

**ASSESSMENT OF PRESENCE OF HEAVY METALS IN
THE BODY FLUIDS OF FEMALES IN SELECTED
DISTRICTS OF BIHAR**

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Forensic Science

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2026

DECLARATION

I, hereby declared that the presented work in the thesis entitled “**Assessment of Presence of Heavy Metals in the Body Fluids of Females in selected districts of Bihar**” in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision **Dr. Tejasvi Pandey**, working as **HoD & Assistant Professor**, in the **Department of Forensic Science/School of Bioengineering & Biosciences** of **Lovely Professional University, Punjab, India**. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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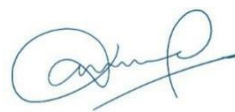
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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled “**Assessment of presence of Heavy Metals in the Body Fluids of Females in selected districts of Bihar**” submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the **Department of Forensic Science/School of Bioengineering & Biosciences**, is a research work carried out by **Radhika Agarwal, 12208293**, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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Abstract

Heavy metal pollution in the Gangetic plains of Bihar, India, has emerged as significant public health crisis, particularly due to chronic exposure to arsenic and lead. These toxic metals pose severe health risks including organ damage, neurological disorders, reproductive system dysfunction, endocrine disruption, and elevated cancer risk. This study, conducted across five arsenic and lead prone districts—Samastipur, Begusarai, Khagaria, Munger, Darbhanga, Katihar, Bhagalpur and control Nalanda (only—presents first evidence of biomagnification of arsenic and lead in lactating mothers and subsequent transmission through breast milk to infants. The research investigates the levels of heavy metals in environmental sources water & staple foods (rice, wheat & potato), biological samples (blood, urine, breast milk), and assesses associated health risks, particularly focusing on mother-infant health. A total of 200 exposed and 50 control maternal samples were analyzed using standardized toxicological methods, while water and food samples were evaluated to identify exposure pathways. Arsenic toxicity was detected in 12% of water samples from Samastipur, Darbhanga, Katihar and Begusarai, with the highest water arsenic concentration reaching 257.5 µg/L, well above the WHO admissible limit of 10 µg/L and 88% samples of water were below acceptable limit. Interestingly, all water samples from Khagaria, Munger, and Nalanda remained within safe limits, indicating that water was not the sole exposure route. Arsenic levels in blood samples showed 76.5% of exposed mothers had values exceeding permissible limits, with maximum level of 379.6µg/L, while 94.5% of breast milk samples in the exposed group contained arsenic concentrations above 1 µg/L, peaking at 458.8µg/L. Similarly, 91% of maternal urine samples exceeded the acceptable threshold of 50µg/L with highest arsenic content of 671.8 µg/L, indicating bioaccumulation & chronic exposure. The source of contamination was attributed primarily to basic foods- wheat, potatoes and rice which absorbs arsenic from contaminated soil and irrigation water, revealing clear evidence of food chain-mediated bio-magnification. In contrast to earlier beliefs that breast milk largely protects infants from arsenic, this study demonstrates significant arsenic transmission via lactation, resulting in high infant exposure. Lead contamination was found in 85.5% of the collected household water samples. In biological samples, it was found to be even more alarming. Among exposed mothers,

85.5% had blood lead levels above 50µg/L, with a maximum value of 1724µg/L in exposed area and in Nalanda it was found in 34% of the blood samples. In breast milk, 94% of the samples surpassed the limit (5 µg/L), with concentrations reaching up to 1309 µg/L in exposed area and in Nalanda 68% of the breastmilk samples were beyond permissible limit. Urine samples showed 71.5% of mothers had lead levels beyond the permissible threshold, with peak levels at 4168 µg/L and in Nalanda, 38% of the urine samples were found above the WHO permissible limit. Household water sources showed lead concentrations within safe limits, indicating that contaminated food, likely from geogenic and anthropogenic soil sources, was the major route of lead exposure. GIS mapping of the affected districts supported this finding and submitted to the government. The lead contamination is also potentially linked to cosmetic adulteration, pesticide residues, and Ayurvedic medicine, with additional risk from industrial activities such as battery recycling and e-waste exposure. Hormonal analysis in exposed mothers revealed significant endocrine disruptions. Estrogen levels were above the normal range (9–289 pg/ml) in 33.5% of samples and below in 9.5%, while progesterone levels were abnormal in 15% of cases, and thyrotropin (TSH) was outside permissible limits in 10% of cases. These hormonal imbalances, influenced by chronic exposure to arsenic, cadmium, mercury, and lead, may adversely affect reproductive aging, ovarian reserve, and thyroid function. Health risk assessments revealed severe consequences for both mothers and their children. Hazard Quotient (HQ) analysis showed that 87% of exposed lactating mothers had HQ values above 1, indicating non-carcinogenic health risks (cardiovascular disease, hepatomegaly and enlargement of spleen, peripheral neuropathy, diabetes, mutilation of the portal vein and cognitive impairment), while Incremental Lifetime Cancer Risk (ILCR) values exceeded the threshold of 0.0001, confirming elevated cancer risk. Children were at even greater risk due to their developmental vulnerability and their reliance on maternal nutrition through breastfeeding. The presence of heavy metals in infants' biological systems, particularly through contaminated breast milk, directly correlated with maternal exposure, creating an alarming scenario of early-life toxic metal accumulation. Arsenic and lead exposure in children has been shown to reduce IQ, impair memory, and damage the nervous system. The current study's findings are consistent with international research linking heavy metal exposure to endocrine

