (0.058 ± 0.006mg/L) was significantly higher (P = 0.004) than the Gr. I (0.028 ± 0.005mg/L). The arsenic concentration of milk of exposed animals ranged from 0.02 to 0.1mg/L.

The result was corroborated with Mahreu *et al.* (1977), who stated that high level of arsenic (117μg/L or 0.117 mg/L) was found to be in cow's milk.

Literature suggests that the As concentration in milk of cattle of exposed group was more than the permissible limit as per standard of International Dairy Federation (1986) wherein, the limit prescribed was 10ng/gm or 0.01mg/L.

The result of arsenic content 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. The value of arsenic in different substrates presented in different table from which it is clear that, the arsenic content in each sample was higher than the animals of non arsenic prone zone (control). The result indicated arsenic exposure and source of spreading contamination in the environment. The result also suggested the presence of As in food chain as indicated its content in milk 0.058 ± 0.006 mg/L which justify the theme of National Agricultural Innovation Project : 'Arsenic in Food Chain: cause, effect and mitigation'.

Table-4: Mean ± S.E. of values of arsenic concentration in milk of cattle of control (Gr. I) and exposed (Gr. II) groups.

Sample / Groups	Milk(mg/L)
Gr. I	0.028 ± 0.005
Gr. II	0.058 ± 0.006
t value	3.29
Significance Level	0.004

P<0.01 indicate significant at 1% level.
P<0.05 indicate significant at 5% level.

Table-5: Mean ± S.E. of values of certain hematological changes of control (Gr. I) and exposed (Gr. II) animals.

Samples / Groups	Hemoglobin (gm/dl)	TEC(10^6/ml)	TLC(10^3/ml)
Gr. I	11.90 ± 0.045	6.58 ± 0.142	6.56 ±0.13
Gr. II	8.44 ± 0.213	5.46 ± 0.186	5.84 ±0.19
t value	6.939	7.232	6.435
Significance Level	<0.001	0.001	0.017

P<0.01 indicate significant at 1% level.
P<0.05 indicate significant at 5% level.

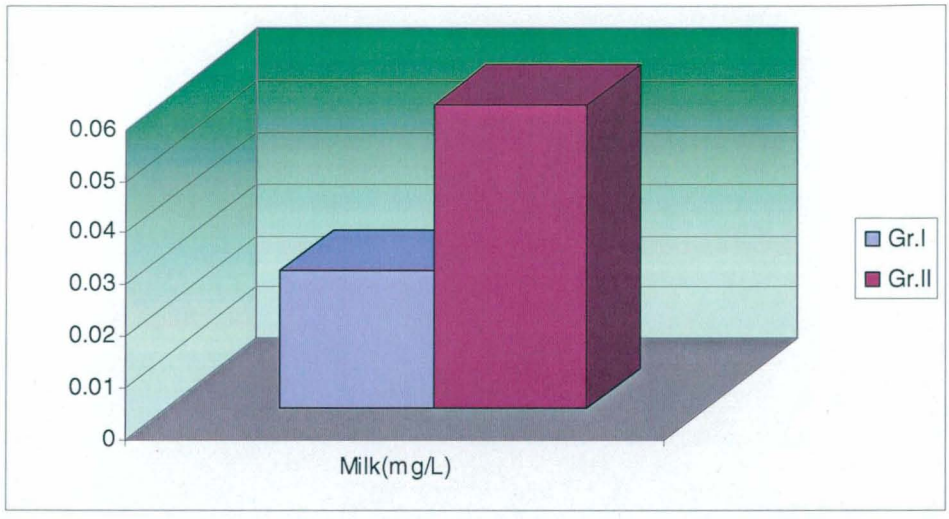


Figure: 5 Shows Comparison of arsenic content in milk of cattle between control (Gr. I) and exposed (Gr. II) group.

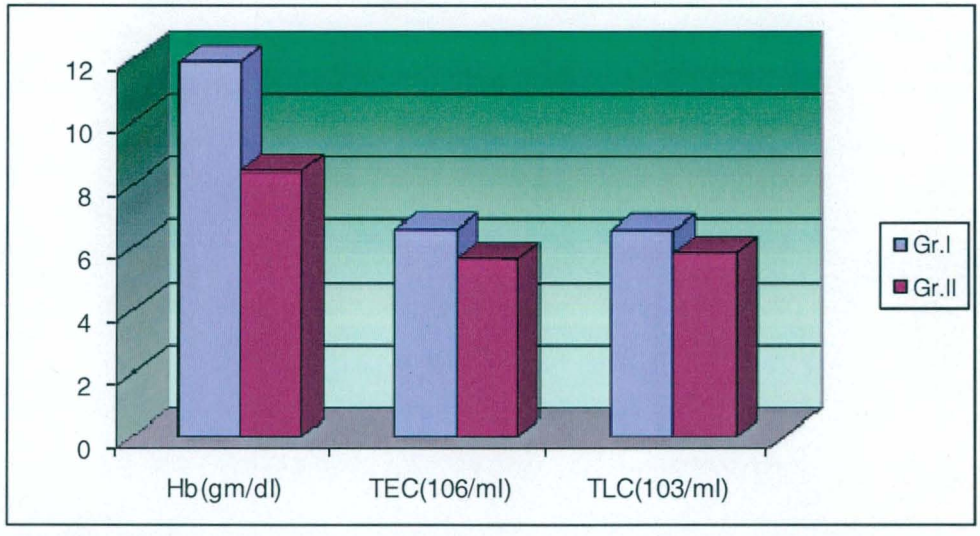


Figure: 6 Shows Comparison of some hematological profiles between control (Gr. I) and exposed (Gr. II) group of cattle.

