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Determination of the Effect of Mushroom on Arsenicosis Patients by Analyzing Physico-Chemical and Biological Parameters

Jahangir, M.

University of Rajshahi

DETERMINATION OF THE EFFECT OF MUSHROOM ON ARSENICOSIS PATIENTS BY ANALYZING PHYSICO-CHEMICAL AND BIOLOGICAL PARAMETERS

A Thesis

Submitted to the
Institute of Environmental Science (IES)
University of Rajshahi, for the Degree
of
DOCTOR OF PHILOSOPHY
IN
ENVIRONMENTAL SCIENCE

By

M. JAHANGIR

Session: 2010-11
ID No. 10201

INSTITUTE OF ENVIRONMENTAL SCIENCE
UNIVERSITY OF RAJSHAHI
RAJSHAHI, BANGLADESH

June, 2016

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Associate Professor
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DEDICATED
TO
MY BELOVED PARENTS WHO LAID THE
FOUNDATION OF MY EDUCATION
AND
PROFESSOR DR. M SHAMSHER ALI
AND
LATE PROFESSOR DR. MD. SARWAR JAHAN
WHO GAVE ME A HAND TO THE RESEARCH WORLD

DECLARATION

I do hereby declare that the thesis entitled "**DETERMINATION OF THE EFFECT OF MUSHROOM ON ARSENICOSIS PATIENTS BY ANALYZING PHYSICO-CHEMICAL AND BIOLOGICAL PARAMETERS"** is prepared by me. The thesis is an outcome of a field and laboratory work in the study area of Bangladesh, where most of all villagers are suffering from arsenicosis disease. We have analyzed the ground water and biological samples, which belonged to them. We applied a previously unknown, single and unique medicine on them. All of these are done under the auspicious supervision of Dr. Md. Redwanur Rahman, Associate Professor of Institute of Environmental Science, University of Rajshahi, Bangladesh. The study was to innovate the curative medicine for the arsenic induced cancer patients. The thesis is being humbly submitted to the Institute of Environmental Science, University of Rajshahi, Bangladesh for the degree of **Doctor of Philosophy in Environmental Science**. It is also declared that the contents of this thesis or any part of it were not submitted to any other institution for achieving any academic degree or diploma.

(M Jahangir)
Ph.D. Research Fellow
Session: 2010-11
ID No. 10201
Institute of Environmental Science
University of Rajshahi
Rajshahi, Bangladesh

June, 2016

Institute of Environmental Science
University of Rajshahi

CERTIFICATE

The thesis entitled “Determination of the effect of Mushroom on Arsenicosis Patients by Analyzing Physic-chemical and Biological Parameters” submitted by M Jahangir, embodies the results of researches carried out by him under my direct supervision and guidance. I certify that this work has not been presented for any degree or prize elsewhere.

Supervisor

(Dr. Md. Redwanur Rahman)
Associate professor
Institute of Environmental Science
University of Rajshahi
Rajshahi- 6205
Bangladesh

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I want to end these acknowledgements by giving glory and thanks to Almighty Allah, with the word of Al-Hadith “It is better than seventy years pray, if you think a while upon the creation of Allah”.

M Jahangir

ABSTRACT

Arsenic is a toxic element for animals and the majority of plants, in spite of evidence that it is also an essential element. There are well-identified regions where arsenic ground water concentrations can reach values higher than 2 mg/L. The long term arsenic exposure via drinking water causes cancer like skin, lungs, heart and blood vessels, kidney and urinary bladder, reproductive organs, leukemia, rather than ceutaneous lesions (Keratosis, melanosis, leucomelanosis *etc.*). *Pleurotus ostreatus* has a medicinal value which can prevent cancer by reducing the arsenic accumulation in the patients. The spectroscopic (GF-AAS) determination of arsenic in biological samples (hair/nail) before and after application of *Pleurotus ostreatus* has given a tremendous solution for arsenicosis treatment.

This study revealed that 42.6% of people were arsenic affected. We have selected 184 arsenic affected patients out of 571 respondents; among them 61.4% female and 38.6% are male patients. In 78.3% are married people, 48.9% female and 29.3% are male; and 7.6% of female and 9.2% of male are in the section of 16.8% unmarried personals. The educational status is interesting. Maximum people are illiterate (56.5%) and no one in university level; others are 18.5% in primary, 19.6% in secondary and 5.4% in higher secondary school. There are only 33.8% are employed, the fields and percentages are respectively group 1 (agriculture and unskilled workers), group 2 (shop keepers, tailors, salesman, carpenter *etc.*), and group 3 (teachers and other services) are respectively 23.4%, 8.2%, 2.2%. Maximum females are house wives (53.3%) and 8.2% are female students. Male students are only 1.6%. Unemployed, old and retired farmers are 3.3%. There are suspected 8 deaths from arsenic induced lung cancer.

The differential studies of vital signs are blood pressure, pulse rate, respiratory rate, body temperature with them two important investigations for arsenicosis patients are BMI and urine's pH value and specific gravity. The study revealed that maximum patients were suffering from low systolic pressure (38.6%) and also from high systolic pressure (35.8%). After treatment no gross changes found in systolic blood pressure.

Diastolic blood pressure had not any countable responses of medicines like SBP. The pulse rate has a similar result like blood pressure. Here the patients from normal range are slightly increased after treatment (from 17.3% to 22.7%). But most of the patients have high pulse rates. In accordance to body temperature, the percentage of the patients in normal range is increased greatly after treatment (from 29.9% to 74.4%). Body temperature is one of the identifying marks for infection or inflammation present in the body. So this condition is reduced in the patients by *Pleurotus ostreatus* treatment. Here respiration rate is more important for arsenicosis patients. Because the arsenicosis patients in this area were dying of lung disease or cancer. Here 96.0% patients have high respiration rate and normal are only in 4.0%. After treatment the normal range is increased to 10.0%. A long time treatment can change the patients to normal. The normal value of BMI was in 51.1% patients and 36.9% were in low BMI. So it can be said that poverty or obesity is not the factor for arsenicosis disease. Maximum pH values of urine of the patients were acidic (91.4%), after treatment it reduced to 86.0%. Long time acidic nature in the body can produce cancer. After a long time *Pleurotus ostreatus* treatment, the possibility of cancer can be reduce by reducing acidic nature in the patients. But the specific gravity of urine was normal in maximum patients (81.3%), after treatment it increased to 82.6%.

We have studied 48 tube-wells, which were used by the arsenic affected people. Among them only one tube-well is in zero level, two up to 50 ppb, 33 found in 201-300 ppb and the highest is 401-500 ppb As found in two tube-wells. It is known that a level of 0.01 mg/L (10 ppb) of As poses a risk of 6 in 10000 chance of lifetime skin cancer risk. Only two months they can drink rain water in a year.

We have divided the patients into two groups. Each of these groups is divided into two sub-groups. Among these four sub-groups only one sub-group's patients' arsenic concentration had reduced tremendously, who are drinking fresh water (DFW) and taking medicine.

In medicinal group, the mean arsenic concentrations (mg/kg) of hair and nail for before and after treatment in two sub-groups (DAW, DFW) were from 8.5935 mg/kg to 16.0974 mg/kg in DAW and from 30.8802 mg/kg to 13.0785 mg/kg in DFW

respectively. There were few notable changes in these two groups that the DAW was increased 200%, but the DFW was decreased to 43%.

In control group, the average arsenic concentrations (mg/kg) in two sub-groups were from 7.2665 mg/kg to 45.9237 mg/kg in DAW and from 9.1822 mg/kg to 20.5333 mg/kg in DFW respectively. Both groups were linearly increasing in values.

The curative rate of full dose, half dose and BLK were respectively 50%, 56% and 8%. In a differential study, the recovery rate of keratosis of half dose was more efficient than full dose, these were 72% and 50%; in melanosis, full dose was somehow had increased rate than half dose, 46% and 43%, in leuco-melanosis, the result of half dose was better than full dose, and was respectively 60% and 54%. In all respect, the half dose was very efficient and effective for Bangladeshi patients.

Photographic variations are demonstrated for keratosis, melanosis and leuco-melanosis of arsenicosis patients. In all respect, all symptoms were reduced, by that the possibility of cancer or ulceration are reduced in many folds.

At the conclusion of this study it is demarcated that the principal achievement of this research is that arsenicosis is now a curable disease indeed. Not a single patient will die with arsenic induced cancer in Bangladesh as well as in the world by the help of this innovated medicine.

Key words: Arsenic, arsenic induced cancer, cross-sectional study, study-control group, biological samples, long term arsenic exposure, *Pleurotus ostreatus*, arsenic treatment.

ABBREVIATIONS

BADC	= Bangladesh Agriculture Research Council
BARD	= Bangladesh Academy for Rural Development
BMI	= Body Mass Index
BWDB	= Bangladesh Water Development Board
CNS	= Central Nervous System
CSTA	= Control and Curative Short-Course Treatment for Arsenicosis
DAW	= Drinking Arsenic Water
DBP	= Diastolic Blood Pressure
DFW	= Drinking Fresh Water
DNA	= Deoxyribonucleic Acid
DTW	= Deep Tube Wells
EHC	= Environmental Health Criteria
FHS	= Fresh Human Samples
GDP	= Gross Domestic Product
GF-AAS	= Graphite Furnace Atomic Absorption Spectrophotometer
GMP	= Good Manufacturing Practice
GOB	= Government of Bangladesh
HTN/HBP	= Hypertension/High Blood Pressure
IARC	= International Agency for Research on Cancer
NBP	= Normal Blood Pressure
NHBT	= Normal Human Body Temperature
NGO	= Non-government Organization
NPR	= Normal Pulse Rate
NRC	= National Research Council
NRR	= Normal Respiration Rate
SBP	= Systolic Blood Pressure
SMR	= Standardized Mortality Ratio
STP	= Sensitivity Test Program
STW	= Shallow Tube Wells
UNICEF	= United Nations International Children Emergency Fund

USATSDR	= U.S. Agency for Toxic Substances and Disease Registry
USEPA	= United States Environmental Protection Agency
WHO	= World Health Organization

Scientific

As	= Arsenic
DMA	= dimethylarsenic acid
IKr-ATP	= Potassium ion (k+) rectifier- Adenosine Tri-phosphate Channel
IKs-ATP	= Potassium ion (k+) sensitive- Adenosine Tri-phosphate Channel
mmHg	= millimeters of Mercury
MMA	= monomethylarsonic acid
pH	= Hydrogen ion concentration or activity
P. ostreatus	= *Pleurotus ostreatus*
PO	= *Pleurotus ostreatus*
ppb	= parts per billion
R^2	= co-efficient of determination

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Chapter One

INTRODUCTION

1.1 Pathway of Arsenic from Ground to Human beings

1.1.1 Relationship in Between Arsenic, Arsenicosis and Cancer

20th century is the witness of an emerging disease, which is arsenicosis. Till now arsenicosis disease and arsenic induced cancers are threat to all mankind, but these diseases have no cure (Das and Sengupta, 2008). Minimum dose and prolonged consumption of metallic arsenic with drinking water leads to different internal as well as external disorders with cutaneous necrosis and cancer, with myriads of internal organ involvement as well as cancers (Kapaj *et al*., 2006). Many researches are performing on this account, but the curing treatments for the patients are not available till today. Only managements and withdrawing arsenic water is prescribed.

A study found that over 137 million people in more than 70 countries are probably affected by arsenic poisoning from drinking water (Ravenscroft, 2007). More affected people of the countries are Argentina, Bangladesh, Cambodia, Chile, China, Hungary, Mexico, Romania, Thailand, the United States of America, and Viet Nam (WHO, 2006). World Health Organization considered the arsenic poisoning in drinking water by the ground of Bangladesh is the worst incident of the world and called it as the "largest mass poisoning of a population in history" (Smith *et al*., 2000 and Wikipedia, 2015). About 59 districts out of 65 of Bangladesh are affected by arsenic.

Non-occupational human exposure to arsenic in the environment is primarily through the ingestion of food and water. Of these, food is generally the principal contributor to the daily intake of total arsenic. In some areas arsenic in drinking-water is a significant source of exposure to inorganic arsenic. In these cases, arsenic in drinking-water often constitutes the principal contributor to the daily arsenic intake. Contaminated soils such as mine tailings are also a potential source of arsenic exposure. The daily intake of total arsenic from food and beverages is generally between 20 and 300 µg/day. Limited data indicate that approximately 25% of the arsenic present in food is inorganic, but this depends highly on the type of food

ingested. Inorganic arsenic levels in fish and shellfish are low (< 1%). Foodstuffs such as meat, poultry, dairy products and cereals have higher levels of inorganic arsenic. Pulmonary exposure may contribute up to approximately 10 µg/day in a smoker and about 1 µg/day in a non-smoker, and more in polluted areas. The concentration of metabolites of inorganic arsenic in urine (inorganic arsenic, MMA and DMA) reflects the absorbed dose of inorganic arsenic on an individual level. Generally, it ranges from 5 to 20 µg As/litre, but may even exceed 1000 µg/litre.

It is known that long-term intake of small doses of inorganic arsenic compounds with drinking water is responsible for the disorders and cancer of lungs, liver, bladder, skin, kidney and reproductive organs cancer, and many other diseases and disorders of vital organs (Chatterjee *et al.*, 1995). There is an evidence of skin ulcer and cancer on the chest found in Sagarkandi of Pabna district and the patients are dying with this skin cancer (Jahangir, 2009). So it is confirmed that death from cancer is the last destination of arsenic affection in human beings.

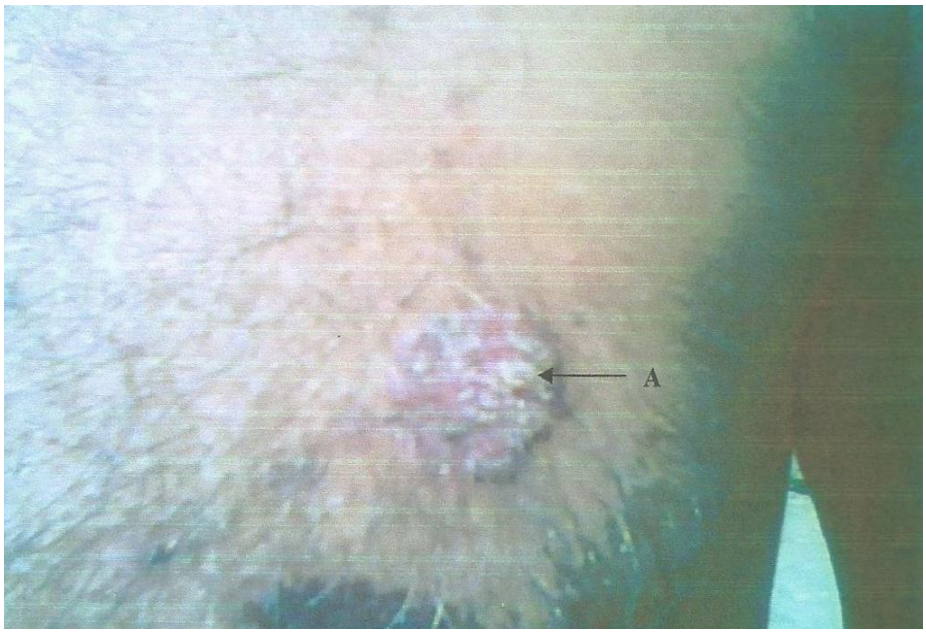


Plate 1.1: A skin ulcer turned to cancer on the left chest over areola on an arsenicosis patient at Sagarkandi, Pabna. (An indication of arsenic induced skin cancer). (Source: Jahangir, 2009)

The consequences is that the skin cancer is found in Sagarkandi, Pabna, Bangladesh (Plate-1.1) (Jahangir, 2009), but also squamous cell carcinoma found in India (Das and Sengupta, 2008) and malignant neoplasms of black foot disease (BFD) is related to continuous exposure to high-arsenic artesian well water in Taiwan (Chen *et al*., 1988). The BFD is also found in Eruine, Laksham of Bangladesh, detail will be found in plate 3.22 of this research.

But the scenario of Laksham of Bangladesh is different. Here no skin cancer was found, instead of that lung cancer is prominent due to arsenic intake through ground water. And there is a similarity found in Canada. Here according to Kusiak *et al*., (1991) the host rock contained 0.1% arsenic in gold mines of Ontario, which produces carcinoma of lungs in the miners.

1.1.2 Different Forms of Arsenic in Nature

Arsenic is heavy metal, naturally found in the ground of Bangladesh. It is also found in soils, groundwater, surface water, air, and some foods (Chou *et al*., 2005). Rocks in the ground are the real home of semi-metallic arsenic. In wetland of southern Australian basin is rich with pyrite and arsenic is one component of pyrite. Like that in some country the earth's crust is the main source of arsenic, but the concentration varies according to the nature of soil, and in some types of rocks and minerals (Chou *et al*., 2005). Its average concentration in earth's crust is about ~5 µg/g (ppm) (Shen *et.al.* 2013). In its pure form, arsenic is a gray-colored, odorless, and tasteless metal; naturally arsenic is inhabited with other elements (Chou *et al*., 2005). The composition of carbon is called "inorganic arsenic."

Ore mining and smelting is the main cause of emission of organic arsenic (arsenate, As^{V}) and inorganic arsenic (arsenites, As^{III}) into the air and then deposited into water and soil during industrial operations, and the other causes are volcanic eruptions and forest fires (Chou *et al*., 2005, Polissar *et al*. 1990 and USEPA, 2000). Wood industries are using huge quantities of arsenic compound as chromated copper arsenate (CCA) to prevent woods and wood products rotting from insects and microbial agent (Chou *et al*., 2005) and (Dang and Chen, 2003). Inorganic arsenic is also found in some Asian folk remedies that claim to relieve constipation during

pregnancy, facilitate delivery in women, and relieve asthma in adults and children (Werner *et al*. 2001) and (Chan, 1994).

In agricultural farms organic (carbon-containing) arsenic (such as, monosodium ethanearsonate and disodium methanearsonate) are used in pesticides (Chou, *et al*., 2005f). Later on, the fruits contain arsenic, which is the direct source to human and the agricultural wastes containing arsenic deposited to the soil. Moreover arsenic contaminated ground water is used in paddy fields in agricultural farms instead of surface water in Bangladesh.

Arsenic is a minor terrestrial element that occurs primarily in association with sulfur-containing minerals such as Realgar (AsS), orpiment (As_2S_3), or arsenopyrite (FeAsS). The mean values of arsenic content in soils, the earth's crust, and sediments are quoted by Sparks as 6, 1.5, and 7.7 mg kg–1, respectively (Sparks, 1995). The natural oxidation of air-exposed sulfide minerals is one of the origins of crustal chemical elements mobilization associated with the generation of acid mine drainage. Mobilization of arsenic in the environment arises also from anthropogenic activities related to mining and ore processing, metallurgy, agriculture, wood preservation, and industry (Clara and Magalhães, 2002).

The major environmental concern about arsenic is not related to its presence in soils and sediments in anomalous amounts, but to its anomalous concentration in surface waters and its availability to living beings. Natural waters, in general, contain low levels of total arsenic as As^{V} and/or As^{III}— 1 to 10 µg/L in normal waters (Willium, 2001).

Arsenic is a metalloid widely distributed in the earth's crust and present at an average concentration of 2 mg/kg. It occurs in trace quantities in all rock, soil, water and air. Arsenic can exist in four valency states: –3, 0, +3 and +5. Under reducing conditions, arsenite (As^{III}) is the dominant form; arsenate (As^{V}) is generally the stable form in oxygenated environments. Elemental arsenic is not soluble in water. Arsenic salts exhibit a wide range of solubility depending on pH and the ionic environment.

In another research Burton (2015) said after study of a wetland located in the Murray-Darling basin in southern Australia that the widespread drainage of wetlands has enhanced the in-situ oxidation of buried iron-sulfide minerals (primarily pyrite,

FeS2). During pyrite oxidation, any pyrite-bound arsenic is released along with iron and sulfate. This leads to the formation of arsenic bearing ferric oxide minerals, such as jarosite, schwertmannite and goethite. These minerals are capable of sequestering much of the arsenic released by in-situ pyrite oxidation and are therefore important controls on arsenic mobility (Figure-1.1) (Burton, 2015).

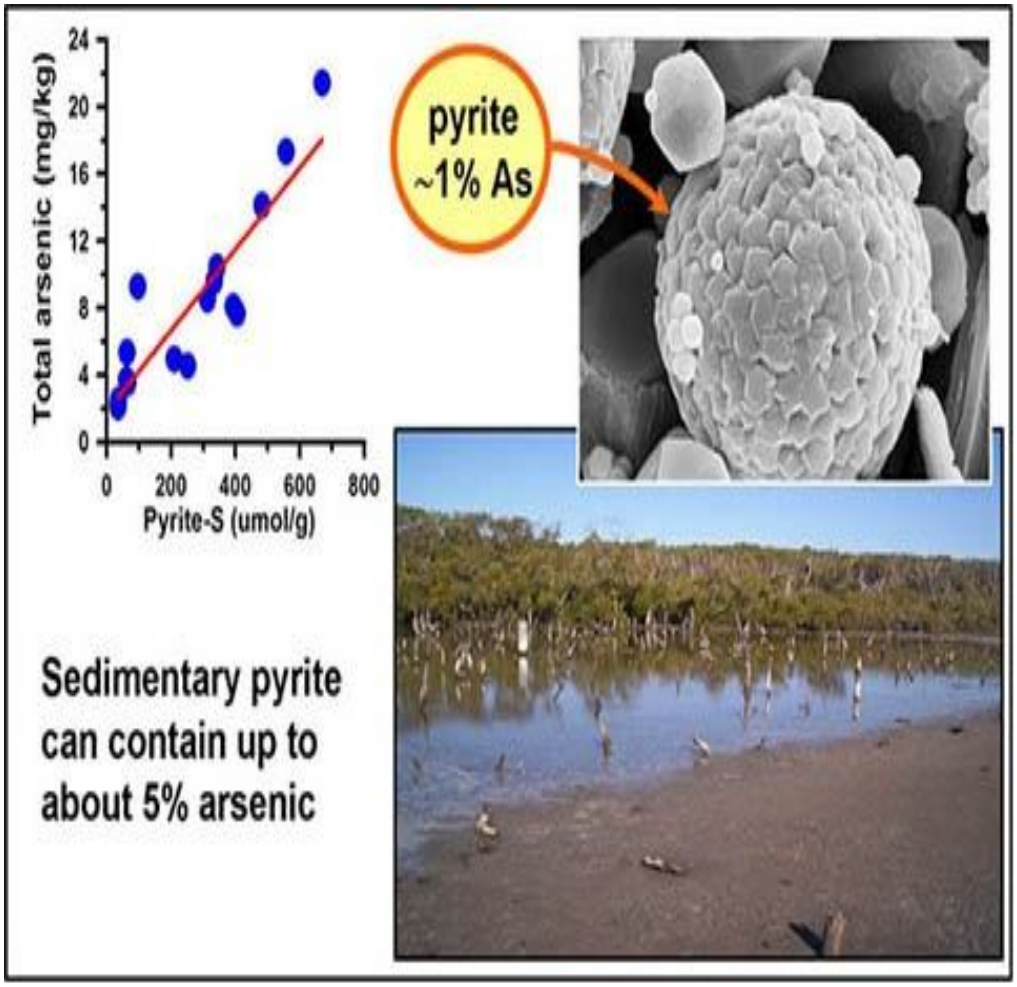


Plate 1.2: Pyrite within undrained wetland soils is often rich in arsenic. (source: Burton, 2015)

1.1.3 Causes of Arsenic in Drinking Water from Ground

Along with other metals arsenic inhibits in the ground. Because of its high toxicity, arsenic is seldom used in the Western world, although in Asia it is still a popular pesticide. Arsenic is mainly encountered occupationally in the smelting of zinc and copper ores. Also Mining techniques such as hydraulic fracturing may mobilize arsenic in groundwater and aquifers due to enhanced methane transport and resulting changes in redox conditions, (Brown *et al.,* 2010) and inject fluid containing additional arsenic (Murcott, 2012) and (Wikipedia, 2015).

No doubt arsenic problem in Bangladesh is due to only the presence of metallic arsenic in drinking water through ground. Before partitioning of India and Pakistan at 1947, there were no deep tube wells, but few shallow tube wells all over Bangladesh. Before liberation at 1971, the population was 70 million (app.) and the use of shallow tube wells were limited. But after liberation huge number of tube wells sunk in last 44 years in the country.

It is well known that the real cause of arsenic in ground water is pulling vigorous quantities of water from the ground for irrigation instead of surface water. The number of shallow tubewells (STW) under operation increased from 93,000 units in 1982–83 to 189,000 in 1987–88, and then expanded exponentially to reach 489,000 units in 1994–95 and 865,000 units in 2000–01. The expansion in the acquisition of minor irrigation equipment can be seen in **Appendix-I**. The number has continued to increase until today. BADC's latest survey of minor irrigation estimates that there were 1,305,000 STWs under operation in the 2007–08 dry season, which were irrigating about 3.2 million ha of land out of a total irrigated area of 5.0 million ha. The number of farmers irrigating land with STWs is estimated at 10.2 million out of 14 million farm households in 2007–08 (Hossain, 2009).

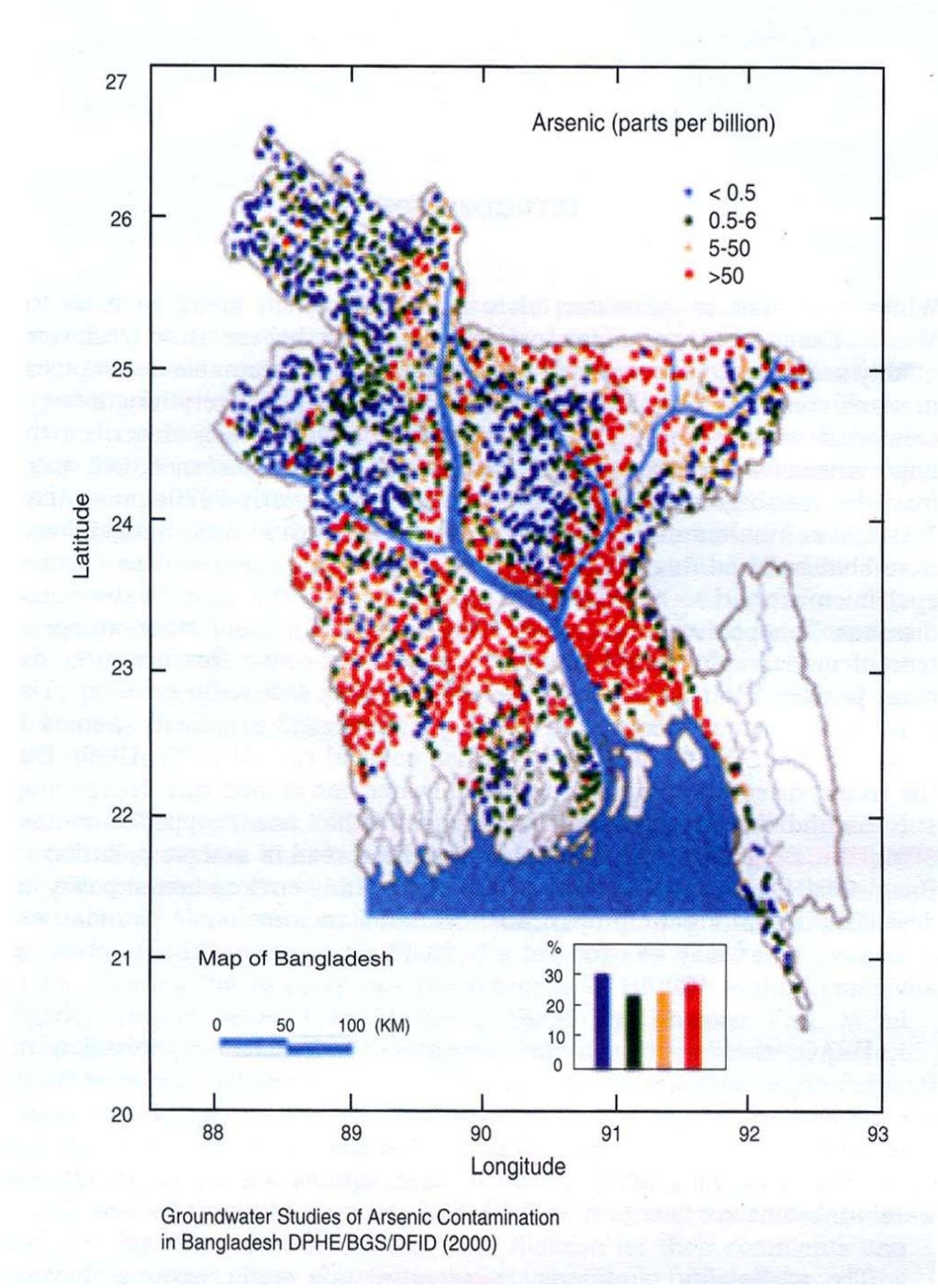
The Bangladesh Water Development Board (BWDB) initiated groundwater irrigation in the early 1960s with the installation of 380 four-cusec capacity deep tubewells (DTW) in Thakurgaon, a northern district. Later, the Bangladesh Academy for Rural Development (BARD) in Comilla experimented smaller capacity tubewells and formed cooperatives of small and marginal farmers. The program was replicated throughout the country. By 1981–82, 12,000 DTWs were under operation and were

irrigating 0.32 million ha of land (Hossain, 2009). Also in 1978, UNICEF gave 300,000 tube-wells in the rural area of Bangladesh (UNICEF, 2013).

Naturally-occurring arsenic contaminated water was first detected in Bangladesh in 1993 at Chapai Nawabganj. According to UNICEF report (2008), A total of 4.7 million tube wells in Bangladesh have been tested and 1.4 million of those were found to contain arsenic above the Government drinking water limit of 50 parts per billion (ppb), mentioned in **Appendix-II**. Combined with another 200,000 unscreened tube wells, which are estimated to also exceed this limit, it means that almost one in five tube wells is not providing safe drinking water. Nationwide, approximately 20 per cent of shallow tubewells are contaminated. There are more than 8,000 villages where 80 per cent of all tube wells are contaminated. About 20 million people in Bangladesh are using tube wells with more than 50ppb of arsenic.

As per WHO report on environmental transport and distribution (1.1.2), arsenic is mainly transported in the environment by water. Sedimentation of arsenic in association with iron and aluminium may sometimes be considerable. In oxygenated water, arsenic usually occurs as arsenate, but under reducing conditions, for instance, in deep well waters, arsenite predominates (WHO, 1981). According to the report, arsenic prospectus in Bangladesh is due to the implementation of tube well in the country instead of using surface water. Now Bangladesh is maintaining a horrible situation. In Bangladesh about 59 districts out of 65 districts are arsenic contaminated (Figure- 1.2). Arsenic has engulfed almost whole Bangladesh. People are dying in severely affected places.

Ground water is used as drinking water in many countries of the world. Recognized ground water pollutants are As, Fe, Pb, Mn, Hg, etc. Arsenic is widely spread in ground of all over Bangladesh. Arsenic can exist in a variety of oxidation states and in organic and inorganic forms in many environmental matrices such as natural water and soil. The predominant oxidation states of arsenic are trivalent arsenite [As(III)] and pentavalent arsenate [As(V)] found in natural waters, and thus in drinking water (Gomez-carminero, *et al.*, 2001). For an accurate assessment of the environmental and biological impact of arsenic in any media, there is a need for laboratory analysis in micro to nano levels for the trace of element.



Map 1.1: Arsenic in ground water of Bangladesh in the year of 2000

1.1.4 Arsenic in Different Foods

It has been found that rice is particularly susceptible to accumulation of arsenic from soil (Kotz, 2011). Rice grown in the US has an average 260 ppb of arsenic according to a study, but U.S. arsenic intake remains far below WHO recommended limits (Sperling, 2005 and Willium *et al*., 2005). China has set a standard for arsenic limits in food (150 ppb) (NAS, 2011), as levels in rice exceed those in water (Evisa News, 2010 and Liang *et al*., 2010). Arsenic is a ubiquitous element present in American drinking water (Ayotte, *et al*. 2011). In the United States, levels of arsenic that are above natural levels, but still well below danger levels set in federal safety standards, have been detected in commercially grown chickens (Tavernise, 2013). The source of the arsenic appears to be the feed additives roxarsone and nitarsone, which are used to control the parasitic infection coccidiosis as well as to increase weight and skin coloring of the poultry (FDA Press Release, 2011).

The potential consequences of exposure to high arsenic groundwater are elevated levels of arsenic in rice grain and straw and, possibly, over time, accumulation of arsenic in soil to levels that are toxic to rice. These outcomes raise the possibility of increased human exposure to arsenic as well as food security concerns if rice yields are reduced (Ross *et al.* 2006).

The arsenic content of unprocessed paddy rice samples ranged from 10 to 420mg/kg (Figure- 1.3). Mean values for the various villages ranged from 108 to 331mg/kg and from 72 to 170mg/kg for boro and aman rice samples, respectively. With the exception of Laksham village in Comilla, mean arsenic contents were higher for boro than for aman rice. The overall mean for boro rice was 1.5 times higher ($p<0.05$) than that for aman rice (183 vs. 117mg/kg). This result is consistent with the hypothesis that arsenic in groundwater is affecting the arsenic content of rice (Duxbury, 2003).

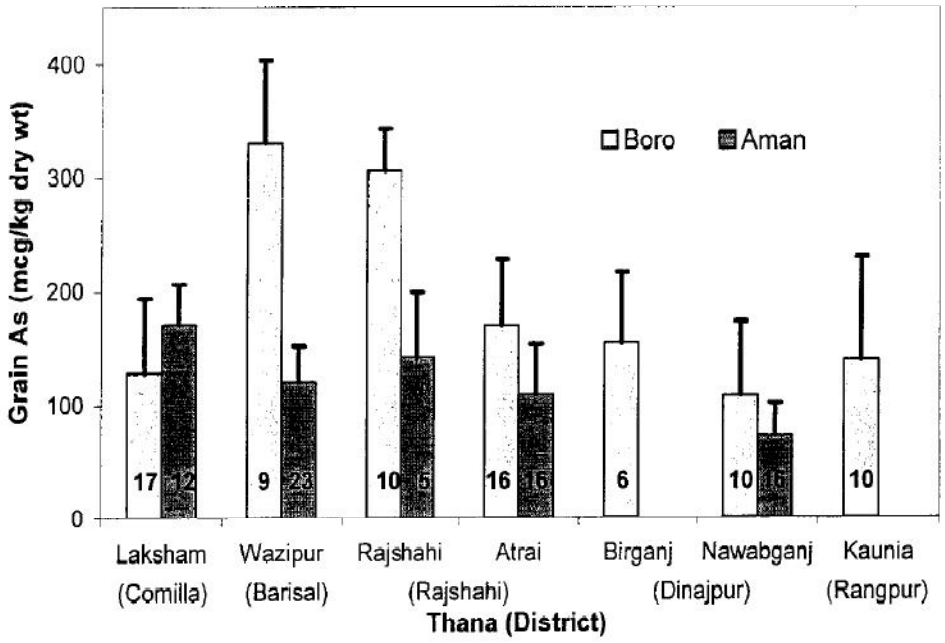


Figure -1.1: Arsenic content of rice from different villages; error bars are SD and values within bars represent the number of samples.

1.1.5 Toxicity of Organic and Inorganic Arsenic

Inorganic forms of arsenic are much more toxic than the organic forms. Inorganic arsenic can be present as either arsenate (As^{V}) or arsenite (As^{III}). Although As^{III} is more toxic, human metabolism of As^{V} involves reduction to As^{III} before undergoing detoxification by methylation (Smith *et al.*1992).

Both inorganic (As^{III}) and organic (As^{V}) forms of arsenic may cause adverse effects in laboratory animals. The effects induced by arsenic range from acute lethality to chronic effects such as cancer. The degree of toxicity of arsenic is basically dependent on the form (e.g. inorganic or organic) and the oxidation state of the arsenical. It is generally considered that inorganic arsenicals are more toxic than organic arsenicals, and within these two classes, the trivalent forms are more toxic than the pentavalent forms, at least at high doses. Several different organ systems are

affected by arsenic, including skin, respiratory, cardiovascular, immune, genitourinary, reproductive, gastrointestinal and nervous systems.