disruption, reproductive challenges, cognitive deficits in children, and carcinogenic effects. The study also confirms a descending order of heavy metal contamination in breast milk across districts: Khagaria > Begusarai > Samastipur > Darbhanga > Munger > Bhagalpur > Katihar > Nalanda. The findings clearly underscore the urgent need for public health intervention. The contamination of breast milk—the primary source of nutrition for infants—poses a dire threat to early childhood development, with long-lasting implications on physical and cognitive health. Chronic exposure in mothers, combined with hormonal imbalance and carcinogenic risk, represents a major public health burden. The study highlights the need for comprehensive, multisectoral policy measures at both the state and central government levels. Recommendations include the provision of arsenic- and lead-free drinking water through community filtration systems, regular screening of food and soil for contaminants, public awareness campaigns on the dangers of contaminated water, food, and cosmetics, and targeted medical interventions for high-risk populations. There is also a critical need to regulate agricultural practices, control industrial emissions, and monitor local markets for adulterated food and cosmetic products. Continued bio monitoring, environmental surveillance, and focused health risk assessments must be institutionalized to safeguard future generations. Hence, this study presents compelling and unprecedented evidence of arsenic and lead bio magnification in mothers and infants residing in the Gangetic plains of Bihar. The transmission of these toxic metals via breast milk directly links maternal exposure to infant health hazards, highlighting an urgent need for immediate action. The study findings provide a scientific basis for formulating targeted health and environmental policies to provide contamination-free water and food for vulnerable population in the rural areas of different districts of Bihar by the government of India and state of Bihar.

Keywords: heavy metal toxicity, female health, chronic exposure, health risks

Acknowledgement

Coming to the end of this work it gives me an earnest pleasure to express my gratitude to all those who have made this thesis a reality and I will always be indebted to all of them....

Above all, I thank God for showering his merciful blessing on me to complete my thesis.

First of all, I am grateful to my HoS, Dr. Neeta Raj, School of Bioengineering & Biosciences. I am profoundly and everlastingly thankful to my supervisor, Prof. (Dr.) Tejasvi Pandey, Head of Department (Forensic Science), School of Bioengineering and Biosciences, Lovely Professional University, Punjab for her invaluable guidance and support during my PhD journey. It has been my privilege to have worked under her valuable and inspiring guidance. I am very deeply thankful to her for teaching a systematic, precise and basic approach to do research work with punctuality, dedication and discipline.

I would also like to acknowledge Prof. (Dr.) Ashok Ghosh, HoD, Research Department, Mahavir Cancer Sansthan & Research Centre, Patna, Bihar for allowing me to work in Research center. I would also like to extend my sincere appreciation to my co-supervisor, Dr. Arun Kumar, Senior Scientist, Department of Research, Mahavir Cancer Sansthan & Research Centre, Patna, Bihar for giving me an opportunity to work under their perceptive guidance that I got during the research period. His expertise, valuable support and cooperation have enriched my research experience that allowed me to thrive for learning. I am truly fortunate to have had the opportunity to work under his supervision. His insightful feedback, constructive criticism and encouragement have been instrumental in my growth as researcher.

I would also like to thank Dr. Mohammad Ali, Senior Scientist, Dr. Abhinav and Dr. Sneha Navin for innate support and mentorship throughout my PhD journey at Mahavir Cancer Sansthan & Research Centre.

I warmly thank to all the research assistants, Shrinu, Neeraj, Mukesh, Prashant & Manishankar at Mahavir Cancer Sansthan & Research Centre for their valuable contributions to the project. Their hard work and commitment have significantly contributed to the success of our research endeavors. A very big thank for teaching me the techniques related to my experiment.

I also extend my appreciation to my lab colleagues, Pratima, Kanhaiya, Kanchan, Deokrishna, Nirmal, Shivam, Nikhil, Pushkar, Mariam & Vidya at Mahavir Cancer Sansthan & Research Centre, for their enthusiasm and collaborative spirit. Working alongside such dedicated individuals has enhanced the overall research environment.

I also would like to thank my college colleagues, Mohd. Hamza, Deeksha & Chitra in forensic department, Lovely Professional University. Your camaraderie and support have created a positive and motivating atmosphere in the college, making the research journey both enjoyable and fulfilling.

Thanks to all the staff of Lovely Professional University and Mahavir Cancer Sansthan & Research Centre for their helping nature.

The work would not have been possible without the sacrifices, support, love and constant cooperation of my parents throughout my academic journey appreciable. They have been pillars of my strength in all highs and low and encouraged me always. I am truly blessed to have you both as my parents.

A very big thanks my sister & my brother-in-law Shivani & Arpit and my brother Mayank for their constant support, understanding, shared joys and my little nephew Sankalp for his innocence and love have been great source of strength for me. Their presence in my life has made my every achievement more meaningful and grateful for the bond we share. I am truly blessed to have a supportive family like this.