4.7 HAEMATOLOGICAL CHANGES:

The results of the analysis of different haematological profiles such as Hb, TEC and TLC were tabulated in Table-5.

4.7.1 HAEMOGLOBIN (Hb):

The mean values of haemoglobin percentage of control and experimental groups were 11.09 ± 0.45gm/dl and 8.44 ± 0.213gm/dl respectively.

From the Table-5 Statistical analysis revealed that there was significance difference ($P<0.001$) of Hb between two groups (Fig.-6).

Low haemoglobin percentage in animals of Gr. II was indicative of anemia. The findings are corroborated with the report of Biswas *et al.* (1993) and Klaassen (2006). Biswas *et al.* (1998) reported the decreased level of Hb in experimentally produced arsenic toxicity in goats.

Low haemoglobin percentage in ruminants suffering from arsenic toxicosis was also reported by Ianchev (2001).

Arsenic exposure has been known to influence the activity of several enzymes of haem biosynthesis, (ferrochelation, ALA synthatase). All eight steps of haem synthesis are catalysed by enzymes which require functional sulfhydryl group for optimal activity. Arsenic has affinity with the functional SH group. The mechanism might have been ascribed for lowering level of haemoglobin in arsenic toxicity (Biswas, 1993; Biswas *et al.*, 2000).

The anaemia or deterioration of the level of Hb and TEC was possibly due to suppression of activity of metabolism and bone marrow as a residue of toxicant.

One of the consequences of chronic exposure to arsenic may include interference with haem synthesis, with an increase in urinary porphyrin excretion, which has been proposed as one of the biomarker of arsenic exposure (Ng *et al.*, 2005)

4.7.2 TOTAL ERYTHROCYTE COUNT (TEC):-

The TEC of control group and experimental group were 6.58 ± 0.142 (10^6/ml) and 5.46 ± 0.186 (10^6/ml) respectively.

The values of TEC in experimental groups dropped significantly (P = 0.001) compared to control groups (Table-5, Fig.-6).

The result is corroborated with the reports of Fusari and Ubaldi (2000) who recorded the decreased level of TEC in arsenic toxicated cows. The reports of present study is also supported by the findings of Biswas *et al.* (1998) in experimentally induced animals, who also recorded decreased level of TEC which was suggestive of suppression of bone marrow.

The decreased level of TEC of affected animals was also possibly due to hypoproteinaemia or nutritional deficiency as a result of continuous inappetance to anorexia by the arsenic exposure.

4.7.3 TOTAL LEUCOCYTE COUNT (TLC):

From the Table-5 it appeared that the mean value of TLC of control group and experimental group were found to be 6.56 ± 0.136($\times 10^3$)/ml and 5.84 ± 0.19($\times 10^3$)/ml respectively.

The values of TLC of control and experimental groups decreased significantly (P=0.017) as presented in Table-5 & Fig.-6. The report was supported by the reports of Biswas *et al.* (1998) and Ianchev (2001).

The dropped values of TLC might be ascribed to suppression of granulopoietic action of bone marrow and destruction of lymphoid tissue such as lymph nodes, spleen and submucosal area of G.I. tract as a result of excessive intake of arsenic through drinking water and feeds.

4.8 BIOCHEMICAL CHANGES:-

The results of the analysis of different biochemical parameters of blood and blood serum such as BGL, TSP, SGPT (ALT), SGOT (AST), BUN and Creatinine of experimental and control group were tabulated in Table-6.

4.8.1 TOTAL SERUM PROTEIN (TSP):

From the Table-6 & Fig.-7 it was evident that the TSP levels were 7.05 ± 0.289 gm/dl and 5.92 ± 0.38gm/dl for control & experimental groups respectively. The levels of experimental groups decreased significantly (P = 0.048) in comparison to control groups.

The findings were corroborated with the report of Biswas (1993) and Rana (2007).

Decrease level of TSP is due to extensive damage to capillaries causing increased permeability and exudation of serum into tissue spaces. Inappetance, malabsorption, hypoproteinemia because of the

interference in the process of synthesis of protein in the liver by the arsenic which has an affinity of sulpher containing amino acids might be the possible contributory factor.

4.8.2 ALBUMIN:

The mean concentration of albumin in Gr. II was 3.70 ± 0.09 g/dl and in Gr. I was 4.6 ± 0.2 g/dl (Table-6, Fig.-7). The analytical result indicated that the albumin level decreased significantly (P = 0.003) in Gr. II.

The present finding supported the findings of Biswas (1993).

The decreased level of albumin might be due to less synthesis of albumin in liver which might be due to inefficiency of the organ as a result of arsenic exposure (Santra *et al.*, 2000).

4.8.3 BLOOD UREA NITROGEN (BUN):

It would be observed from the Table-6 & Fig.-7 that, the level of BUN was14.68 ± 0.51 mg/dl & 23.14 ± 0.47 mg/dl for control & experimental groups respectively.

The BUN level of experiment groups increased significantly (P<0.001) in comparison to control groups. The rise of urea might be the indication of incompetencey to cope up with the sudden increasing demand by the kidney to remove metabolic products. The finding was corroborated with the report of Rana (2007) and Faires (2004) who also recorded the increased level of BUN in inorganic arsenic toxicosis in a beef herd.

4.8.4 SERUM CREATININE:

From the Table-6 & Fig.-7 the creatinine level of Gr. I and Gr. II were 0.754 ± 0.044 mg/dl and 0.964 ±0.063 mg/dl.