Several animal carcinogenicity studies on arsenic have been carried out, but limitations such as high dose levels, limited time of exposure and limited number of animals make these inconclusive.

However, a recently reported animal model may be a useful tool for future carcinogenicity studies. In that study, female C57B1/6J mice exposed to arsenic in drinking-water containing 500 μg As^V/litre over 2 years was associated with increased incidence in tumours involving mainly lung, liver, gastrointestinal tract and skin. Inorganic arsenic does not induce point mutations. However, arsenic can produce chromosomal aberrations *in vitro*, affect methylation and repair of DNA, induce cell proliferation, transform cells and promote tumours. One study has indicated that DMA may cause cancer of the urinary bladder in male rats at high doses.

1.2 Arsenicosis as a Environmental Problem

1.2.1 Geographic Description of the Ground of Bangladesh

The arsenic pollution of groundwater has become a major disaster for Bangladesh. The alluvial aquifer that underlies the Ganges- Brahamputra river basin contains arsenic in mineral form and has been widely tapped for obtaining drinking and irrigation water. Over a period of about 20-25 years about four million wells have been installed to utilize the groundwater from deeper aquifer layers, typically less than 200m deep (UNICEF, 1999). Exploitation of groundwater from these wells has resulted in mobilizing the arsenic and led to mass poisoning in the region, which is defined by the generic term arsenicosis (Rahman *et al*., 2001).

Bangladesh is a tropical country with a total surface area of about 144,000 km2 and an estimated population of 129 million as of July 2000 (Smith, A.H. *et al.*, 2000). Of the surface area available, about 70% is arable and about 10-15% comprises forests and woodlands. World Bank estimates put the contribution of the agricultural sector to national GDP at about 25%, while a vast majority (~76%) of the population lives in rural settings (BAMWSP, 1999). Mostly, this rural population is the victim of arsenic poisoning. The gross cause of arsenic contamination is metallic arsenic in ground water, which they drink through tube-well. But the important thing is, all the

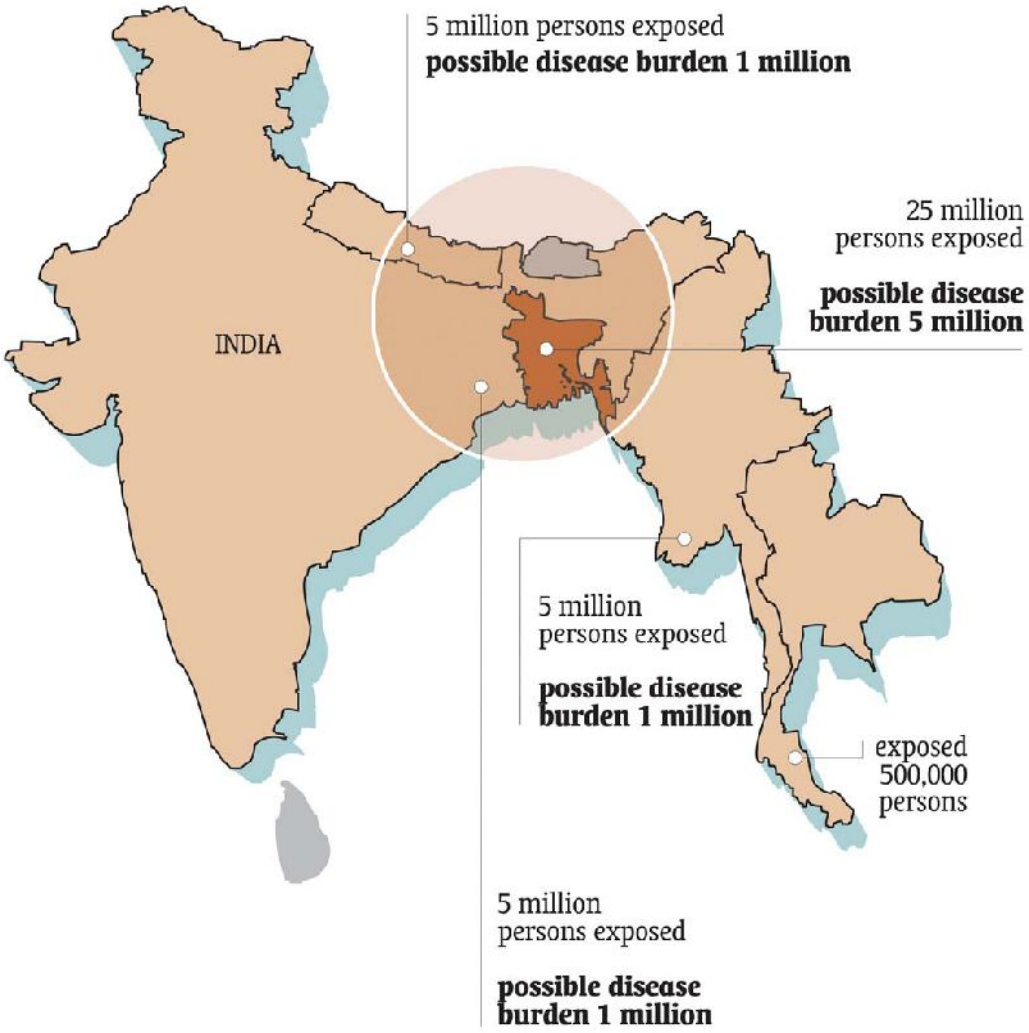
family members are not getting affected by this arsenic contaminated water. In fact, poor are affecting more than the rich. According to the angle of vision of WHO, anti-oxidants are supplied to the contaminated people. But the result is not satisfactory. Now people are getting afraid from NGO people, because they are dying from their medicines. In rural area, who are living well; some are getting affected, some are not. So, it is decided that arsenic free drinking water is the main precaution from arsenic contamination. But still no treatment is available for affected persons.

1.2.2 Arsenicosis Problem in Bangladesh

World Health Organization indicates the 'largest mass poisoning of a population in history' for Bangladesh because of the worst condition of arsenic poisoning through ground water (WHO, 2013). The research by Allan H. Smith, professor of epidemiology at the University of California at Berkeley, said that between 33 and 77 millions of Bangladesh's 125 million people are at risk. Smith predicted a big increase over the coming years in the number of cases of disease caused by arsenic. These ranged from skin lesions to cancers of the bladder, kidney, lung and skin to cardiovascular problems. Bangladesh is grappling with the largest mass poisoning of a population in history because groundwater used for drinking has been contaminated with naturally occurring inorganic arsenic. According to Smith, the scale of this environmental disaster is greater than anything seen before. It is beyond the accidents at Bhopal, India, in 1984 and Chernobyl, Ukraine, in 1986 (Smith, *et al*., 2000).

1.2.3 Epidemiology of Arsenicosis in South-East Asia

The South-East Asia Region contains a natural arsenic-rich eco-belt formed by arsenic-laden alluvium or sediments deposited in the Brahmaputra-Gangetic river basins millions of years ago. Countries of South-East Asia that are in this belt include Bangladesh, parts of India, Myanmar and Nepal. Anthropogenic mining activities in one province of Thailand have also been responsible for arsenic contamination. Groundwater from tubewells is a predominant source of drinking water in many of the Member Countries in which contamination of groundwater often exceeds either the WHO guideline values or the respective prevailing national standards. It is estimated that some 30 million persons may be at risk for arsenic-related diseases by virtue of consuming arsenic contaminated water in the region (Caussy, 2006).

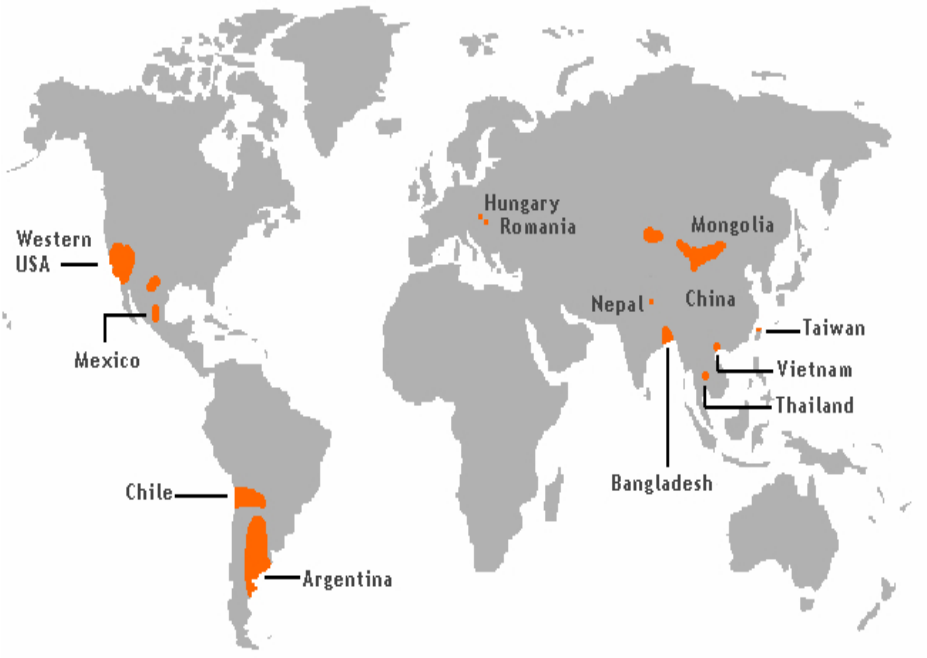


Map 1.2: Epidemiology of arsenicosis in South-East Asia

Cassay (2006) wrote in his guide line that the arsenic exposure in this subcontinent indicates a serious epidemiological effect. Only in Bangladesh, there are 25 million peoples are exposed and 5 million for possible disease burden. Kolkata of India, Nepal and Myanmar are in same rank. Their exposing and burden rates are 5 million and 1 million. And in Thailand, there are exposed 500,000 people. But in Pakistan and Afghanistan, arsenic scenario is different.

1.2.4 Worldwide Arsenicosis Scenario

Arsenicosis, the crippling disease is posing a threat to many poor people worldwide. As we know over 137 million people in more than 70 countries are probably affected by arsenic poisoning from drinking water (Ravenseroft, 2007). The prevalence and magnitude of arsenicosis is high in countries like Taiwan (Tseng, *et al.*, 1968), Thailand (Foy, *et.al.*, 1992), Argentina (Biagini,1974), Chile (Zaldivar, 1974) and Bangladesh (Tondel, *et.al.*, 1999), where it is claimed to be endemic in the country. Bangladesh seems to be one of the largest examples of mass arsenic poisoning: 30-70 million people in 41-64 districts may have been consuming arsenic polluted water containing >50 µg/L arsenic for a long time (DCH, 1998), which exceeded the guideline value of WHO. The World Health Organization (WHO), the European Union, the United States, and many other countries' governments have established 0.05 mg/L (50ppb) arsenic as the maximum contaminant level for total arsenic in potable water. However, there is evidence of adverse health effects at lower exposure levels, and WHO promoted 0.01mg/L (10 ppb) arsenic as the new guideline value for arsenic in potable water (Williams, 2001).



Map 1.3 : Arsenic risk area worldwide, 2012; Source- Wikipedia;
Web: http://en.wikipedia.org/wiki/File:Arsenic_contamination_areas.jpg

Arsenic is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) and the U.S. Environmental Protection Agency (EPA). Chronic exposure to elevated concentrations of arsenic has also been associated with the increased risk of a number of noncancerous effects. Although the adverse health effects arising from exposure to arsenic have been well-recognized, the mechanism(s) of action responsible for the diverse range of health effects are complicated and poorly understood (Shen *et al*., 2013).

1.2.5 WHO Concept for Arsenicosis in Human

It is confirmed that arsenicosis is a deadly disease for this century, which has no cure till now. Inorganic arsenic damages the vital organs of the body. It creates necrosis and ulcers in the cells and tissues of different parts of the body. Keratosis, melanosis, leuco-melanosis are the primary indications of arsenic affection. Secondly, it affects and forms cancer in lungs, kidney, liver, heart, reproductive system, nervous system and blood cells. There are so many evidences for cancer by inorganic arsenic by drinking ground water and inhalation of air in mine area worldwide.

WHO representatives for Environmental Health Criteria 224 (EHC-224) Gomez-Caminero *et.al.* (2001) described that soluble inorganic arsenic is acutely toxic, and ingestion of large doses leads to gastrointestinal symptoms, disturbances of cardiovascular and nervous system functions, and eventually death. In survivors, bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy may be observed. Long-term exposure to arsenic in drinking-water is causally related to increased risks of cancer in the skin, lungs, bladder and kidney, as well as other skin changes such as hyperkeratosis and pigmentation changes. These effects have been demonstrated in many studies using different study designs. Exposure–response relationships and high risks have been observed for each of these end-points. The effects have been most thoroughly studied in Taiwan but there is considerable evidence from studies on populations in other countries as well. Increased risks of lung and bladder cancer and of arsenic-associated skin lesions have been reported to be associated with ingestion of drinking-water at concentrations ≤50 µg arsenic/litre (Gomez-Caminero, *et.al.* 2001). In fact many researches of China, Taiwan had found the skin lesions, as well as skin cancers are the cause of arsenic in drinking water. But in Bangladesh, here respiratory cancer is the main effect of arsenicosis.

1.3 Victims of Arsenic Poisoning

1.3.1 Acute Effects of Arsenic Poisoning

Symptoms of arsenic poisoning begin with headaches, confusion, severe diarrhea, and drowsiness. As the poisoning develops, convulsions and changes in fingernail pigmentation called leukonychia striata may occur. When the poisoning becomes acute, symptoms may include diarrhea, vomiting, blood in the urine, cramping muscles, hair loss, stomach pain, and more convulsions. The organs of the body that are usually affected by arsenic poisoning are the lungs, skin, kidneys, and liver (Mayo clinic, 2012). The final result of arsenic poisoning is coma and death.

The acute minimal lethal dose of arsenic in adults is estimated to be 70 to 200 mg or 1 mg/kg/day (Dart, 2004).

1.3.2 Chronic Effects of Arsenic Poisoning

Chronic high level exposure to arsenic in drinking water (up to over 500 µg/L) was associated with an increased incidence of skin lesions in children, including hyperpigmentation (dark colorations) and keratosis (a local overgrowth of skin, like a callous) (Maharjan, *et al*., 2005 and Ahmed, *et al*., 2006). Studies of skin lesions and exposure to arsenic performed in India and Bangladesh suggested that a possible confounder in these studies was malnutrition of the children (Rahman, *et al*., 2001) and arsenic exposure may be a factor in malnutrition as well (Minamoto, *et al*., 2005). However, another study in Chile demonstrated that the incidence of skin lesions was associated with arsenic exposure regardless of nutritional state of the children (Smith, e*t al*., 2000). Two other studies in the U.S. found no arsenic-associated skin lesions in children associated with concentrations of arsenic in drinking water up to 90 µg/L (Haupert, e*t al*., 1996).

1.3.3 Pathogenesis of Arsenic Toxicity

Arsenic is consumed mainly in 2 forms, arsenite (As^{+3}) and arsenate (As^{+5}). The absorption takes place mainly through ingestion of water, food, beverage, medicine, and in mining areas, swallows and inhales arsenic with particulate matter (Gomez-

Caminero, *et al*. 2001). It is widely accepted that methylated metabolites of inorganic arsenic are less reactive and less genotoxic; metabolism is regarded as a bio-inactivation mechanism. Following metabolism, arsenic is rapidly cleared from blood, and only 0.1% of the arsenic remains in the plasma 24 hours after dosing. Urine is the most common route of elimination. As much as 45% to 75% of the dose is excreted in the urine within a few days to a week (Vahter and Norin, 1980). The trivalent state of arsenic, As^{3+}, is widely distributed by virtue of its binding with sulfhydryl groups in keratin filament and has a tendency to accumulate in the skin, hair, nails, and mucosa of the oral cavity, esophagus, stomach, and the small intestine. On the other hand, arsenate (As^{5+}) is the predominant form deposited in the skeleton because of its ability to replace phosphate in the apatite crystal in bones; as a result of this it is retained there for a longer time (Lindgren *et al*. 1982). The mechanism of toxicity depends on the arsenic species and their valence state.

Arsenic interferes with cellular longevity by allosteric inhibition of an essential metabolic enzyme pyruvate dehydrogenase (PDH) complex, which catalyzes the oxidation of pyruvate to acetyl-CoA by NAD^+. With the enzyme inhibited, the energy system of the cell is disrupted resulting in a cellular apoptosis episode. Biochemically, arsenic prevents use of thiamine resulting in a clinical picture resembling thiamine deficiency. Poisoning with arsenic can raise lactate levels and lead to lactic acidosis. Low potassium levels in the cells increases the risk of experiencing a life-threatening heart rhythm problem from arsenic trioxide. Arsenic in cells clearly stimulates the production of hydrogen peroxide (H_2O_2). When the H_2O_2 reacts with certain metals such as iron or manganese it produces a highly reactive hydroxyl radical. Inorganic arsenic trioxide found in ground water particularly affects voltage-gated potassium channels (Zhou, *et al*., 2007), disrupting cellular electrolytic function resulting in neurological disturbances, cardiovascular episodes such as prolonged QT interval, neutropenia, high blood pressure (Konduri, *et al*., 2009), central nervous system dysfunction, anemia, and death.

Tissue culture studies have shown that arsenic blocks both IKr and Iks channels and, at the same time, activates IK-ATP channels. Arsenic also disrupts ATP production through several mechanisms. At the level of the citric acid cycle, arsenic inhibits pyruvate dehydrogenase and by competing with phosphate it

uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD+, mitochondrial respiration, and ATP synthesis. Hydrogen peroxide production has also increased, which might form reactive oxygen species and oxidative stress. These metabolic interferences lead to death from multi-system organ failure, probably from necrotic cell death, not apoptosis. A post mortem reveals brick red colored mucosa, due to severe hemorrhage. Although arsenic causes toxicity, it can also play a protective role (Klaassen, 2003).

1.3.4 Manifestations of Arsenicosis

Manifestations are the main characteristic of the disease [**Appendix-III**]. The cutaneous manifestations are mainly melanosis, leuco-melanosis, keratosis, ulceration and malignant lesions. In case of diagnosis, it can be diagnosed on the basis of its cutaneous (clinical) manifestations.

According to Caussy (2006), other non-cutaneous manifestations are as follows -

The most common systemic manifestations include neurological, haematological, gastrointestinal and respiratory complications.

Complications of the central and peripheral nervous systems are neuropathy characterized by paresthesias and numbness. Studies from Taiwan have documented the presence of black-foot disease, a unique peripheral arterial disease characterized by severe systemic arteriosclerosis as well as dry gangrene and spontaneous amputations of affected extremities at end stages. Though the incidence of leg pain or intermittent cramp in the leg muscles is not uncommon, dry gangrene is less frequently seen in the Indo-Gangetic Basin.

Haematological complications include leukopenia, anaemia and spleenomegaly.

Gastrointestinal complications include symptoms like anorexia, vague abdominal pain or chronic diarrhoea; liver enlargement with or without non-cirrhotic portal fibrosis are also seen.

Respiratory complications include chronic cough or bronchitis.

1.3.5 Arsenicosis and Cancer

Hutchinson (1887) identified arsenic as a carcinogen because of the high number of skin cancers occurring on patients treated with arsenicals. The International Agency for Research on Cancer (IARC, 1980) classified inorganic arsenic compounds as skin and lung (via inhalation) carcinogens. In the period following this classification, concerns have grown over the possibility of arsenic in drinking water causing a number of other cancers.

The strongest epidemiological evidence on skin cancer effects comes from studies of arsenic contamination of drinking water in China (Province of Taiwan). Villages in south-western China (Province of Taiwan) switched from surface water to arsenic contaminated well water for drinking in the 1920s. An early study by Tseng *et al*. (1968) found evidence of a dose response relationship between concentration of arsenic in drinking water and prevalence of skin cancer. IPCS (1981) estimated skin cancer risk from life-time exposure to arsenic in drinking water at 5% for 0.2 mg of arsenic per litre, based on the findings of Tseng *et al.* (1977). Based on the increased incidence of skin cancer observed in the population in China (Province of Taiwan), the US Environmental Protection Agency (1988) has used a multistage model that is both linear and quadratic in dose to estimate the lifetime skin cancer risk associated with the ingestion of arsenic in drinking-water. With this model and data on males, the concentrations of arsenic in drinking-water associated with estimated excess lifetime skin cancer risks of 10-4, 10-5, and 10-6 are 0.0017, 0.00017, and 0.000017 mg/l respectively. Considering other data and the fact that the concentration of arsenic in drinking-water at an estimated skin cancer risk of 10-5 is below the practical quantification limit of 0.01 mg/l as well as a view to reducing the concentration of arsenic in drinking-water, provisional guideline value of 0.01 mg/l is recommended (WHO, 1996). The guideline value is associated with an excess life-time risk for skin cancer of 6 × 10-4 (i.e. six persons in 10,000).

High levels of arsenic in drinking water are also associated with a number of internal cancers. However, it is difficult to quantitatively establish risk in many of the studies, due to problems in measuring exposure to arsenic. Chen *et al*. (1985) calculated standardized mortality ratios (SMRs) for a number of cancers in 84 villages in southwestern China (Province of Taiwan). Mortality from 1968-1986 was

compared with age and sex adjusted expected mortality. Significantly increased mortality was observed among both males and females for bladder, kidney, lung, liver and colon cancers.

However, the authors were not able to directly estimate arsenic concentrations in well water. Chen and Wang (1990) were able to use data on arsenic concentrations in 83,656 wells in 314 precincts and townships, collected from 1974-1976 in China (Province of Taiwan). The authors used a multiple regression approach to control for socioeconomic confounding factors, and compared age adjusted mortality rates with average arsenic concentrations in each township. They found a significant relationship with arsenic concentration and mortality from cancers of the liver, nasal cavity, lung, bladder and kidney for both sexes. One problem with this study for the purpose of quantitative risk assessment, is that the authors do not report the methodology used for calculating the average arsenic concentration for each township or precinct. Hopenhayn-Rich *et al*. (1998) examined SMRs for bladder, kidney, lung, liver and stomach cancers for 1986-1991 for 26 counties in the Cordoba Province in Argentina. The authors stratified counties into low, intermediate and high exposure groups based on arsenic levels in their drinking water. The low and intermediate exposure counties were defined by the authors. Data for arsenic levels in the two high exposure counties were given. These levels ranged from 0.04 mg/l to 0.43 mg/l. SMRs were calculated using age and sex specific national rates for Argentina. Significant exposure-response relationships were found for the cancer in the bladder, lung and kidney. It is unlikely that smoking is a confounding factor, as deaths from chronic obstructive pulmonary disease (indicative of smoking) were not related to arsenic concentrations.

The above-mentioned studies all utilized an ecological design and are thus susceptible to bias from confounding factors. However, the bladder and lung cancer results of these studies are also confirmed by cohort studies which may be less susceptible to this form of bias. These studies are also useful in providing data on the latency period of internal cancers. Cuzick *et al*. (1992) studied a cohort of patients treated with Fowler's solution (potassium arsenite) in England from 1945-1969. In the follow up until 1991, a significant excess of bladder cancer mortality occurred. In addition, a subset of patients had exhibited skin lesions when examined in 1970. It was found that all patients who subsequently died of bladder cancer had also suffered

skin lesions. Even after stratifying this subset according to dose group, the finding that all cases had skin lesions was highly significant. The authors suggested this provided evidence that skin lesions are a useful biomarker for susceptibility to internal cancers. The period between first exposure and death from bladder cancer varied from 10 years to over 20 years. Tsuda *et al.* (1995) followed up a cohort of 454 residents in Japan who had used industrially polluted water for five years. The authors separated the cohort into low, medium and high exposure groups, based on arsenic concentration in local wells. The low group was exposed to less than 0.05 mg/l, the medium exposure group 0.05-0.99 mg/l and the high exposure group greater than 1 mg/l. A significant excess of cancers occurred only in the group exposed to an arsenic concentration greater than 1 mg/l.

This finding may be because the small sample size is unable to detect significant excess deaths in the medium exposure group. There may also be underestimation of the effect due to the relatively short period of exposure. Significant excess deaths from lung cancer (nine deaths) and urinary tract cancer (two from bladder cancer and one renal pelvis cancer) were observed in the high exposure group. In contrast to the findings of Cuzick *et al.* (1992), the authors found excess cancer mortality among those both with and without skin lesions present: "The results demonstrate that negative skin signs are no assurance of low risk for cancer development." (Tsuda *et al.*, 1995). The authors noted that the period from first exposure to death from lung cancer varied from 11 to 35 years, with a mean of 26.7 years.

To conclude, the results from studies of cancer indicate strong evidence that exposure to arsenic is related to skin, lung and bladder cancer, although more established assessment on health effect of arsenic is being prepared by IPCS/EHC. It is likely that arsenic causes a number of other cancers, but thus far epidemiological evidence has not been consistent for other sites in the body.

1.3.6 Non-Cancer Health Effects

According to the report of National Research Council (NRC, 1999): "Arsenic exposure interferes with the action of enzymes, essential actions, and transcriptional events in cells in the body, and a multitude of multisystemic non-cancer effects might

ensue". The most widely noted non-cancer effects of chronic arsenic consumption are skin lesions. The first symptoms to appear after initiation of exposure are hyperpigmentation (dark spots on the skin) and hypopigmentation (white spots on the skin). Some physicians collectively refer to these symptoms as melanosis. Hyperpigmentation commonly appear in a raindrop pattern on the trunk or extremities, but also on mucous membranes such as the tongue (Yeh, 1973).

Over time arsenic exposure is associated with keratoses on the hands and feet. Keratosis is a condition where the skin hardens and develops into raised wart-like nodules. These nodules become more pronounced over time, sometimes reaching 1 cm in size (National Research Council, 1999). Tseng *et al*. (1968) noted that skin cancers often appear at the sites of existing keratoses. The time from exposure to manifestation is debated in the literature (National Research Council, 1999). It is likely that differing exposures to arsenic accounts for the heterogeneity in observations. The youngest age reported for patients with hyperpigmentation and keratosis is 2 years (Rosenberg, 1974). For Bangladesh, Guha Mazumder *et al*. (1998) suggests a minimum time gap of five years between first exposure and initial cutaneous manifestations. The distinctive appearance of these skin lesions has meant they have been used as indicators of arsenic exposure, when it has not been possible to ascertain arsenic concentrations in well water.

Arsenic has been associated with a multitude of other non-cancer health effects. Arsenic is associated with peripheral vascular disease (blackfoot disease) in China (Province of Taiwan) (Tseng, 1977). This condition results in gangrene in the extremities and usually occurs in conjunction with skin lesions. Other cardiovascular problems such as hypertension (Chen, *et al*. 1995) and ischemic heart disease have been found to be associated with arsenic (Tsuda *et al*. 1995). Research of organ damage has concentrated mainly on the liver. Guha Mazumder *et al.* (1988) found evidence of liver enlargement and non cirrhotic portal fibrosis among a small sample of severely affected arsenic patients in West Bengal. In a later study, Guha Mazumder *et al.* (1997) also suggested pulmonary health effects. They found restrictive lung disease among 53% of a small sample of severely affected arsenic patients in West Bengal.

In terms of haematological effects, anaemia is commonly cited (NRC, 1999). Another widely suggested health effect is diabetes mellitus. Rahman *et al*. (1998) found a significant dose response relationship between arsenic exposure and diabetes mellitus among those suffering from keratoses in Dhaka, Bangladesh.

1.3.7 Prevalence of Arsenicosis

As arsenic compounds have long been recognized as toxicants (Ghazy, 1995), it creates necrosis in cells and tissues, external, as well as internal of the body. In excessive amounts, arsenic causes gastrointestinal damage and cardiac damage. Chronic doses can cause vascular disorders such as black foot disease (Chen, *et.al.*, 1985). These non-dermatological disorders are not highlighted as clinical criteria for diagnosis of arsenicosis, but these are frequent accompaniment of arsenicosis (**Appendix IV**) (Kapaj, *et.al*., 2006). Arsenic and its compounds are reported to be carcinogenic, mutagenic and teratogenic in nature. The toxicity, availability and environmental mobility of arsenic are very much independent on their chemical forms. The arsenic toxicity is affecting all vital organs of the body. The manifestations of different systems are as- respiratory, Hepato-biliary, neurological, peripheral vascular, gastro intestinal are major and endocrinal, cardiac, ocular, renal, obstetrical, hematological, constitutional are minor in severity of affection (**Appendix IV**) (Kapaj, *et.al.*, 2006). In all aspects, finally all the diseases turn to cancer and death. According to WHO estimation arsenic will cause 200,000-270,000 deaths from cancer in Bangladesh alone (WHO, 2001). WHO is very much annoyed for the arsenicosis situation in Bangladesh. WHO announced 'the number of people drinking arsenic-rich water in Bangladesh has increased dramatically since the 1970s due to well-drilling and population growth' and also said, 'the most commonly manifested disease so far is skin lesions. Over the next decade, skin and internal cancers are likely to become the principal human health concern arising from arsenic'.

1.3.8 Prevention and Management of Arsenicosis

The preventive measures of arsenicosis revolve around the positive role of raising public awareness by information, education, and communication strategies. Arsenicosis is one of those diseases for which no effective therapy is known till date

(Das and Sengupta, 2008). Considering the fact that a patient once affected may not recover even after remediation, the principal focus rests on prevention of the problem.

There are few selected managements for arsenic in water and human. WHO has recommended 5 key approaches (Caussy, 2006) in the management of arsenicosis:

i) Cessation of exposure to drinking water-

As there is no known specific treatment for arsenicosis, the 7y6 prudent intervention is to stop consumption of arsenic-contaminated water. Appropriate counseling for safe water options and health consequences of consuming arsenic contaminated water should be supported through standard Information, Education and Communication (IEC) strategies. In general, the water supply option for an area will depend on the availability, quality and development potentials of available alternative water sources in a given area. A single option may not be suitable or affordable for people with different social and economic conditions. Some of the main strategies for safe water should include:

a) Treatment of surface water,
b) Rain water harvesting,
c) Deep tube well, and
d) Treatment of Arsenic contaminated water.

ii) Administration of nutritional supplements-

Administration of non-specific nutritional supplements or anti-oxidants directed at hastening recovery or averting disease progression has been undertaken in many countries. Some commonly used anti-oxidants include beta carotene, vitamin E and vitamin C. Presently, there is no large-scale randomized-controlled double-blinded trial to evaluate the efficacy of these treatment regimens. Their use depends on the national policy and the recommendations of the concerned medical bodies in respective countries.

As per Niloy Das and Sengupta, (2008) the nutritional supplements are-

a) People should be encouraged to take food of high calorific value from either animal or plant source, so that in spite of drinking arsenic-contaminated water, they can survive its consequences.

b) Consumption of polyphenols and extracts of green and black tea results in efficient reduction of As III-induced DNA damage in human lymphocytes and also enhanced recovery of DNA damage by virtue of its antioxidant effects.
c) Animal study has shown that N-acetyl-L-cysteine (NAC), which modulates hepatic glutathione hydroxylase GSH level, reduces the liver injury produced by arsenic. Thus supplementation of NAC can be explored in future to reduce the arsenic-induced hepatic damage.
d) It needs to be emphasized that antioxidants (vitamin A, alpha-tocopherol, ascorbic acid) when taken along with arsenic-free water showed significant improvement in arsenicosis.
e) Selenium (Se) in diet was shown to ameliorate the effect of arsenic toxicity.
f) Spirulina, a microscopic blue green algae, alone or in combination with zinc, is found to be effective in improving symptoms of arsenicosis.

iii) Provision of non-specific therapy-

Symptomatic treatment for patients with keratosis or keratosis and melanosis includes the application of keratolytic agents. Presently, 5-10% of salicylic acid and 10-20% of urea-based ointment for the treatment of keratotic lesions is the most common prevailing practice, as evidenced by literature review. Higher doses need further evaluation.

iv) Secondary prevention of latent effect-

Secondary prevention of latent effects should be done through medical surveillance. The management of arsenic-associated cancer patients should follow the prevailing national standards and practice for the management of cancer patients in general.

v) Counseling and education-

Counseling and education address the psychosocial sequelae of the illness and provide appropriate rehabilitation. Programs should be implemented on educating patients and other community members about basic public health aspects of arsenicosis and to dispel misconceptions that may lead to stigmatization, family and occupational disruption and other social hardship.

1.4 Vital Signs Analysis of the Patients

It is considered that the standard for monitoring a patient for most of the cases, the vital signs (blood pressure, pulse rate, respiratory rate and temperature) recording are very important for the patients in hospitals (Cretikos, *et al*., 2008). In case of arsenicosis, these vital signs are neglecting by many researchers. But according to environmental health criteria 18 for arsenic of WHO (1981), long term arsenic exposure via drinking water can cause several arsenic induced cancers, such as- in skin, lungs, heart and blood vessels, kidney and urinary bladder, reproductive organs, leukemia; and also non-cancerous skin lesions (Keratosis, melanosis, leucomelanosis *etc.*) and oedema of extremities. As arsenic can cause diseases in vital organs, so we have chosen to study these vital signs, as well as skin lesions of arsenicosis patients for our research, and beside of them, three more important investigations- BMI, pH value and specific gravity of urine, which are closely related with cancer and arsenicosis patients.

As we know arsenicosis is the disease of poor, estimation of BMI has an important role for the patients. Body obesity, especially body weight gaining or loosing, can be detected by BMI analysis (WHO, 2016). For BMI estimation, we have noted body weight and height of the patients. On the other hand, pH value is very much important for many disease formations, but it may be accurate for initial investigation for arsenic induced cancer formation in the patients. When cancer is not formed, but the body field is favorable, this condition can be detected by pH value, the bodily buffer system. Acidic pH is having the features of cancer (Aredia and Scovassi, 2014). The excreting fluid of the body is urine, so we have chosen the urine pH for the analysis. Another important diagnosis is specific gravity of urine for arsenicosis patients. Specific gravity measures the kidney's ability to concentrate or dilute urine in relation to plasma. All of their information and data are collected and noted in black and white on questionnaire cum data collection form in **Appendix-V**, which is mainly prepared for arsenicosis patients of this research. These are the aims and objectives of this part of the research.

1.4.1 Study of Blood Pressure

Blood pressure is a physiological condition, by which blood is circulating in the body to carry out the nutrients and oxygen to all cells and tissues, to maintain body temperature, to regulate the immune system, *etc*. Heart is pumping the blood to every periphery of capillaries. Arsenic in drinking water comes into blood circulation after digestion and absorption in intestines and mesentery. Blood also carry out arsenic with other nutrients and minerals. Finally arsenic settle down as a residue in different transportation areas of the body, such as- capillary to cell in palm and feet forming keratosis, artery to vein vessels in lungs, kidneys, liver, spleen, placenta and heart muscle forming necrosis and cancer, *etc.* and then death of the patient.

If any difficulty arises in the circulating system, then blood pressure shows hypotension or hypertension. Distance between diastole and systole indicates different problems in the heart. We have to observe our patients, whatever their blood pressure shows, that will be important and this diagnosis will be an epoch-making for arsenic researchers.

In circulatory system, blood pressure is the force of blood pushing against the walls of the arteries as the heart pumps out blood in one diversion. In physiology, inadequate blood pressures are divided into two types; one- high blood pressure, which is abnormal increase rate of blood pressure, called “hypertension” and other- low blood pressure, that is abnormal decrease rate of blood pressure, called “hypotension”.

Normal range of blood pressure is 120/80 millimeters of mercury (mm Hg), that is systolic 120 and diastolic is 80. Systolic more than 120 associate with increasing diastole rate is considered as **hypertension** (e,g,- 160/100 mm Hg) and diastolic less than 80 associate with systolic rate is **hypotension** (e,g,- 90/50 mm Hg).

Hypertension

High blood pressure is a common condition in which the force of the blood against artery walls is high enough that it may eventually cause health problems, such as heart disease. High blood pressure (hypertension) for years with any symptoms, or

even without symptoms, can damage to blood vessels. Uncontrolled high blood pressure increases the risk of serious health problems, including heart attack and stroke (Mayo clinic, 2015).

Causes of Hypertension

Most of the time, no cause of high blood pressure is found. This is called essential hypertension.