Lastly, I want to show my gratitude to **Indian Council of Medical Research (ICMR)** to provide me this opportunity to work under the esteemed project titled “**Assessment of female reproductive health and child health risks in heavy metal exposed population of Bihar**” with project grant no. **F.No. 5/10/FR/79/2020-RBMCH (I.D No. 2020-3381)**.

Ms. Radhika Agarwal

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List of Abbreviations

As- Arsenic	GIS- Geographic information system
Pb- Lead	WMA- World Medical Association
PCOS- polycystic ovarian syndrome	SPSS- Statistical Package for Social Sciences
WHO- World Health Organization	GF-AAS- Graphite Furnace Atomic Absorption Spectrophotometer
GMB- Ganga-Meghna-Brahmaputra	ELISA- Enzyme Linked Immunosorbent Assay
UPR- Unfolded Protein Response	HCl- Hydrochloric Acid
DNA- Deoxyribonucleic Acid	HNO ₃ - Nitric Acid
ALT- Alanine Transaminase	EDTA- Ethylenediaminetetraacetic acid
ALP- Alkaline Phosphatase	HClO ₄ - Perchloric Acid
AST- Aspartate Transaminase	NH ₄ H ₂ PO ₄ - ammonium dihydrogen phosphate
ROS- Reactive Oxygen Species	Mg(NO ₃) ₂ - Magnesium Nitrate
T2DM- Type 2 Diabetes Mellitus	Pd- Palladium
MAPK- Mitogen-activated protein kinase	NIOSH- National Institute for Occupational Safety and Health
ATSDR- Agency for Toxic Substances and Disease Registry	FAO- Food and Agriculture Organization
CDC- Centers for Disease Control and Prevention	ADD- Average Daily Dose
IHME- Institute for Health Metrics and Evaluation	RfD- Oral Reference Dose
DALYs- Disability-adjusted life years	CSF- Cancer Slope Factor
UNICEF- United Nations Children's Fund	IR- Ingestion rate
BLL- Blood lead level	ED- Exposure duration
NASEM- National Academies of Sciences, Engineering, and Medicine	EF- Exposure frequency
EFSA- European Food Safety Authority	BW- Body weight
ADHD- attention deficit hyperactivity disorder	AT = Average time
IQ- Intelligent Quotient	ILCR- Incremental Lifetime Cancer Risk
ALAD- Delta-Aminolevulinic Acid Dehydratase	C- Concentration
ATO- arsenic trioxide	GSI- Geological Survey of India
NGO- Non-profit Government Organization	ICMR- Indian Council of Medical Research
8-OHdG-8-hydroxy-2'-deoxyguanosine	PTE- potential toxic elements

N7-MeG- N7-methylguanine	ICP-OES- Inductively Coupled Plasma - Optical Emission Spectrometry
iAs- inorganic arsenic	HI- Hazard Index
WQI- Water Quality Index	THI- Total Hazard Index
HPI- Heavy Metal Pollution Index	SWAN- Soil, Water, Air & Noise
CD- Contamination Degree	FSH- Follicle Stimulating Hormone
HEI- Heavy Metal Evaluation Index	SHBG- Sex Hormone-Binding Globulin
3SLS- Three-Stage Least Squares	AMH- Anti-Mullerian Hormone
INR- Indian Rupee	FMP- Final Menstrual Period
HQ- hazard quotient	LH- Leutinizing Hormone
CR- carcinogenic risk	TAC- total antioxidant capacity
GBC- gallbladder cancer	MDA- Malondialdehyde
CPT- Continuous Performance Test	8-iso-PG- 8-isoprostaglandin
SDT- Signal Detection Theory/ Symbol Digit Test	IL-6- Interleukin-6
PMT- Parent Management Training/ Perceptual Maze Test	TNF- α - Tumor Necrosis Factor-alpha
SAB- Spontaneous Alternation Behavior	LDL- Low-Density Lipoprotein
ANOVA- Analysis of Variance	WBC- White Blood Cells
EPA- U.S. Environmental Protection Agency	IVF-ET- In Vitro Fertilization & Embryo Transfer
Pxrf- Portable X-ray Fluorescence	NHANES- National Health and Nutrition Examination Survey
ICP-MS- Inductively Coupled Plasma - Mass Spectrometry	BMI- Body Mass Index
PbIC- Lead Isotopic Compositional analysis	DMSA- dimercaptosuccinic acid
TSH- Thyroid-Stimulating Hormone	DMPS- dimercapto-propanesulfonic acid
T3- Triiodothyronine	T4- Tetraiodothyronine