The value of creatinine level was significantly increased (P = 0.027) as comparison to control animal. The increased level of creatinine also rccorded by Faires (2004) in arsenic toxicated cattle. The action of arsenic on renal capillaries, tubules and glomeruli may cause severe renal damage (Klaassen, 2006). However, the level of creatinine of Gr. II (affected) did not cross the normal physiological range which was indicative of the optimum functioning of kidney tubule inspite of the exposure.

4.8.5 BLOOD GLUCOSE LEVEL (BGL):

From the Table-6 it appeared that the mean values of glucose of control and experimental groups were 47.70 ± 0.70 mg/dl and 51.02 ±0.77 mg/dl respectively.

The analytical results of the analysis indicated the increase level of blood glucose significantly (P = 0.013) which has been given in Fig-8. The present findings were closely similar with the report of Rana (2007) and also with the report of Biswas *et al.* (1998) who also recorded the increased level of blood glucose in experimentally produced arsenic toxicity in goats.

The augmented level of blood glucose in spite of inappetance might be attributed to stress factors. The increased release of glucocorticoids secretion by the adrenal cortex, receiving the stimulation from anterior pituitary and there by it caused

Table-6: Mean ± S.E. of values of certain biochemical changes of control (Gr. l) and exposed (Gr. II) animals.

Parameter / Group	Total Serum Protein (gm/dl)	Albumin (gm/dl)	BUN(mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)	AST(IU/L)	ALT(IU/L)
Gr. I	7.05 ±0.28	4.6 ±0.09	14.68 ± 0.51	0.754 ± 0.04	47.70 ± 0.70	26.36 ±1.02	6.61±0.03
Gr. II	5.92 ±0.38	3.70 ± 0.2	23.14 ± 0.47	0.964 ± 0.06	51.02 ± 0.77	34.29 ± 0.69	7.85±0.03
t value	2.335	1.90	12.02	2.711	3.183	6.422	2.77
Significance Level	0.048	0.003	<0.001	0.027	0.013	<0.001	0.024

P<0.01 indicate significant at 1% level.
P<0.05 indicate significant at 5% level.

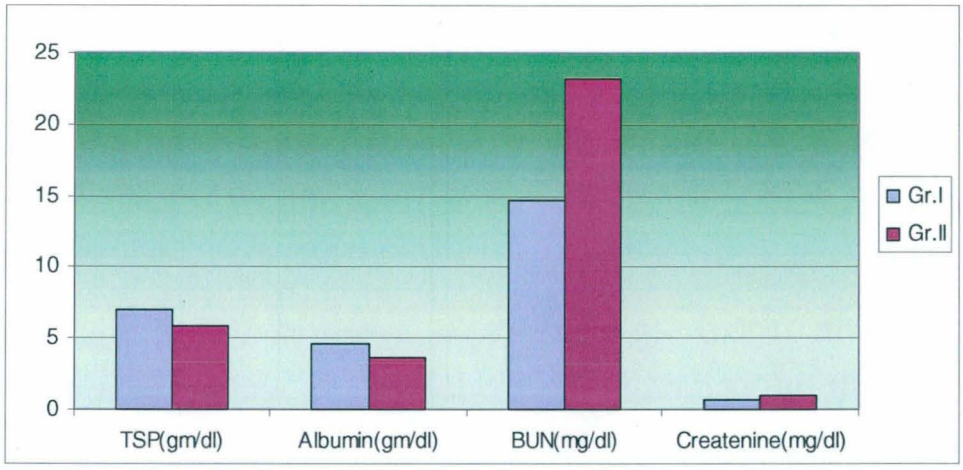


Figure: 7 Shows Comparison of total protein, albumin, BUN & Creatinine level between control (Gr. I) and exposed (Gr. II) group of cattle.

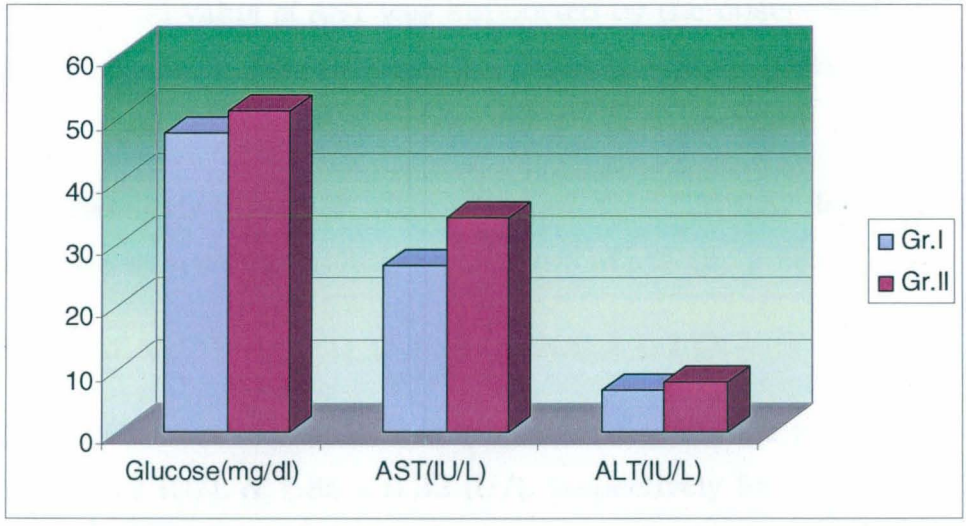


Figure: 8 Shows Comparison of serum glucose, AST & ALT level between control (Gr. I) and exposed (Gr. II) group of cattle.

The increased value of ALT was supported by the observation of Biswas *et al.* (2000) in experimentally produced arsenic toxicity in goats. Mazumder *et al.* (1998) also reported the elevated level of alanine amino transferase in human patients of arsenic poisoning.