Another medical condition for high blood pressure is caused by taking medicines that is called secondary hypertension. According to Mayo Clinic (2016), secondary hypertension may be due to:

Diabetes complications (diabetic nephropathy, Polycystic kidney disease, Disorders of the adrenal gland (such as pheochromocytoma, Cushing syndrome, Thyroid problems, Hyperparathyroidism, Pregnancy or preeclampsia, Coarctation of aorta or Narrowed artery that supplies blood to the kidney (renal artery stenosis), Glomerular disease, Renovascular hypertension, Aldosteronism, Sleep apnea, Obesity, Medications such as birth control pills, diet pills, some cold medicines, and migraine medicines.

Hypotension

The abnormally low blood pressure, especially in the arteries of the systemic circulation is known as hypotension. It is often associated with shock, though not necessarily indicative of it. If it is lower than normal, then it is called **low blood pressure** or hypotension. Hypotension is generally considered to be systolic blood pressure less than 90 millimeters of mercury (mm Hg) or diastolic less than 60 mm Hg. However in practice, blood pressure is considered too low only if noticeable symptoms are present (Wikipedia, 2016).

For some people who exercise and are in top physical condition, low blood pressure is a sign of good health and fitness (BUPA, 2016) For many people, low blood pressure can cause dizziness and fainting or indicate serious heart, endocrine or neurological disorders. Severely low blood pressure can deprive the brain and other

vital organs of oxygen and nutrients, leading to a life-threatening condition called shock (Wikipedia, 2016).

Causes of Hypotension

Severe hypotension can be caused by sudden loss of blood (shock), severe infection, heart attack, or severe allergic reaction (anaphylaxis).

According to Chan, M.A. (2015), certain medicines and substances can lead to low blood pressure, Including these conditions, as- Alcohol, Anti-anxiety medicines, Certain antidepressants, Diuretics, Heart medicines, including those used to treat high blood pressure and coronary heart disease, Medicines used for surgery and Painkillers.

Other causes of low blood pressure are as follows-

Nerve damage from diabetes, changes in heart rhythm (arrhythmias), not drinking enough fluids (dehydration) and Heart failure.

These are usual, but arsenic can cause serious damage or stenosis in heart, arteries and veins. It can be possible that hypotension or hypertension is the primary sign of arsenic affection in heart, artery and vein. So our research needs to do work on them.

1.4.2 Study of Pulse Rate

Heart is a vital part of the body. Heart pumps blood in all parts of the body through arteries. Foetal heart sound found by ultrasound fetoscope at the 8^{th} to 14^{th} weeks of gestation (pregnancy) (Nicolaides, *et al.*, 2001 and Hernadi *et al.*, 1997). So heart started its work before a child born. If there is any difficulties arise in heart muscle that could be detected by abnormal rate of pulse.

Cardiac diseases, such as ischemic heart disease, arrhythmia and hypertension are the features of arsenicosis patients also (Kapaj, 2006). There are many diagnostic instruments (according to the severity of the disease) are present, but pulse (heart) rate study is the key to all.

Normal pulse rate is 72 beats per minutes (bpm) in adults. In an average resting adult, a healthy heart beats approximately 60 to 100 times per minutes. It may be increase or decrease in relation to the heart disease.

Tachycardia

It is a heart rate that exceeds the normal range. Tachycardia can be caused by various factors which often are benign. However, tachycardia can be dangerous depending on the speed and type of rhythm (Wikipedia, 2016). Arsenicosis patients have many signs and symptoms which may cause tachycardia.

Tachycardia is caused by something that disrupts the normal electrical impulses that control the rate of the heart's pumping action. Many things can cause or contribute to problems with the heart's electrical system. According to the experts of Mayo clinic (2014), the factors of tachycardia are as follows-

Damage to heart tissues from heart disease, Abnormal electrical pathways in the heart present at birth (congenital), Disease or congenital abnormality of the heart, Anemia, Exercise, Sudden stress, such as fright, High blood pressure, Smoking, Fever, Drinking too much alcohol, Drinking too many caffeinated beverages, Medicinal side effects, Abuse of recreational drugs, such as cocaine, Imbalance of electrolytes, mineral-related substances necessary for conducting electrical impulses, Overactive thyroid (hyperthyroidism).

In arsenicosis patients heart can be damaged by metallic arsenic in the blood vessels. Because of that the patients may form tachycardia. So it is necessary to detect pulse rate of the arsenicosis patients.

Bradycardia

It is a slower heart rate than normal. The heart usually beats between 60 and 100 times a minute in an adult at rest. When bradycardia happens, the heart beats fewer than 60 times a minute. Bradycardia can be a serious problem if the heart doesn't pump enough oxygen-rich blood to the body (Mayo Clinic, 2014).

In bradycardia, the brain and other organs may not get sufficient supply of oxygen they need. As a result, one may face bradycardia. The symptoms of bradycardia are as follows:

Near-fainting or fainting (syncope), Dizziness, Weakness, Fatigue, Shortness of breath, Chest pains, Confusion or memory problems, Easily tiring during physical activity.

Bradycardia is caused by something that disrupts the normal electrical impulses controlling the rate of the heart's pumping action. According to experts of Mayo Clinic (2014), many things can cause or contribute to problems with the heart's electrical system, including:

Heart tissue damage related to aging, Damage to heart tissues from heart disease or heart attack, High blood pressure (hypertension), Heart disorder present at birth (congenital heart defect), Infection of heart tissue (myocarditis), A complication of heart surgery, Underactive thyroid gland (hypothyroidism), Imbalance of mineral-related substances necessary for conducting electrical impulses (electrolytes), Repeated disruption of breathing during sleep (obstructive sleep apnea), Inflammatory disease, such as rheumatic fever or lupus, The buildup of iron in organs (hemochromatosis), Medications, including some drugs for other heart rhythm disorders, high blood pressure and psychosis.

As arsenic has a nature to damage the vital parts of the body. Keeping in mind these causes and symptoms, we can assume that if heart muscle is damaged by inorganic arsenic, the arsenic affected patient can suffer from tachycardia or bradycardia. Proper diagnosis can assure about the arsenic induced heart diseases.

1.4.3 Study of Body Temperature

Body temperature is one of the vital sign as a diagnosis. It is not only an important sign in fever (high temperature or hyperthermia), but also essential in low temperature or hypothermia. Hypothermia usually occurs from exposure to low temperatures (Wikipedia, 2016) (atmospheric or diving in cold water). High temperature is not only found in general fever, but also cancer has an identification sign of local temperature. In case of arsenic induced cancer patients, temperature recording can be a valuable diagnosis for the study.

It is known that heat is primarily generated in muscle tissue, including the heart and in the liver, while it is lost through the skin (90%) and lungs (10%)

(Wikipedia, 2016). The vital organs muscle tissue, heart, liver, skin and lungs all are affecting in arsenicosis patients. So it is so much important to investigate temperature in arsenicosis patients.

Hyperthermia

It is elevated body temperature due to failed thermoregulation that occurs when a body produces or absorbs more heat than it dissipates. Extreme temperature elevation then becomes a medical emergency requiring immediate treatment to prevent disability or death (Wikipedia, 2016).

Causes of Hyperthermia

The most common causes include heat stroke and adverse reactions to drugs. The former is an acute temperature elevation caused by exposure to excessive heat, or combination of heat and humidity, that overwhelms the heat-regulating mechanisms. The latter is a relatively rare side effect of many drugs, particularly those that affect the central nervous system. Malignant hyperthermia is a rare complication of some types of general anesthesia. Those working in industry, the military and first responders, may be required to wear personal protective equipment (PPE) against hazards such as chemical agents, gases, fire, small arms and even Improvised Explosive Devices (IEDs). PPE includes a range of hazmat suits, firefighting turnout gear, body armor and bomb suits, amongst others (Wikipedia, 2016).

Hypothermia

It is a medical emergency that occurs when your body loses heat faster than it can produce heat, causing a dangerously low body temperature. Normal body temperature is around 98.6 F (37 C). Hypothermia occurs as your body temperature passes below 95 F (35 C) (Mayo clinic, 2014).

Causes of Hypothermia

Cold exposure- When the balance between the body's heat production and heat loss tips toward heat loss for a prolonged period, hypothermia can occur. Accidental hypothermia usually happens after cold temperature exposure without enough warm, dry clothing for protection. Mountain climbers on Mount Everest avoid

hypothermia by wearing specialized, high-tech gear designed for that windy, icy environment.

However, much milder environments can also lead to hypothermia, depending on a person's age, body mass, body fat, overall health, and length of time exposed to cold temperatures.

Other causes- Certain medical conditions such as diabetes and thyroid conditions, some medications, severe trauma, or using drugs or alcohol all increase the risk of hypothermia.

Hyperthermia and hypothermia, both are important for arsenic induced skin, lungs, liver kidney and cancer patients. As in all cancer cases hyperthermia may occur; on the other hand, hypothermia in other diseases. Generally arsenicosis patients are very weak, with that protein deficiency may cause hypothermia to them. So, both could be happened in initial and later cases. A keen observation can give a good result by investigating all arsenicosis patient.

1.4.4 Study of Respiration Rate

Lungs cancer is an important characteristic of arsenicosis disease. In Bangladesh, most of the arsenicosis patients are suffering from respiratory problems. When lungs are affected the intake and outlet of air will be hampered in the system and study of respiratory rate will notice it.

The average total lung capacity of an adult human male is about 6 litres of air, but only a small amount of this capacity is used during normal breathing. (**Appendix-VI**) Always some air remains in the lungs, which are residual volume. (**Appendix-VII**) When we take a long breath or a run or sneeze, then the lungs gets more oxygen by expelling residues. The average human respiratory rate is 30-60 breaths per minute at birth, decreasing to 12-20 breaths per minute in adults (Wikipedia, 2016).

The tidal volume, vital capacity, inspiratory capacity and expiratory reserve volume can be measured directly with a spirometer. These are the basic elements of a ventilatory pulmonary function test.

The establishment of the tidal volume and pattern of respiration in normal individuals is a complicated process. Recognizing alterations in these factors is an important early clue of disease recognition. While frequently it is nonspecific, in many instances it can lead directly to a diagnosis. Careful observation of the respiratory rate and pattern is a crucial part of the physical examination.

Mechanical properties also are altered by diseases. Interstitial disease probably enhances the stretch receptor response, leading to rapid shallow ventilation. This is efficient because the lung with interstitial disease is less compliant, requiring more distending pressure per unit of volume than normal. By breathing at lower tidal volumes, the work of breathing is diminished.

The respiratory center feedback from the higher cortical centers can also be modulated with diseases. Anxiety can increase the respiratory rate and pattern. The acute hyperventilation syndrome is an example where drive from higher centers can maintain high minute ventilation in face of an elevated pH. Increased intracranial pressure leads to a rapid and deep breathing pattern. This pattern is frequently seen with head trauma. Pain contributes to a rapid respiratory rate. A fractured rib produces pain on inspiration and therefore leads to a low-volume, rapid-rate pattern. Tachypnea is commonly part of any chest pain and is partly modulated through higher cortical input.

The central controlling center can be affected directly. Any central nervous system depressing drug will reduce the respiratory rate and pattern. It will also blunt the response to other neural inputs. The patient with obstructive lung disease who receives a narcotic frequently will elevate the $PaCO_2$ even further. The same is true for many drug overdoses. If the central nervous system is depressed by drugs, the depression of the respiratory center leads to CO_2 retention.

In 1993, Fieselmann and colleagues reported that a respiratory rate higher than 27 breaths/minute was the most important predictor of cardiac arrest in hospital wards (Fieselmann, *et.al.,* 1993). Subbe and colleagues found that, in unstable patients, relative changes in respiratory rate were much greater than changes in heart rate or systolic blood pressure, and thus that the respiratory rate was likely to be a better

means of discriminating between stable patients and patients at risk (Subbe, *et al*., 2003). Goldhill and colleagues reported that 21% of ward patients with a respiratory rate of 25–29 breaths/minute assessed by a critical care outreach service died in hospital. Those with a higher respiratory rate had an even higher mortality rate (Goldhill, *et al*., 2004). In another study, just over half of all patients suffering a serious adverse event on the general wards (such as a cardiac arrest or ICU admission) had a respiratory rate greater than 24 breaths/minute. These patients could have been identified as high risk up to 24 hours before the event with a specificity of over 95% (Cretikos, *et al.*, 2007).

If any arsenicosis patient suffers from respiratory problem, we have to take a full phase case study of the patient. There are many investigations for respiratory problem. Respiratory rate is the key to all.

1.4.5 Study of BMI

As it is assumed that the arsenicosis disease is for the poor's disease, riches are not affecting, so body mass index is very much important to investigate for the arsenicosis patients. Many researchers targeted to protein deficiency, as a sign of arsenicosis in poor. Protein deficiency, malnutrition and no obesity in the body, these are the main constituents of a poor. On this view, estimation of body mass index (BMI) is a correct investigation for arsenicosis patients.

The body mass index (BMI), is a heuristic proxy for human body fat based on an individual's weight and height. BMI does not actually measure the percentage of body fat. Body mass index is defined as the individual's body weight divided by the square of his or her height. The formulae universally used in medicine produce a unit of measure of kg/m^2. There are other units rather than this SI unit. These are mentioned in **Appendix-VIII**. BMI can also be determined using a BMI chart (Wikipedia, 2016), which displays BMI as a function of weight (horizontal axis) and height (vertical axis) using contour lines for different values of BMI or colours for different BMI categories.

BMI Category:

A frequent use of the BMI is to assess how much an individual's body weight departs from what is normal or desirable for a person of his or her height. The weight excess or deficiency may, in part, be accounted for by body fat (adipose tissue) although other factors such as muscularity also affect BMI significantly (see discussion below and overweight). The WHO regards a BMI of less than 18.5 as underweight and may indicate malnutrition, an eating disorder, or other health problems, while a BMI greater than 25 is considered overweight and above 30 is considered obese (Wikipedia, 2016). These ranges of BMI values are valid only as statistical categories. The categories with BMI values are mentioned in **Appendix-IX**.

1.4.6 Study of Urine (pH and specific gravity)

Urinary bladder cancer, due to arsenic is an important case of arsenicosis. In many countries, bladder cancers are found in arsenicosis patients. If cancer is formed in bladder, the pH value and specific gravity of urine will be changed. As well as acidic pH is one of the sign of cancer in situ. So it is important to diagnose urine pH and specific gravity.

1.4.6.1 pH of Urine

pH: The symbol relating the hydrogen ion concentration or activity of a solution to that of a given standard solution.

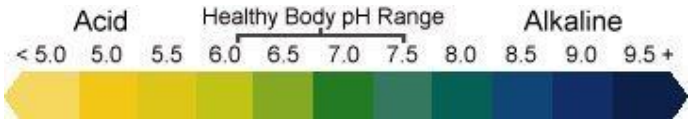
pH is a measure of acidity and alkalinity. On a scale of 1-14, 7 represents neutrality, lower numbers indicate increasing acidity and higher numbers increasing alkalinity. Each unit of change represents a tenfold change in acidity or alkalinity. The pH of urine can range from an extremely unhealthy low of 4.5 to a high of 8.5. A high pH value may indicate the body is over buffering to compensate for a physiological system that is too acidic.

The hydrochloric acid in the stomach is required for digestion and high acidic levels in the body actually inhibit the proper production of stomach acids. When the body pH is too acidic the body's response is to compensate by secreting less acidic fluids and a shortage of proper stomach acid is the result. Balance is the key. Too

alkaline is as undesirable as too acidic (though easier to tolerate). In a pH balanced body, urine is slightly acid in the morning (after fasting), with a pH range of 6.5 - 7.0, generally becoming more alkaline, pH = 7.5 - 8.0, by evening as the body digests food and releases electrolytes.

Water-

Water is the most abundant compound in the human body, comprising 70% of the body. The body has an acid-alkaline (or acid-base) ratio called the pH which is a balance between positively charges ions (acid-forming) and negatively charged ions (alkaline-forming.) The body continually strives to balance pH. When this balance is compromised many problems can occur.



It is important to understand that we are not talking about stomach acid or the pH of the stomach. We are talking about the pH of the body's fluids and tissues which is an entirely different matter.

Urine-

Urine testing may indicate how well your body is excreting acids and assimilating minerals, especially calcium, magnesium, sodium and potassium. These minerals function as "buffers." Buffers are substances that help maintain and balance the body against the introduction of too much acidity or too much alkalinity. Even with the proper amounts of buffers, acid or alkaline levels can become extreme. When the body ingests or produces too many of these acids or alkalis, it must excrete the excess. The urine is the perfect way for the body to remove any excess acids or alkaline substances that cannot be buffered. If the average urine pH is below 6.5 the body's buffering system is overwhelmed, a state of "auto-toxication" exists, and attention should be given to lowering acid levels.

Most people who suffer from unbalanced pH are acidic. This unbalanced pH condition forces the body to borrow minerals, including calcium, sodium, potassium and magnesium from vital organs and bones to buffer (neutralize) the acid and safely

remove it from the body. Because of this strain, the body can suffer severe and prolonged damage due to high acidity—a condition that may go undetected for years.

Mild acidosis can cause such problems as:

1) Cardiovascular damage, including the constriction of blood vessels and the reduction of oxygen. 2) Weight gain, obesity and diabetes. 3) Bladder and kidney conditions, including kidney stones. 4) Immune deficiency. 5) Acceleration of free radical damage, possibly contributing to cancerous mutations. 6) Hormone concerns. 7) Premature aging. 8) Osteoporosis; weak, brittle bones, hip fractures and bone spurs. 9) Joint pain, aching muscles and lactic acid buildup. 10) Low energy and chronic fatigue. 11) Slow digestion and elimination. 12) Yeast/fungal overgrowth.

1.4.6.2 Specific Gravity of Urine

Specific gravity, in the context of clinical pathology, is a urinalysis parameter commonly used in the evaluation of kidney function and can aid in the diagnosis of various renal diseases.

Renal Function:

The role of the kidneys in humans and other mammals is to aid in the clearance of various water-soluble molecules, including toxins, toxicants, and metabolic waste. The body excretes some of these waste molecules via urination, and the role of the kidney is to concentrate the urine, such that waste molecules can be excreted with minimal loss of water and nutrients. The concentration of the excreted molecules determines the urine's specific gravity. In adult humans, normal specific gravity values range from 1.010 to 1.020.

Specific Gravity and Disease:

Increased specific gravity (hypersthenuria, i.e. increased concentration of solutes in the urine) may be associated with dehydration, diarrhea, emesis, excessive sweating, glucosuria, renal artery stenosis, hepatorenal syndrome, decreased blood flow to the kidney (as a result of heart failure), and excess of antidiuretic

hormone caused by Syndrome of inappropriate antidiuretic hormone. A specific gravity greater than 1.035 is consistent with frank dehydration. In neonates, normal urine specific gravity is 1.003. A specific gravity of > 1.015 is considered hypovolemia (Wikipedia, 2014).

Decreased specific gravity (hyposthenuria, i.e. decreased concentration of solutes in urine) may be associated with renal failure, pyelonephritis, diabetes insipidus, acute tubular necrosis, interstitial nephritis, and excessive fluid intake (e.g., psychogenic polydipsia)

1.5 Drinking Water Analysis

The result of minerals dissolving from weathered rocks and soils are the main source of arsenic in the ground. Several types of cancer have been linked to arsenic in water. If somebody found with keratosis in palm or feet, or arsenic found in nail or hair, it is essential to find out the source of this arsenic. Then first priority is to analyze the drinking water, mainly ground water.

1.6 Biological Samples (Hair and Nails) Analysis

Arsenic is normally found in higher concentrations in keratin-rich tissues, such as human hair and nails than in other parts of the body (Ya´ n˜ ez, *et al*, 2005). The SH-groups of keratin may bind trivalent arsenic. Due to the high affinity of arsenic for keratin, arsenic concentrates in hair much higher than in other tissues or biological fluids (Hindmarsh, 2000). A normal concentration of arsenic in hair ranges from 0.08 to 0.25 mg g_1 in an unexposed population. In contrast, in chronically exposed populations, concentrations ranging from 1 to greater than 9 mg g_1 have been reported (Hindmarsh, 2002).

The level of arsenic in hair was found to be less than 1 mg/kg in more than 80% of 1000 persons examined in a study using neutron activation analysis (Smith, 1964). The average level was 0.81 mg/kg and the median 0.51 mg/kg. Liebscher & Smith (1968) found a log-normal distribution of arsenic concentrations in over 1200 hair samples from residents of the Glasgow area in Scotland. They performed neutron

activation analysis on the samples and found arsenic levels ranging from 0.02 to 8.17 mg/kg dry weight with a geometric mean of 0.46 mg/kg. Geometric mean arsenic concentrations in hair of about 0.3 mg/kg were reported by Boylen and Hardy (1967).

The value in hair and nails is not known with certainty. On review of the literature it can be assumed that arsenic concentration of greater than 1 mg/kg of dry hair and arsenic concentration of more than 1.5 mg/kg of nail may be considered as indicative of exposure to an unsafe dose of arsenic within the preceding 11 months (Caussy, 2006).

The World Health Organization asserts that a level of 0.01 mg/L (10ppb) poses a risk of 6 in 10000 chance of lifetime skin cancer risk and contends that this level of risk is acceptable (WHO, 2012).

1.7 Mushroom as a Medicine (*Pleurotus ostreatus*)

Although mushrooms are divided into three parts, one- edible, two- medicinal and, three-poisonous. But all the mushrooms have a disease curing ability, even cancer. Generally *Pleurotus ostreatus* is an edible mushroom, along with it is a medicinal one also. According to Nuhu Alam and others, *Pleurotus ostreatus* has a strongest chelating capacity (85.66%) of the acetone extract toward ferrous ions was investigated (Alam, *et al.*, 2010). In another research of Wu JY and others was revealed that, human colorectal adenocarcinoma and a human monocytic leukemia were decreased with the protein extracts of *Pleurotus ostreatus* (Wu, *et al*., 2011). So it is important to study with *Pleurotus ostreatus* in arsenic cases.

1.7.1 General Information of *P. ostreatus*

Pleurotus ostreatus, commonly known as oyster mushroom (Zhenuk mushroom in bangla), is a popular edible mushroom in Bangladesh. Oyster mushroom is cultivating all over Bangladesh for last 10-15 years and selling them to China and Thai food restaurants and also in local restaurants for making soup. People are purchasing them (powder and fresh) from different departmental stores and shops, like Agora, Shopno, Taja bazaar, Mina bazaar *etc*. Health conscious people are taking powdered mushroom for high cholesterol and heart diseases. It is recommended for

reducing cholesterol for containing alkaloids, statins and lovastatin (Gunde-Cimerman and Cimerman. 1995).

Name and Synonym

Both the Latin and common names refer to the shape of the fruiting body. The Latin *pleurotus* (sideways) refers to the sideways growth of the stem with respect to the cap, while the Latin *ostreatus* (and the English common name, oyster) refers to the shape of the cap which resembles the bivalve of the same name. Many also believe that the name is fitting due to a flavor resemblance to oysters (Wikipedia, 2016). Here also in Bangla, these are named 'Zhenuk Mushroom', (literally oyster mushroom), due to the shape of oyster. In Japanese, it is called 'Hiratake' that means 'Flat Mushroom'. In Chinese, they are called *píng gū* (literally "flat mushroom"). In Vietnam, the mushroom is known as *nấm sò* or *nấm bào ngư*. It is called *chippikkoon* in Malayalam (EOL, 2015). In Iran it is called "Sadafi" ("*sadaf*" meaning oyster in Persian) (Asef, 2012).

Classification of *Pleurotus ostreatus*

Classification of *Pleurotus ostreatus* are mentioned according to Wikipedia, free encyclopedia, (2016).

Domain: Eukarya
Kingdom: Fungi
Phylum: Basidiomycota
Class: Agricomycetes
Order: Agricales
Family: Pleurotaceae
Genus: Pleurotus
Species: *Pleurotus ostreatus*

Pleurotus ostreatus (roughly translating to 'beside-the-ear oyster shaped) predominantly grows on hardwoods throughout North America, Europe and Asia. It has a pale lilac-gray spore print and a soft fleshy fruiting body that ranges in color

from white to gray, brown or even blackish. There's some variability among the species due to the wide distribution and reproductive isolation between continents.

Description:

Michael Kuo mentioned the *Pleurotus ostreatus* mushroom (Kuo, 2005) as follows-

Ecology: Saprobic; growing in shelf-like clusters on dead logs and living trees (primarily hardwoods, but sometimes on conifers); causing a white rot; fall, winter, and early spring; common; widely distributed in North America.

Plate 1.3: Fungi (*Pleurotus ostreatus* mushroom) have it's cap, gills and a stem, (Photo by- Researcher, 29/09/2013)

Cap: 4-15 cm; convex, becoming flat or somewhat depressed; kidney-shaped to fan-shaped, or nearly circular if growing on the tops of logs; somewhat greasy when young and fresh; smooth; pale brown to dark brown; the margin inrolled when young, later wavy, never lined.

Gills: Running down the stem; close; whitish or with a gray tinge, sometimes yellowish in age; often filled with black beetles, in my collecting areas.

Stem: Usually rudimentary and lateral (or absent) when the mushroom is growing from the side of a log or tree. When it grows on the tops of logs or branches, or at an angle, however, it may develop a substantial and thick stem that is dry and slightly hairy near the base.

Flesh: Thick; white.

Odor and Taste: Odor distinctive but hard to describe (see above); taste mild.

Spore Print: Whitish to grayish or lilac. Be sure to check out George Barron's photo and essay on what can only be called the Mother of All Spore Prints, produced by an oyster mushroom.

Microscopic Features**:** Spores 8-10.5 x 3-3.5 µ; smooth; cylindrical to narrowly kidney-shaped. These are the measurements given by Petersen & Krisai-Greilhuber (1994) for an epitype collection of *Pleurotus ostreatus*, and they match measurements supported by various mating studies. Field guides quote a large range of measurements, conflating *Pleurotus ostreatus* with other members of the species complex.

Culinary uses

According to Wikipedia (2013), the oyster mushroom is frequently used in Japanese, Korean and Chinese cookery as a delicacy: it is frequently served on its own, in soups, stuffed, or in stir-fry recipes with soy sauce. Oyster mushrooms are sometimes made into a sauce, used in Asian cooking, which is similar to oyster sauce. The mushroom's taste has been described as a mild with a slight odor similar to anise. The oyster mushroom is best when picked young; as the mushroom ages, the flesh becomes tough and the flavor becomes acrid and unpleasant.

Mushrooms have been used as food supplement from times immemorial not only for their flavour, aroma and nutritive values but also for their medicinal properties as evident from ancient literature. In the present day world they are known

for culinary values due to their high-quality proteins, vitamins, fibres and many medicinal properties and accordingly they are called nutraceuticals. *Pleurotus* as health promoter and environmental restorer is gaining more importance as compared to other medicinal mushrooms resulting in an upsurge in their R and D activities during the past two decades. The chemical nature of the bioactive compounds present in this mushroom includes: polysaccharides, lipopolysaccharides, proteins, peptides, glycoproteins, nucleosides, triterpenoids, lectins, lipids and their derivatives (Patel, *et al.*, 2012).

1.7.2 Antioxidant Activities of *P. ostreatus*

Pleurotus ostreatus is a good source of dietary fiber and other valuable nutrients. This mushroom has some active ingredients, which have biological and medicinal important, such as- influencing and regulating immune system, decrease blood lipid density, lowered high blood pressure and pervert atherosclerosis, as well as hypoglycemic and antithrombotic activities (Alam, *et.al*., 2010).

Tissue damage in the vital organs of the body caused by free radicals due to some diseases, as atherosclerosis, diabetes, cirrhosis and cancer (Halliwell and Gutterige, 1984), can be repaired by antioxidants food supplements like mushroom. Except them, bodily systems are insufficient to protect them against oxidative damage (Simic, 1988).

1.7.3 Tyrosinase Inhibitory Effects of *P. ostreatus*

In molecular biology, Tyrosinase refers to an oxidase, which is the rate limiting enzyme for controlling the production of melanin (Wikipedia, 2013). Tyrosinase is also called polyphenol oxidase (Zawistowaki, *et.al.*, 1991), is a copper-containing mono-oxygenase present in a diverse range of organisms and is responsible for melanization in animals and enzymatic browning of fruit. (Robb, 1984). Due to its action on melanin synthesis, it is possible to reduce melanosis and leuco-melanosis in arsenicosis patients.

1.7.4 Ferrous Chelating Ability of *P. ostreatus*

In a laboratory study Nuhu Alam (Alam, *et al.*, 2010) reported that the chelating ability of the acetone, methanol and hot water extracts at five different concentrations (0.063, 0.125, 0.250, 0.500, and 1.000 mg/mL) from the *P. ostreatus* fruiting bodies toward ferrous ions was investigated. Butylated hydroxytoluene (BHT) and α-tocopherol (TOC) were used as ferrous ion standards. The chelating capacity of the extracts increased with increasing concentration. The strongest chelating effect (85.66%) was obtained with the acetone extract at 1.0 mg/mL. So, it is possible that the fruiting body of *P. ostreatus* can chelate arsenic from human body.

1.7.5 Cancer Protection by *P. ostreatus*

In a study of Wu JY and others was revealed that, cell viabilities were evaluated for a human colorectal adenocarcinoma cell line (SW480 cells) and a human monocytic leukemia cell line (THP-1 cells). Apoptotic mechanisms induced by the protein extracts of *Pleurotus ostreatus* (PO) and *Volvariella volvacea* (VV) were evaluated for SW480 cells. The viabilities of THP-1 and SW480 cells decreased in a concentration-dependent manner after 24 h of treatment with the protein extracts of *P. ostreatus*. Apoptosis analysis revealed that the percentage of SW480 cells in the $SubG_1$ phase (a marker of apoptosis) was increased upon PO and VV protein-extract treatments, indicating that oligonucleosomal DNA fragmentation existed concomitantly with cellular death. So, the protein extracts of these mushrooms could be considered (Wu, *et al*., 2011).

1.7.6 International Recommended Doses as a Medicine

More or less same medicinal doses are recommended in different literatures and scientific papers. Christopher hobbs describes in his paper, the doses for *Ganoderma lucidum* is 10 ml 3x/day (tincture), *Pleurotus ostreus* is 3-9 gm. (dried)/day, *Flammulina velutipes* is 8-9 gm. (dried)/day and *Lentinula ododes* is 6-16 gm. (dried)/day and 90 gm. (fresh) (Bo and Yun-sun, 1980).

He also suggested that for mild to moderate immune support 2 capsules morning and evening. For specific immune-suppressed conditions 2-3capsules a day,

when placed in '00' size capsules, average amount of powdered mushroom is about 400 mg.

A GMP registered nutritional company, Neutro Health International, registration no. 52993309L in Singapore manufacturing mushroom capsules. They recommended the doses for-

1. Normal health maintenance- 2 capsules / day,
2. Diabetes and skin disease patients- 2 capsules twice a day,
3. More aggressive cancer treatment and before and after chemo treatment- 3 to 4 capsules twice a day.

Each capsule contains: 550 mg 100% pure *Agaricus blazei murill* (ABM) and ABM extracts.

Best taken ABM extract is with a glass of warm water before meals.

1.8 Pertinent Issues and Rationale for the Research

Although there are so many usual treatments, food supplements, chelating therapy etc. but they are not so effective indeed. It seems that it is vitamin- A deficiency disease in poor. But the supplements become inappropriate. Patients are dying off in villages of Bangladesh, as well as different countries of the world. It is possible that there are some anomalies in between aetiology of the disease and treatments. So it is important to find out the actual cause and/or treatment, which we needed even now.

1.9 Research Gap

A number of gaps in the scientific knowledge exist; only a few of them are described here. **Firstly,** There is no remedy to prevent arsenic induced cancers. In these cases, medicinal research is very much important for treatment. But according to Das and Sengupta "no effective therapy is known till date" (Das and Sengupta, 2008) Considering no active medicine is identified till now, we are proceeding to give a new medicine to prevent cancer, as well as treatment of arsenicosis. **Secondly,** vital signs of arsenic affected people are not diagnosed previously. It is important to know their vital signs as well as respiration rate, cardiac (pulse) rate, blood pressure, general temperature of the body, BMI and urine for pH and specific gravity. These investigations are important for arsenicosis. **Thirdly,** fungus (Medicinal Mushroom)

is used as a medicine from years, but is not tried in case of arsenicosis. Some of these issues highlight the need for carefully conducted scientific studies that describe the fate of arsenicosis and give a better solution to arsenic poisoning in human health.

1.10 Aim and Objectives of the Study

The present study was undertaken to obtain a population based estimation of arsenicosis prevalence in Eruine, a high prevalence village in Bangladesh. Comparing the result of this study with the sentinel surveillance will throw some light on the true situation of Eruine village and modify the future control and curative short-course treatment for arsenicosis patients (CSTA) in Bangladesh, as well as all over the world.

General Objective of the research is to save the life of arsenic affected people from the consequences (lungs affection, liver diseases, respiratory diseases, skin ulcers and cancers *etc.*) of arsenocosis.

Specific Objectives:

Accordingly, the following specific objectives are aimed:

i. To assess the present status of arsenicosis and its treatment;
ii. To find out the arsenic load in ground water of affected area ;
iii. To find out the arsenic load in different parts (nail and/or hair) of the patients by using AAS (Atomic Absorption Spectrophotometry).
iv. To find out the efficacy of mushroom on arsenic affected people for prevention and treatment purposes.

Chapter Two
MATERIALS AND METHODS

2.1 General Statistics of the Field and Respondents

This population-based survey experiment is administered on a representative population sample of the village Eruine. The survey of information of households, age groups, marital status, education, occupation, death records and counseling are the main resource samples of the study for arsenicosis patients. As arsenicosis disease is the disease of poor, so this descriptive cross-sectional population-based survey is very much important for this study.

2.1.1 Description of the Study Area

The study is adopted for the sample design in a rural area of Bangladesh. Laksham upazila of Comilla district is consists of an area of 429.34 sq km. It is bounded by Comilla sadar and Barura upazila on the north, Chatkhil, Begumganj and Shenbagh upazilas on the south, Nangolcot and Choddogram upazilas on the east, Barura and Shahrasti upazilas on the west. Main rivers are Dakatia and Shoto Feni (Map-2.4).

2.1.2 Site Selection

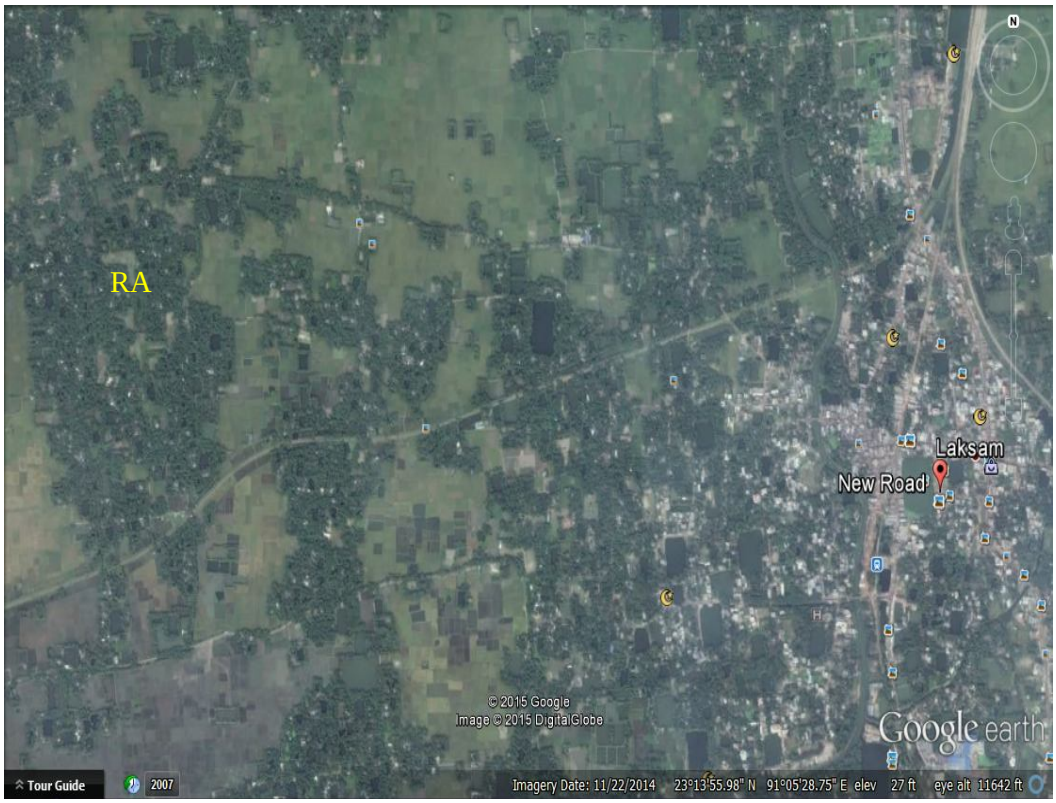
The main authorized government organization for arsenicosis disease in Bangladesh is Community Care Hospital, Mogbazar, Dhaka. As we know 59 districts out of 65 are arsenic contaminated in Bangladesh. The hospital authority selected the village Eruine for the study, because very recently they have visited the village. The

health situations of the villagers are very poor. Maximum people of the houses are arsenic affected. Even some people already died in the area. People are suffering severely with arsenic induced cancer and skin lesions. Drinking water situation is also too much deteriorated. Each and every tube wells are arsenic contaminated except one. So the prevalence of arsenicosis situation is posing a threat to the affected people and survivals of the village. For these circumstances we thought this is the perfect place for a good study.