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Chapter 1

1. Introduction

In the recent years the groundwater pollution has led to serious health hazards among the exposed population globally. The heavy metals human exposure has magnified the pollution through groundwater, food and other sources and the heavy metals have entered the food chain through geogenic and anthropogenic changes. Arsenic and lead are the heavy metals which in the recent years are the prone human health risk contaminants. Body fluids are the most feasible route for administering the heavy metals in any human body. Blood is one of the most important because they transfer oxygen and essential nutrients to other organs which can also include heavy metals and they interact with other cellular tissues and system. Particularly in females, hormones play a significant role in each process so if heavy metals interfere with the production or secretion of hormones it can lead to many issues like infertility, PCOS, pregnancy issues and many more. Chronic exposure to low levels of arsenic gradually manifests through distinct dermatological changes. The most characteristic signs include hyperpigmentation, marked by darkened patches of skin, and occasionally hypopigmentation, resulting in lighter areas—often appearing in an alternating, mottled pattern. Over time, some individuals experience a diffuse thickening of the skin on the palms and soles, while others develop localized keratotic lesions, forming small, hardened areas. These visible cutaneous alterations serve as early clinical indicators of long-term arsenic toxicity (Martínez-Castillo et al., 2021). In about 5% of cases, distinctive pale bands known as Mees' lines appear across the fingernails (Kliegman et al., 2007). Prolonged arsenic exposure has been unequivocally linked to a heightened risk of malignancies across multiple organ systems, including the skin, lungs, liver, bladder, prostate, and vascular tissues (Kliegman et al., 2007; Chen & Costa, 2021). Among these, cutaneous squamous cell carcinoma in situ remains the most prevalent manifestation of arsenic-induced carcinogenesis. This form of cancer typically develops after a latency period ranging from two to twenty years, reflecting the insidious and cumulative nature of arsenic toxicity (Soni et al., 2024). Such findings underscore arsenic's role not merely as an environmental contaminant but as a potent, long-term carcinogenic threat to human health. So, the present research work

emphasizes the detrimental effects of arsenic and lead exposure on female health, highlighting its impacts on various bodily fluids in females including the blood, breast milk, urine and endocrine system.

1.1 Arsenic calamity around the globe:

Water is a vital part of sustaining life on Earth. Only 2.5 % of Earth's water resource consists of freshwater. As the world's population grows, so does the need for clean water, leading to an increased reliance on groundwater. This escalating population, combined with the effects of global warming and declining water quality, exacerbates the need for safe drinking water (Mishra, 2023). The World Health Organization (WHO) reports that more than 700 million people worldwide do not have access to clean water (Lal, 2019). Relying solely on surface water supplies will be insufficient to cope with the escalating demand for freshwater (Mishra, 2023). Rural communities face significant obstacles in obtaining potable water for both personal and irrigation purposes, including groundwater depletion, contamination from heavy metals and other toxic chemicals, flooding, salinity, and drought (Mishra, 2023). Heavy metals are extremely dangerous due to their toxic, persistent, and bio-accumulative properties (Ali et al., 2019). Even in minute quantities over prolonged periods, these carcinogenic contaminants can cause a range of health issues, including hypertension, abdominal pain, anorexia, kidney and nerve damage, and cardiovascular diseases. Health complications linked to the ingestion of this metal include high blood pressure, abdominal pain, anorexia, kidney damage, nerve damage and heart disease etc.

Arsenic, with an atomic number of 33, is a metalloid falling into group 15 of the Periodic Table (Binkowski, 2019). It can exist in 4 oxidation states (-3, 0, +3, and +5), with the pentavalent form being predominant under normal oxygenated environmental conditions (Verma et al., 2023). In aqueous systems, inorganic arsenic is typically found in 2 oxidation states: pentavalent arsenate and trivalent arsenite (Jha et al, 2017).

a) Arsenate: it is the oxidized form of arsenic, with a (+5) oxidation state. It has an unfilled 3d electron orbital and five valence electrons (Nayek et al., 2024). Arsenate is less soluble in water compared to arsenite, resulting in lower mobility in groundwater.

Arsenate is generally more stable and less prone to redox reactions (Mueller, 2017). The concentration of arsenate in groundwater is less sensitive to pH compared to arsenite (Fig 1.1). Arsenate is still toxic, but is recognized as being less toxic than arsenite (Valskys et al., 2022). It is less readily taken up by cells, resulting in lower potential health risks compared to arsenite. Under oxygen rich conditions, pentavalent arsenic is stable and occurs as a monovalent (H_2AsO_4^-) or divalent (HAsO_4^-) anion (Chaudhary et al., 2024).

b) Arsenite: Arsenite is the reduced form of arsenic, with a (+3) oxidation state. It has a filled 3d electron orbital and three valence electrons (Xiang et al., 2025). Arsenite is more soluble in water compared to arsenate, making it highly mobile in groundwater. Arsenite is chemically more reactive and can undergo redox reactions more easily (Hassan & Westerhoff, 2024). It can readily enter biological cells and disrupt various cellular processes, leading to adverse health effects. it exists as uncharged (H_3AsO_3) or anionic species (H_3AsO_3^-) (Chaudhary et al., 2024).