Santra *et al.* (2000) reported the hepatic damaged caused by chronic arsenic toxicity in experimental animals and there was elevated level of serum ALT & AST.

The significant lowered level of ALT might be attributed to inhibition of enzymes synthesis and damage of excretory & secretory tissues as result of necrosis of the cell. Alteration of cell metabolism increases the enzyme activity and necrosis of the cell decreases the enzyme activity.

EXAMINATION OF ANIMALS:

During total survey period the animals of both, exposed and non exposed groups were considered thoroughly to find out any abnormalities relating to arsenic exposure.

The animals reared in the same environment were critically examined for the identification of classical symptoms as expected in humane beings such as depigmentation, melanosis, leucomelanosis, keratosis and hyperkeratosis, non pitting edema, ulceration and corn (Guha Mazumder, 2003). The detailed examination indicated no characteristic clinical signs as diagnostic point in animals. However, non specific symptoms like stunted growth, unthriftiness, loss of hair, inappetance to anorexia, loss of milk production, delayed estrous, anoestrous; post parturient anoestrous etc. were detected. Specific

difference between toxicities of one element with other is confusing (McDowell, 1976).

The animals of control group (Gr. I) was apparently healthy. The rate, rhythm and character of respiration and pulse were normal. Body coats were glossy in appearance. Posture and gait were normal. The conjunctival mucous membrane was pink to roseate in color in all animals. The observations were similar with the observations of Kelly (1979) in cattle.

However, the clinical examination of cattle under arsenic exposure of Dakshin Panchpota village revealed some deviation from normal as in recording of health status such as increased rate of respiration and pulse with symptoms of dyspnoea. The cattle had dry hair coat and congested mucous membrane with occasional diarrhea. Observations also pointed out that some animals were emaciated and hide bound condition. Occasional diarrheic symptom might be due to alimentary tract lesions which is the most susceptible of arsenic toxicosis. Alimentary tract mucosa is rich in oxidative enzyme system, which is affected by arsenic (Radostits *et al.*, 2000).

Some unorthodox symptoms in comparison to healthy control animals indicated the chronic arsenic exposure in animals through water and feed contained more arsenic than the permissible limit as recorded in present study. This warrants immediate detailed study of arsenic exposure in cattle.

The quantification of arsenic in faeces, urine, milk and hair indicated higher level of this metalloid to justify its presence in food chain without showing clinical signs in animals. This might be ascribed factors like duration of chronic exposure, concentration of arsenic below the threshold limit in the system required for producing

MITIGATION MEASURE:

1. The victimized livestock owners were advised to supply surface water rather than tube well water for drinking purpose.
2. They were also advised to supplement concentrate added with feed additives in arsenic exposed animals to increase protein content.
3. There was a recommendation for public awareness that the milk containing high arsenic content at least should not be given to children.
4. Faecal samples and if possible urine samples also to be disposed of in the pits to avoid environmental contamination as far as practicable.
5. Sodium thio-sulphate @ 40 mg/kg body weight I.V. in 10% solution at every 6 hours interval for 3 days followed by at every 12 hrs. Interval for another 3 days had been proved to be effective against experimentally induced arsenic toxicity in goats (Biswas, 1993).
6. 2,3 Dimercaprol / British Anti-Lewisite (BAL) would be also effective against arsenic toxicity in large animal @ 3 mg/kg body weight I.V. (5% sol. in a 10% sol. of benzyl benzoate) at every 4 hours for the first 2 days, every 6 hours the 3rd day, and twice a day for next 10 days until recovery (Sandhu and Brar, 2000).

CHAPTER-V

SUMMARY AND CONCLUSIONS

SUMMARY

A detail investigation was undertaken (from Jan, 2008 to July, 2008) at the Dakshin Panchpota Mouza of Chakdha block of Nadia district, which is one of the nine arsenic affected districts of West Bengal. Simultaneously non arsenic affected village, Akna of Polba block of Hooghly district was also selected for control study and comparison of the data.

From both the village i.e. arsenic exposed and non exposed (control) drinking water and feed samples of cattle as well as substrate like milk, urine, faeces and hair of cattle were collected for estimation of arsenic content.

The arsenic content of water and feed of arsenic exposed cattle were 0.031 ± 0.001mg/L and 1.161 ± 0.191mg/kg respectively. In comparison to samples of water (0.0124 ± 0.002 mg/L) and feed (0.403 ± 0.057 mg/kg) of control group, the arsenic content was higher in both the profiles of exposed group as indicated by statistical analysis.

The arsenic content of milk of exposed cattle was 0.058 ± 0.006 mg/kg which was higher than the permissible limit of milk (10 ng/gm or 0.01 mg/kg) as per International Dairy Federation (1986). The higher concentration of arsenic in milk might cause public health hazards and definitely a cause of concern of children's exposure.

The concentration of arsenic in urine, faeces and hair of exposed cattle was 0.2125 ± 0.029mg/L, 0.687 ± 0.06mg/kg and 4.0782 ± 0.371mg/kg respectively where as in control animals the contents of

these profiles was 0.057 ± 0.004 mg/L, 0.1889 ± 0.033 mg/kg and 0.8713 ± 0.124 mg/kg respectively. The values of arsenic in urine, faeces and hair of exposed and control cattle differed significantly when the procedure of statistical analyses was adopted.

During the survey period all the selected animals were examined thoroughly for detection of any abnormalities relating to arsenic exposure. The external manifestation of exposed animals recorded were increased respiration rate, pulse rate, congested mucous membrane, occasional diarrhea with unusual weight loss.

Blood samples were collected from the cattle of both the groups for the estimation of hematological as well as biochemical changes.