The village Eruaine of Kandirpar union is bounded by Bakoi union on the north, Gobindopur and Moisatua union on the south, Mozaffargonj union on the west and Laksham pouroshava on the east (Map-2.4). According to the Community Care Hospital description all the villagers are arsenic affected. We have selected a part of them. The selected 184 arsenicosis patients (113 females and 71 males) are of 113 houses of 15 bari (residential area) of Eruain village (Map-2.5).

The satellite photograph showing (Map 2.5) the white and ash coloured boxes of pakka houses in Laksham bazaar, in between them string like roads in bazaar; on the north of bazaar there is a thick string divided into two way is rail lines, one to Chittagong in the east, another to Chandpur in the west. In the west side, there is a dense big green area is Eruain village. That is our research spot. This is 27 ft high from the sea level. Because of that no ponds are seen in the photograph, maximum are dried in winter. Some water found at the bottom in the rainy season.

Map 2.4: Laksham upazila with it's union porishads, the study area is mentioned by marked the sign of in the map; Source: Maps of Bangladesh (2011), Web: http://mapofbangladesh.blogspot.com/2011/11/laksam-upazila.html



Map 2.5: Satellite photograph showing the location of Research Area (RA) in Eruain village and Laksham Pouroshava. The longitude and latitude of the area is 23º13'55.98"N and 91º05'28.75"E. (Imagery Date: 22/11/2014)

2.1.3 Respondent Survey for Arsenicosis Patients

Respondent survey for the arsenicosis patients includes all personal particulars of them , such as their quantity, life style, age, occupation, education, marital status, death records before study, drinking water quality, food quality and nutritional status; and also noted other important information that the patients knowledge about arsenic and arsenicosis, safe drinking water, previous medications *etc*. These information will be collected and noted in black and white on questionnaire form by an oral interview of the patients. Questionnaire is in **Appendix-V**.

2.1.4 Study Design and Sampling Method

The present study is a descriptive cross-sectional population based study conducted in the village Eruain of Laksham upazilla of Comilla district in Bangladesh. Each and every personal of selected 113 houses of 15 Bari's (residential area) were the principal samples. Then the samples were divided into two parts: one- arsenic affected personals and, another- arsenic free people. Primarily they were separated by observing the presence of cutaneous symptoms of arsenicosis. Among the arsenic affected people, we have selected 150-200 fresh human samples (FHS) for the study.

Fresh human samples (FHS) were not taking any drugs and nutritional supplements from hospitals or any other NGO's. All the samples were divided into two parts: a) drinking arsenic water (DAW), and b) drinking fresh water (DFW). Again they were divided into two parts: 1) for medicinal group, and 2) for control group.

2.1.5 Statistical Analysis for the Population Based Survey

Statistical analysis was done using Epi-info (ver. 3.5.1, 2008) and SPSS 15.0 (SPSS, Inc., Chicago, IL. USA). Univariate and bivariate analysis of socio-demographic and other variables were performed. The pearson's chi-square test, the adjusted Mantel Haenszel's test were used to analyze the differences in the categorical data. A p-value <0.005 was considered significant.

2.1.6 Methods for Data Collection and Processing

2.1.6.1 Data Collection

A semi structured interview schedule was developed by researcher and filled by the data collectors on direct questioning by the researcher after obtaining informed consent. Information collected include socio-demographic profile, risk behavior profile and test results. Data collectors are the junior physicians, and other assistants are trained for this study **(Appendix-X)**.

A work plan was made for performing the whole work smoothly and in time at the beginning of the study. The work plan is mentioned in **Appendix- XI**.

This prospective explorative study included persons who observed cutaneous arsenicosis symptoms with their drinking water analysis found arsenic from August, 2013 – October, 2013. All those of any age who gave their consent are included for the study. Field work lasted up to October, 2014.

A standard proforma in the form of an interview schedule with close-ended questions was developed by the researcher and filled in a questionnaire form (**Appendix-V**) by direct questioning by the interviewers (researcher and data collectors). Subsequently the interview lasted for an hour for each person.

2.1.7.2 Data Processing

All questionnaires were completed in the field by data collectors with the help of two local assistants. The questionnaires were examined by the researcher and rechecked by the supervisor.

2.3 Vital Signs Analysis Methods

There are some medical instruments used by the physicians in the hospitals and in private practices. More or less all patients have a need to estimate these vital signs in the respect of every case.

2.3.1 Blood Pressure Estimation Method

A blood pressure (BP) reading appears in two numbers. The first and higher of the two is a measure of systolic blood pressure (SBP), or the pressure in the arteries when the heart beats and fills them with blood. The second number measures diastolic blood pressure (DBP), or the pressure in the arteries when the heart rests between beats.

Estimation of BP is essential to know about cardiac diseases and impurities in blood serum. Normal average of adult BP is about 120/80 mm Hg for systolic and

diastolic (Wikipedia, 2016). As for our study we have divided these SBP and DBP in 4 categories. These are low, normal, over and more over. The SBP and DBP of these categories are respectively >119 and >76, 120-130 and 77-87, 131-145 and 88-99, and, <146 and <100.

Measuring Utensil

1. Blood pressure Monitor (Digital)- Bioland Medical Technology ltd.
   S/N: 10A16619, Model No. 2003, Made in USA.

2.3.2 Pulse (Heart) Rate Estimation Method

Pulse rate is important to know about the heart's physical and functional condition. Normal pulse rate (PR) is 71-76 beats per minute. For the sake of study, we have taken three more abnormal pulse rates to and fro of this normal pulse rate. The categories are as respectively low, normal, over and more over; and the pulse rates are >70, 71-76, 77-90 and 91-120.

Measuring Utensil

1. Blood pressure monitor (Digital)- Bioland Medical Technology ltd.
   S/N: 10A16619, Model No. 2003, Made in USA.

2.3.3 Body Temperature Estimation Method

Bodily outer surface temperature (T) varies man to man, due to body constitution and climate. But internal body temperature (IBT) varies a little. Normal human body temperature (NHBT) in adults is 34.4 – 37.8 ℃ (94-100 °F). As Bangladesh is a climatic country, here 37.8 °C or 100 °F is counted as fever. In the study we have selected 36.5-37.5 °C (97.7-99.5 °F) as normal body temperature. Other very low temperature and very high temperatures are disease conditions. We are considering four categories of body temperatures for the study. These are lower, normal, over and more over. The under tongue temperatures for these categories are respectively 95.0 - 97.6 °F and 35.0 - 36.5 °C, 97.7 - 99.5 °F and 36.5 – 37.5 °C, 99.6 - 100.5 °F and 37.6 - 38.0 °C, and, 100.6 - 100.9 °F and 38.1 - 38.3 °C.

Hypothermia, hyperthermia and hyperpyrexia are severe conditions of the temperature, due to severe thermal condition of the nature or artificial. So these conditions are discarded from the research.

Measuring Utensil

1. Thermometer- BSMI thermometer (mercury scale), China.

2.3.4 Respiration Rate Estimation Method

Estimating respiratory rate (RR) is the introductory examination of the lung and heart. An abnormal RR predicts any disease condition in lungs or heart. Normal RR is 12-20 breaths per minute. In our study, we have chosen three other categories to determine an abnormality. The categories are low, normal, over and more over and the RR are respectively <12, 13-20, 21-30 and 31-40.

Measuring Utensil

1. Stethoscope, ALPK2 Double Head, type- FT 801, Made in Japan.
2. Measuring tape, Plastic made, showing inches and centi-meters.

2.3.5 BMI Measurement Method

2.3.5.1 Weight Count

Weight is measured with a digital weight scale (TANITA glass digital bathroom scale, HD-380, Japan), which measures the body weight in kg.

2.3.5.2 Height Count

Height is measured by height measuring scale in meter (LEND steel tape, JC-379W of China).

2.3.5.3 BMI Estimation

Body mass index, or BMI, is a calculation of an individual's height to weight ratio. A BMI less than 18.5 is considered underweight by the American Diabetic

association, while a BMI between 18.5 to 25 is considered normal weight (Paula, 2016).

$$\mathrm{BMI} = \frac{\mathrm{mass\ (kg)}}{(\mathrm{height(m)})^2}$$

Measurement Techniques-

1. Weight is measured for every patient.
2. Height is also measured for all.
3. BMI calculated individually and noted in tabulated sheet.

2.3.6 Sample Collection and Analytic Method of Urine

2.3.6.1 Urine pH

We have considered one normal and two unhealthy (acidic and basic) pH values for the study. The conditions and pH values are respectively acidic (4.6-6.8), normal (6.9-7.1) and basic (7.2-8.0).

Measuring Utensil-

1. Urine pH is measured with a digital pH meter, (EZODO, PH5011 of Taiwan).
2. Buffer solution.

2.3.6.2 Urine Specific Gravity

Specific gravity measures the kidney's ability to concentrate or dilute urine relation to plasma. Because urine is a solution of minerals, salts and compounds dissolved in water. The more concentrated urine has the higher specific gravity. Normal specific gravity of urine is 1.034

Measuring Utensil-

1. Urinometer, GERA, Specific gravity meter for urine.
2. Test tube, size- 8 inch. X 1 inch.
3. Wash bottle (plastic) with jet tube.

2.4 Methods for Water Samples Analysis

2.4.1 Water Sample Collection Method

Water samples were collected from tube wells used by the participants visiting the household of each family. Maximum tube wells were found shallow tube wells. Approximately 5 (five) liters of water were pulled out, then water samples was collected from each tube well. Water samples were collected in polyethylene bottles which was prewashed with aqueous nitric acid (1:1). Water samples were analyzed by the proposed and reported method immediately after the collection at the field.

2.4.2 Analytic Method for Arsenic in Water Samples

In this method, water samples (tube well water and filtered water) to be used in the test procedure.

WHO standard for arsenic in drinking water is >10 µg/l (10 ppb) and >50 µg/l (50 ppb) for Bangladesh.

Measuring Utensil-

4. Wagtech Digital Arsenitor with multipack, UK.
5. Graduated flat bottom flasks (Pyrex),
6. Stoppers, tubing, pipettes, graduating measuring cylinders,
7. Wash bottle (plastic) with jet tube and sample collection bottles.

Chemicals-

1. Distilled and de-ionized water,
2. Reagent 1 (sulphamic acid) and reagent 2 (borohydride tablet) were from Wegtech Instrument ltd. UK.

Measurement Techniques-

1. Taken 50 ml water sample in the reaction vessel of the Wagtech Digital Arsenator.
2. Prepared the bung of the Wagtech Digital Arsenator.

3. Added 1 sachet of reagent 1 to the sample in the reaction vessel and dissolved the acid sufficiently by swirling the reaction vessel.
4. Added 1tablet from reagent 2 to the contents of the reaction vessel and inserted the bung onto the mouth of the reaction vessel immediately.
5. Waited for 20 minutes.
6. Removed the bung and detached the black slide from the bung.
7. Matched the colour of black slide with the colour chart and noted the reading.
8. Inserted the black slide onto the arsenator (for bellow 100 µgm/l) and noted the reading.

2.5 Methods for Biological Samples

2.5.1 Biological Samples Collection and Preparation

In this study biological samples (hair and nail) are collected from arsenicosis patients and preserved them in 4°C in the laboratory of the Institute until the Laboratory works would be done.

Biological samples standard for hair and nail are >1mg/kg and 1.5mg/kg.

Measuring Utensil-

1. 50 ml Borosil glass flask,
2. Hot plate heater with magnetic stirrer,
3. Watch glass, pipettes, graduating measuring cylinders and beakers of 50 ml,
4. Wash bottle (plastic) with jet tube and sample collection plastic packets.
5. Millipore membrane (0.45µm) filtering apparatus,
6. Gloves, goggles and masks.

Chemicals-

1. Distilled and de-ionized water,
2. Concentrated nitric acid.

Measurement Techniques-

a) Hair Samples Preparation-

1. 0.05 to 0.1 gm of hair sample was taken in a 50 ml Borosil glass flask,
2. 5 ml concentrated nitric acid was added and dissolved the samples,

3. Brown coloured liquid sample then heated on a hot plate with a watch glass at the top of the beaker at temperature of 90-100°C for a few minutes.
4. Then heating discontinued and kept for overnight.
5. Next morning, sample was evaporated at about 100°C in an exhaust chamber.
6. Nitric acid was added if necessary till the colour of the solution turned into pale-yellow.
7. On reaching a final volume of about 1 ml, heating was discontinued.
8. The pale-yellow liquid was diluted with de-ionized water and filtered through a Millipore membrane (0.45µm) filtering apparatus, then adjusted to a fixed volume (5-10 ml) and putted in the beaker with serial numbers.

b) Nail Samples Preparation-

1. 0.05 to 0.1 gm of hair sample was taken in a 50 ml Borosil glass flask,
2. 5 ml concentrated nitric acid was added and dissolved the samples,
3. Yellow coloured liquid sample then heated on a hot plate with a watch glass at the top of the beaker at temperature of 90-100°C for a few minutes.
4. Then heating discontinued and kept for overnight.
5. Next morning, sample was evaporated at about 100°C in an exhaust chamber.
6. Heating was continued with time to time addition of a known volume of concentrated HNO_3 until the colour of the solution turned to almost colorless.
7. On reaching a final volume of about 1 ml, heating was discontinued.
8. The pale-yellow liquid was diluted with de-ionized water and filtered through a Millipore membrane (0.45µm) filtering apparatus, then adjusted to a fixed volume (5-10 ml) and putted in the beaker with serial numbers.

Then the samples were prepared for analyzing by AAS with Electro-thermal atomic absorption (flameless method),

2.5.2 Biological Samples Analysis by Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS)

Measuring Utensil-

1. Atomic Absorption Spectrophotometer with Electro-thermal atomic absorption (flameless method),
2. Glass ampoules and beakers of 50 ml.

Chemicals-

1. Distilled and de-ionized water,

Measurement Techniques

Electro-thermal atomic absorption

According to Shimadzu Corporation, the atomization method using a flame is still popularly used as the standard atomization method due to good reproducibility of measured values and easy use. However, a major defect of the flame method is the atomization rate out of all sample quantity used is about 1/10 and the remaining 9/10 is discharged to the drain. Therefore, it has been pointed out that atomization efficiency is low and analysis sensitivity is not so high.

Electro-thermal atomic absorption (flameless method), using a graphite tube to elevate sensitivity 10 to 200 times as much. This method was originated by Dr. L'vov of Russia.

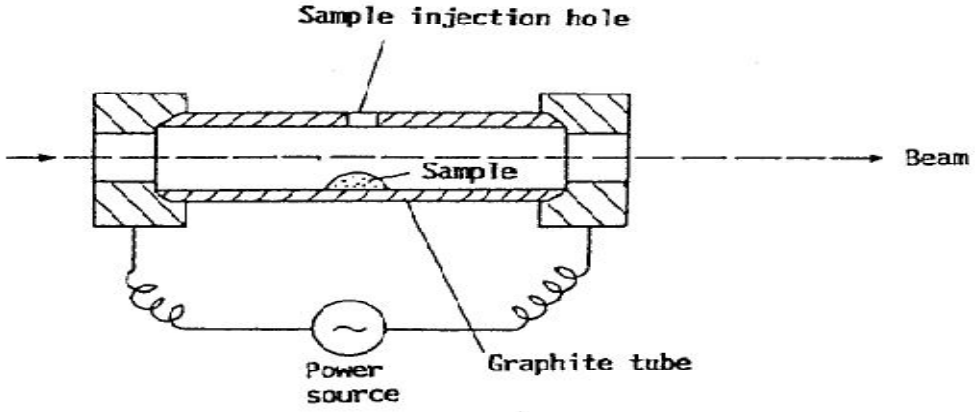


Plate 2.4: Flameless atomizer, (technical design)

In the electro-thermal atomic absorption method, the sample is injected in the formed graphite tube and an electric current of 300 ampere (maximum) is applied to the tube. The graphite is heated to a high temperature and the elements in the sample are atomized. If light from the light source is sent through the tube, light is absorbed when they are atomized. In an actual measurement, after the sample is injected in the tube, heating is done in three stages as shown in Fig. 1.6. That is, in the drying stage, the tube is heated to about 100°C and water in the sample evaporates completely. Then, in the ashing stage, the tube is heated to 400°C to 1000°C and organic matter and other coexistent matter dissolve and evaporate.

Lastly, in the atomizing stage, it is heated to 1400°C to 3000°C and metallic salts left in the tube are atomized. Heating is usually done by changing the temperature in steps shown by the solid line in Fig. 1.6 (step heating). Depending on the sample, when the decomposition temperature of coexistent matter is close to its atomization temperature, heating is done by changing temperature continuously (ramp mode heating). Heating must be done under the conditions (temperature, heating time, and temperature raising method), which suit the type of element and composition of the sample to be measured. If heating is started after the optimum conditions are set on the equipment in advance, the tube is automatically heated according to the set temperature program (Shimadzu Corporation).

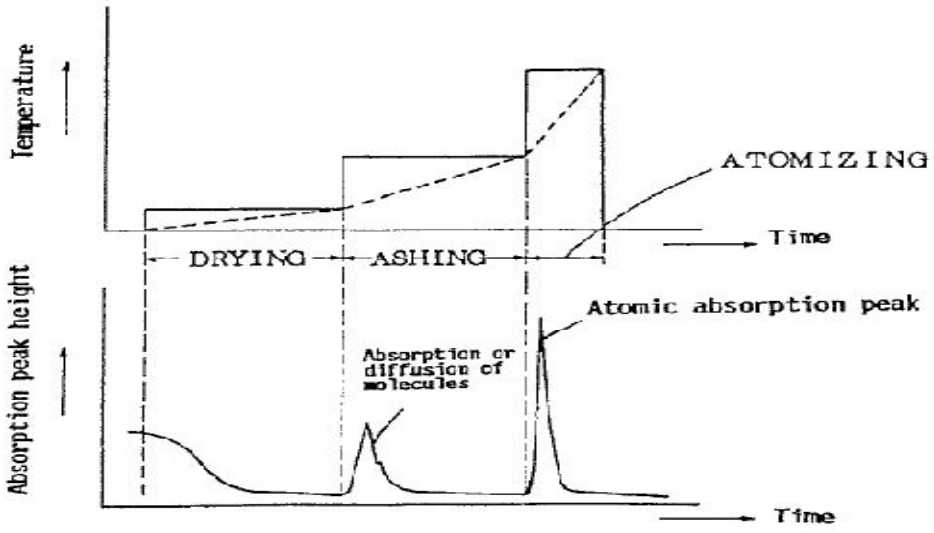


Plate 2.5: Heating program and absorption curve according to electro-thermal atomic absorption, (technical design)

Determination of Arsenic in Hair and Nail-

1. The following concentrations of working standards were prepared by serial dilution: 0; 4; 12; 16 and 20 µg $As.L^{-1}$
2. Each and every prepared biological sample was placed serially after five working standards.
3. Specially made ampoules for AAS are filled with serially arranged prepared standards and biological samples and fixed in the tray (of AAS).
4. Then, placed the tray on AAS.
5. When everything checked normal, then AAS started to analyze the samples.
6. Arsenic analysis results are shown in AAS monitor and preserved in computer; result data are- Sample ID, True value (ppb), Concentration (ppb), Absorbance, Position and % RSD.

2.6 Analytical Methods for Medicinal Source

2.6.1 Oyster mushroom (*Pleurotus ostreatus*) Identification Method

Oyster mushroom is easily identifiable due to its ear or oyster shape white cap. There are decurrent gills beneath the cap. Decurrent means that the gills are attached to and run directly down the short stem (Plate-2.6). They may not have a long stem. If they do it will often be stubby and off-center if the mushroom is growing on the side of a log. Usually they are edible, white and light gray in colour. There are some varieties of oyster mushrooms, these are -

Pleurotus citrinopileatus - The Golden Oyster, *Pleurotus eryngii* - King Oyster, *Pleurotus pulmonarius* - Phoenix Oyster, *Pleurotus tuberregium* - King Tuber and some others.

Plate 2.6: Gills of Oyster mushroom**,** runs directly to the stem

2.6.2 Medicinal Source (*Pleurotus ostreatus*) Collection and Preparation

Samples of mushrooms were collected from The National Mushroom Development and Extension Center, Sobhanbag, Savar, Dhaka-1340 and Center for Mass Education in Science (CMES), Dhanmondi, Dhaka-1209. Mushrooms were grown using the standard tray system under controlled conditions and were harvested at the optimum maturation stage with closed caps that were 2.0-2.5 inches in diameter.

Specialty mushrooms were harvested using standard mycology protocols and harvested on peak production days. Harvested mushroom crops were randomly sampled, cleaned, sliced, and stored at 0°C for 24 h. Samples were later freeze-dried, ground to a fine powder, and sieved through a 16 mesh screen. Mushroom powders were collected in sterile sample bags (Fisher Scientific, Pittsburgh, PA) and stored in the dark at room temperature.

Empty hard gelatin capsules, size # 0, quantity- 1.00 Lac, color- maroon opaque (cap and body) and next 1.00 Lac, color- maroon opaque (cap) and rich yellow opaque (body) was purchased from Global Capsules Ltd. Barisal, Bangladesh.

Empty capsules were filled with powdered mushroom (*Pleurotus ostreus*) and powdered fried wheat (Placebo) at Helal & Company Homoeo Laboratories Ltd. Khilgaon, Dhaka-1219, a manufacturing company of homoeopathic and biochemic medicines.

2.6.3 Selected Medicinal Doses for the Study

Finally we have selected the dose for our research is-

i) Capsule size- ‘0’
ii) Each capsule contains: 0.80 gm powered mushroom (*Pleurotus ostreatus*),
iii) Full dose: 1 capsule x twice / day for extremely affected patients,
iv) Half dose: 1 capsule x once / day for less affected patients, and
v) Placebo: 1 capsule x twice / day for random selected patients.

2.6.4 Study Design

This treatment criterion is named as "Curative Short-course Treatment for Arsenicosis (CSTA) patients. Whole research program was designed in 3 phases which took a total period of 18 months. First 6 months for physical examinations of the patients and laboratory analysis of drinking water, hair and nails. Then, the current study, second 6 months and third 6 months are for medicinal application. In between second and third phase, there is identification of intermediate improvement (III/Triple I). If III is positive, research will be run as per plan. Otherwise medicinal part should be change for better prospect.

All sample population {150-210 patients, (app.), because some patients may discontinue in between treatment period} are divided into 2 groups, the

a) Experimental groups, that is- Arsenicosis + Mushroom (AM) and
b) Control groups, that is- Arsenicosis + Wheat (AW).

Each groups again regrouped into 2 groups:

a) Experimental groups-
   I. Patients with arsenic free drinking water = 100 patients (app.)
      (50% Full dose and 50% half dose)
   II. Patients with drinking arsenic water = 50 patients (app.) (Full dose)
b) Control groups-
   I. Patients with arsenic free drinking water = 30 patients (app.) (Placebo)
   II. Patients with drinking arsenic water = 30 patients (app.) (Placebo)

Capsules of powdered *P. ostreatus* and wheat were ensured for each individual of AM and AW by the responsible field workers daily by home visits.

2.6.5 Statistical Analysis

The recorded data of the patients were analyzed by standard statistical methods using computer software, SPSS package programme. Data was presented as means ± SE. Comparisons between baseline characteristics of each group were made by using Student's Paired and Unpaired 't' test. Means were significantly difference at p value <0.05 at 95% confidence limit.

Chapter Three

RESULTS AND DISCUSSION

3.1 Population Based Survey Results

3.1.1 Households (HH) and Respondents Survey

Eruain village is a big village and very low land. Several ponds are situated in the land. Most of the year, ponds are dry, except rainy season. Water of the ponds is dirty, due to wash of the land and full of plankton, that is unhygienic for health to drink (Plate-3.7). A narrow river is running beside the union parishod of Laksham upazila with mostly black color of water due to waste of the town and a big bazaar (Plate-3.8). But the water becomes slight better in rainy season.

People of the village are underprivileged, poor, mostly uneducated and unhygienic; lives with agriculture, few are working in Laksham bazaar. They are always intended for government and NGO's help in health and other sectors.

Plate 3.7: Pond of Eruain village, unhygienic to drink

Plate 3.8: The Dakatia river is in rainy season, situated beside the town Laksham; only surface water source in the study area. Engineering student (in the photograph) is detailed to analyze surface water.

Eruain village of Laksham upazila is highly contaminated with arsenic in the ground. It is a big village, but we have selected only 113 houses of 15 Bari for our study. We have chosen 184 arsenicosis patients for the study, who are severely affected. But there are 571respondents in these houses and 243 arsenic affected people are found (Table-3.1).

The highest affected Bari was Jomodder bari (100%) and less affected bari is Mosjid bari (22.2%). Average percentage of arsenic affection in the selected houses was 42.6%. So the situation was very critical in Eruain village.

The whole area is arsenic contaminated in ground water. The people of this research area had no other alternative to drink. They collect rain water in the rainy season and drink it for a month after rain. Except rainy season, rest of the year they are engulfing arsenic contaminated water of the ground. So they are accumulating arsenic in their body every day in a year. As per arsenic situation in ground, the arsenic contamination percentage (42.6%) is increasing day by day.

Table-3.1
Affected People (%) in the study area

Sl.No	Bari	Houses	Total Population	Affected People (%)	Selected Patients
01	Master Bari	14	91	37 (40.6)	26
02	Kazi ,,	8	43	14 (32.6)	10
03	Bain ,,	12	60	29 (48.3)	20
04	Dewan Ali ,,	12	48	25 (52.1)	17
05	Mosjid ,,	3	18	4 (22.2)	4
06	Jomoddar ,,	1	6	6 (100.0)	6
07	Choukidar ,,	18	86	45 (52.3)	38
08	Mayeri Baper ,,	11	49	14 (28.6)	11
09	Aijjar Baper ,,	5	22	7 (31.8)	6
10	Hazi ,,	15	69	30 (43.5)	21
11	Miazi ,,	7	40	21 (52.5)	15
12	Dolla ,,	3	21	6 (28.5)	6
13	Hakim Ali ,,	1	4	1 (25.0)	1
14	Akbor Ali Master ,,	2	10	3 (30.0)	2
15	Thakur ,,	1	4	1 (25.0)	1
	Total =	**113**	**571**	**243 (42.6)**	**184**

(Source: field survey)

Socio-demographic characteristics such as age, sex, marital status, occupation, education are studied and given in tables- 3.2, 3.3, 3.4 and 3.5. A total of 184 (71 male and 113 female) respondents were interviewed for this census.

3.1.2 Arsenic Affected People by Different Age groups

This village is a semi-dense populated area, where the people are very busy in their works for sunrise to sunset. Different aged people are found everywhere. Children are playing and walking along the road, aged people are in the local tiny tea stall. Mainly middle age people are working in fields and shops in Laksham. The respondent of different age are classified in the table bellow-

In this socio-demographic study of a population, males are more than one third (38.6%) of total respondents. But the female are nearly double of them (61.4%). Maximum arsenic affected people belong to 20-29, 30-39 and 40-49 age groups in both male and female. Less affected people are in below 19 and over 70 age groups. In relation to the sociological factors the qualitative aspects of these two groups are somehow depends on nutritional deficiency. It is assumed that the anatomical

structure of the muscular tissue of the children below 19 are more elastic and cell pore of old people over 70 are loose than the adult, because of that arsenic affection is less in these two groups. Chi-squire test results of age groups were statistically not significant as p- 0.492.

Table-3.2

Distribution of respondent (%) by age groups

Age	Male		Female		Total		Chi-Squire	df	p-Value
	Count	Table %	Count	Table %	Count	Table %			
< 19	3	1.6%	13	7.1%	16	8.7%			
20-29	23	12.5%	20	10.9%	43	23.4%			
30-39	12	6.5%	23	12.5%	35	19.0%			
40-49	15	8.2%	33	17.9%	48	26.1%	3.41	4	0.492
50-59	7	3.8%	9	4.9%	16	8.7%			Not-significant
60-69	7	3.8%	10	5.4%	17	9.2%			
70 <	4	2.2%	5	2.7%	9	4.9%			
Total =	**71**	**38.6%**	**113**	**61.4%**	**184**	**100.0%**			

3.1.3 Marital status of Arsenicosis Patients

As they were less educated, they usually married in early age. In fact, their earning was insufficient, houses are wood and tin made with some floors were cemented. On the other hand the number of females were more than male. The marital status is given in the table below.

In the study population only $1/6^{th}$ were unmarried (16.8%), among them they could understand their husband easily. As per socio-demographic factors, the study reveals that the wives demands were very limited, sometimes zero. Even they are satisfied with cheap foods and two piece of cotton saree. For their good understandings, there were no separation or divorced found. Due to the death of husband, widows were found 4.9%. It is notable that the marital status was not quite significant between male and female ($p>0.05$).

Table-3.3

Distribution of respondent (%) by marital status

Marital Status	Male		Female		Total		Chi-Squire	Df	p-Value
	Count	Table %	Count	Table %	Count	Table %			
Single	17	9.2%	14	7.6%	31	16.8%	5.99	2	0.05 Not-quite significant
Married	54	29.3%	90	48.9%	144	78.3%			
Separated	0	-	0	-	0	-			
Divorced	0	-	0	-	0	-			
Widowed	-	-	9	4.9%	9	4.9%			
Total	**71**	**38.6%**	**113**	**61.4%**	**184**	**100%**			

3.1.4 Education of Arsenicosis Patients

Educational condition was not so good in the research area. Children and parents both were away from the academic qualification. There may be the one factor were timing. They couldn't think that one would spoil their 10 to 15 years in schools without earning money. The educational status was mentioned in the table below.

Table-3.4:

Distribution of respondent (%) by educational status

Education	Male		Female		Total		Chi-Squire	df	p-Value
	Count	Table %	Count	Table %	Count	Table %			
Illiterate	37	20.1%	67	36.4%	104	56.5%	10.9	3	0.0123 Significant
Primary School (1 to 5 Classes)	14	7.6%	20	10.9%	34	18.5%			
Secondary School (6 to 10 Classes)	15	8.2%	21	11.4%	36	19.6%			
College (HSC)	5	2.7%	5	2.7%	10	5.4%			
University	0	0.0%	0	0.0%	0	0.0%			
Total	**71**	**38.6%**	**113**	**61.4%**	**184**	**100%**			

There was an unbelievable result found in education. More than half of all population was illiterate (56.5%). The cause is that they were not much cautious about education. Only 18.5% spend their 5 years in primary, 19.6% in high school giving 10 years and few crossed the college life (5.4%). No university student found in the field (0.0%). Primary education is free with the books. As they had not to spend money for it; so they had grown interest in it. But notable is that the literacy rate was found significant (p>0.05).

Sociologically mass population education quality is not satisfactory. Although there is not found any direct relation between arsenicosis and education, but the educational level and income level runs parallel; and it is found that low income people are suffering with arsenicosis. So education has an indirect relation with arsenicosis.

3.1.5 Occupation Relation of Arsenicosis Patients

The people were very caring about working in field or doing job in shops. From the very beginning, children worked with their father in the crop field. Female worked in the house. As they were very needy, they thought that earning is the best profession even that could be in the crop field.

Table-3.5:

Distribution of respondent (%) by Occupation

Occupation	Male		Female		Total		Chi-Squire	Df	p-Value
	Count	Table %	Count	Table %	Count	Table %			
Agriculture/Unskilled workers and Drivers (Auto/Taxi/Rickshaw)	43	23.4%	-	-	43	23.4%	18.887	4	0.0008 Significant
Shop keeper/ Tailor/ Salesman/Electrician/ Carpenter	15	8.2%	-	-	15	8.2%			
Teacher/other Services	4	2.2%	-	-	4	2.2%			
Retired Farmer	6	3.3%	-	-	6	3.3%			
Housewife	-	-	98	53.3%	98	53.3%			
Student	3	1.6%	15	8.2%	18	9.8%			
Total	**71**	**38.6%**	**113**	**61.4%**	**184**	**100%**			

Physical labor based jobs were the main occupation in the study field. Unskilled or half skilled males were doing in agriculture field and taxi driving (23.4%). But the maximum occupied people were housewives (53.3%). They were doing nothing, but household deals. Third position was for students (9.8%). Then came the time for shopkeepers, tailors, salesmen, electricians and carpenters (8.2%) and very small percentage was for the teachers and service holders (2.2%). Another unoccupied class was for retired farmers (3.3%). They were also doing nothing, but gossiping in tea stall. Chi-squire test result was statistically significant of the respondents as p-0.0008.

Occupation has a great value in relation with arsenicosis. Still it is assumed that protein deficiency is one of the causes of arsenicosis. Where income is low, protein deficiency usually occurs. Here maximum male workers are unskilled or half skilled and their income is very insufficient for their families. So the socio-economic factor occupation is very much important for the study of arsenicosis.

3.1.6 Study of Death Records in the Field

Several NGO's arsenic awareness programs are done in the village. Eight people died by arsenic induced cancer in 113 houses of the research area (Table- 3.6). According to table-3.1, 42.6% people are arsenic affected. They are assumed as psychological patients. If they find keratosis on their hands and feet, and feels chest pain; they think that 'they will die soon, because the disease became hereditary in them'. If we look to the history for last 10 years in the field, people are living with anxiety all the time. Basically who are suffering from lung problems, they are going to Dhaka for treatment for the fear of cancer of lung and death. Some people have already shifted to Dhaka, Chittagong, Comilla and Sylhet for the fear of death.

Within 8 (eight) death 3 (three) are male and 5 (five) are female; the ratio is 3:5 for male and female. But I think, it may be vice versa or equal. Because it depends on the consumption arsenic in the body is the main. Another important thing is that the males who are working in town (Laksham), they are less affecting than the females, who are drinking always arsenic contaminated drinking water in the village. In the initial time, people went to the hospital with lung affection, as- cough, pain etc. Hospital diagnose as lung disease, because they have no idea about arsenic problems

are in this village. Later on they send the patients to Dhaka cancer hospital, they diagnosed it as cancer. Causing death is depending on the consumption rate of arsenic, as well as their age and the energy in the body. Average population base death percentage is 13.9%; that is very high for locality area. But after four years of research, the arsenic induced cancer death become within 0.0%.

Table- 3.6:

Death record of the people in the study area

Name of Bari	Respondent Name	Age	Cause of Death	Total Respondent of Bari	Percentage of Death
Hazi ,,	1. Wage Ali	75	Lung Cancer	74	6.75
	2. Adjan Bibi	60	Lung disease		
	3. Rajjaben Nessa	75	,,		
	4. Hurmatun Nessa	65	,,		
	5. Ali Azam	90	,,		
Jomoddar,,	1. Asmot Ali	65	,,	8	25
	2. Ankuren Nessa	60	Lung Cancer		
Mojumder,,	1. Ferdausi Begum	35	,,	10	10

3.1.7 Counseling

Counseling had a great value for arsenicosis patients in this research area. There were three important factors for counseling here.

First- the patients were facing the disease, but no recovery.

Second- hospital had no sufficient treatments for them.

Third- patients were dying in front of them, but they are hopeless.

Plate 3.9: Single subject counseling for arsenicosis treatment. Here the physician and researcher M. Jahangir is explaining the fate of arsenicosis to a patient in Mojumder Bari, Name- Amena Begum (60), patient ID- 0405-171. Date- 12/09/2011.