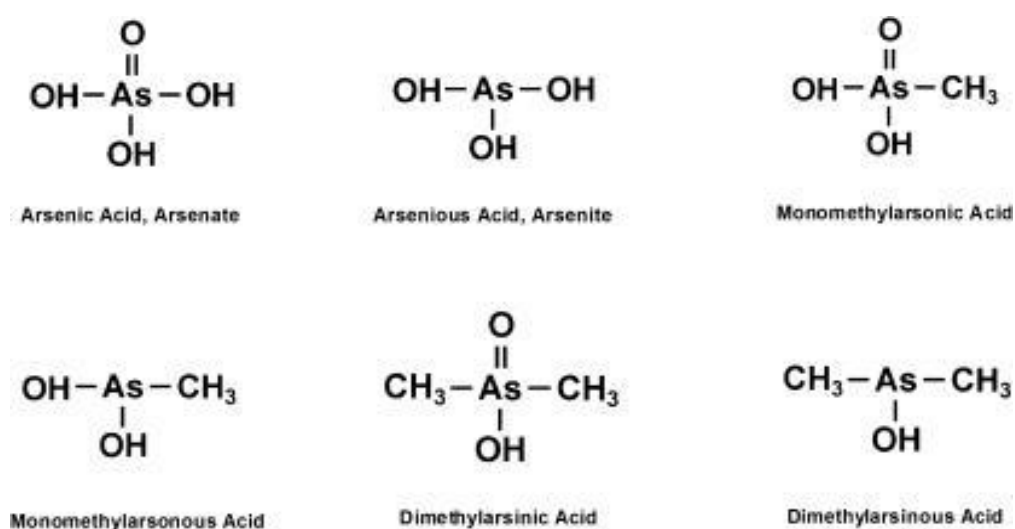


Figure 1.1 Different molecular structure of arsenic binding (Image Courtesy- Hughes et al., 2011)

1.2 Arsenic in Environment:

Arsenic holds the 20th position in large quantity within the Earth's crust, ranks 14th in seawater, and 12th in human body, underscoring its pervasive presence across natural and biological systems. (Chaudhary et al., 2024). The chemical properties of

arsenic exhibit resemblances to those of nitrogen and phosphorus, which falling to same group in periodic table (Börner & Zeidler, 2023). The structural resemblance between phosphate (PO_4^{3-}) and arsenate (AsO_4^{3-}) ions leads to a high pick-up of arsenic (Hassan, 2019). This may also be the reason to explain the high concentration of inorganic arsenic in marine organisms and plants (Reitsema et al., 2018). As contamination of groundwater results from both natural and human-related sources, which harms human health and ecology (Eid et al., 2024). Chronic exposure to inorganic arsenic causes arsenicosis, a disease characterized by skin pigmentation, patches on the palm of hands and feet, and skin lesions (Flora, 2020). Drinking groundwater with high arsenic contents exposes a significant proportion of individuals globally to high levels of arsenic (Chakraborti et al., 2017). Additionally, indirect exposure to arsenic occurs through the utilization of contaminated food. Arsenic is lethal if the ingested quantity is above 2 mg/kg (200–300 mg) (Dueñas-Laita et al., 2005). Many criminals are also using heavy metals (As, Pb, Th, Zn, etc.) for homicidal poisoning, therefore, suicidal poisoning cases are rare to find and accidental poisoning cases are found generally in children if they lick any paint or substance containing these heavy metals.

1.3 Arsenic Global Scenario

The global arsenic contamination in groundwater affects 300 million people in the world (Fig 1.2). Besides agriculture, mining activities leads to arsenic arising in some area like south Dakota, Alaska, Nevada and California (Pye & Patrick, 1983). Before 2000, only four Asian countries were arsenic affected namely India, China and Bangladesh. From 2000-2005 As-related problems were reported in south east Asian countries (Vietnam, Thailand, Taiwan, Bangladesh, Cambodia, China, India, Pakistan, Burma, and Nepal) (Hasan et al., 2019 & Mishra et al., 2016). Apart from that some chronic arsenic exposure is also reported in Kurdistan province of Western Iran and Vietnam (Solgi & Jalili, 2021). According to WHO (1993) standards, drinking water's maximum allowable level of arsenic was lowered from 50 $\mu\text{g/L}$ to 10 $\mu\text{g/L}$ (Frisbie & Mitchell, 2022). However, due to treatment implementation and analytical attainability, the allowable limit for arsenic is 50 $\mu\text{g/L}$ in several developing nations, involving India, Pakistan and Bangladesh (Taiwo et al., 2023). The government's

negligence and poor policies will worsen the arsenic situation. About 57 million people in Bangladesh consume water contaminated with arsenic, and another 35 million drink water whose arsenic concentration is higher than the Bangladeshi norm (Parvin et al., 2022). China is the one of severely affect countries since 1960s and also accounts for the 50% production of arsenic globally (Zhang et al., 2020). Extensive industrialization and other human activities including mining, are the reasons behind sharp increase in containment concentration in potable water during 1990s. Consequently, regions such as China have reported arsenic concentrations in groundwater far exceeding the permissible safety thresholds (Izah et al., 2016). On a global scale, groundwater arsenic contamination has emerged as a critical environmental and public health concern, with over 107 countries documenting arsenic levels surpassing the World Health Organization's (WHO) guideline value of 10 µg/L. The most severe incidences are concentrated in Asia and Europe, followed by notable occurrences in Africa, North and South America, and Australia (Medunić et al., 2020). Moreover, nations such as Mexico, Chile, Hungary, Argentina and several regions across the African continent continue to grapple with chronic arsenic pollution. This pervasive contamination poses a grave global health burden, with an estimated 150 million people worldwide currently exposed to arsenic levels that threaten both human well-being and environmental sustainability. This number is expected to rise as new areas experiencing arsenic contamination are continually identified (Rahman & Singh, 2019). Anthropogenic sources like the mining industry, livestock farming and industrial waste cause arsenic contamination in groundwater from sedimentary aquifers arsenic in agricultural irrigation (Olson & Chau, 2022).