The hematological profiles like hemoglobin, total erythrocyte count (TEC) and total leucocyte count (TLC) were estimated as well as serum biochemical profiles like serum glucose, serum total protein, albumin, AST, ALT, blood urea nitrogen and creatinine were also estimated as per conventional methods.

The level of hemoglobin, total erythrocyte counts (TEC) and total leucocyte counts (TLC) was decreased significantly in exposed animals in comparison to control animals.

The estimation of serum biochemical profiles in arsenic exposed cattle revealed that the level of glucose, serum enzymes such as AST and ALT increased significantly than the control animals where as total serum protein and albumin were decreased. There was also increased level of blood urea nitrogen and creatinine.

CONCLUSION

1. From the study it can be concluded that the drinking water and feeds, provided to the cattle as per socio economic condition was contaminated with arsenic.

2. There was clean indication of arsenic exposure in cattle where the content of the same was significantly high in all profiles such as milk, urine, faeces and hair as compared to the value of cattle of arsenic safe (control) zone. The high content of arsenic in milk further indicate its presence in food chain which justify the concept of project entitled "Arsenic in food chain: cause, effect and mitigation" sponsored by World Bank through ICAR.

3. Some non specific symptoms like increased rate of pulse and respiration with sometimes dyspnoea, congested mucous membrane, with occasional diarrhea was marked and recorded during survey work.

4. The arsenic exposed animals were anaemic as supported both by clinical examination and laboratory examination.

5. Biochemical alteration was also recorded with the increase of serum glucose, AST, ALT, BUN and creatinine and decrease of total protein and albumin when compared to normal.

6. From the study, a conclusion can be drawn that the arsenic content in hair and urine may be considered as biomarker for exposure.

CHAPTER-VI

FUTURE SCOPE OF RESEARCH

Future Scope of Research

1. To investigate the arsenic load in ruminants, birds and ducks and their subsequent physiological changes due to arsenic accumulation in their body.

2. To investigate health condition and pathophysiological changes in human, ruminants in arsenic endemic areas through food chain.

3. Arsenic content in the soil, plant and animal system throughout the year should be studied to find out the influence of season on it.

4. To find out a possible suggestive measure to mitigate the menace.

5. To see the impact on man being exposed of arsenic as proved its presence in food chain i.e. in milk and on environment.

6. Epidemiological research in the arsenic prone areas to characteristic and quantify the arsenic related health hazard in animals.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abedin, M.J., Cresser, M.S., Meharg, A.A., Feldmann, J., Cotter-Howells, J. (2002). Arsenic accumulation and metabolism in rice (Oryza sativa). *Environ. Sci. Technol.* **36**(5):962- 968.

Adak, S.K., Mandal, B.K. and Sanyal, S.K. (2002). Yield of potato as influenced by arsenic contaminated irrigation water. Potato, Global Research & Development. **2**:926-928.

Alsono, M.L., Benedito, J.L., Miranda, M., Castillo, C., Hernandez, J. and Shore, R.F. (2004). Cattle as Biomonitors of soil Arsenic, Copper, and Zinc concentrations in Galicia (NW Spain). Archieves of Environmental Contamination and Toxicology. **43**(1):103-108.

Anderson, W.A.D. (1953). Pathology. 2nd edition. St. Louis, The C.V.Mosby Company. 150-151.

Anderson, N.V. (1992). Veterinary Gastroenterology, 2nd ed. Philadelphia, Lea & Febiger, London. 780-781.

Andrews, A.H., Blowery, R.W., Boyd, H. and Eddy, R.G. (1992). Bovine Medicine, disease and Husbandry of Cattle. 1st ed. Oxford Blackwell Scientific publications, London Ed. Burgh Boston. 613.

Aposhian, H.V. and Aposhian, M.M. (2006). Arsenic toxicology: Five questions. *Chem Res Toxicol.* **19**:1-15.

Bahri, L.E., Romdane, S.B., (1991). Arsenic poisoning in livestock. *Vet Hum Toxicol.* **33**:259–264.

Belon, P., Banerjee, P., Choudhury, S.C., Banerjee, A., Biswas, S.J., Karmakar, S.R., Pathak, S., Guha, B., Chatterjee, S., Bhattacharjee, N., Das, J.K. and Khuda-Buksh, A.R. (2006). Can Administration of Potentized Homeopathic Remedy, Arsenicum Album, Alter Antinuclear Antibody (ANA) Titer in People Living in High-Risk Arsenic Contaminated Areas? I. A Correlation with Certain Hematological Parameters. Advance Access Publication. **3**(1):99-107

Biswas, B.K., Dhar, R.K., Samanta, G., Mandal, B.K., Chakraborti, D., Faruk, I., Islam, K.S., Chowdhury, M.M., Islam, A. and Roy, S. (1998). Detailed study report of Samta, one of the arsenic-affected villages of Jessore District, Bangladesh. *Curr Sci.* **74**(2):134–145.

Biswas, U. (1993). Studies on metabolism, toxicosis, effect, immunoglobulin status and therapy of experimentally induced arsenic toxicity in goats. M.V.Sc thesis submitted to the Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India.

Biswas, U., Sarkar, S. and Bhowmik, M.K. (1998). Clinicopathological profile of induced chronic arsenic toxicity in goats. *Indian journal of Animal Sciences.* **68**(4): 320-323.

Biswas, U., Sarkar, S., Bhowmik, M.K., Samanta, S.K., Biswas, S. (2000). Chronic toxicity of arsenic in goats:

clinicobiochemical changes, pathomorphology and tissue residues. *Small Rumin Res.* **38** (3):229-235.

Browers, L.D. (1980). *Clin. Chem.* **26**:551.