Plate- 3.10: Multiple subject counseling for arsenicosis patients of Bain Bari. Name of the patients are (from the left) Jobeda (45), Kursia begum (48), Atorun Nessa (55), Lal Moti Nessa (45) and back, Hafeja (30).

Figure- 3.11: Multiple mixed subject counseling for arsenicosis patients of Bain Bari, patients are- Lal Moti Nessa (45), Rahima Begum (25), Ambor Ali (62) and Rojjober Nessa (30).

After few months of the research started, some patients of Bain Bari declared that they will withdraw their name form the program and they are not interested to take medicine. I (researcher) myself and my assistants attended there and heard them. They have said that they had seen many NGO's to give them medicines, but they can't change their disease. Patients had died in different houses. So leave them on their way. We assured them, "we are not NGO. We are university team. If we get result, then it will be supplied by the NGO's. And you will be the pioneer to get rid from this disease". Then they again agreed to take medicine (Plate-3.9, 3.10 and 3.11).

3.2 Results of Vital Signs Analysis

3.2.1 Evaluation of Blood Pressure

Blood pressure is the indicator of the functional and morphological changes in blood vessels and heart. We had taken the systolic and diastolic blood pressure separately to evaluate them individually. Although somewhere they got meanings in counting together for individual patients; as a big difference between systolic and diastolic in a patient means valvular disease.

The tables indicate the four groups (low, normal, over and more over) in blood pressure according to its intensity and age groups were divided into seven parts.

Table- 3.7:
Systolic blood pressure (mmHg) of different age groups of the patients, before Treatment.

Sl. No.	Different age groups	Number of Patients in Different Systolic Blood Pressure Groups (mmHg)				Total
		Low >119	Normal 120-130	Over 131-145	More over <146	
1	15-19	6	5	6	0	17
2	20-29	**21**	11	5	1	38
3	30-39	**18**	7	6	1	32
4	40-49	12	**18**	10	2	42
5	50-59	7	4	6	0	17
6	60-69	6	2	**11**	**11**	30
7	70 <	1	0	0	**7**	8
	Total	**71(38.6%)**	**47(25.5%)**	**44(23.9%)**	**22(11.9%)**	**184(100%)**

Systolic Blood Pressure

In differential study of the table-3.8, among 184 patients 71(38.6%) were in low blood pressure group, was the biggest patients group. The over blood pressure group (23.9%), it was considered as high blood pressure group was nearer to normal group (25.5%). Systolic blood pressure (SBP) is more or less same in all SBP groups of (15-19) ages, except more over pressure. There are 38 patients in (20-29) age group; in them maximum patients are 21 (55%), in low group. Similarly, in (30-39) age group, 18 (56%) within 32 are maximum, in low group. In (40-49) age group, normal pressure counted in 18 (42.8%), within 42 patients. In (50-59) age group, patients are distributed more or less equally in all pressure groups. But in (60-69) and (70<) age groups, 11 (36.6%) and 11 (36.6%) patients within 30; and 7 (87.5%) within 8, in over and more over pressure groups. In a total, maximum patients are suffering in low pressure or hypotension, 71 (38.6%) within 184 patients.

After one year of medicinal treatment, the Blood pressure (BP) data were taken again to know the changes in BP at 03/09/2014 to 11/09/2014 from the field.

Table- 3.8:

Systolic blood pressure (mmHg) of different age groups of the patients, after treatment.

Sl. No.	Different age groups	Number of Patients in Different Systolic Blood Pressure Groups (mmHg)				Total
		Low >119	Normal 120-130	Over 131-145	More over <146	
1	15-19	**13**	0	4	0	17
2	20-29	**23**	10	5	0	38
3	30-39	**20**	5	5	2	32
4	40-49	**25**	8	4	5	42
5	50-59	**12**	4	1	0	17
6	60-69	6	7	7	**10**	30
7	70 <	0	2	6	0	8
	Total	**99(53.8%)**	**36(19.6%)**	**32(17.4%)**	**17(9.2%)**	**184(100%)**

The scenario of SBP became to maximum patients in low pressure group (53.8%), then the term came of normal (19.6%) and over (17.4%) groups; and lastly more over (9.2%) group. And according to the age groups, patients all about nearer in all age groups, as- (15-19)(20-29)(30-39)(40-49)(50-59), except (60-69) and (70<) groups in low pressure group. Among them, middle aged groups had the maximum low pressure suffering patients. That may be for the physical weakness of the arsenicosis patients (general phenomenon). But the heart disease indicated in over and more over groups, maximum people in (60-69) and (70<) old age groups in both table-3.7 and table-3.8.

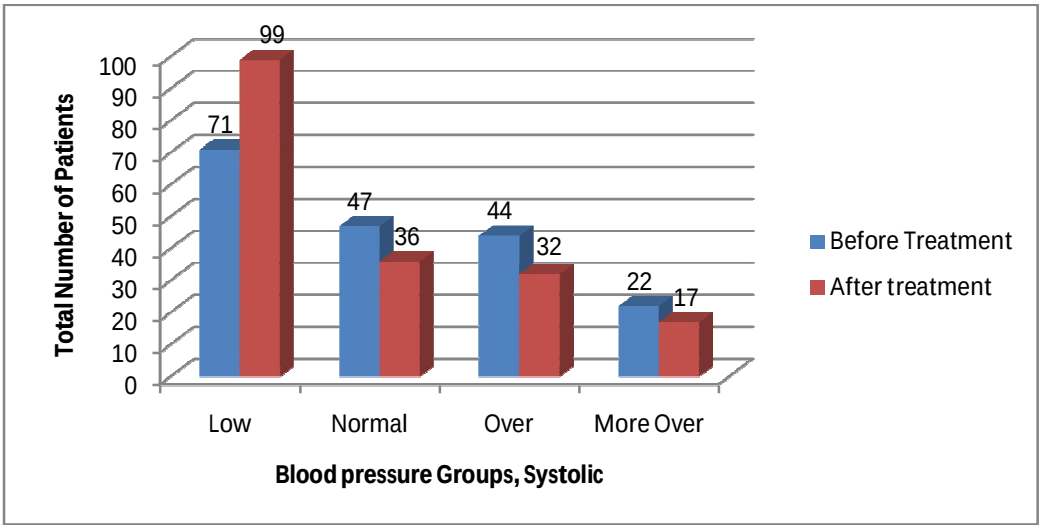


Figure- 3.2: Changes in systolic blood pressure

According to figure-3.2, the after treatment scenario was greatly changed in below normal and after normal pressure groups. The low pressure grouped patients were increased after treatment. But the other BP groups decreased over all. So the heart disease risk was decreased in old age people.

Diastolic Blood Pressure

Like SBP, there is a similarity in low blood pressure in diastolic blood pressure (DBP). Here maximum age groups are suffering with low blood pressure, except 70< age group.

Table- 3.9:

Diastolic blood pressure (mmHg) of different age groups of the patients, before treatment.

Sl. No.	Different age groups	Number of Patients in Different Diastolic Blood Pressure Groups (mmHg)				Total
		Low >76	Normal 77-87	Over 88-99	More over <100	
1	15-19	**10**	6	1	0	17
2	20-29	**23**	14	1	0	38
3	30-39	**19**	11	2	0	32
4	40-49	**24**	10	5	3	42
5	50-59	**11**	6	0	0	17
6	60-69	**12**	8	6	4	30
7	70 <	1	**5**	1	1	8
	Total	**100(54.3%)**	**60(32.6%)**	**16(8.7%)**	**8(4.3%)**	**184(100%)**

In differential study of before treatment of diastolic blood pressure (DBP), the maximum patients (54.3%) were in low blood pressure in all age groups (15-19), (20-29), (30-39), (40-49), (50-59) and (60-69), except age (70<) group; and 32.6% patients were counted in normal DBP, where the remarkable age groups were (20-29), (30-39) and (40-40), the middle aged groups. There were very few patients (8.7% and 4.3%) with high blood pressure (HBP/HTN) and they were mainly in (60-69) age group (Table- 3.9).

Table- 3.10:

Diastolic blood pressure (mmHg) of different age groups of the patients, after treatment.

Sl. No.	Different age groups	Number of Patients in Different Diastolic Blood Pressure Groups (mmHg)				Total
		Low >76	Normal 77-87	Over 88-99	More over <100	
1	15-19	**13**	4	0	0	17
2	20-29	**23**	12	3	0	38
3	30-39	**17**	**14**	0	1	32
4	40-49	**24**	11	6	1	42
5	50-59	**12**	5	0	0	17
6	60-69	7	**13**	10	0	30
7	70 <	2	**6**	0	0	8
	Total	**98(53.3%)**	**65(35.4%)**	**19(10.3%)**	**2(1.0%)**	**184(100%)**

Comprising the scenario of after treatment results of DBP of the tables- (3.9) and (3.10) were found a general improvement in every heads. The low pressure patients were reduced to 53.3% from 54.3% (before treatment). In the HBP groups, there two differences were found, in over group- the patients were increased (10.3% from 8.7%) due to the patients came from more over group. So the differences in BP were over increased and more over decreased (1.0% from 4.3%).

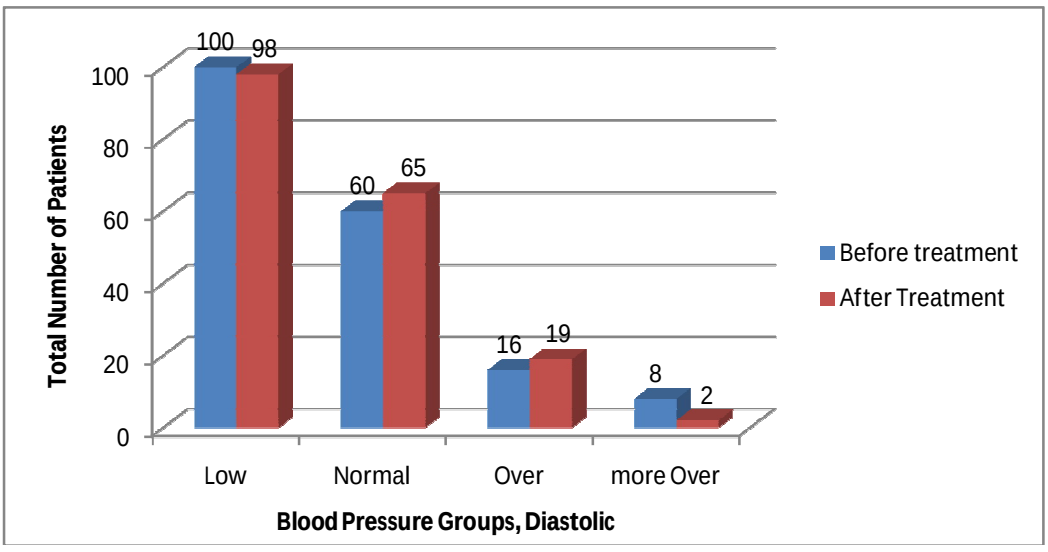


Figure-3.3: Changes in diastolic blood pressure

A dramatic change found in the diastolic blood pressure, maximum patients were demonstrating low than in normal ranges. Two important facts are supporting this finding. First, low blood pressure or hypotension was the sign of physical weakness in the arsenicosis patients. Bar chart analysis reveals that the HBP/HTN groups were reduced after treatment, that the patients were more or less safe from the risk of heart disease due to the treatment of the patients (Figure- 3.3).

Differential Diagnosis for Hypotension

According to the clinical study of arsenicosis patients, they may have postural hypotension (PO), which is a type of low blood pressure. It can happen for various reasons, such as being overly fatigued, always lack of food and dehydration, medication, psychological factors, and acute triggers, such as infection and allergy (Beckerman, 2015). All of these signs and symptoms were present in arsenicosis patient. Usually they were very weak, but they had to do laborious work and fatigue for their poverty, protein deficiency was common in them; dehydration, due to arsenic phobia to drink water; and could also be influenced by arsenic infection in capillaries and tissues, recommending to postural hypotension. So, arsenicosis is one of the causes of PO.

On a keen investigation at blood vessels and capillaries- rupture, necrosis and thrombosis can occur due to hyperkeratosis in arsenicosis patients. PO is considered a failure of the cardiovascular system or nervous system (Beckerman, 2015). For this reason, cardiovascular, as well as, nervous failure and necrosis is possible in patients of arsenicosis, due to accumulation of arsenic tri-oxide in capillaries.

There is a dramatic co-incise of blood pressure data of arsenicosis patients with orthostatic hypotension (OH). Analysis in arsenicosis patients detect that 71 patients out of 184 (38.6%) are counted bellow 119 mmHg in systolic blood pressure; (Table- 3.7) and 100 patients out of 184 (54.3%) are below 76 mmHg in diastolic blood pressure (Table- 3.9). That is 119/76 mmHg in an average patient; and an increase heart rate is 67.4% (42.0+25.4) (Table- 3.11). This is nearer to orthostatic hypotension. A OH is a drop in 20 mmHg of systolic pressure (and a 10 mmHg drop in diastolic pressure in some facilities) and a 20 beats per minute increase in heart rate (Wikipedia, 2016). The p-values of the patents after treatment of SBP, DBP and PR/HR were found extremely significant statistically.

Table- 3.11:

Characteristics of arsenicosis cases and postural hypertension (control)

Parameters After treatment	**Patients (N=184)**			**Controls Values**	***p*-value**
	N	**%**	**Mean value**		
Systolic pressure				**(120-130)**	
Low	99	53.8	105	125	
Normal	36	19.6	125	125	0.0001
Over	32	17.4	138	125	
More over	17	9.2	170	125	
Diastolic pressure				**(77-87)**	
Low	98	53.3	68	82	
Normal	65	35.4	82	82	0.0001
Over	19	10.3	94	82	
More over	2	1.0	110	82	
Heart rate				**(71-76)**	
Low	20	10.0	65	74	
Normal	40	22.7	74	74	0.0001
Over	91	49.3	84	74	
More over	33	18.0	105		

3.2.2 Evaluation of Pulse (Heart) Rate

Pulse or heart rate counting is essential for arsenicosis patients. Pulse rate can show the difficulties in heart, arteries and veins. We had selected 184 patients for this analysis.

As in Table- 3.12, we had found maximum patients were suffering from high pulse rate, the percentage is 67.4% (42.0+25.4). But very few were in normal pulse rate (17.3%). Only 15.3% patients were in low pulse rate group.

Table-3.12:
Pulse (Heart) rate (/min.) of different age groups of the patients (%), before treatment.

Sl. No.	Different age groups of the patients	Number of Patients in Different Pulse Rate				Total Patients (%)
		Low (%)	Normal (%)	Over (%)	More over (%)	
		>70/min.	71-76 /min.	77-90 /min.	91-120 /min.	
1	>19	3	3	5	6	17
2	20-29	8	6	16	8	38
3	30-39	5	7	12	8	32
4	40-49	5	10	17	10	42
5	50-59	3	6	4	4	17
6	60-69	5	3	16	6	30
7	70<	0	0	6	2	8
	Total	**29 (15.3)**	**35 (17.3)**	**76 (42.0)**	**44 (25.4)**	**184 (100)**

There may be a link between high pulse rate and high blood pressure in account of heart disorders. In case of our study, it can be possible that some of them were suffering from heart disorders from metallic arsenic in heart muscle or due to regular fatigue, losing extra weight, anxiety and stress which are common in arsenicosis patients; and high pulse rate develops tachycardia (increase pulse rate), i.e.- the increased heart rate leads to increased work and oxygen demand in the heart, which can lead to rate related ischemia (Wikipedia, 2016). In differential diagnosis, patients of (20-29), (30-39), and (40-49) age groups were more suffering with tachycardia. It is said that an increase in sympathetic nervous system stimulation causes the heart rate to increase and this is due to physical or psychological stress (Barker, 1999). The stress on arsenicosis patients of Eruine village as well as Bangladesh depends on many causes. Two causes are important. One- poverty and another- fear of arsenicosis disease.

After one year of medicinal treatment (fruiting body of mushroom, *Pleurotus ostreatus*), the changes of pulse rate were taken from all human samples and noted in data sheet. The analysis of pulse rates was mentioned in table below.

Table-3.13:

Pulse (Heart) rate of different age groups of the patients (%), after treatment.

Sl. No.	Different age groupsof the patients	Number of Patients in Different Pulse Rate				Total Patients (%)
		Low (%)	Normal (%)	Over (%)	More over (%)	
		>70/min.	71-76 /min.	77-90 /min.	91-120 /min.	
1	>19	0	0	13	4	17
2	20-29	3	10	20	5	38
3	30-39	3	5	21	3	32
4	40-49	7	9	20	6	42
5	50-59	3	4	4	6	17
6	60-69	4	7	10	9	30
7	70<	0	5	3	0	8
	Total	**20 (10.0)**	**40 (22.7)**	**91 (49.3)**	**33 (18.0)**	**184 (100)**

Comprising with the table-3.12 and 3.13, the pulse rate of more over group was reduced tremendously (25.4% to 18.0%), it is good improvement of the patients. But the over pulse rate group was increased (49.3% from 42.0%), may due to the patients came from more over group. And the low pulse group was decreased (15.3% to 10.0%) and developed normal group 22.7% from 17.3%. That is very impressive result after medicinal treatment.

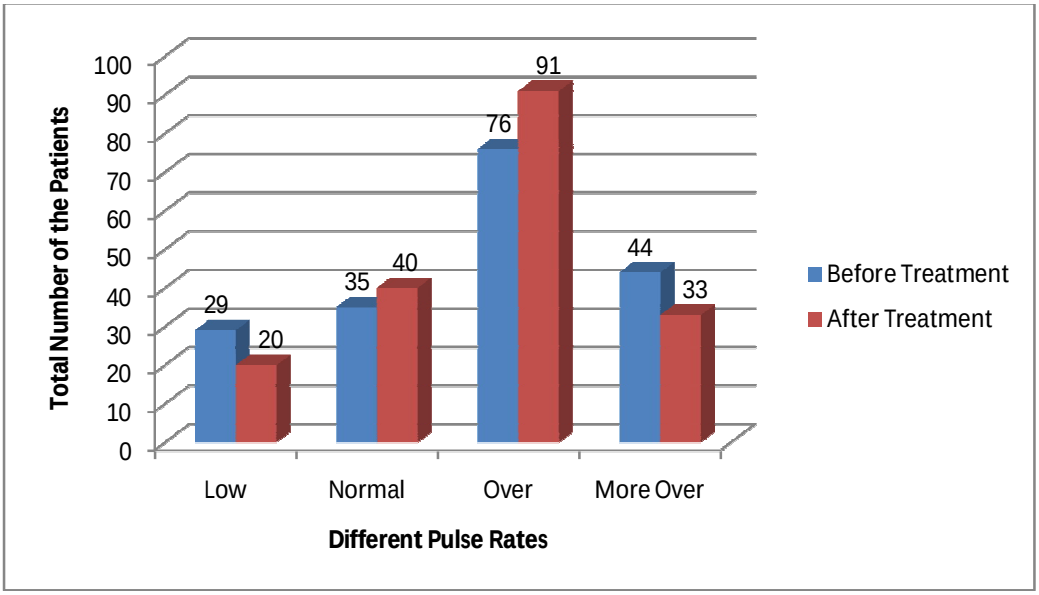


Figure-3.4: Different of Pulse Rates of before and after treatment

According to figure-3.4, the after treatment scenario had accelerated result found in normal and over groups. On the other hand, the number of patients were also increased in over group. The low and more over group patients were decreased after treatment. Over all different pulse rate groups from before and after treatment were showing better. But the heart disease risks were present in over and more over groups.

Most instances of increased pulse (heart) rate are remedied with healthy diet and regular exercise. Losing extra weight and quitting smoking always help to increase health and lower the risk of heart disease, anxiety and stress[p]. But when one has had over weight, rich diet, smoking habit, anxiety and stress, then pulse rate are rises. As we know arsenicosis is the disease for poor, they can't have a healthy diet. Protein deficiency is common in them. However, they have anxiety and stress always, due to their economic crises, which can cause high pulse rate in them.

3.2.3 Evaluation of Bodily Temperature

As there is no variation of temperature in age groups, so we have taken all the patients in a group. Only different scale of temperature is taken for the study. The temperatures of the patients are noted in black and white before and after treatment.

Table- 3.14:

Body temperature variation in all patients (%), before treatment

Sl. No.	Number of Patients in Different Bodily Temperature (° F)				Total Patients
	Low	Normal	Over	More over	
	>97.9° F	98-99° F	99.1°-100° F	100.1° F<	
1	125	55	4	0	184
Total	125(67.9%)	55(29.9%)	4(2.2%)	0	184(100%)

Table- 3.14 mentioned that the maximum patients (67.9%) were suffering with hypothermia before treatment. Hypothermia usually occurs from exposure to low body temperature. There are many causes of hypothermia. But hypothermia in arsenicosis patients is different. Arsenicosis patients have some other decreased rate of vital signs, i.e.- blood pressure, respiratory rate, except pulse rate. Here pulse rate is

increased. But as a whole, these results of vital sign break down the physiological system in the patient (Wikipedia, 2016). This condition is known as severe type of hypothermia. Although some patients were maintaining normal temperature (29.9%) and a negligible quantity were found in over group (2.2%).

Table- 3.15:

Body temperature variation in all patients (%), after treatment.

Sl. No.	Number of Patients in Different Bodily Temperature				Total Patients
	Low	Normal	over	More over	
	>97.9° F	98-99° F	99.1°-100° F	100.1° F<	
1	36	137	6	5	184
Total	36(19.9%)	137(74.4%)	6(3.3%)	5(2.7%)	184(100%)

Significant changes found in case of normal body temperature after one year of treatment, according to table-3.14 and 3.15. The percentages in normal value of before and after treatment were 29.9% to 74.4% and also the low temperature improvement found from 67.9% to 19.6%. It was a great change by the treatment of mushroom. The over and more over values were very less in number, but the meaningless values found in more over group.

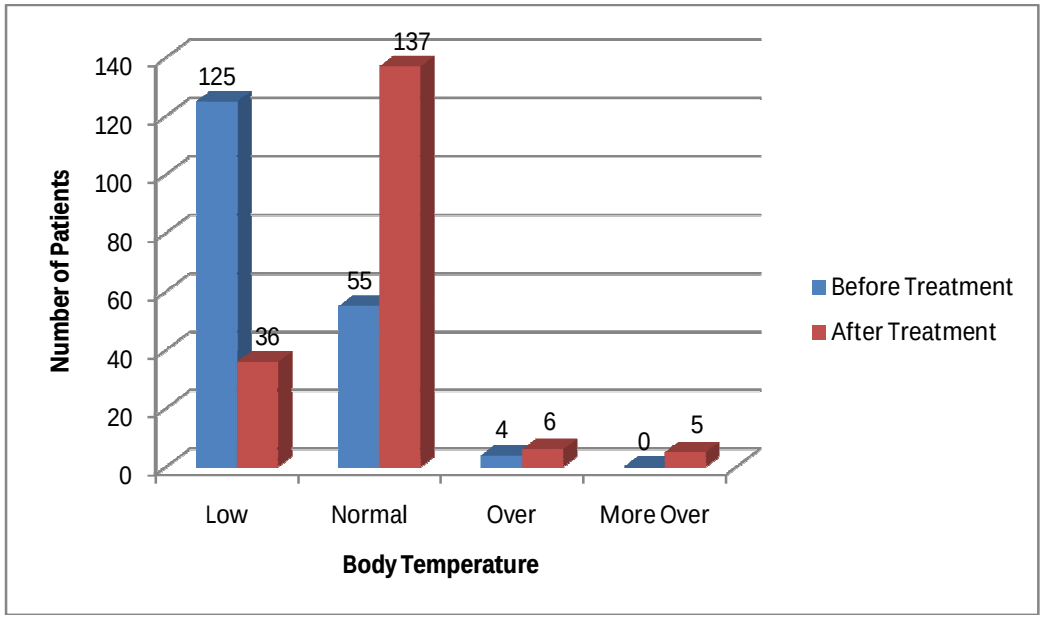


Figure-3.5: General body Temperature (before and after) of the patients

Figure-3.5 shows that the bar chart of low temperature of before treatment was reduced in many fold at after treatment (125 to 36) and simultaneously an opposite scenario found in normal temperature. The value of normal temperature was increased after treatment (55 to 137). Very low bars from few patients were found in over and more over groups of temperature.

Normal body temperature of the body is 98.4 °F and heat primarily generated in muscle tissue, including the heart and liver, while it is lost through the skin (90%) and lung (10%) (Hanania and Zimmerman, 1999). But in an arsenicosis patient muscle tissue, lungs, kidney, heart and liver are affected by metallic arsenic. It is possible that heat generation is hampering in the muscle tissues and other organs, due to arsenic effect. Another thing is sepsis. Sepsis could be the cause for low body temperature and sweating. Sepsis affects all the major organs in the body including the central nervous system, and in some cases, pushes the body into shock (Contente, 2015).

Many changes to physiology occur as body temperature decreases. These occur in the cardiovascular system leading to the Osborn J wave and other dysrhythmias, decreased CNS electrical activity, cold dieresis and non-cardiogenic pulmonary edema (Marx, 2010). So arsenicosis patients may have the chance to suffer with these diseases.

3.2.4 Evaluation of Respiration Rate

Respiration is the key to lungs well-being. Counting respiration rate (RR) one can estimate the difficulties in lungs. We have taken four types of respiration rates (RR) from different age groups. The rates are given bellow table.

Among the systems used to activate medical emergency response teams, such as outreach and medical emergency teams, the definition of an “abnormal” respiratory rate for adults varies from over 14 to over 36 breaths/minute (Subbe, *et al*., 2003). Recent evidence suggests that an adult with a respiratory rate of over 20 breaths/minute is probably unwell, and an adult with a respiratory rate of over 24 breaths/minute is likely to be critically ill (Cretikos, *et al*., 2007 and Gold hill, and McNarry. 2004).

Table- 3.16:

Different respiration rate of different age groups of the patients (%), before treatment.

Sl. No.	Different age groups of the patients	Number of Patients in Different Respiration Rate				Total
		Low	Normal	Over	More over	
		>12/min	12-20 /min	20-30 /min	30-40 /min	
1	>19	0	4	**13**	0	17
2	20-29	0	2	**34**	2	38
3	30-39	0	2	**29**	1	32
4	40-49	0	2	**39**	1	42
5	50-59	0	0	**17**	0	17
6	60-69	0	0	**30**	0	30
7	70<	0	0	**8**	0	8
	Total	**0**	**10(4.0%)**	**170(93.3%)**	**4 (2.7%)**	**184 (100%)**

So we have selected the normal respiration rate is 12-20/min. After counting the RR of 184 patients, we found that there were none in below normal range, few in normal (4.0%) and more over (2.7%) rate. But over normal group rates (93.3%) were accelerated in every age group.

According to Fieselmann and colleagues (1993) report, a respiratory rate higher than 27 breaths/minute was the most important predictor of cardiac arrest (Fieselmann *et al*., 1993). As the study report, the table shows that 93.3% patients are in 20-30 breaths/minute group and they have a chance for cardiac arrest. Although there were few patients died with lung disease and lung cancer before our study in Eruain village. After our research, taking medicines by the patients, no casualties were found in the village with lung disease and cancer.

The results obtained after medicinal treatment are mentioned in the table 3.17.

Comprising with the table-3.16 and 3.17, the accelerated rate of over group was reduced in some instance (93.3% to 73.3%). But the reverse action found in more over group, it was increased (2.7% to 16.6%) after treatment in table-3.20. But the physical response to well-being was better for all patients. Also the patients of normal group were increased in 2.5 time, that was 4.0% to 10.0%.

Table- 3.17:

Different respiration rate of different age groups of the patients (%), after treatment.

Sl. No.	Different age groups of the patients	Number of Patients in Different Respiration Rate				Total
		Low	Normal	Over	More over	
		>12/min	12-20 /min	20-30 /min	30-40 /min	
1	>19	0	6	**8**	3	17
2	20-29	0	5	**33**	0	38
3	30-39	0	0	**19**	13	32
4	40-49	0	5	**32**	5	42
5	50-59	0	1	**16**	0	17
6	60-69	0	4	**20**	6	30
7	70<	0	0	**5**	3	8
	Total	**0**	**21(10.0%)**	**133(73.3%)**	**30(16.6%)**	**184(100%)**

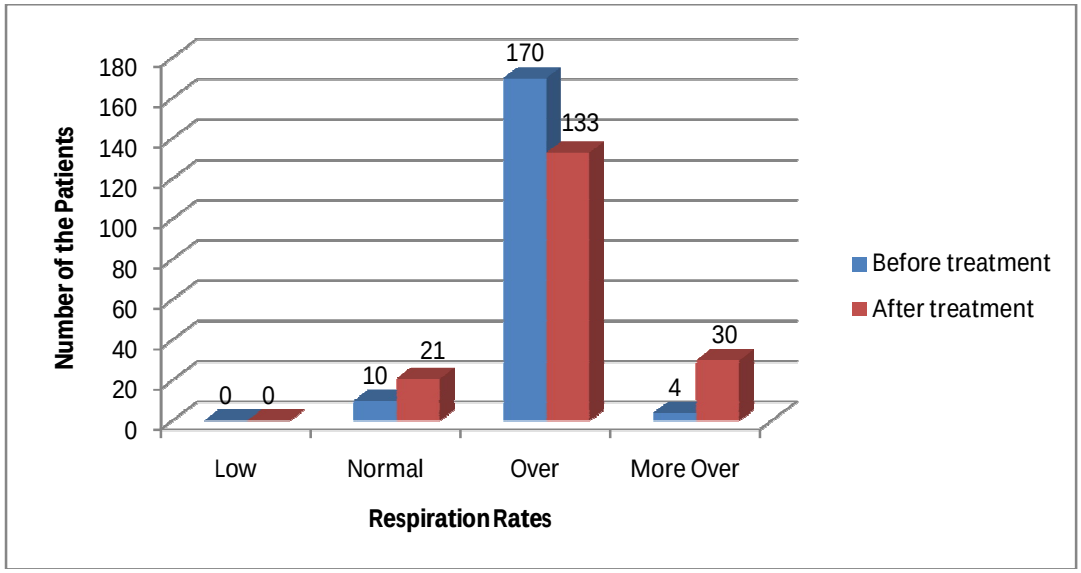


Figure-3.6: Different respiration rates, before and after treatment of the patients

According to figure-3.6, the bar chart shows that the length of over group is very high, but the others are very low, somewhere negligible. Important thing is that the height of over group is reduced after medicinal treatment. It is important that high respiration rate is responsible for infection or sepsis in the lungs with an increased

concentration of hydrogen ions, which leads to increased CO_2 production. In effect, the respiratory rate is an important indicator of a severe derangement in many body systems, not just the respiratory system, and is therefore a key predictor of adverse events (Cretikos, *et al.*, 2008). In accordance with the study, maximum arsenicosis patients are suffering with respiratory derangements. It is nothing but the accumulation of metallic arsenic effect in the lungs.

3.2.5 Analysis Result of BMI

It is said that arsenic affected people are suffering from malnutrition, mainly protein deficiency. But it differs from our results. The WHO regards a BMI of less than 18.5 as underweight and may indicate malnutrition, an eating disorder, or other health problems, while a BMI greater than 25 is considered overweight and above 30 is considered obese (WHO, 2013).

As per our study (table-3.18), total underweight people were only 37.0% (2.7 + 4.9 + 29.4) in the population of 184 and maximum from middle age groups, (30-39) and (40-49). But the 51.1% of total population was in normal group from every age group. Although there were 11.9% people in overweight and maximum from middle age groups, (30-39) and (40-49). So the result is that, these people were maintaining their body fat (adipose tissue) properly and slight over.

Table- 3.18:

BMI of different age groups of the patients (%), before treatment.

Sl. No.	Different age groups of the patients	BMI						Total (%)
		Low (%)			Normal (%)	Over (%)		
		>15	15-16	16-18.4	18.5-25	26-30	31-35	
1	>19	0	0	6	**11**	0	0	17
2	20-29	0	3	6	**25**	4	0	38
3	30-39	0	1	**12**	**15**	4	0	32
4	40-49	1	1	**14**	**16**	**10**	0	42
5	50-59	1	0	5	**10**	1	0	17
6	60-69	2	1	7	**17**	3	0	30
7	70<	1	3	4	**0**	0	0	8
	Total	**5(2.7)**	**9(4.9)**	**54(29.4)**	**94(51.1)**	**22(11.9)**	**0**	**184(100)**

According to our result, we had 37.0% people were in low BMI. And in according to Mayo Clinic, underweight individuals are at an increased risk of developing poor bone health and osteoporosis or reduced bone density, due to calcium and vitamin D deficiency (IOF, 2007 and Paula, 2016).Many factors contribute to low BMI. Having a diet in low folic acid and iron increase the risk of developing anemia; underweight individuals are at increased risk of more severe and frequent infection due to weakened immune system and there are other factors. Metallic arsenic can affect the bodily tissue due to weakened immune system. On the other hand, it is revealed that folic acid supplement can reduce arsenic from the blood. According to Dr Gamble, lead researcher of the study, the folic acid increases the methylation of arsenic increasing the detoxification of arsenic in the body. This methylation process allows the body to change the methyl arsonic (MMA) acid (very toxic) to a form that could more easily be excreted from the body (Gamble, *et al*., 2007). So low BMI may not be the cause of arsenicosis. There should be other cause behind it.

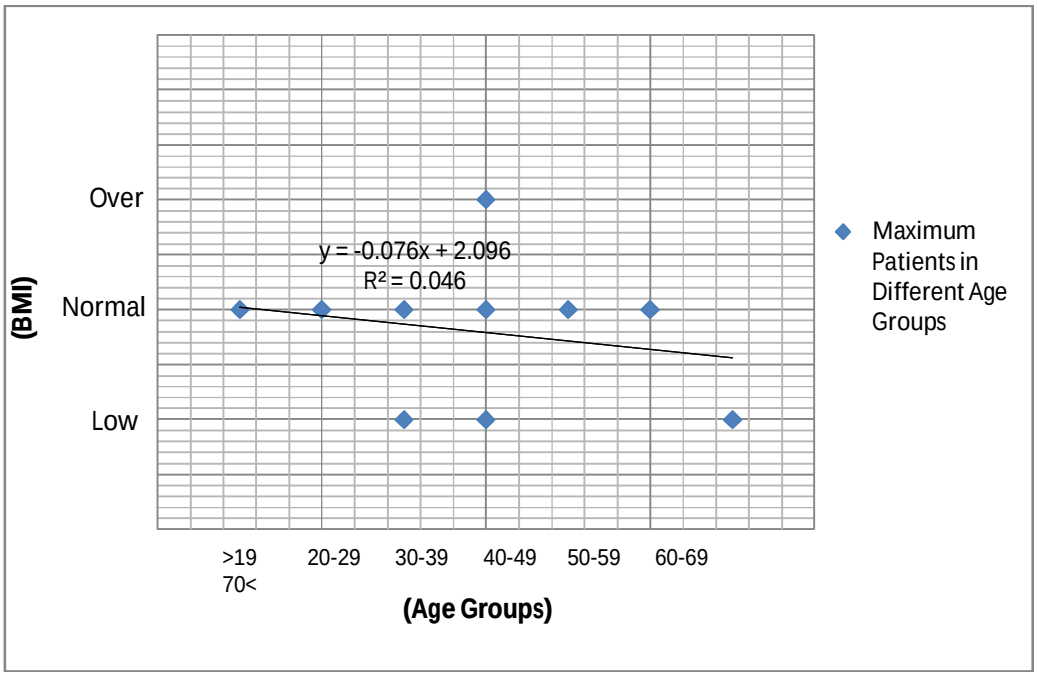


Figure-3.7: Trend of BMI Rates in Arsenicosis Patients (before treatment)

The trend analysis reveals that the maximum patients are in normal range (before treatment) and the trend runs to slight lower to the normal value. The R^2 value is not near to 1.0, that indicates that the model fits the data as normal (Figure- 3.7).

Finally, we can say that the malnutrition is not the cause of arsenic affection. The real cause is different. But in fact, poor and middle earning people are suffering from arsenicosis.