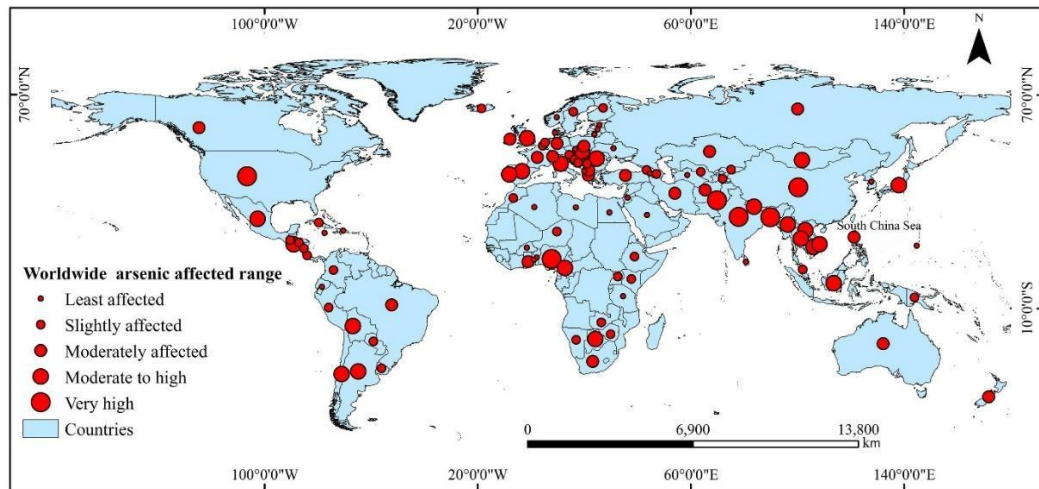


Figure 1.2. Worldwide distribution arsenic contamination (Image- Shaji et al., 2021)

1.4 Arsenic Indian Scenario:

Currently, an estimated 70 million people in India are at risk due to arsenic-contaminated groundwater (Fig. 1.3). Numerous research groups have extensively documented the prevalence of arsenic contamination across the country, with a particular concentration of studies focused on the Ganga basin region (Rajan et al., 2024). Covering approximately 26% of landmass in India, the Ganga River basin ranks as one of the world's most populous and productive agricultural regions (Sati, 2021). Currently Ganga is the most polluted river in the world and contain several toxic contaminants like cadmium, lead, arsenic, mercury, microbial pathogens and pesticides (Chakraborti et al., 2018). In India first report of groundwater contaminated by arsenic was came from north Indian city Chandigarh and second case came from lower Gangetic plain region of West Bengal (Mishra et al., 2016). Reports followed from many more states including Bihar, UP, Jharkhand, Punjab, Himachal Pradesh, Chandigarh, Arunachal Pradesh, Assam, Manipur, Nagaland, Uttarakhand (out of 13 districts only 5 districts are affected), Mizoram, Tripura and Andhra Pradesh. Recently some other states like Gujarat, Madhya Pradesh, Haryana, Telangana, Karnataka, Orissa and four union territories like Jammu and Kashmir, Daman and Diu, Pondicherry and Delhi are also found to be affect by groundwater arsenic contamination (Kulkarni et al., 2017; Chatterjee et al., 2017; Chakraborti et al., 2003,

2017; Kumar et al., 2016b; Nickson et al., 2007; Mukherjee et al., 2006; Ministry of Jal Shakti, 2022).