Browning, E. (1969). Toxicity of Industrial Metals, 2nd ed. Appleton-Century Croft, New York.

Buck, W.B., Osweiler, G.D. and Van Gelder, G.A. eds. (1976). Clinical and Diagnostic Veterinary Toxicology. 2nd ed. Kendall Hunt, Dubuque.

Chakravarty, A.K. and Saha, K.C. (1987). Arsenical dermatoses from tube well water in West Bengal. *Indian J. Med. Res.* **85**(3):326-334.

Chandra Sekhar, K., Chary, N.S., Kamala, C.T. and Anjaeyulu, Y. (2003). Proc. Indian natn. *Sci. Acad.* **B70**(1):13-30.

Chatterjee, A., Das, D., Mandal, B.K., Chowdhury, T.R., Samanta, G. and Chakraborti, D. (1995). Arsenic in ground water in six districts of west Bengal, India: the biggest arsenic calamity in the world .Part1.Arsenic species in drinking water and urine of the affected people. *The Analyst.* **120**:643-650.

Chen, C.J., Hsu, L.I., Wang, C.H., Shih, W.L., Hsu, Y.H., Tseng, M.P. (2005). Biomarkers of exposure, effect and susceptibility of arsenic-induced health hazards in Taiwan. *Toxicol Appl Pharmacol.*, 206:198-206.

Clarke, E.G.C. and Clarke, M.L. (1975). Veterinary Toxicology. The English Language Book Society, Baillere Tindall. 2nd ed. 34-42.

Fusari, A. and Ubaldi, A. (2000). Haematological and Biochemical abnormalities in Dairy cows with chronic arsenic poisoning: Preliminary Results. European Society for Veterinary Clinical Pathology, 2nd Annual Scientific Meeting.

Garland, T. (2007). Arsenic. In: Veterinary Toxicology. Gupta, R.C. (eds). 1st ed. Elsevier, New York. 418-421.

Ghosh, K., Das, I, Saha, S., Banik, G.C., Ghosh, S., Maji, N.C. and Sanyal, S.K. (2004). Arsenic Chemistry in Groundwater in the Bengal Delta Plain: Implications in Agricultural System. *journal of Indian Chemical Society.* **81**:1063-1072.

Goodman Gilman, A., Rail, T.W., Nies, A.S. and Taylor, P. (1990). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 8th ed. Pergamon Press. New York, Oxford, Beijing, Frankfurt, Sao Paulo, Sydney, Tokyo and Torento. 1602-1605.

Guha Mazumder, D.N. (2003). Chronic Arsenic toxicity: Clinical features, epidemiology and treatment, Experience in West Bengal; *Journal of Environmental Science and Health.* **38**(1):141-163.

Hatch, R.C. (1982). Poisons Causing Abdominal Distress or Liver or Kidney Damage, In: Veterinary Pharmacology & Therapeutics, 6th ed. 1102-1107.

Hatch, R.C. and Funnel, H.S. (1969). *Can. Vet. J.* **10**:112-119.

Hellen, C.J. and Cook, J.G.H. (1971). *Clin. Chem. Acta.* **35**: 33.

Hindmarsh, T. (2002). Caveats in hair analysis in chronic arsenic poisoning. *Clin Biochem.* **35**:1-11.

Hopenhayn, C. (2006). Arsenic in drinking water: Impact on human health. *Elements.* **2**:103-107.

Howard, J.l. and Smith, R. A. (1999). Current Animal Practice. 4th ed. W.B Saunders Company, Philadelphia, London. 627.

Hungerford T. G. (1975). Diseases of Livestock. 8th ed. McGraw-Hill Book Company, Sydney. 489, 801.

Ianchev, I. (2001). Influnce of some geo-chemical ecological factors on some blood haematological characteristics in sheep from Chiprovitei. Zhivotnov, dni-Nauki. **38**(6):41-43.

IARC (International Agency for Research on Cancer) (1987). Monographs, Suppl. **6**:71-76.

IARC (International Agency for Research on Cancer) (1980). Summaries & Evaluations: Arsenic and Arsenic Compounds. **23**:39.

International Dairy Federation (1986). Questinnaire 2386/E. Brussels IDF.

Jackson, M.L. (1967). Soil chemical analysis. 1st ed. Prentice Hall of India Pvt. Ltd. New Delhi.

Jones, J. S. and Hatch, M. B. (1945). *Soil Sci.* **60**:277.

Kelly, W.R. (1979). Veterinary Clinical Diagnosis. 2nd ed. Bailliere Tindale, London.

Kent, C. (1998). Basis of toxicology. 1st ed. John Wiley & sons, INC.197.

Kirchgessner, M., Friesecke, H. and Koch, C. (1967). Nutrition and the Composition of Milk. Lippincott and Co. Philadelphia. PA.

Klaassen, C.D. (2006). Heavy Metals and Heavy Metals Antagonists, In: Goodman & Gilman's The Pharmacological Basis of Therapeutics. Bruton, Laurence, L., Lazo, J.S., Parker, K.L. (eds.) 7th ed. McGraw-Hill Companies, New York. 1763-1766.

Korenovska, M. (2001). Arsenic in cereals, oilseeds and forages produced in Slovakia in 1997-1999. Bulletin-Potravinarskeho-Vyskumu.**40** (2):145-150.

Lakso, J.U. and Peoples, S.A. (1975). Preliminary studies on lead, cadmium and arsenic contents of feed, cattle and food animal origin coming from different farms in Saxony. *Journal of Agricultural and Food Chemistry.* **23**(4):674-676.

Liu, J., Zheng, B., Aposhian, H.V., Zhou, Y., Chen, M., Zhang, A. and Waalkes, M.P.(2002). Chronic arsenic poisoning from burning high arsenic containing coal in Guizhou, China. *Environ Heath Perspect.* **110**(2):119-122.