3.2.6 Analysis Results of Urine-

3.2.6.1 Result of Urine pH Value

Measuring acidity and alkalinity of bodily fluid is essential for forming a disease condition in the body, which is neglecting by medical diagnostic laboratories. Finding a particular disease in laboratory is a delay process in diagnostic system. But pH value indicates the primary influence of a disease, even cancer. So we have taken the three divided parts of pH value for all age groups of patients. The results of pH value are mentioned in tables below.

Table- 3.19:

Values of urine pH of different age groups of the patients (%), before treatment.

Sl. No.	Different age groups of the patients	Number of Patients in Different Level of Urine pH Value			Total (%)
		Acidic (%)	Normal (%)	Basic (%)	
		4.6-6.8	6.9-7.1	7.2-8.0	
1	>19	**17**	0	0	17
2	20-29	**35**	3	0	38
3	30-39	**27**	5	0	32
4	40-49	**37**	5	0	42
5	50-59	**16**	1	0	17
6	60-69	**27**	3	0	30
7	70<	**8**	0	0	8
	Total	**167 (91.4)**	**17 (8.6)**	**0 (0.0)**	**184 (100)**

According to the table- 3.19, maximum patients (91.4%) were found acidic in nature, which indicate many types of disease formation in the body, such as- cardiovascular damage, bladder and kidney disease, bone and joint disease, immune deficiency and free radical damage which produces cancer *etc*. other (8.6%) were in normal group. Not a single patient was found in basic group.

Table- 3.20:
Values of urine pH of different age groups of the patients (%), after treatment.

Sl. No.	Different age groups of the patients	Number of Patients in Different Level of Urine pH Value			Total (%)
		Acidic (%)	Normal (%)	Basic (%)	
		4.6-6.8	6.9-7.1	7.2-8.0	
1	>19	**14**	3	0	17
2	20-29	**35**	1	2	38
3	30-39	**27**	3	2	32
4	40-49	**33**	8	1	42
5	50-59	**14**	3	0	17
6	60-69	**26**	4	0	30
7	70<	**7**	1	0	8
	Total	**156 (86.0)**	**23 (12.0)**	**5 (2.0%)**	**184 (100)**

The after treatment scenario was slightly changed. Some patients (2.0%) found in basic group, some increased values (8.6% to 12.0%) in normal group and some decreased rate (91.4% to 86.0%) in acidic group.

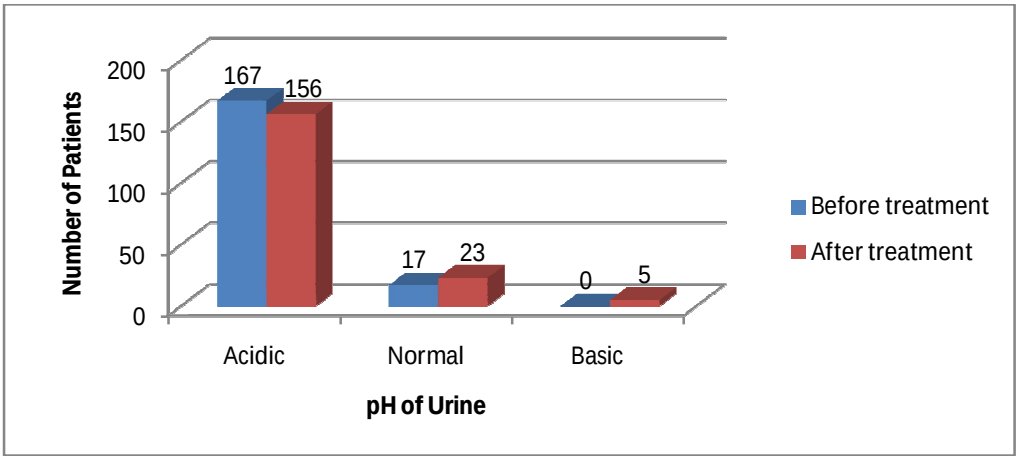


Figure-3.8: Urine pH (before and after treatment) of the patients

It is found that arsenic can create acidic nature in the body. The body excretes excess acids or alkalis with urine, it is the real excretion process. But arsenic forms a gross amount of acid in the body, which can't be neutralized by bodily buffer system. The neutralization is influenced a little bit by applying medicines (*Pleurotus ostreatus*) that the normal value is increased and acid value is decreased.

3.2.6.2 Result of Urine Specific Gravity

Specific gravity of urine is another demarcation of diseased condition of the body. Both the increased or decreased types of specific gravity indicate different renal and other diseases. So we have chosen low, high and normal value of specific gravity of urine for the research. And we have mentioned the values in tables below.

Table- 3.21:
Values of specific gravity of urine of different age groups of the patients (%), before treatment.

Sl. No.	Different age groups of the patients	Number of Patients in Different Level of Specific Gravity of Urine			Total (%)
		Low Sp.Gr (%)	Normal Sp.Gr (%)	High Sp.Gr (%)	
		>1.003	1.003-1.035	1.036<	
1	>19	0	**17**	0	17
2	20-29	11	**27**	0	38
3	30-39	3	**29**	0	32
4	40-49	8	**34**	0	42
5	50-59	7	**10**	0	17
6	60-69	3	**27**	0	30
7	70<	0	**6**	2	8
	Total	**32 (17.3)**	**150 (81.3)**	**2 (1.4)**	**184 (100)**

According to table-3.21, arsenic can't change the specific gravity of urine or somehow remain in normal range. Here the maximum patients (81.3%) were in normal specific gravity, few in low (17.3%) and very few (1.4%) in high specific gravity level.

Considering table-3.22 with table-3.21 the result exhibit that the medicine was increased the level in normal range (81.3% to 82.6%). The low value was decreased from the previous value (17.3% to 13.6%) and high specific gravity was increased a little bit (1.4% to 3.8%) after treatment.

Table- 3.22:

Values of specific gravity of urine of different age groups of the patients (%), after treatment.

Sl. No.	Different age groups of the patients	Number of Patients in Different Level of Specific Gravity of Urine			Total (%)
		Low Sp.Gr (%)	Normal Sp.Gr (%)	High Sp.Gr (%)	
		>1.003	1.003-1.035	1.036<	
1	>19	1	**16**	0	17
2	20-29	7	**31**	0	38
3	30-39	1	**31**	0	32
4	40-49	7	**32**	3	42
5	50-59	6	**11**	0	17
6	60-69	3	**26**	1	30
7	70<	0	**5**	3	8
	Total	**25 (13.6)**	**152 (82.6)**	**7 (3.8)**	**184 (100)**

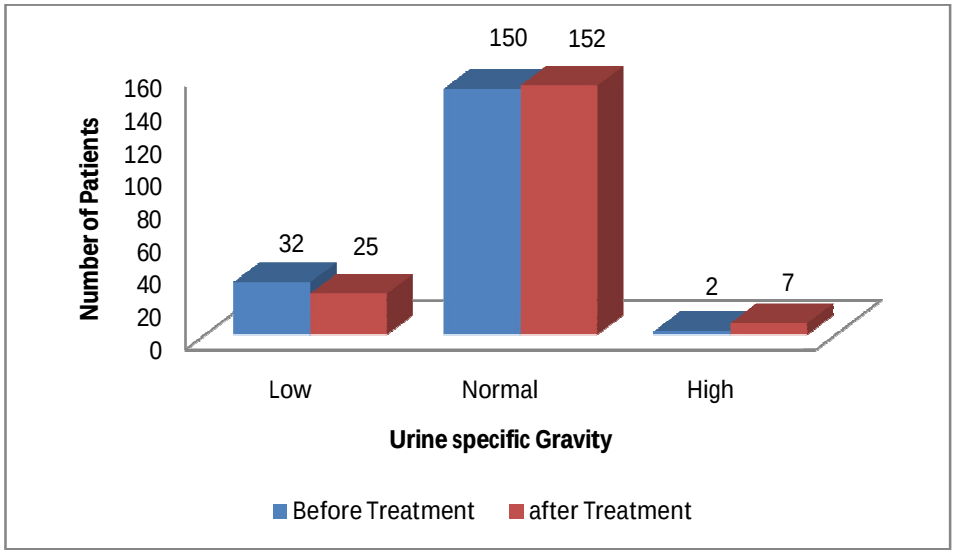


Figure-3.9: Observation of Urine Specific Gravity (before and after treatment) of the patients

Bar chart of figure-3.9 revealed that the arsenicosis patients had no response with specific gravity. Maximum patients of urine specific gravity found in normal group. But the bar chart of figure-3.8 showed that the pH value became lowered in arsenicosis patients. So the arsenic affected patients were acidic in nature.

By the keen investigation, it was found that the arsenicosis patient had more chance to suffer severe and prolonged damage due to high acidity and on the other hand, 32 patients had hyposthenuria, the low specific gravity. These hypostenuria patients may had renal failure or/and different types of nephritis. Considering both pH and specific gravity together, severe cardiovascular damage and seldom necrosis may have in the kidneys.

3.3 Drinking Water and Biological Samples Analysis Results

3.3.1 Arsenic Concentration Results of Tube Well Water

We have analyzed 48 tube-wells from 113 houses. Among them 3 tube-wells were bellow and equal to normal level, the accepted rate for our country. Other 45 tube-wells were in danger level for arsenic concentration. Maximum people were drinking arsenic contaminated water from these tube-wells founding no other alternatives.

Table-3.23:

Arsenic Concentration in Tube-wells in the Research Field Area

Sl.No.	Arsenic Quantity (µgm / L) or ppb	Number of Tubewells
1	>500	0
2	401-500	2
3	301-400	2
4	201-300	33
5	101-200	4
6	51-100	4
7	1-50	2
8	0	1
Total		48

Discussions

Determination of arsenic in drinking water is the most important part of the research to save people in arsenic affected area. In our research area, we have studied 48 tube-wells, which are used by the arsenic affected people. Among them only one tube-well is in zero level, two up to 50 ppb, 33 found in (201-300) ppb and the highest is (401-500) ppb As found in two tube-wells. As we know The World Health Organization asserts that a level of 0.01 mg/L (10 ppb) As poses a risk of 6 in 10000 chance of lifetime skin cancer risk and contends that this level of risk is acceptable (WHO, 2012). As per considerable level of arsenic in drinking water, our research area poses full risk of arsenicosis for all people who are drinking tube-well water. Some of them are using 'Sono filter' to purify tube-well water. There is a condition to use the filter; that is to alter in every six months, the sands and chemical chamber in between it. But nobody did it. As a result, one filter found 85 ppb and other one is (201-300) ppb As in them.

Comparing the arsenic concentrations in drinking water with other area of the earth, it is found that there were 75 and 1250 ppb (0.075 and 1.25 mg L^{-1}), respectively found from two villages (Esquin˜a and Illapata) of the Atacama Desert, Chile (Ya´n˜ez,*et.al.*, 2005). Here two different picture were found in two places. The ground water of Esquin˜a had a consistency with Eruaine village. The ground water of Eruaine village was ranging from 0 to 500 ppb. But the ground water of Illapata village was very high others.

The diameter of the study area (Eruine village) is about 3km. X 3km. This is very low land; approximately 23-36 ft from the sea level (showing satellite map). In this area, there are no alternative drinking water sources, except tube well water. There are some ponds in this area, but water become mostly empty or waterless in winter (Plate- 3.11) and ponds are filled with water in rainy season, but this field wash water is dirty, poisonous and unhealthy. The Dakatia river is flowing beside of Laksham bazaar and is 2.5 km from the village Eruine. Its width is 25-30 meters. The branch of Dakatia in Eruine is dried up in winter. The upazila city waste is released through this river. In the rainy season, ponds and paddy fields are filled with this dirty and unhygienic water.

People only drink the tube well water in all seasons and in rainy season they collect rain water to drink (Plate- 3.12). First they collect them in a small cooking cauldron, then they reserve them to a big 'mait' (soil made burned vessel) in the house. Some people were using filters for fresh water (Plate-3.13).

Plate 3.12: Dirty, scanty and unusable water in the ponds bottom

Plate 3.13: Rain water harvesting by a village woman

Plate 3.14: Few households are filtering the tube well water to make them arsenic free

Only one river Dakatia is lying down from Laksham bazar. That is 2.5 km from this Eruine village. A narrow channel and few ponds are dried in winter season. People harvest rain water in a 'mait', a big soil made burned pot. The rain water is preserved for the winter season. After that people have to drink tube-well water, due to fresh water scarcity. For the reason people are suffering with arsenicosis. Many died in last 10 years by arsenic induced respiratory diseases along with lung cancer. So decreasing arsenic level in drinking water to acceptable levels is essential to save the people in arsenic affected area.

3.3.2 Arsenic Concentration Results of Biological Samples, before Study

We had randomly selected 20 human samples for biological study. Among them, there were 8 hair and 12 nail samples. And 9 were drinking arsenic water and 11in fresh water group. We had divided them again into two groups, one- medicinal group and another- control group. Medicinal group contains 16 samples and control group contains 4 samples.

Table-3.24:

Arsenic Concentration (mg/kg) in hair and nail, Results of all groups (Before treatment), consistency with <1.0 gm of Sample weight

Sl.No.	Patient ID	Sample ID	Sample Type	Medicine / Placebo	DAW/DFW	Arsenic Concentration (mg/kg)
01	1105/153	B-1	Hair	++	DAW	6.8552
02	1107/158	B-2	‘’	++	DFW	39.3139
03	1402/075	B-3	Nail	BLK	DAW	6.2550
04	1401/062	B-4	‘’	BLK	DFW	9.1822
05	1015/150	B-5	Hair	++	DFW	101.2992
06	1006/143	B-6	‘’	+	DFW	6.3103
07	1013/148	B-7	‘’	++	DFW	5.1697
08	0905/071	B-8	Nail	++	DFW	14.2769
09	0810/091	B-9	‘’	OFF	DAW	7.9596
10	0801/034	B-10	‘’	+	DAW	8.8824
11	0713/107	B-11	‘’	OFF	DAW	7.5849
12	0715/113	B-12	‘’	+	DAW	6.9702
13	0706/076	B-13	Hair	+	DFW	9.1140
14	0707/081	B-14	Nail	+	DAW	10.9996
15	0713/108	B-15	‘’	+	DAW	9.0417
16	0402/172	B-16	‘’	+	DAW	8.8121
17	0405/171	B-17	Hair	++	DFW	10.2304
18	0411/170	B-18	Nail	++	DFW	7.6078
19	0406/163	B-19	Hair	++	DFW	10.4782
20	0705/073	B-20	Nail	+	DFW	105.0012

We had unequal sample weights and equalized mathematically 1.0 gm to all sample weights and the result also equally calculated with the ratio to the sample weight. Medicinal group had two types of deviations; we had selected two capsules daily for a person. But some patients had some complains like- headache, nausea, diarrhea *etc.*, we discarded one capsule from their requisite. Maximum arsenic level

had found in the nail (105 mg/kg), which was 70 times greater than the normal value (1.5 mg/kg) and the lowest one was 5 mg/kg in hair, it was 5 times greater than normal value (1.0 mg/kg).

Comparing with two villages (Esquin˜a and Illapata) of the Atacama Desert, Chile were found that on average, the total arsenic concentrations in hair from individuals of Esquin˜a and Illapata were 0.7 and 6.1 μg g^{-1} (mg/kg) respectively (Ya´n˜ez, *et.al.*, 2005). But there was symmetry with the patients of Illapata village with Eruaine village rather than Esquin˜a, that was the opposite of the rate of drinking water arsenic concentration. We had a concentration from 5 to 105 mg/kg of the hair and nail of the patients of Eruaine village. But the maximum was from 5 to 10 mg/kg with few exceptions (Table-3.24).

3.3.3 Arsenic Concentration Results of Biological Samples, after Study

The same selected 20 biological samples were again collected and analyzed after one year of medicinal treatment of the patients. The obtained results were mentioned in table below-

Discussion

Generally, we know that arsenicosis is the disease of poor people. But no perfect ground is detected. We see, only poor are affecting rather than rich. Rich are very rare. Scientists thought about different deficiencies; i.e.- nutrition, anti-oxidants, selenium *etc*. But all are in vein. No true cause is detected till now. According to the field record, there are some differences between arsenic affected and non-affected persons in a (poor) family. All the family members of these families are eating a part of food, which they have cooked in a dish. Usually they eat same foods in a day or week or month or even year. In a keen observation we can see, children were not affecting too much (boys-1.6% and girls-7.1%), young and old are getting affected in a family, mentioned in Table-3.2. Here one factor is time. A long time consumption of arsenic is responsible for chronic arsenic poisoning. Another factor is morphologically children's muscle fiber and bone hardness are soft and mucelinase. Physiologically they are more active than the old one. These may be the other cause to release or digest metallic arsenic.

Table-3.25:

Arsenic Concentration (mg/kg) in hair and nail, Result of all groups (After Treatment), consistency with <1.0 gm of Sample weight

Sl.No.	Patient ID	Sample ID	Sample Type	Medicine / Placebo	DAW/DFW	Arsenic Concentration (mg/kg)
01	1105/153	A-1	Hair	++	DAW	8.7803
02	1107/158	A-2	‘’	++	DFW	10.2558
03	1402/075	A-3	Nail	BLK	DAW	63.2245
04	1401/062	A-4	‘’	BLK	DFW	20.5333
05	1015/150	A-5	Hair	++	DFW	5.2726
06	1006/143	A-6	‘’	+	DFW	21.3267
07	1013/148	A-7	‘’	++	DFW	2.8976
08	0905/071	A-8	Nail	++	DFW	6.0797
09	0810/091	A-9	‘’	OFF	DAW	4.6358
10	0801/034	A-10	‘’	+	DAW	11.3775
11	0713/107	A-11	‘’	OFF	DAW	69.9110
12	0715/113	A-12	‘’	+	DAW	28.7628
13	0706/076	A-13	Hair	+	DFW	7.2588
14	0707/081	A-14	Nail	+	DAW	12.7123
15	0713/108	A-15	‘’	+	DAW	13.5771
16	0402/172	A-16	‘’	+	DAW	21.3744
17	0405/171	A-17	Hair	++	DFW	6.8709
18	0411/170	A-18	Nail	++	DFW	6.7055
19	0406/163	A-19	Hair	++	DFW	6.6687
20	0705/073	A-20	Nail	+	DFW	74.5107

According to Jahangir (2009), the cross section of keratosis shows that the malformation of epithelial cells around arsenic molecule create destruction of cells and then malfunction of the cells and forms cancer, mentioned in plate 3.14. In plate A, the microscopic photograph of keratosis (magnification x 100) shows seven germ keratoses with a hole in between them and more black coloured little germ keratoses. In plate B, (magnification x 400) normal keratin cells are found everywhere with few dark coloured destruction of keratin cells due to arsenic in situ. In plate C, (magnification x 1000) shows a black coloured hyperkeratosis with an oval shaped hole in the centre. In plate D, (magnification x 1000) shows a hyperkeratosis with an unruptured centre, the home of arsenic molecule.

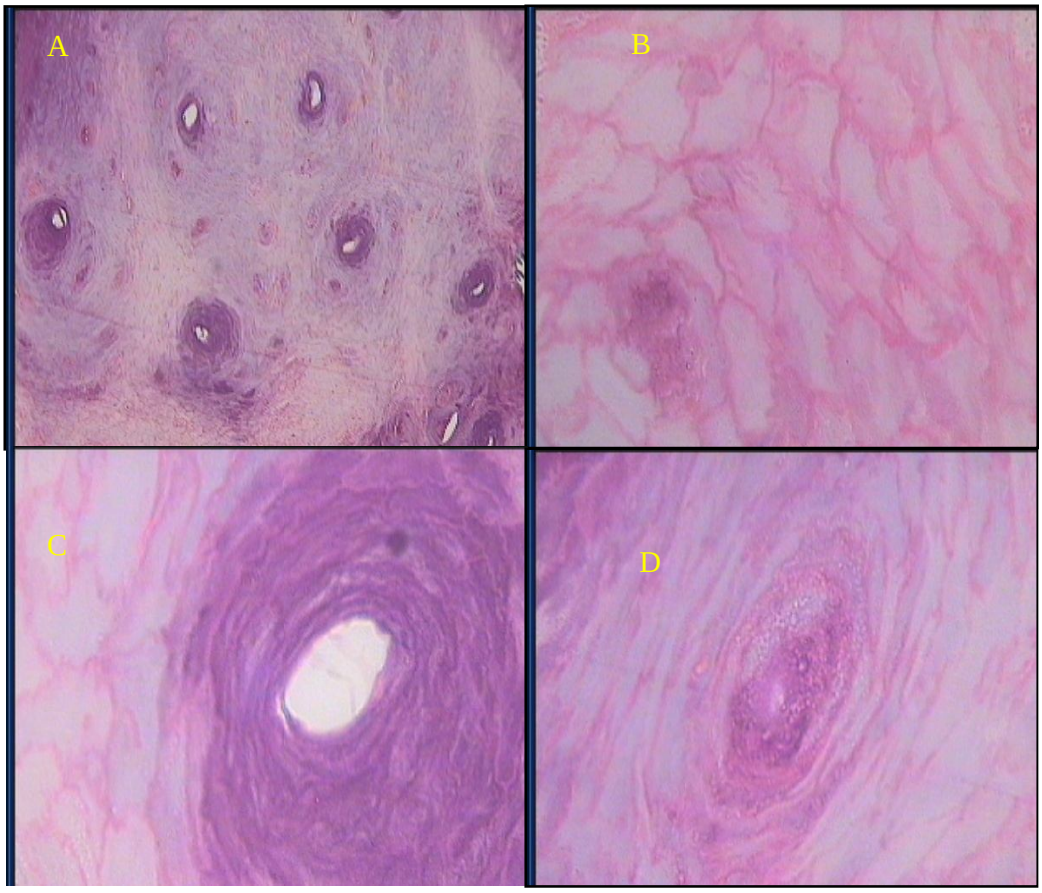


Plate 3.15: Metallic arsenic is destructing the keratins of human tissues (4 microscopic photos); Source- M.Phil. Thesis of M Jahangir, Consequences of arsenic contamination in human beings and their prevention by applying homoeopathic principles, 2009, p- 62,63, 65 and 67.

According to the study of Angela D'Amico, arsenic exposure can cause a low birth length and weight, altered musculature, and decreased locomotor activity found in killifish (*Fundulus heteroclitus*) (D'Amico, 2012). Another research shows that arsenic tri-oxide (As_2O_3) induced inhibition in myotube formation and muscle-specific protein expression was reversed by transfection with the constitutively active form of Akt phosphorylation and typical changes of injury and regeneration after local glycerol injection in mice (Yen, 2010).

There is a correlation in between these three studies on arsenic effect. First- the cross section of human keratin cell, second- the cross section of killifish, and lastly-

the cross section of muscle of mice. In every case the same destructive nature of arsenic tri-oxide on keratin or muscular tissue was found.

Mushroom, *Pleurotus ostreatus* have been used as a food supplement and medicinal properties, such as- polysaccharides, lipopolysaccharides, proteins, peptides, glycoproteins, nucleosides, triterpenoids, lectins, lipids and their derivatives. Somewhere oyster mushroom have found as a metal remover from the body. But in case of metallic arsenic, it is found less effective to release arsenic from the body. In our study, we found arsenic concentration in hair and nail before and after medicinal application for both DAW and DFW groups in table-3.26.

Table- 3.26:

Arsenic Concentration (mg/kg), of Before and After Medicinal Treatment Groups

Sl.No.	Patient ID	Medicine / Placebo	DAW/DFW	Arsenic Concentration (mg/kg)	
				Before Treatment	After Treatment
01	1105/153	++	DAW	6.8552	8.7803
02	1107/158	++	DFW	39.3139	10.2558
03	1402/075	BLK	DAW	6.2550	63.2245
04	1401/062	BLK	DFW	9.1822	20.5333
05	1015/150	++	DFW	101.2992	5.2726
06	1006/143	+	DFW	6.3103	21.3267
07	1013/148	++	DFW	5.1697	2.8976
08	0905/071	++	DFW	14.2769	6.0797
09	0810/091	OFF	DAW	7.9596	4.6358
10	0801/034	+	DAW	8.8824	11.3775
11	0713/107	OFF	DAW	7.5849	69.9110
12	0715/113	+	DAW	6.9702	28.7628
13	0706/076	+	DFW	9.1140	7.2588
14	0707/081	+	DAW	10.9996	12.7123
15	0713/108	+	DAW	9.0417	13.5771
16	0402/172	+	DAW	8.8121	21.3744
17	0405/171	++	DFW	10.2304	6.8709
18	0411/170	++	DFW	7.6078	6.7055
19	0406/163	++	DFW	10.4782	6.6687
20	0705/073	+	DFW	105.0012	74.5107

BLK = Blank or Placebo, ++ = Double dose, + = Single dose.
DAW = Drinking Arsenic Water, DFW = Drinking Fresh Water.

In differential study, Patients who were drinking arsenic water and taking medicines were not improving too much. But who were drinking fresh water and taking medicines had a positive result. Rest of all had negative results. Because they were accumulating too much arsenic through drinking water.

Table- 3.27:
Average arsenic concentrations (mg/kg), before and after treatment

Group	Drinking Water	Average arsenic concentration (mg/kg)		Increasing or Decreasing Rate (%)
		Before treatment	After treatment	
Medicinal	DAW	8.5935	16.0974	↑ 200%
	DFW	30.8802	13.0785	↓ 43%
Control	DAW	7.2665	45.9237	↑ 642%
	DFW	9.1822	20.5333	↑ 222%

In medicinal group, the mean arsenic concentrations (mg/kg) of hair and nail for before and after treatment in two groups (DAW, DFW) were from 8.5935 mg/kg to 16.0974 mg/kg in DAW and from 30.8802 mg/kg to 13.0785 mg/kg in DFW respectively. There were few notable changes in these two groups that the DAW was increased 200%, but the DFW was decreased to 43% (Table- 3.27).

In control group, the average arsenic concentrations (mg/kg) in two groups were from 7.2665 mg/kg to 45.9237 mg/kg in DAW and from 9.1822 mg/kg to 20.5333 mg/kg in DFW respectively (Table-3.27). Both groups were increased in values, but DAW was more increased. Bar charts are showing the differences between medicinal and control groups in figure- 3.10 and 3.11.

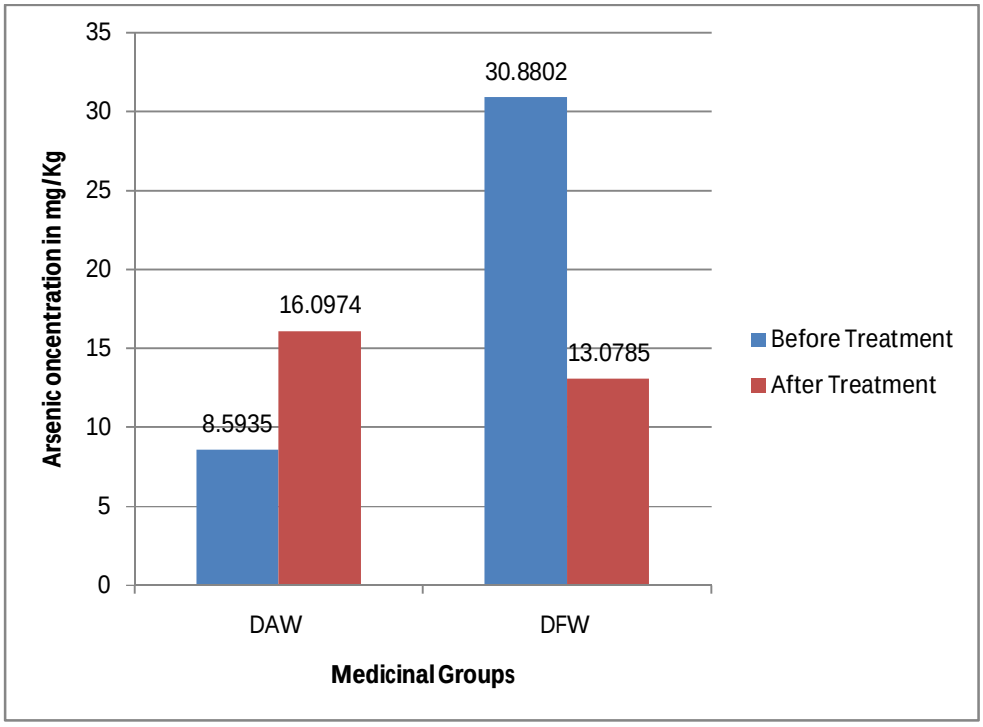


Figure- 3.10: Arsenic Concentration in Medicinal Group
DAW = Drinking Arsenic Water, DFW = Drinking Fresh Water

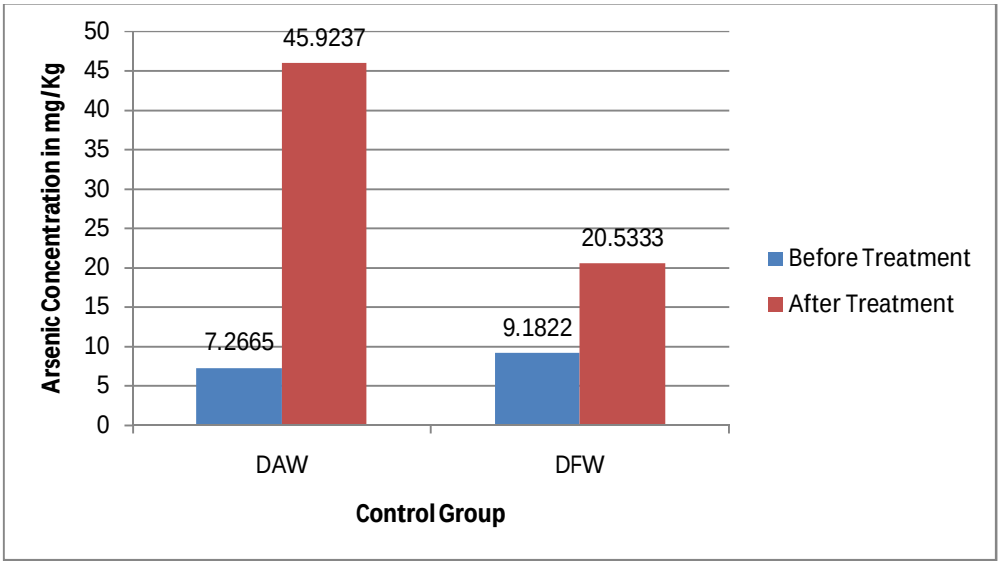


Figure-3.11: Arsenic Concentration in Control Group
DAW = Drinking Arsenic Water, DFW = Drinking Fresh Water

Comprising both medicinal and control groups together, only decreasing rate is found in DFW of medicinal group (figure-3.10). So it is important that the medicine alone can't significantly reduce the arsenic level in the body, if the person is drinking arsenic water. DFW and medicine is the solution to treat the patients. On the other hand, medicinal dose can be increased to tolerable position. As we know, most of the tube-wells were arsenic contaminated, among them 33 tube-wells out of 45 had the arsenic range (201-300) ppb. Some were more and bellow than that level, and only 3 were below 50 ppb level (table-3.23).

3.4 Dose Related Results of Cutaneous Symptoms

3.4.1 Results of Sensitivity Test Program

As this is the first time study with *P. ostreatus* on arsenicosis patients, we did not have any literature or research or clinical trial papers about the prognosis of the study and it is a yearlong medicinal application program. For this reason, we have taken a sensitivity test program (STP) with the medicine (*P. ostreatus*) for three months (two months- application of medicines and one month- results analysis) upon the patients from October, 2012 to December, 2012. After three months we have found some good and bad results. Bad results areas; few patient complains of about diarrhea. After keen observation we found that the complains come on them who are old in age and taking full doses. We have reduced the dose of week and old patients into half for the yearlong medicinal application program (January, 2013-December, 2013). One patient complains about dyspnoea with oedema of foot. We have also reduced the dose into half. The patients who are half dose from the beginning, they have found no complicacy. But the good news is that few patient symptoms are reduced. The recovering symptoms are weakness, vertigo, appetite and melanosis in some cases.

Comprising international standard for oral application of *P. ostreatus* with our sensitivity test (STP) study in human body, finally we have selected two capsules/day is a full dose, one capsule/day means a half dose and a control (null or blank) dose.

The randomly selected patients from 16 bari for full, half and control dose, the medicinal response in one month is a very criterion for initial study. We have selected 50 persons for control group, then the rest of the patients are divided into two parts, mostly equal, 65 and 69 persons. They are all together 184 in number; mentioned in table-3.28.

Table- 3.28:
Selection of Full, Half and Null Doses for the Patients (before treatment)

Bari	Full Dose	Half Dose	BLK	Absent/ Discontinued/Died	Total
Master Bari	12	11	4	0	27
Kazi Bari	6	0	2	0	8
Bain Bari	5	7	8	0	20
Dewan Bari	6	4	4	0	14
Mosjid Bari	0	3	1	0	4
Jomodder Bari	2	3	1	0	6
Choukider Bari	2	21	14	0	37
Mayeri Baper Bari	3	5	3	0	11
Aijjar Baper Bari	1	7	1	0	9
Hazi Bari	11	5	6	0	22
Miazi Bari	11	1	2	0	14
Dolla Bari	3	0	3	0	6
Hakim Ali Bari	1	0	0	0	1
Akbar Ali Master Bari	2	0	1	0	3
Thakur Bari	0	1	0	0	1
Khondoker Bari	0	1	0	0	1
16 (Sixteen)	65	69	50	0	184

(Note: Bari- a group of houses)

At the conclusion of the study we found that the STP is not same for every person of the study. According to our study, children, weak and old persons are sensitive to a massive dose (two capsules/day), so they need a single dose (one capsule/day). The complicacies are diarrhoea, in most of the cases. Finally, we changed the number of patients in each head, as- full, half and BLK dose, mentioned in table-3.29.

Table-3.29:

Variation of Numbers of the Patients after Medicinal Treatment for 12 (twelve) Months

Bari	Full Dose	Half Dose	BLK	Absent/Discontinued/Died	Total
Master Bari	11	10	3	3 (1 =Full, 1 =Half, 1 = BLK)	27
Kazi Bari	6	0	2	0	8
Bain Bari	5	6	6	3 (1 =Half, 2 = BLK)	20
Dewan Bari	6	4	4	0	14
Mosjid Bari	0	3	1	0	4
Jomodder Bari	1	3	1	1 (Died)	6
Choukider Bari	2	14	12	9 (7 = Half, 2 =BLK)	37
Mayeri Baper Bari	1	4	3	3 (2 =Full, 1 =Half)	11
Aijjar Baper Bari	1	6	1	1 (1=Half)	9
Hazi Bari	9	4	6	3 (2 =Full, 1 =Half)	22
Miazi Bari	10	1	2	1 (1 =Full)	14
Dolla Bari	3	0	3	0	6
Hakim Ali Bari	1	0	0	0	1
Akbar Ali Master Bari	1	0	1	1 (1 =Full)	3
Thakur Bari	0	1	0	0	1
Khondoker Bari	0	1	0	0	1
16 (Sixteen)	57	57	45	25	184

(Note: Bari- a group of houses)

The most important thing is that the humans are not guinea pigs. In a study, guinea pigs are taken in a case or room. They can't do anything without researchers wish. But the researches with human beings are difficult. We can't observe the humans' daily works, intakes or behaviors for a yearlong time in a hospital. They have human right to stop the treatment in between the study time, if he wishes. This is very dangerous for a study. The result and economy both can hamper with that decision. So it is important to do a STP before a study with human. After all of these complicacies the result is changed in table-3.29. The total numbers of the patients are same, but the full dose, half dose and control dose changed as 57, 57, 45 and 25 for discontinue group.

3.4.2 Results of Cutaneous Changes of Experimental Group

As per our STP study, the selected patients for a full dose are 57 in number for an experimental group. Among them they have keratosis in 38, melanosis in 48 and leuco-melanosis in 39 patients, mentioned in table-3.30. In the remark, we have decided to mark good result for <50%, <75% is better and <90% is best; and negligible is >25% and worst for >10%.