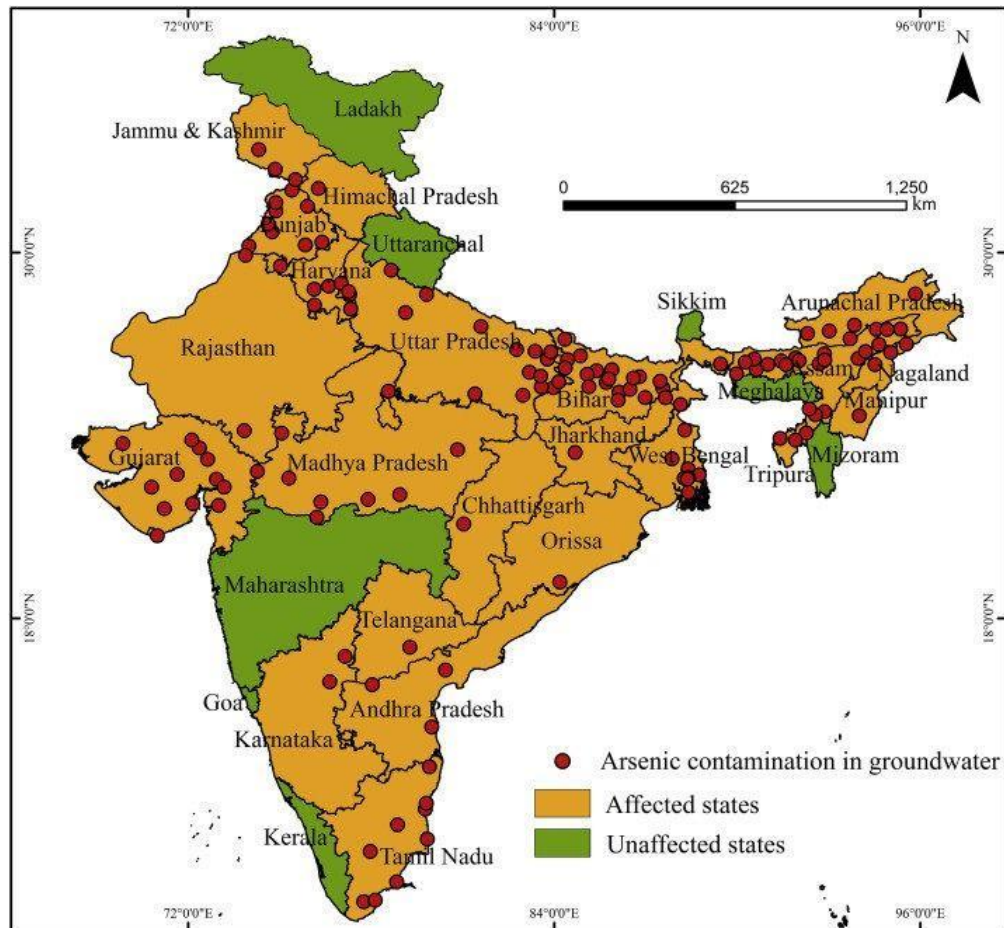


Figure 1.3. Groundwater arsenic contamination in India (Image- Shaji et al., 2021)

1.5 Arsenic Bihar Scenario

Bihar, a state in eastern India situated within the Ganga–Meghna–Brahmaputra (GMB) basin, faces a severe public health crisis due to arsenic contamination. Approximately 150 million people living in this region are affected by arsenic exposure. In this predominantly agrarian state, groundwater serves as the primary source of drinking water, meeting over 80% of rural water needs. Alarming, around 10 million residents of Bihar are directly exposed to the harmful effects of arsenic poisoning (Fig. 1.4) (Kumar et al., 2021a). Multiple studies have documented the widespread health hazards associated with arsenic toxicity in the region. Notably, arsenic contamination in Bihar’s groundwater was first reported in 2003 (Chakraborti

et al., 2003). The existence of arsenic in groundwater is categorized in two: first is alluvial terrain which accounts for the sources of 90% As in India and second is hard rock terrain which is responsible for 10% As which includes states like Karnataka and Chandigarh (Ranjan, 2019). In 2002, Bihar was reported that groundwater in two villages in the Bhojpur district, which is in the central Gangetic plain, was contaminated by arsenic. Out of Bihar's 38 districts, 32 have been found to contain groundwater with arsenic concentrations exceeding 10 µg/L (Chakraborti et al., 2002). Approximately 9 million people are consuming water contaminated beyond the WHO permissible limit. The exposed populations commonly exhibit dermatological symptoms, including hyperkeratosis, melanosis, and pigmentation disorders (Kumar et al., 2021b). Prolonged arsenic exposure has further been linked to an increased incidence of cancer among these communities (Kumar et al., 2023^{a, b}).



Figure 1.4. Arsenic contamination in groundwater in Bihar (Image- Jal Shakti Mission, Govt of India, 2022)

1.6 Effect of Arsenic exposure on human:

The adverse effect of arsenic on human health depends strongly upon duration of exposure, dose of As and the nutritional statue of exposed individual (Milton et al., 2017). The most commonly manifested clinical symptoms of chronic exposed people by arsenic poisoning are melanosis (pigmentation change), skin lesions and keratosis (de Carvalho & Dinis-Oliveira, 2023). The chronic exposure of arsenic can cause cardiovascular, respiratory, reproductive, neurological, hepatic, hematological, diabetic effect in human (Fig 1.5). its exposure also recognized as cause of, lung cancer, skin and bladder cancer (Martinez-Castillo et al., 2021).