Liu, J., Goyer, R.A. and waalkes, M.P. (2008). Toxic effects of metals. In: Casarett and Doull's Toxicology – The Basic Science of Poisons. Klaassen, C.D. (eds). 7th ed. McGraw-Hill companies, New York. 936-939.

Mahreu, H., Jaouen, J.C., Luquet, F.M., Mouillet, L. (1977). Comparative study of the composition and contamination

of milk from cows, ewes and goats. *Lait. French.* **57**(568): 561-571.

Mazumder, D.N., Das Gupta, J., Santra, A., Pal, A., Ghose, A., Sarkar, S. (1998). Chronic arsenic toxicity in west Bengal--the worst calamity in the world. *J Indian Med Assoc.* **96**(1): 4-7, 18.

McArthur, J.M., Banerjee, D.M., Hudson- Edwards, K.A., Mishra, R., Purohit, R., Ravenscroft, P., Cronin, A., Howarth, R.J., Chatterjee, A., Talukder, T., Lowry, D., Houghton, S. and Chadha, D.K. (2004). Natural organic matter in sedimentary basins and its relation to arsenic in anoxic ground water: the example of West Bengal and its worldwide implications. *Appl Geochem.* **19**:1255-1293.

McDowell, L. R. (1976). Mineral deficiencies and toxicities and their effect on beef production in developing countries, Symposium on Beef Cattle production in developing countries. University of Edinburg Scotland. 216-241.

Mitranescu, E., Ciocarlie, N., Gavrila, G., Tudor, L. and Diaconnescu, L. (2003). Observations regarding the correlation between arsenic concentration in soil and hair simples from young bulls bred in the neighbourhood of a chemical fertilizer plant. *Revista-Romana-de-Medicina-veterinara.* **13**(1):89-92.

Morales, K.H., Ryan, L., Kuo, T.L., Wu, M.M. and Chen, C.J. (2000). Risk of internal cancers from arsenic in drinking water. *Environ Health Perspect.* **108**:655-661.

Mukherjee, S., Das, D., Darbar, S., Mukherjee, M., Das, A. S., and Mitra, C. (2004). Arsenic trioxide generates oxidative stress and islet cell toxicity in rabbit. *Current Science.* **86**(6):854-857.

NRC (National Research Council) (1977). Committee on Medical and Biologic Effects of Environmental Pollutants. Distribution of arsenic in the environment. In: Arsenic. National academy of science. Washington, DC. 16-79.

Ng, J.C., Wang, J.P., Zheng, B., Zhai, C., Maddalena, R., Liu, F. and Moore, M.R. (2005). Urinary porphyrins as biomarkers for arsenic exposure among susceptible populations in Guizhou province, China. *Toxicol Appl Pharmacol.* **206** (2):176-84.

NHMRC and ARMCANZ (National Health and Medical Research Council, Agricultural and Resource Management Council) Australia and New Zealand (1996). Australian drinking water guidelines, National Water Quality Management Strategy. Canberra: NHMRC/ARMCANZ (amended 2001).

Nielberle and Paul, C. (1966). Text book of Special pathological Anatomy of domestic animals, 1st English ed. Pergamon Press. 363, 483.

Osweiler, G.D. (1996). Toxicology. Philadelphia: Lippincott Williams and Wilkins. 181–184.

Pal. A., Nayak, B., Das, B., Hossain, M.A., Ahamed, S. and Chakraborti, D. (2007). Additional danger of arsenic exposure through inhalation from burning of cow dung cake laced with arsenic affected villages in Ganga-Meghna-

Brahmaputra plain. *Journal of environmental monitoring.* **9**(10):1067-70.

Pandey, P. K., Yadav, S., Nair, S. and Bhui, A. (2002). Arsenic contamination of the environment: a new perspective from central-east India. *Asstrocl Environ Int.* **28** (4):235-245.

Peoples, S. A. (1964). Arsenic toxicity in cattle. *Ann. N.Y. Acad. Sci.* **111**: 644-649.

Radostits, O.M., Gay, C.C., Blood, D.C., Hinchcliff, K.W. (2000). Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th ed. W.B. Saunders Company, London, Philadelphia. 1585-1589.

Rahaman, M.M., Sengupta, M.K., Ahamed, S., Lodh, D., Das, B., Hussain, M. A., Nayak, B., Mukherjee, S.C., Pati, S., Saha, K.C., Palit, S.K., Kaies, I. Barua, A.K. and Asad, K.A. (2005). Murshidabad-one of the nine ground water arsenic-affected districts of West Bengal, India. Part: magnitude of contamination and population at risk. *Clin Toxicol.* **43**(7):823-834.

Rahman, M.A., Hasegawa, H., Rahman, M.M., Miah, M.A.M. and Tasmin, A. (2007a). Arsenic accumulation in rice (Oryza sativa L.). Human exposure through food chain. *Ecotoxicol. Environ. Safety.*, Article in press, doi:10.1016/ j.ecoenv.2007.01.005.

Rana, T. (2007): A survey work on the effect of toxicity in cattle under highly arsenic prone zone in Haringhata block of Nadia district of West Bengal. M.V.Sc thesis submitted to

West Bengal University of Animal & Fishery Sciences, West Bengal.

Ratnaike, R.N. (2003). Acute and chronic arsenic toxicity. *Postgraduate Medical Journal.* **79**:391-396.

Reitman, S. and Frankel, S. (1957). *Am.J.Clin.Path.* 28, 56.