Table-3.30: Results of Full Dose taken by the Patients after Medicinal Treatment of 8 (Eight) Months

Bari	Full Dose	Keratosis		Melanosis		Leucomelanosis		Remarks
		Prev.	.*Reco.+(%)	Prev.	.*Reco.+(%)	Prev.	.*Reco.+(%)	
Master Bari	11	6	3 (50)	10	6 (60)	6	4 (67)	Good
Kazi Bari	6	5	5 (100)	6	3 (50)	4	3 (75)	Better
Bain Bari	5	3	0 (0)	5	1 (20)	4	1(25)	Negligible
Dewan Bari	6	5	2 (40)	5	0 (0)	3	1(33)	,,
Mosjid Bari	0	0	0	0	0	0	0	None
Jomodder Bari	1	0	0	0	0	1	1 (100)	Better
Choukider Bari	2	2	1 (50)	2	1 (50)	2	1 (50)	Good
Mayeri Baper Bari	1	1	1 (50)	1	1 (50)	0	0	Good
Aijjar Baper Bari	1	1	0 (0)	1	0 (0)	1	0	Worst
Hazi Bari	9	7	4 (57)	8	5 (63)	6	4 (67)	Better
Miazi Bari	10	5	2 (40)	8	4 (50)	10	5 (50)	Good
Dolla Bari	3	2	0 (0)	1	1 (100)	1	1 (100)	Better
Hakim Ali Bari	1	0	0	0	0	1	0 (0)	Worst
Akbar Ali Master Bari	1	1	1 (100)	1	0 (0)	0	0	Better
Thakur Bari	0	0	0	0	0	0	0	None
Khondoker Bari	0	0	0	0	0	0	0	,,
16 (Sixteen)	57	38	19 (50)	48	22 (46)	39	21 (54)	Good

Note:- Prev.= Previous; Reco.= Recovery.

We have good results in master bari, choukider bari, mayeri baper bari and miazi bari; also better results in kazi bari, jomodder bari, hazi bari and dolla bari. No best results in deed. We have also negligible and worst results in bain bari, dewan bari, aijjar baper bari and hakim ali bari. The recovery rate for keratosis is 50%, melanosis is 46% and leuco-melanosis is 54%.All together the result goes to an appreciation remark, this is good and the recovery rate is more than 49.2%, mentioned in table-3.30.

Table-3.31: Results of Half Dose taken by the Patients after Medicinal Treatment of 12(twelve)Months

Bari	Half Dose	Keratosis		Melanosis		Leucomelanosis		Remarks
		Prev.	.*Reco.+(%)	Prev.	.*Reco.+(%)	Prev.	.*Reco.+(%)	
Master Bari	10	6	6 (100)	9	6 (67)	6	5 (83)	Best Result
Kazi Bari	0	0	0	0	0	0	0	Nil
Bain Bari	6	3	2 (67)	5	1 (20)	6	3 (50)	Better
Dewan Bari	4	3	1 (33)	3	1 (33)	2	2 (100	,,
Mosjid Bari	3	0	0	3	0 (0)	2	0 (0)	Worst
Jomodder Bari	3	1	0 (0)	1	0 (0)	3	3 (100)	Better
Choukider Bari	14	7	5 (71)	12	5 (42)	10	5 (50)	,,
Mayeri Baper Bari	4	1	1 (100	4	1 (25)	2	1 (50)	,,
Aijjar Baper Bari	6	3	3 (100)	4	2 (50)	3	2 (67)	,,
Hazi Bari	4	3	2 (67)	4	3 (75)	2	1 (50)	,,
Miazi Bari	1	1	1 (100)	1	0 (0)	0	0	Good
Dolla Bari	0	0	0	0	0	0	0	Nil
Hakim Ali Bari	0	0	0	0	0	0	0	,,
Akbar Ali Master Bari	0	0	0	0	0	0	0	,,
Thakur Bari	1	1	1 (100)	1	1 (100)	0	0	Better
Khondoker Bari	1	0	0	0	0	1	0 (0)	Worst
16 (Sixteen)	57	29	21 (72)	47	20 (43)	37	22 (60)	Good

Note:- Prev.= Previous; Reco.= Recovery.

The result of half dose for 57 respondents for keratosis, melanosis and leuco-melanosis are mentioned in table-3.31. There are 29 patients in keratosis, 47 in melanosis and 37 in leuco-melanosis. And the recovery rates are 72% in keratosis, 43% in melanosis and 60% in leuco-melanosis. All together the result goes to good (55.75%), as a remark.

3.4.3 Results of Cutanous Changes of Control Group

As per our STP study, we have selected 45 patients for a control group. Among them keratosis is in 20, melanosis in 43 and leuco-melanosis in 29 patients, mentioned in table-3.32. In the remark, we have decided to mark good result for <50%, <75% is better and <90% is best; and negligible is >25% and worst for >10%.

Table-3.32: Results of BLK taken by the Patients after Medicinal Treatment of 12 (twelve) Months

Bari	Full Dose	Keratosis		Melanosis		Leucomelanosis		Remarks
		Prev.	.*Reco.+(%)	Prev.	.*Reco.+(%)	Prev.	.*Reco.+(%)	
Master Bari	3	0	0	3	0 (0)	0	0	Worst
Kazi Bari	2	0	0	2	0 (0)	0	0	,,
Bain Bari	6	2	0 (0)	6	0 (0)	3	0 (0)	,,
Dewan Bari	4	0	0	4	0 (0)	2	0 (0)	,,
Mosjid Bari	1	0	0	1	0 (0)	1	0 (0)	,,
Jomodder Bari	1	0	0	1	1 (100)	0	0	Better
Choukider Bari	12	8	0 (0)	11	3 (27)	9	1 (11)	Negligible
Mayeri Baper Bari	3	3	0 (0)	3	0 (0)	2	0 (0)	Worst
Aijjar Baper Bari	1	0	0	1	0 (0)	0	0	,,
Hazi Bari	6	3	0 (0)	6	1 (17)	6	0 (0)	Negligible
Miazi Bari	2	2	0 (0)	2	0 (0	2	0 (0)	Worst
Dolla Bari	3	2	0 (0)	2	0 (0)	3	0 (0)	,,
Hakim Ali Bari	0	0	0	0	0	0	0	Nil
Akbar Ali Master Bari	1	0	0	1	1 (100)	1	0 (0)	Better
Thakur Bari	0	0	0	0	0	0	0	Nil
Khondoker Bari	0	0	0	0	0	0	0	,,
16 (Sixteen)	45	20	0 (0)	43	6 (14)	29	1 (3)	Negligible

Note:- Prev.*= Previous; Reco.+= Recovery.

As per our study, no one was cured in between 20 patients in keratosis group. Only 14% and 3% of the patients were cured in melanosis and leuco-melanosis groups. All together the remark was negligible.

3.4.4 Comparison of the Results of All Groups

As per our STP study result, we had 159 patients for the study of experimental and control groups; rest 25 patients were absent or/and discontinued (Table-3.33).

Table-3.33: Total Results summary of Full, Half and BLK Doses of the Patients after Treatment of 12 (twelve) Months

Doses	No. of Patients	Keratosis Recovered	Melanosis Recovered	Leucomelanosis Recovered	Total Recovered (%)
Full Dose	57	38 19(50)	48 22(46)	39 21 (54)	125 62 (50)
Half Dose	57	29 21(72	47 20(43)	37 22 (60)	113 63 (56)
BLK (Blank/Null)	4	20 0(0)	43 6(14)	29 1 (3)	92 7 (8)
Total Disease Wise	159	87 40(46)	138 102(74)	105 44 (42)	330 (132 (40)

In the result of the study, the curative rate of full dose, half dose and BLK were respectively 50%, 56% and 8%. In a differential study, the recovery rate of keratosis of half dose was more efficient than full dose, these were 72% and 50%; in melanosis, full dose was somehow increase rate than half dose, 46% and 43%, in leuco-melanosis, the result of half dose was better than full dose, and was respectively 60% and 54% (Table-3.33). In all respect, the half dose was too much efficient for Bangladeshi patients.

3.5 Results of the Treatment by Photographic Demonstrations

3.5.1 Results of Keratosis Development

Formation of keratosis is one of the most important characteristic for arsenicosis. According to the Table-3.33, considering all groups (full dose, half dose and BLK dose), the keratosis patients are lower (87) than other two verities-melanosis (138) and leuco-melanosis (105). But the keratosis can be turn to ulcers and cancer rather than melanosis and leuco-melanosis in case of arsenicosis.

In plate 3.16a and 3.16b, a woman named Monjuma (40) of Hazi Bari, ID No. 1016-203. Here Bari number is 10, House number is 16 and patient's serial number is 203. Monjuma is a house-wife, usually she is doing all house works and helping his husband in crop field. Initially she had some keratosis nodes on her feet with some ulceration on the front and back of feet from years. She was suffering with heel pain on keratoses. So she had to cut them some times. She had also ulceration on her feet, marked by yellow colored circle. First the photograph was taken on 22-03-2012. Again photo had taken on 05-09-2013 prior to the end of the application of medicine program. Then she was cured the keratosis many fold and softened, smoothness came on skin of foot. The ulceration was recovered completely.

Surjo Ban is a woman of age 45 years, living in Miaji Bari, the patient ID No. 1106-210. She is lean and thin, non-fleshy woman. Her husband is earning by a lower class business, she also helping him. But she was suffering with arsenicosis from 10 years approximately. Her palm of the hand and soles of the feet were filled with hyperkeratosis with pain in sole and physical weakness. Her legs were covered with leuco-melanosis. The photograph of her hands before treatment were taken on 22-03-2012 and given in plate 3.17a.

After 1(one) year of treatment with the fruiting body of *Pleurotus ostreatus* by orally, most of the keratoses are recovered from the palm and sole. Her sole pain and weakness are gone. Now she is energetic, doing household and outside works everyday happily. After treatment photograph is taken on 05-09-2013, mentioned in plate 3.17b.

In Plate 3.18a, Minoara Begum, age 30 years of Mayeri baper Bari, patient ID No. 0810-091, was suffering from hyperkeratosis turned to ulceration in her sole from 7/8 years. Her soles were becoming black, which is called black-foot disease due to arsenicosis in China (Taiwan). In Taiwan many people are died with this arsenic induced black-foot disease (Chen, 1985). Minoara was also in severe condition due to black-foot disease on her foot. Black colored keratins of foot were breaking, loosing tonicity and destroying the hard sole. Any time it may turn to cancer.

After 1 (one) year of treatment, tremendously the black nature of sole was recovered and her foot pain was gone. Now she is free from the risk of cancer. The ulceration of foot was diminishing slowly, because she usually walks without shoes or

sandals. We suggested her to take sandals always. Photograph is taken on 20-08-2013, plate no. 3.18b.

In Plate 3.19a, Ali Ashraf of age 70 years of Master Bari, patient ID No. 0106-007 was suffering from skin pulling from 5/6 years. His skin of the palm of the hands were pulling like snake skin. His palm skin was very thin. We have no evidence of any case before like this. It is exceptional one indeed, in Bangladesh. So, we have given a special attention on him. Usually he hides his hands from others. As we know, metallic arsenic accumulates in keratins of hands and feet. So, it is possible that that this skin pulling can be happening by arsenic. Photograph is taken on 21-06-2012.

We thought it will remain as usual after treatment, but After 1 (one) year, astonishingly he was fully cured by the treatment of P.O. fruiting body. Photograph is taken on 20-08-2013, mentioned in plate 3.19b.

In Plate 3.20a, Amena Begum of age 60 years of Mojumder Bari, patient ID no. 0405-171. She was suffering from few nodes of keratosis on her palm. Her both palm skin were hard. Actually she was not worried about it. But she feels pain when holding something or doing household works. Photograph is on 12-07-2012.

After 1 (one) year of treatment, she become happy, when she found the keratoses were recovered, only one spot left after removal and the skin of the palm become soften. Plate 3.20b, photograph is on 10-04-2015.

3.5.2 Results of Melanosis Development

Melanosis is one kind of brown pigments, usually found on the chest and back of arsenicosis patients. Sometimes it is found with leuco-melanosis (white pigment). Generally, melanosis is common in all arsenicosis patients, rather than leuco-melanosis and keratosis. Here we are mentioning a patient, who is severely affected with melanosis.

In Plate 3.21a, M Hanif of age 25 years of Dewan ali Bari, patient ID No. 0405-175. He is a farmer. He had severe melanosis on his chest and back, although he had no other complaints. Photograph dated 09-06-2012.

After 1 (one) year of treatment, the melanosis was reduced in many folds. After keen observation, it was found that the brightness of color of melanosis became faded, the roughness of skin became smoother. Photograph is taken on 10-04-2015, the plate no. 3.21b.

3.5.3 Results of Leuco-melanosis Development

Leuco-melanosis is white pigments found in chest, back, arms and legs of arsenicosis patients. Here mentioning a sever type of leuco-melanosis patient among other leuco-melanosis patients.

In Plate 3.22a, Rubel of age 22 years, patient ID No. 0601-161 of Jomodder Bari. He was suffering with profuse melanosis and leuco-melanosis on his back and chest. He had few keratoses on his palm with hardness of palm. It is important that in his family he is elder, he had three sisters and one brother. Recently his father was died with arsenic induced lung cancer. His mother also died with the same disease. Previously it was unknown that the actual cause of lung disease of this area. Now it is declared that the arsenicosis is endemic in this area and pandemic in Bangladesh.

After 1 (one) year of treatment, the melanosis and leuco-melanosis both are reduced in many folds. Roughness of the skin and hardness of palm are recovered, shown in Plate 3.22b.

3.5.4 Results of Black Pigmentation Development

Black pigments are special cases of arsenicosis found in China, especially on the feet, considered as black-foot disease. But in Bangladesh, we found in feet, forehead, chest, arms and back in arsenicosis patients.

In Plate 3.23a, a woman named Tohura Begum, age 24 years of Hazi Bari, patient ID No. 1014-149, photograph dated 17-02-2012. She had a round shaped black pigment with protruded crust on her forehead. No other complicacy was found.

After 5 (five) months of treatment, at the date of 17-07-2012, the crust of black pigment dried up and started falling from one side, plate 3.23b. As the round shape is demonstrated the disease ring worm, that will not cure until anti-bacterial treatment applied.

Another black pigmentation mentioned in Plate 3.24a,a male patient named Joshim of the age of 45 years of Akbor Ali Master Bari, patient ID no. 1401-189, photograph dated 22-03-2012. Usually he is ferrying vegetables in houses. He had few patches of pigments on his chest and both arms, demonstrated in photograph. The pigment of right chest was very big and bright black color with rough surfaces.

After 1 (one) year of treatment, at the date of 17-02-2013, the black pigmentations are reduced in many folds, the color of the centre of the big patch and the centre of the patch of right shoulder were changing to skin color. Other patches were mildly reduced in color. At the centre of the chest, the brown color is for the sun burn. Other few white spots of leuco-melanosis were found in plate 3.24b.

3.5.5 Results of Degenerative Changes in Blood

Perpura haemorrhagica is an exceptional case for arsenicosis found in Bangladesh. It isa rare complication of equine strangles and is caused by bleeding from capillaries which results in red spots on the skin and mucous membranes together with oedema (swelling) of the limbs and the head.

In Plate 3.25, In this case bright red spots of blood are found in palm and arms of Ferdausi, age 20 years, patient ID No. 1102-155 of Miaji Bari. She also had swelling of hands and legs.

After 1 (one) year of treatment, no gross changes were found. Red spots are remaining as usual, due to degenerative change disease in blood. But her physical and mental wellbeing are present.

3.5.6 Result of Pedal Swelling Development

Pedal swelling is one of the rare cases for arsenicosis. But found in many countries. Painless swelling of the feet and ankles is a common problem, especially among older people. Abnormal buildup of fluid in the ankles, feet, and legs can cause swelling. But that is exceptional in arsenicosis disease.

We have few cases of pedal swellings, but no good result found from the treatment with *Pleurotus ostreatus* fruiting body.

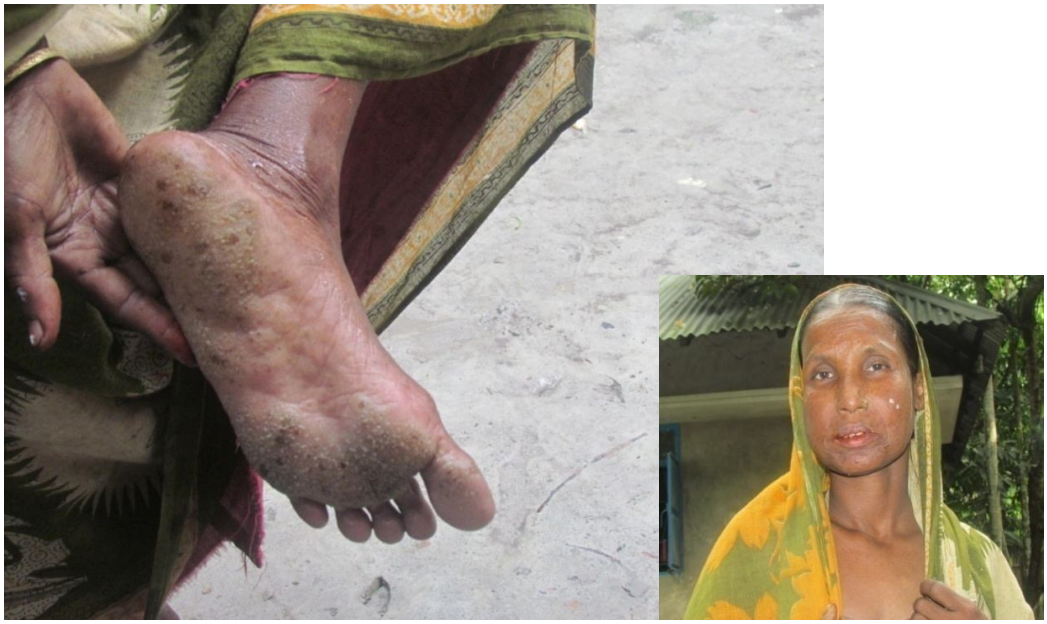


Plate-3.16a: Keratoses and ulceration on feet of a woman, named Monjuma (40) of Hazi Bari, Patient ID. No. 1016-203. Dated: 22-03-2012.

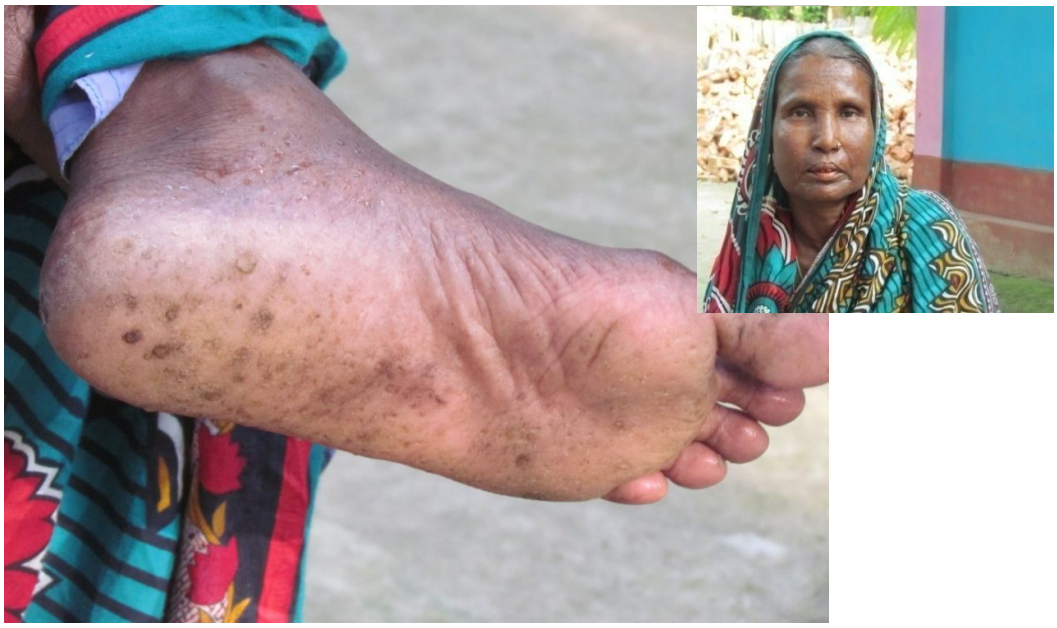


Plate-3.16b: Keratosis and ulceration of Monjuma are reduced tremendously after 1 year's of medicinal treatment, Photo on 05-09-2013.

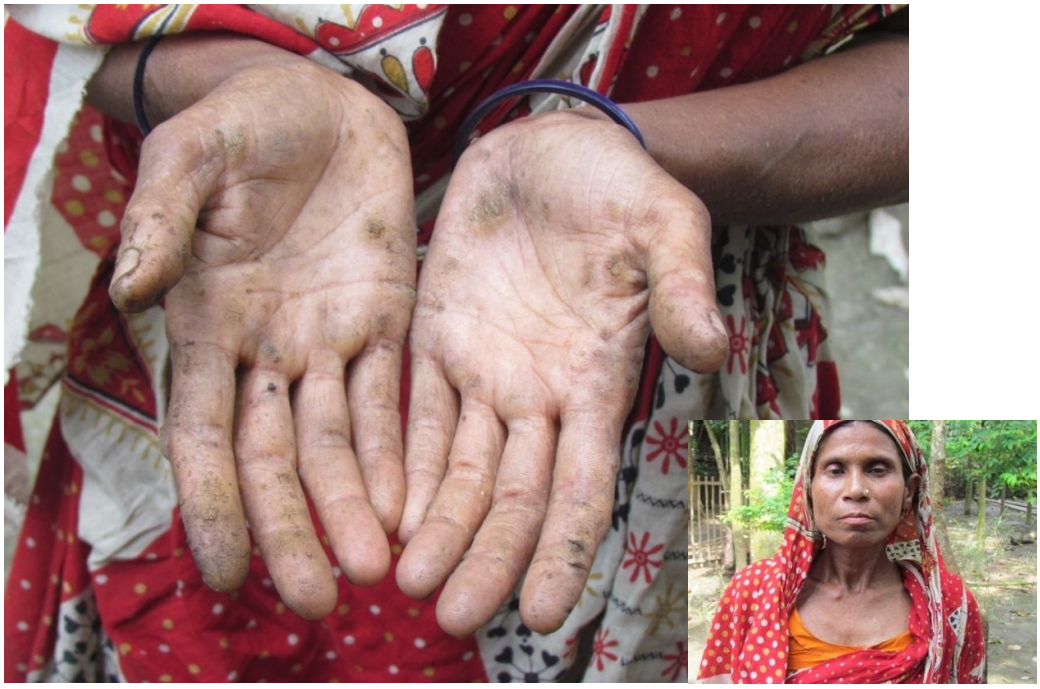


Plate- 3.17a: Keratosis and white nodes on palm of a patient, named Surjo Ban (45) of Miaji Bari, ID No. 1106-210, before treatment (Photo taken on 22-03-2012)

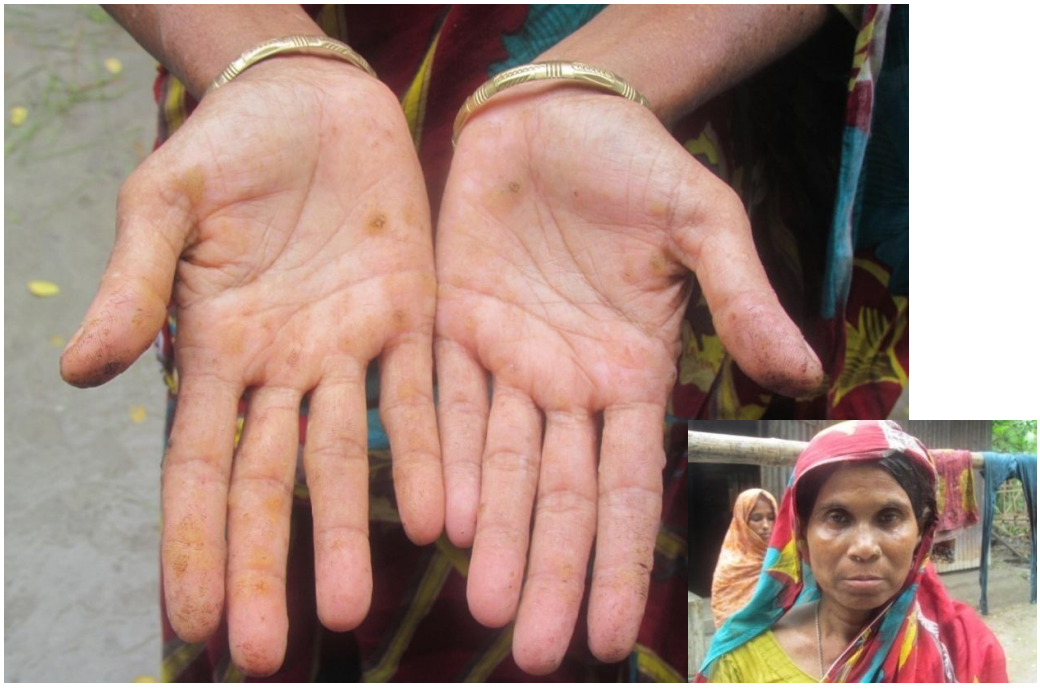


Plate-3.17b: Improvement of Keratosis and nodes of Surjo Ban are soften and reduced in many times, after 1year's of treatment (Photo taken on- 05-09-2013)

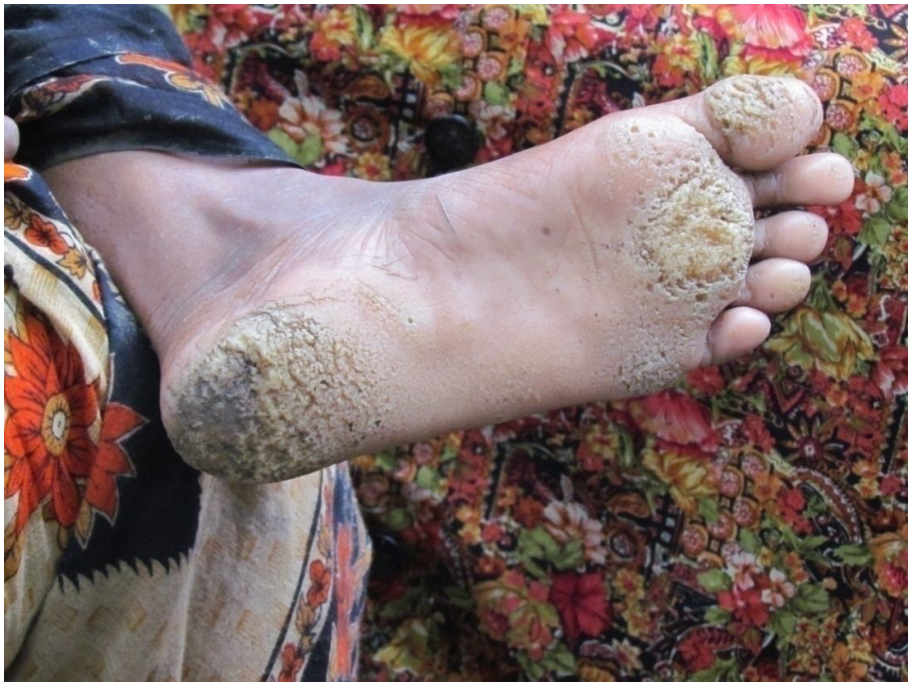


Plate-3.18a: Hyperkeratosis and wet feet ulcers on the toe of the feet of a patient, named Minoara Begum (30) of Mayeri Baper Bari, ID No.0810-091, before treatment, (Dated-22-03-2012).

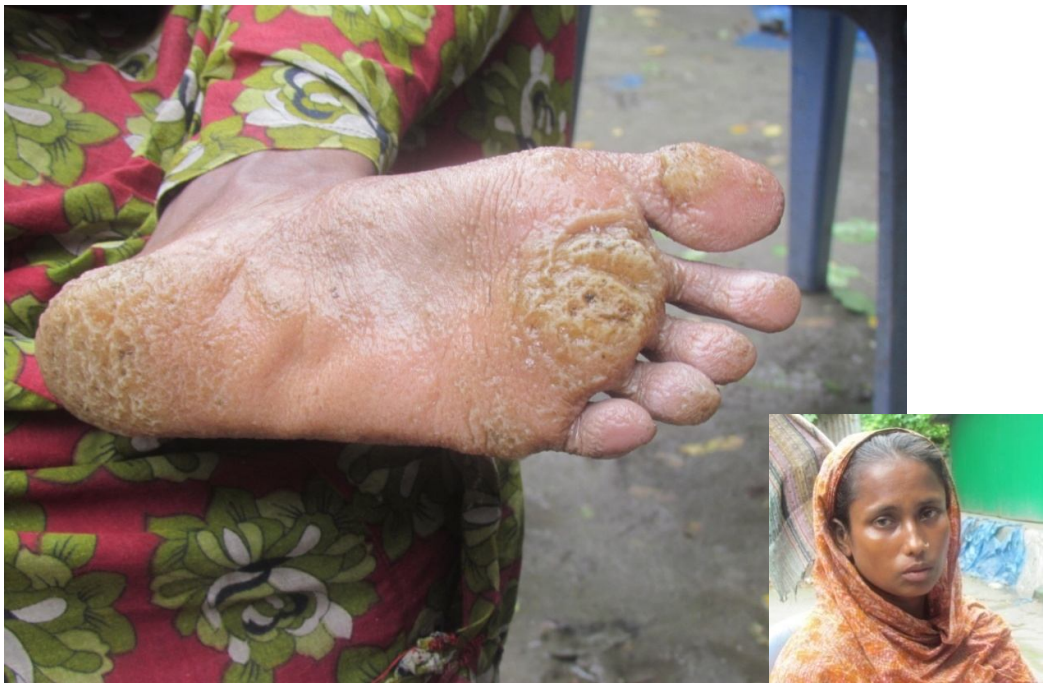


Plate-3.18b: No hyperkeratosis on the toe of the patient Minoara Begum, after 1 year treatment. Only wet feet ulcers are present in whole feet, (Dated-20-08-2013).

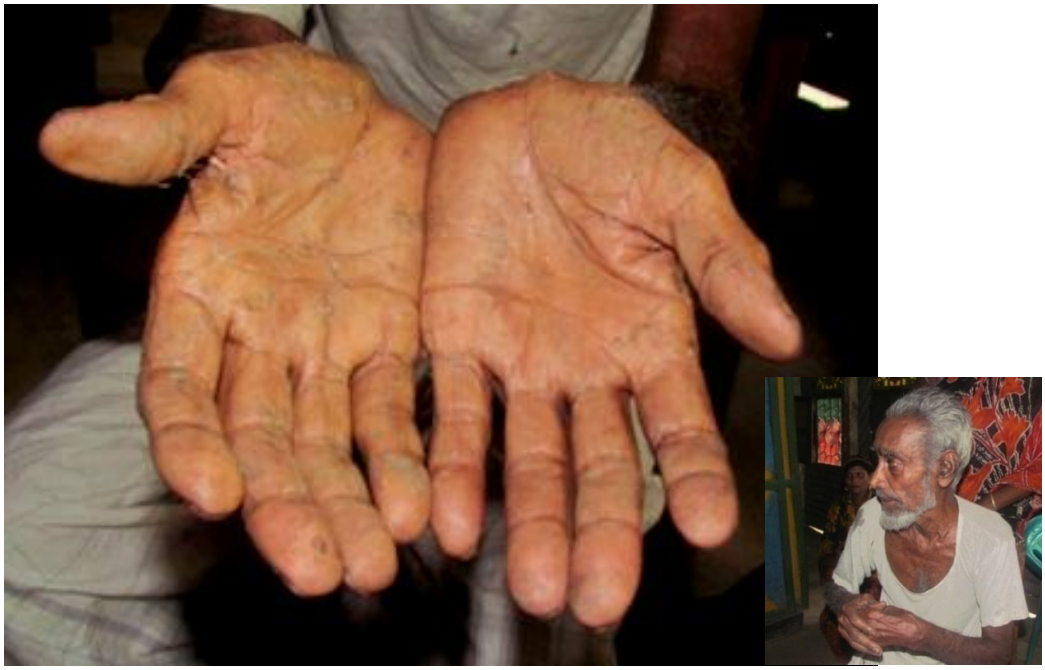


Plate-3.19a: Ulceration on the palm with skins pulling out of a Patient, named Ali Ashraf (70) of Master Bari, Patient ID. No. 0106-007, dated 21-06-2012.

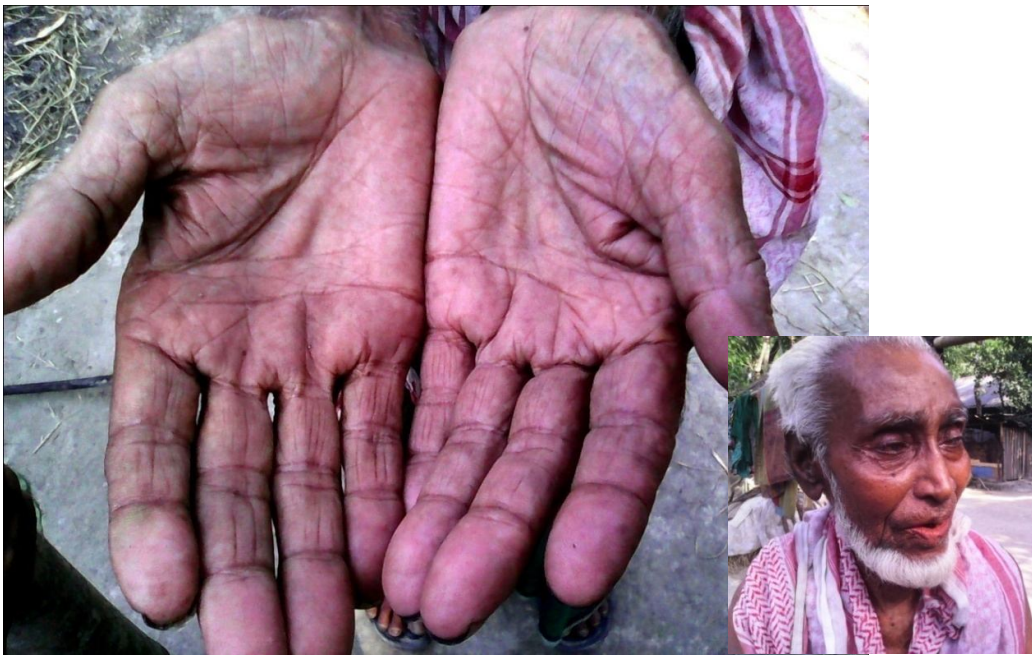


Plate-3.19b: Ulcerations are fully cured after 1 year of treatment and palms are cleaned of Ali Ashraf, dated 20-08-2013.

Plate-3.20a: Few Keratosis on the palm of left hand of Amena Begum (60) of Mojumder Bari, Patient ID. No. 0405-171. Photo at 12-07-2012.