Riviere, J.E., Boosinger, T.R., Everson, R.J. (1981). Inorganic arsenic toxicosis in cattle. *Mod Vet Pract.* **62**(3):209-11.

Robertson, J.E., Boosinger, T.R. and Everson, R.J. (1981). Inorganic arsenic toxicosis in cattle. A review of selective cases. *Vet. Bull.* **51**(8):5189.

Rosas, I., Belmont, R., Armienta, A. and Baez, A. (1999). Arsenic concentrations in water, soil, milk and forage in Comarca Lagunera, Mexico. Water, Air, and Soil Pollution **112**:133–149.

Sahli, B.P. (1982). Arsenic contamination in cattle liver, kidney and milk. *Veterinary and Human toxicology.* **24**(3):173-174.

Sandhu, H. S. and Brar, R. S. (2000). Textbook of Veterinary Toxicology. In: Metals, 1st edition, Kalyani Publishers, New Delhi. 86.

Santra, A., Maiti, A., Das, S., Lahiri, S., Charkaborty, S.K. and Guha Mazumder, D.N. (2000). Hepatic Damage Caused by Chronic Arsenic Toxicity in Experimental Animals. *Clinical Toxicology.* **38**(4):395–405.

Sanyal and Dhillon (2005). Arsenic and selenium dynamics in water-soil-plant system: A threat to environmental Quality,

Indian Society of Soil Science. ICSWEQ-Proceedings. 239-263.

Sarder, P. (2004). Chronic arsenicosis in chicken and common carp (Cyprenius carpio L.) and its remedial measures through nutritional manipulation. A PhD thesis submitted to West Bengal University of Animal & Fishery Sciences, West Bengal.

Sastry, G.A., and Rao, P.R. (2002). Veterinary pathology. 7th ed. CBS Publishers & Distributors. 134-135.

Satoskar, R.S. and Bhandarkar, S.D. (1993). Pharmacology & Pharmacotherapeutics, 13th ed. Popular Prakashan, Bombay. 901-902.

Satyanarayana, U. (2002). Biochemistry. 2nd ed. Books & Allied (P) Ltd. 354, 675.

Schalm, O.W., Jain, N.C. and Corroll, E.J. (1986).Veterinary Haematology, 4th ed. Lee and Fibiger, Philadelphia.

Selby, L.A., Case, A.A., Dorn, C.R. and Wagstaff, D.J. (1974). Public health hazards associated with arsenic poisoning in cattle. *Journal of the American Veterinary Medical Association.* **165**(11): 1010-1014.

Sharma, H.L. and Sharma, K.K. (2007). Principles of Pharmacology. 1st ed. Paras Publishing. 899.

Singh, A.K. (2006). Chemistry of arsenic in groundwater of Ganges, Brahmaputra river basin. *Current Science.* **91**(5):599-606.

Smith, A.H., Lingas, E.O. and Rahman, M. (2000). Contamination of drinking water by arsenic in Bangladesh: a public health emergency. Bull. WHO, **78** (9): 1093-1103.

Smith, H.A., Jones T.C. and Hunt R.D. (1972). Veterinary Pathology. 4th ed. Lea & Febiger, Philadelphia. 848-850.

SOES (The School of Environmental Studies, 2006). Study on groundwater arsenic contamination in West Bengal - India (20 years study); Till September 2006. Jadavpur University.

Styblo, M., Drobna, Z., Jaspers, I., Lin, S. and Thomas, D.J. (2002). The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. *Environ Health Perspect.* **110** (Suppl 5): 767-771.

Suzuki, K.T., Mandal, B.K., Ogra, Y. (2002). Speciation of arsenic in body fluids. Elisevier Science, B.V.Talanta, Amsterdam, Natherlands. **58** (1): 111-119.

Trinder, P. (1969). *Annals. Clin. Bio Chem.* **6**: 24

USEPA (United States Environmental Protection Agency) (1973). Water Quality Criteria. Ecological Research series. Washington, DC.

Walsh, L.M. and Keeney, D.R. (1975). Behavior and phytotoxicity of inorganic arsenicals in soils. In: Arsenical pesticides. Woolson, E.A., eds. Washington, DC, American Chemical Society, (ACS Symp.Ser. No. 7).

Wang, J.P., Qi, L., Zheng, B., Liu, F., Moore, M.R. and Ng, J.C. (2002). Porphyrins as early biomarkers for arsenic

exposure in animals and humans. *Cell Mol Biol.* **48**:835-843.

Webster, O. (1977). *Clin. Chem.* **21**:1159N.

WHO (World Health Organization) (1993). WHO Guidelines for Drinking Water Quality, Vol. 2. Health Criteria and Other Supporting Information. WHO, Geneva, Switzerland.

WHO (World Health Organization) (2005). Water sanitation and health. Guidelines for drinking water quality. 3rd ed.

Winek, C.L. and Shanor, S.P. (1977). Toxicology Animal. Vol. 2. 1st ed. Marcell Dekker, INC. New York and Basel. 8.

Woolson, E.A. (1983). Man's perturbation of the arsenic cycle. In: Arsenic: industrial, biomedical and environmental perspectives. Lederer, W.H. and Fensterheim, R.J., (eds.) Proceedings of the Arsenic Symposium, Gaithersburg, MD. New York, Van Nostrand Reinhold.

WRI (World Resource Institute) (2000). World Resources 2000-2001 – People and Ecosystem: The Fraying Web of Life, World Resources Institute, Washington, DC.

Zheng, Y., Stute, M., van Geen, A., Gavrieli, I., Dhar, R., Simpson, H.J., Schlosser, P. and Ahmed, K.M. (2004). Redox control of arsenic mobilization in Bangladesh groundwater. *Appl Geochem.* **19**:201-214.