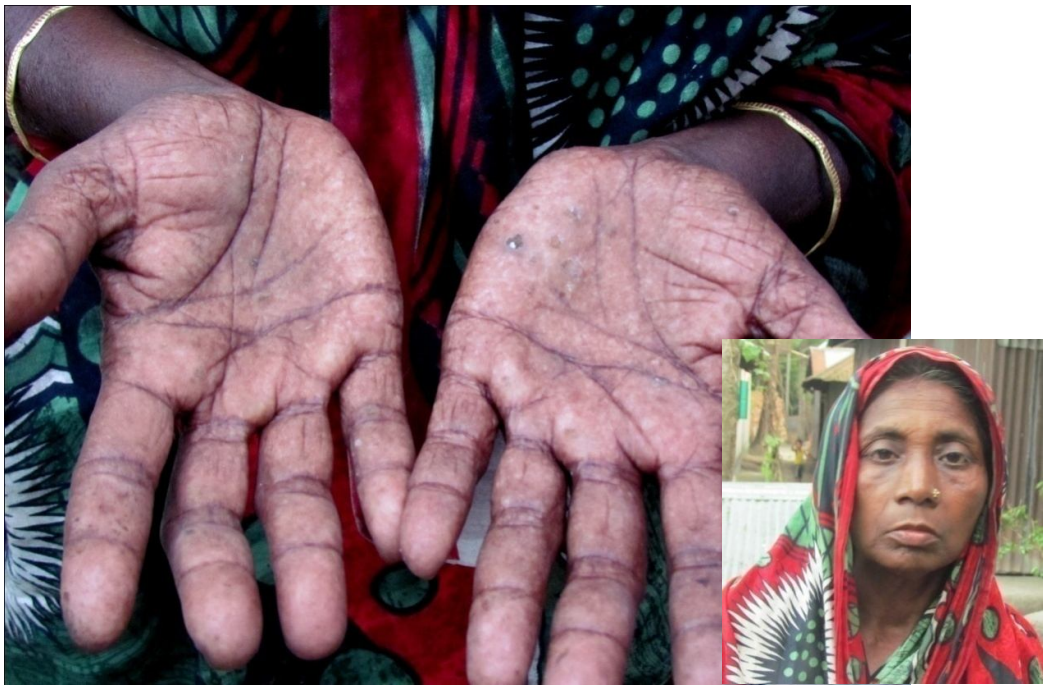


Plate-3.20b: All Keratoses are gone except one, after 1 year of treatment of Amena Begum, Photo at 10-04-2015

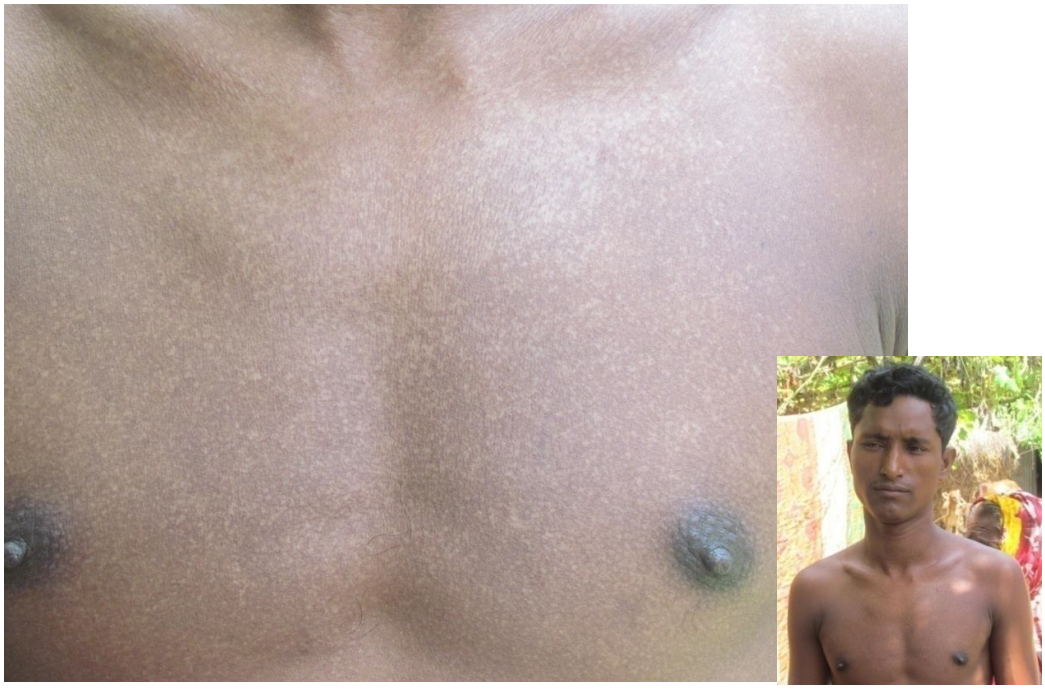


Plate- 3.21a: Profuse Melanosis on Chest of M Hanif (25) of Dewan Ali Bari, Patient ID. No. 0405-175. Photo Dated 09-06-2012.

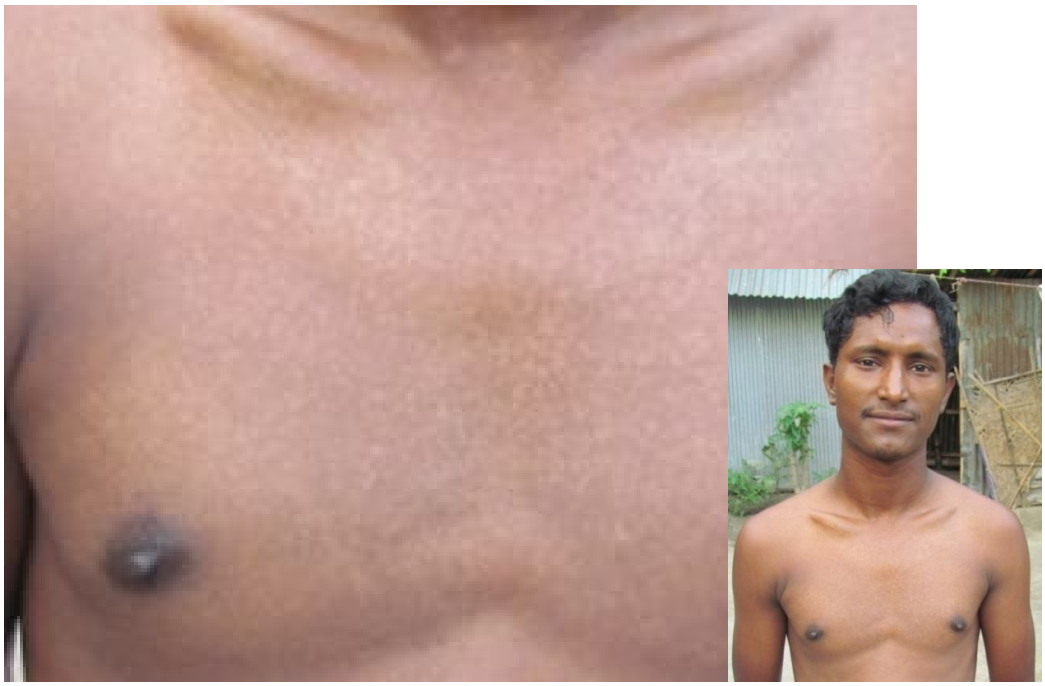


Plate-3.21b: Melanoses are reduced many fold of the chest of M Hanif, Photo at 10-04-2015.

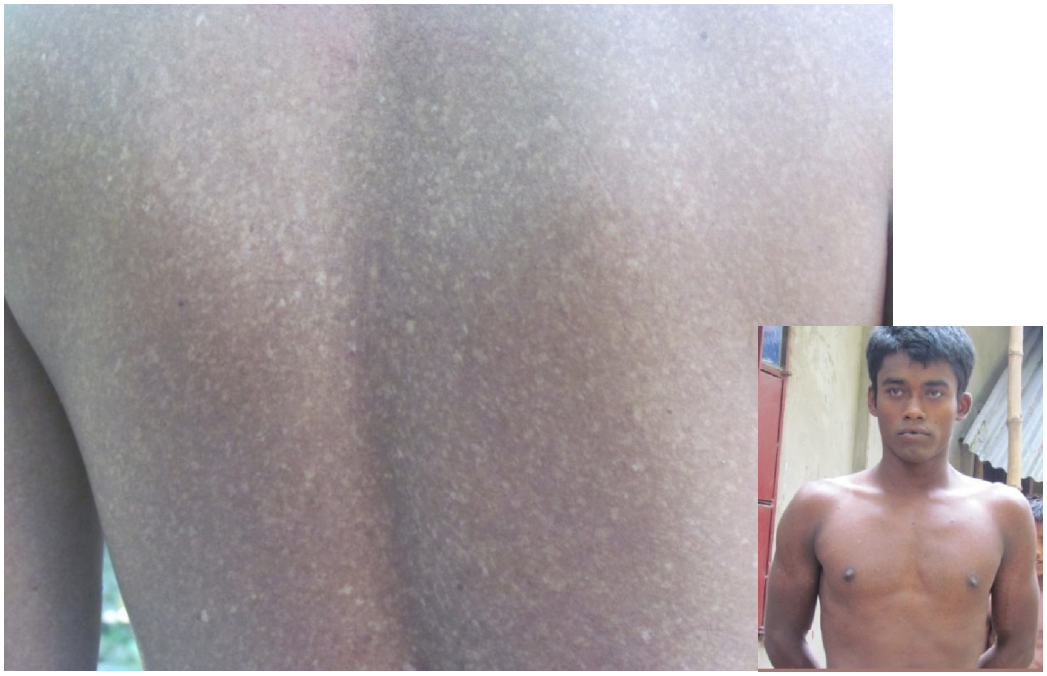


Plate-3.22a: A severe case of mixed Leuco-melanosis and Melanosis on a patient's back, named Rubel (22), Patient ID.No.0601-161 of Jomodder Bari, before treatment (Dated- 22-03-2012).

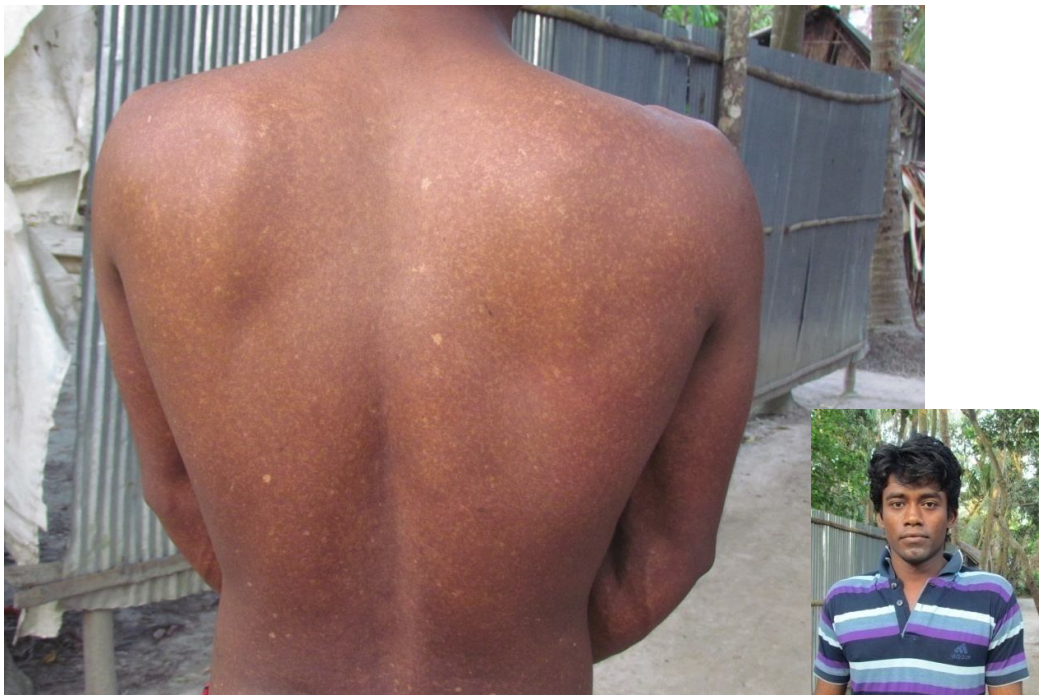


Plate-3.22b: Both Leuco-melanosis and Melanosis are present, but the intensity are reduced of Rubel, after 1 year of treatment. Photo at 10-04-2015.

Plate-3.23a: A round shaped black pigmentation on forehead of a women, named Tohura (24) of Hazi Bari, Patients ID. No. 1014-149, before treatment, dated 17-02-2012.

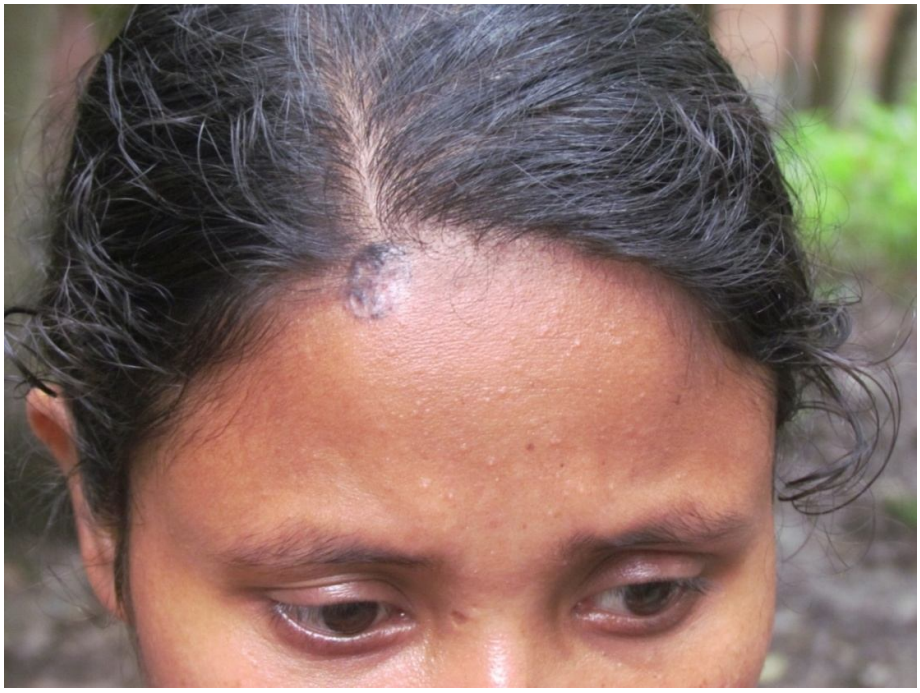


Plate-3.23b: The black pigmentation of forehead of Tohura are almost 40% recovered after 3 month of medicinal treatment (Dated- 17-07-2012)

Plate-3.24a: Few big patches of black pigmentation on chest of a male patient, named Joshim (45) of Akbar Ali Master Bari, ID No. 1401-189, Photo before treatment, (Dated- 22-03-2012)

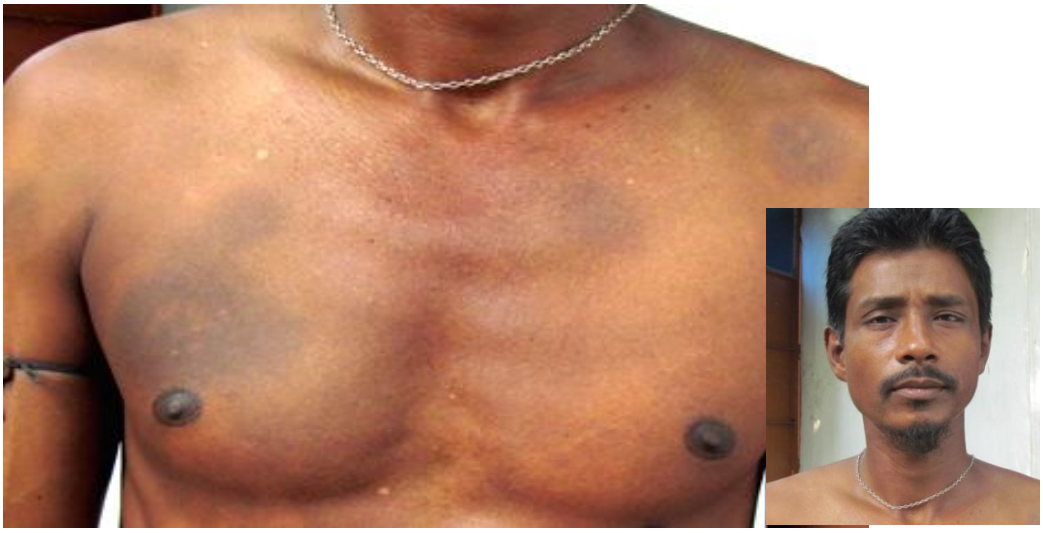


Plate-3.24b: The color of black pigments are becoming fate, after 1 year of treatment of Joshim (Photo at 17-02-2013)

Plate-3.25: Bloody red spotted accumulation (Perpura) on palms of a woman, named Ferdousi (20) of Miaji Bari, ID No. 1102-155. Photo on 22-03-2012.

Chapter Four

CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

Arsenicosis is a crucial disease in these days worldwide. The people of more than 70 countries are affected by the disease. More affected people of the countries are China, Bangladesh, Chili, Argentina, Thailand and Taiwan. The numbers of countries are increasing day by day. In Bangladesh and its adjoining part of west Bengal (India) alone, ~100 million people are at risk of arsenic induced cancer and other related diseases. It is known that long-term intake of small doses of inorganic arsenic compounds with drinking water is responsible for the disorders and cancer of lungs, liver, bladder, skin, kidney and reproductive organs cancer, and many other diseases and disorders of vital organs. There is an evidence of skin ulcer and cancer on the chest found in Sagarkandi of Pabna district and the patients are dying with this skin cancer. But the scenario of Eruine village is different. Here the people are getting affected by lung disease and lung cancer. So it is confirmed that death from cancer is the last destination of arsenic ingestion in human beings.

In this study it was highlighted the importance of vital signs in the research of arsenicosis patients and the healing of keratin cells in cutaneous tissue, as well as reducing capacity of arsenic by the mushroom *Pleurotus ostreatus* in situ. This is therefore the first comprehensive and unique documentation of the activity of mushroom upon the arsenicosis patients.

The study reveals that the arsenic in ground water level is very high in the research field of Eruaine village. People are drinking arsenic water every day without any alternative. It is important to alter the drinking water instead of ground water. Biological samples reveal that the arsenic levels of the patients are reducing by the treatment, along with drinking fresh water. Only withdrawing arsenic water is not an effective measure for the patients, where arsenic is still remaining in the body.

In the study of vital signs, it is found that the respiratory rate, pulse rate, body temperature and pH value of urine were in high range initially. That is reduced after the treatment by mushroom. But the BMI had no more importance in the case of arsenicosis.

Application of fruiting body of *Pleurotus ostreatus* as a medicine on arsenicosis patients is an exclusive research to save them. Withdrawal of arsenic water and counseling is the only manifesto of WHO to perform worldwide. Here we applied mushroom as a medicine directly upon the patients. We achieved a marvelous result from that. First- no one died during and after our research by arsenic induced cancer in the field. Second- internal as well as external symptoms of arsenicosis are vanished from the patients. Third- only those patients are affected, who are acidic.

The principal achievement of this research is that the arsenicosis is now a curable disease indeed. Not a single patient will die with arsenic induced cancer. Thus, the research is expected to be used as a guide to facilitate towards appraising the saving lives in Bangladesh as well as the arsenicosis affected people of the world, more comprehensively in future.

Though the research was constrained by time, resource, adequate replications, till then it has opened a new avenue for thinking over the problem. In this perspective, this research is only a modest beginning. Further researches should be initiated for refinement of the methods tested in this analysis.

4.2 Recommendations

Arsenicosis disease as well as arsenic induced cancer is the most life hazardous problem for the people of the world. It is necessary to cure the patients at any cost and any means. A number of recommendations are put forward in the light of these findings. These are as follows-

(i) To combat the situation, include increasing awareness of facilities and treatment for arsenicosis disease with the medicine '*Pleurotus ostreatus*' to save the people of the country from arsenic induced cancers.

(ii) Awareness should be built up and regulate the safe water to the community level.

(iii) Knowledge of proper balanced and basic in pH diet are recommended instead of only protein diet.

(iv) Community health center should be established in the arsenic rich ground water area to communicate with the people and grading them for the treatment.

4.3 Further Research

The research, based on a small sample from a single area study, has created a milestone by generating treatment and protecting the people. There are some future researches that can be done in this ground-

1. To increase the wider applicability of the research, a larger analysis all over the country is needed.
2. The same research can be done with the active ingredient of mushroom *Pleurotus ostreatus*.
3. To search a better result, it may be done with other mushrooms.

Chapter Five

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Chapter Six

APPENDICES

Appendix-I

Operation of minor irrigation equipment, 1976/77–2007/08

Year	Shallow tubewells	Deep tubewells	Low lift power
pumps	(000)	(000)	(000)
1976/77	7	4.5	35
1982/83	93	13.8	38
1987/88	189	20.3	51
1989/90	260	22.6	51
1991/92	309	25.5	50
1994/95	489	26.7	57
1998/99	736	26.7	73
2004/05	1129	27.2	99
2006/07	1203	29.2	107
2007/08	1305	31.3	139

Source: Government of Bangladesh, 2008.

Appendix-II

Key statistics for arsenic situation in Bangladesh by UNICEF (2008)

Basic Data	Number	Percentage
Estimated number of tube wells in Bangladesh	8,600,000	100
Tube wells tested for arsenic	4,750,000	55
Tube wells marked green (safe)	3,300,000	39
Tube wells marked red (unsafe)	1,400,000	16
Estimated total villages in country	87,319	100
Villages screened 54,041 62	54,041	62
Villages where < 40% of the wells are contaminated	70,610	81
Villages where 40-80% of the wells are contaminated	8,331	10
Villages where 80-99% of the wells are contaminated	6,062	7
Villages where ALL wells contaminated	2,316	3
Actions taken by people to avoid arsenic contamination*		
Using arsenic free tubewell water		55
Using treated pond, canal or river water		21
Using filtered water		5
Using rain water tanks or sand filtered water		5
No action		32

Appendix-III

Characteristic clinical and laboratory criteria for diagnosis of arsenicosis	
Clinical (Cutaneous) Manifestations	
1. Melanosis	**a.** Fine freckled or spotted pigmentation (Rain drop pigmentation) **b.** Diffuse or generalized hyperpigmentation **c.** Guttate hypopigmentation on normal or hyperpigmented background (Leucomelanosis) **d.** Mucosal pigmentation (esp. oral mucosa)
2. Keratosis	**a.** Mild: Minute papules (<2mm) with slight thickening of palms and soles associated with grit-like texture detected primarily by palpation **b.** Moderate: Multiple keratotic papules (2-5mm) present symmetrically on palms and soles. **c.** Severe: Large discrete papule (>5mm) or conß uent keratotic elevation with nodular, wart-like or horny appearance.
3. Malignant/ Pre-malignant lesions:	**a.** Bowen.s disease (Squamous cell carcinoma in-situ): Multi-centric Bowen.s disease in non sunexposed areas **b.** Squamous cell carcinoma and Basal cell epithelioma

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Appendix-IV

Systemic manifestations in chronic arsenicosis

Systems	Category	Clinical manifestations
Respiratory	Major	1. Chronic bronchitis 2. Pulmonaryfibrosis 3. Bronchiectasis 4. Chronic obstructive airwaydisease
Hepato-biliary	Major	1. Non-cirrhotic portal fibrosis 2. Cirrhosis of liver(± ascites) 3. Portal Hypertension 4. Hepatocellular carcinoma (very rarely)
Neurological	Major	Predominantly peripheral sensory neuropathy
Peripheral vascular	Major	1. Raynaud.s phenomenon 2. Cramp 3. Claudication 4. Gangrene
Gastro-intestinal	Minor	1. Anorexia 2. Nausea 3. Chronic diarrhea
Endocrinal	Minor	Diabetes mellitus
Cardiac	Minor	1. Ischemic heart disease 2. Arrythmia 3. Hypertension
Ocular	Minor	Conjuctivitis (congested/injected eye)
Renal	Minor	1. Proximal tubule degeneration 2. Papillary and cortical necrosis 3. Renal carcinoma (very rarely)
Obstetrical	Minor	Spontaneous abortion/ Still birth/ Pre-term birth
Hematological	Minor	Anemia/ Leucopenia/ Thrombocytopenia
Constitutional	Minor	1. Weakness/ Headache 2. Pedal edema

Appendix-V

PATIENTS FORMAT

'DETERMINATION OF THE EFFECT OF MUSHROOMS ON ARSENICOSIS PATIENTS BY ANALYZING PHYSICO-CHEMICAL AND BIOLOGICAL PARAMETERS'

A PhD Research of the Institute of Environmental Science, University of Rajshahi

Researcher

M. Jahangir
PhD Research Fellow
Roll no.10201, Session. 2010-11
Institute of Environmental Science
University of Rajshahi

Supervisor

Dr.Md. Redwanur Rahman
Associate Professor
Institute of Environmental Science
University of Rajshahi

Institute of Environmental Science
University of Rajshahi

PATIENT AND NON-PATIENTS DATA IN ARSENIC AFFECTED AREA

Date- ……..

1. Identity (Patient / Non-Patient): Serial no. /

1. Name-……(M / F) (M /U) Age-……..
2. Father / Husband's Name-……..……
3. Address-……..
   ……
4. Occupation-……..Income (Monthly)……..
5. Telephone-…….Mobile-……..
6. Total family members and serial no.'s-(……)……./……./……/
   ……../……../……../……/……/……../……..
7. Arsenicosis affected members sl.no.- ……/……/……/……../

2. Drinking Water Quest:

1. Tube well (Deep / Shallow) / Well / Pond / River / Pipe line / Bottle / ……...
2. Duration of Consuming Tubewell Water-……..
3. Tubewell water Analysis for Arsenicosis- ……...

3. Physical Quest:

1. Blood Pressure- ……..	2. Pulse Rate- ……
3. Temperature- ……..	4. Respiration Rate- ……...
5. Height- ……...	6. Weight- ……
7. BMI- ……..	

4. Chemical Quest:

1. **pH value**- Blood serum- ……Urine- ……

5. Patient's Symptoms:

a) Habit and Mind-

Habit- Smoking / Tea / Coffee / Pan / Jorda / Alcohol ……(Duration-…….) Drinks water- More / Normal / Less	**Mind-** Anxiety / Cool /Quarrelsome Sleep- Better / Cat's nape / Insomnia Weakness- Physical / Mental / Both

b)Skin (Before Treatment)-

A. Melanosis : Duration-…….
   Spotted (Rain-drop)……...
   Diffuse…….
   Leuco-melanosis ……...
   Mucosal ……
B. Keratoses: Duration-…….
   Punctate (Pitted) keratoses ……..……..
   Diffuse (Hyper-) keratosis ……
C. Ulceration on ……..…….... Duration-……
D. Cancer in/on ……..……... Duration-……

c) Other Complicacy (Before Treatment):

1. Respiratory- ……..... …. Duration ……...
2. Cardiac- ……..……..... Duration …….
3. Renal- ……..……..……..... Duration …….
4. Hepato-biliary …… …..……..... Duration …….
5. Gastro-Intestinal- ……..……..Duration …….
6. Neurological- ……. Duration …….
7. Male genital / Obstetrical-...…..……..……. Duration ……..……..
8. Endocrinal- ……..... Duration …….
9. Ocular- …… Duration …….
10. Hematological- ……..Duration …….
11. Others (Wasting)- …… Duration-……...

6. Arsenic Analysis by AAS (Before Treatment):

Nail-	Hair-

7. Administration of Mushrooms and Other:

1. Mushroom- Reishi / Shitake / White Button / Oyster / Or- Placibo
2. Dose- ( ) 5 Capsules / Day, Or- ( ) 3 gm. / thrice daily or twice daily
3. Starting Date- ……...
4. Cessation (Temporary / Permanent), if any- ……
5. End Date- ……..
6. Gross Result- ……..

8. a) Skin (After Treatment)-

Skin- Melanosis ……. (T I / P I / Un)* / Leucomelanosis ……. (T I / P I / Un)* / Keratosis .. ……. (T I / P I / Un)* / Hyperkeratosis … ……..... (T I / P I / Un)* / Ulceration .. ……. (T I / P I / Un)* / Cancer ... ……..…(T I / P I / Un)*

***** T I=Total Improved; P I= Partial Improved; Un= Unchanged

b) Other Complicacy (After Treatment):

1. Respiratory-
   ……..…….....
2. Cardiac-
   ……..……..…….....
3. Renal-
   ……..……..…….....
4. Hepato-biliary ……
   …..……..…….....
5. Gastro-Intestinal-
   ……..……..……
6. Neurological-
   ……..……
7. Male genital / Obstetrical-
   ...…..……..…….....
8. Endocrinal-
   ……..…….....
9. Ocular- ……
10. Hematological- ……

9. Arsenic Analysis by AAS (After Treatment):

Nail-	Hair-

10. Questionnaire for Interviewee:

1	If your drinking / cooking water is not arsenic safe, then why you are still consuming?	1. Safe water is far away 2. Water is not of good quality 3. Option is not under operation 4. No arsenic safe TW 5. There will be no disease by the grace of God 6. Others (specify----------)
2	Are you or any member of your family suffering from arsenicosis?	1. Yes 2. No 3. No response
3	If yes, what are the manifestations? (do not read answers)	1. Dark spots on skin 2. Wart-like hardening of palm/sole 3. Ulcer 4. Gangrene 5. Cancer 6. Other(specify)
4	Who has detected you / your family member as arsenicosis patient?	1. Relative 2. Neighbor 3. Government health staff 4. NGO staff 5. Researcher 6. Others-
5	Have you/your family member got any treatment for arsenic problem?	1. Yes 2. No 3. No response
6	If no, mention the reason (please mention three important reason)	1. 2. 3.
7	Does female patient face any problem during treatment?	1. Yes 2. No 3. Don't know 4. No response
8	If yes, specify the problems (please mention three important problems)	1. 2. 3.
9	Have you face/ faced any economic problem for this disease?	1. Yes 2. No 3. Don't know 4. No response
10	If yes, please mention three important problems	1. 2. 3.
11	Have you face/ faced any social problem because of this disease?	1. Yes 2. No 3. Don't know 4. No response
12	If yes, please mention three important problems	1. 2. 3.

13	Did you or any family member face any problem during receiving the health care for arsenicosis treatment?	1. Yes 1. No 2. No response
14	If yes, please mention three important problems.	1. 2. 3.
15	What type of treatment you are availing /availed	1. Local pharmacy 2. Village doctor 3. Govt.Hospital 4. Homoeopathy 5. Unani 6. Aurvedy 7. NGO 8. University Researcher 9. Other_____ 10. No where
16	What are the reasons for taking treatment from other than government or NGO facility (mention the important three)	1. 2. 3.
17	What kind of treatment you are receiving or received?	1. Vitamins 2. Vitamins + ointment 3. Surgery 4. Cancer therapy 5. Herbal Medicine 6. Homoeopathic Medicine 7. Research drugs 8. Other_____
18	How much times you are receiving the treatment?	1. Months_____ 2. Years _____
19	What result you have achieved?	1. Better 2. Partial recovery 3. As usual

Patients Name (Signature):

Interviewer:

MONTHLY RESULT CHART

1. MONTH:

A. Skin:

1. Total Improved ……
2. Partial Improved ……..
3. Unchanged ……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

2. MONTH:

A. Skin:

1. Total Improved ……
2. Partial Improved ……
3. Unchanged ……

B. Other Complicacy:

1. ……
2. ……
3. ……
4. ……
5. ……
6. ……

3. MONTH:

A. Skin:

1. Total Improved ……
2. Partial Improved ……
3. Unchanged ……

B. Other Complicacy:

1. ……

2. ……
3. ……
4. .....……
5.……..
6. ……..

4. MONTH:

A. Skin:

1. Total Improved ……
2. Partial Improved ……..
3. Unchanged ……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

5. MONTH:

A. Skin:

1. Total Improved ……
2. Partial Improved ……..
3. Unchanged ……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

6. MONTH:

A. Skin:

1. Total Improved ……
2. Partial Improved ……..
3. Unchanged ……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

Researcher's Remark:

……
……
……
……
……

7. MONTH:

A. Skin:

1. Total Improved ……
2. Partial Improved ……..
3. Unchanged ……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

8. MONTH:

A. Skin:

1. Total Improved ……
2. Partial Improved ……..
3. Unchanged ……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

9. MONTH:

A. Skin:

1. Total Improved ……
2. Partial Improved ……..
3. Unchanged ……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

10. MONTH:

A. Skin:

1. Total Improved
……
2. Partial Improved
……..
3. Unchanged
……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

11. MONTH:

A. Skin:

1. Total Improved
……
2. Partial Improved
……..
3. Unchanged
……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

12. MONTH:

A. Skin:

1. Total Improved
……
2. Partial Improved
……..
3. Unchanged
……...

B. Other Complicacy:

1. ……..
2. ……..
3. ……..
4. ……..
5. ……..
6. ……..

Researcher's Remark:

……
……
……
……
……

Appendix-VI

Lung capacities in healthy adults

Volume	Litres		Derivation
	In men	In Women	
Vital capacity	4.6	3.1	IRV plus TV plus ERV
Inspiratory capacity	3.5	2.4	IRV plus TV
Functional residual capacity	2.3	1.8	ERV plus RV
Total lung capacity	5.8	4.2	IRV plus TV plus ERV plus RV

Appendix-VII

Average lung volume in healthy adults-

Volume	Values (Litres)	
	In Men	In Women
Inspiratory reserve volume (IRV)	3.0	1.9
Tidal volume (TV)	0.5	0.5
Expiratory reserve volume (ERV)	1.1	0.7
Residual volume (RV)	1.2	1.1

Appendix-VIII

Different units for measuring BMI

SI units	$\mathrm{BMI} = \frac{\mathrm{mass\ (kg)}}{(\mathrm{height(m)})^2}$
Imperial/US Customaryunits	$\mathrm{BMI} = \frac{\mathrm{mass\ (lb)} \times 703}{(\mathrm{height(in)})^2}$
	$\mathrm{BMI} = \frac{\mathrm{mass\ (lb)} \times 4.88}{(\mathrm{height(ft)})^2}$
	$\mathrm{BMI} = \frac{\mathrm{mass\ (st)} \times 9840}{(\mathrm{height(in)})^2}$

Appendix-IX

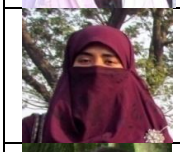
BMI values in different categories

Category	**BMI range – kg/m^2**	**BMI Prime**	**Mass (weight) of a 1.8 metres (5 ft 11 in) person with this BMI.**
Severely underweight	less than 16.0	less than 0.66	less than 51.8 kilograms (8.16 st; 114 lb)
Underweight	from 16.0 to 18.5	from 0.66 to 0.73	between 51.8 and 59.9 kilograms (8.16 and 9.43 st; 114 and 132 lb)
Normal	from 18.5 to 25	from 0.74 to 0.99	between 59.9 and 81.0 kilograms (9.43 and 12.76 st; 132 and 179 lb)
Overweight	from 25 to 30	from 1.0 to 1.19	between 81.0 and 97.2 kilograms (12.76 and 15.31 st; 179 and 214 lb)
Obese Class I	from 30 to 35	from 1.2 to 1.39	between 97.2 and 113.4 kilograms (15.31 and 17.86 st; 214 and 250 lb)
Obese Class II	from 35 to 40	from 1.4 to 1.59	between 113.4 and 129.6 kilograms (17.86 and 20.41 st; 250 and 286 lb)
Obese Class III	over 40	over 1.6	from 129.6 kilograms (20.41 st; 286 lb)

Appendix-X

Data collectors and other field assistants worked in this study group

Participant physicians in this arsenicosis research are considered essential for identifying an arsenicosis patient, filling the questionnaire of the patients, household survey, testing tube-wells for arsenic analysis and analyzing different vital signs of the patients.
Local participants in the field are the full time visitors in the field. They are inhabitant of the village. They have the close relation with the patients. They are giving time to time reports of the patients to the researcher.

Photographs	Name and Address	Qualification	Contribution in Research
	Dr. M. Aminul Islam	BHMS(DU)	Worked in the field as a Research assistant.
	Dr. Yasir Arafat Arif Billah	BHMS(DU)	Worked in the field as a Research assistant.
	Dr. Ruhul Amin Rahi	BHMS(DU)	Worked in the field as a Research assistant.
	Dr. Sobuj-Al-Mamun	BHMS(DU)	Worked in the field as a Research assistant.
	Dr. Selina Jahan	DHMS	Worked in the field as a Research assistant.
	Dr. Amanullahis Sakira	BHMS(DU)	Worked in the field as a Research assistant.
	Dr. Akhteruz Zaman Ovi	BHMS(DU)	Worked in the field as a Research assistant.
	Morshed Alam	B.Com	Worked as a Field worker.
	Rahima Begum	Eight	Worked as a Field worker.

Appendix-XI

WORK PLAN

Sl.No.	Works	Time Duration
01	Site selection and Field survey	September, 2011-October, 2011
02	Work Plan and Questionnaire Making	November, 2011
03	Questionnaire Filled up	December, 2011-March, 2012
04	Vital Sign analysis	January, 2012- April, 2012
05	Drinking Water analysis	May, 2012 – July, 2012
06	Biological samples Collection and Analysis (Before Treatment)	August, 2012 – September, 2012
07	Sensitivity Test Program (STP)	October, 2012 – December, 2012
08	Application of Medicine and Symptoms collection	January, 2013 – December, 2013
09	Biological samples Collection and Analysis (After Treatment)	January, 2014 – February, 2014
10	Thesis Writing and References Collection	March, 2014 – October, 2014
11	Academic Preparation and Pre-Submission Seminar	November, 2014 – December, 2014
12	Thesis Analysis and Submission	January, 2015 – June, 2015