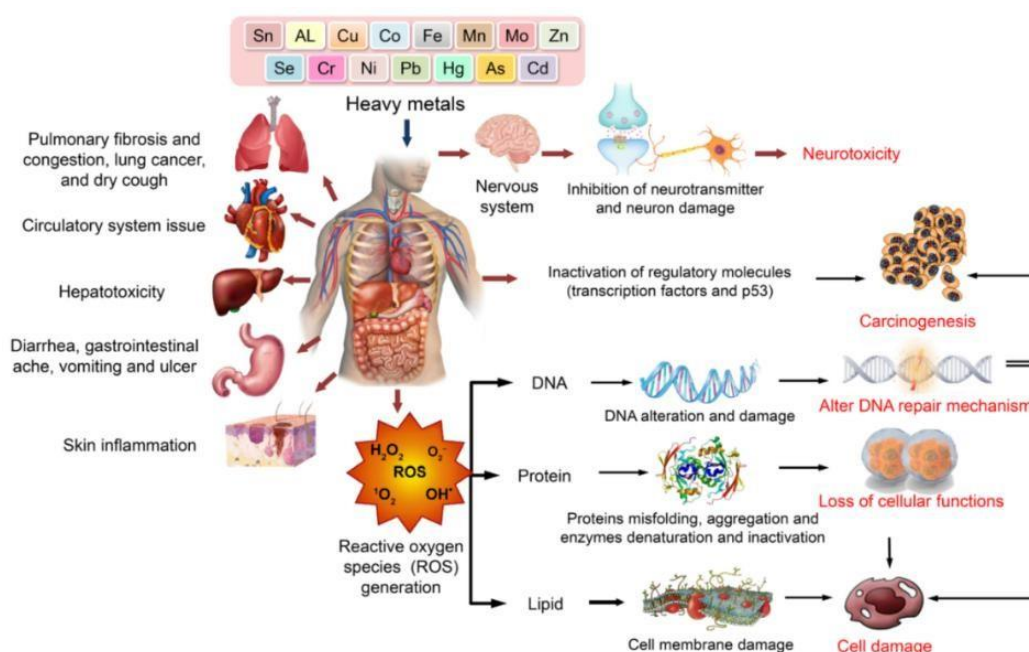


Figure 1.5. Arsenic and other heavy metals toxicity in the human body (Courtesy: Abd Elnabi et al., 2023)

1.6.1 Effect of arsenic on nervous system: Because arsenic can easily pass the blood brain barrier, the brain is a prominent target organ for toxicity associated with As, that affects learning and focus. The hypophysis (pituitary gland) showed the greatest accumulation of arsenic (Ishaq et al., 2021). Arsenic preferentially damages neurons with long axons, while sensory nerves are notably more vulnerable than motor nerves (Anushree & Ahsan, 2021). Commonly reported symptoms include paraesthesia, numbness in the soles of the feet, and neuropathic pain, arising from the neurons'

limited capacity to neutralize reactive oxygen species (ROS) and synthesize glutathione (Srivastava & Flora, 2020). One of the main causes of the neurotoxicity caused by arsenic is thought to be oxidative stress (Fig 1.6). The disorganization of the cytoskeletal structure, which can happen either by changing the protein configuration of the cytoskeleton or by hyper phosphorylation of proteins, is the leading cause of As-induced neurotoxicity (Ariafar et al., 2023). A recent study from West Bengal, India, reported that individuals consuming arsenic-contaminated groundwater most commonly developed peripheral neuropathy as a primary neurological manifestation (Pal et al., 2024).

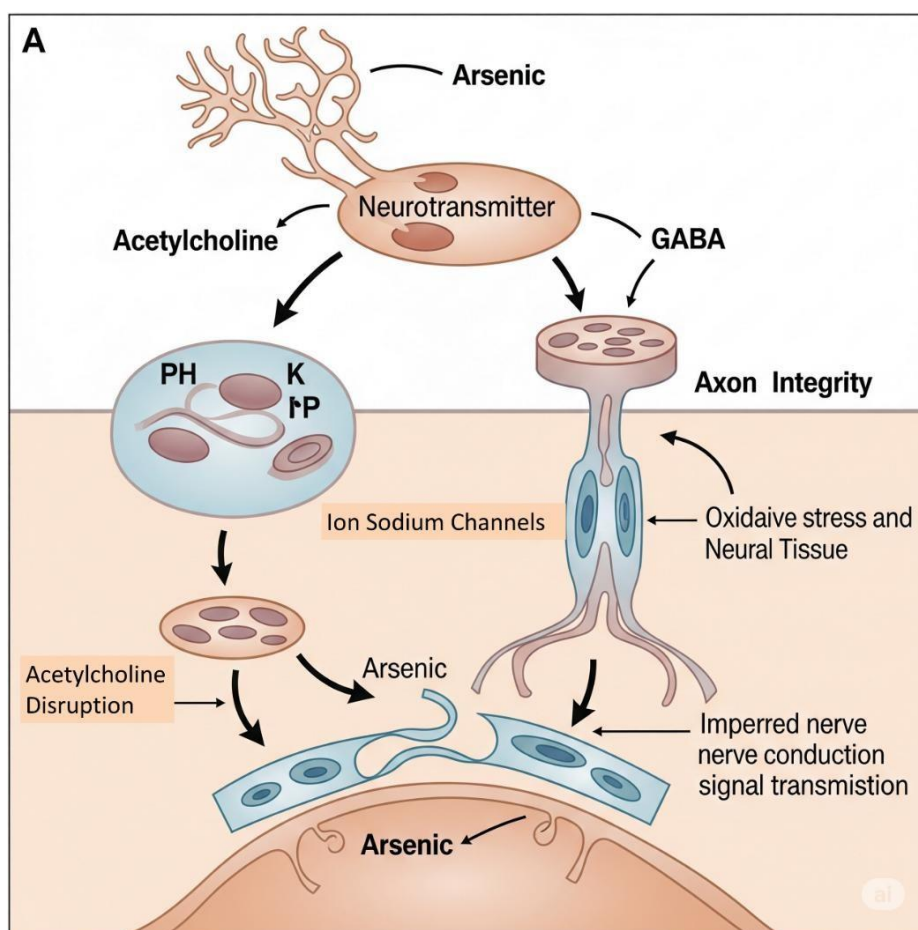


Figure 1.6. Effects of arsenic toxicity in the human nervous system

1.6.2 Effect of arsenic on immune system: Humans and experimental animals have exhibited several disruptive innate immunity signs as a consequence of inorganic arsenic exposure (Parvez et al., 2019). There was a quantitative correlation between

immune suppression and arsenic exposure. Depending on the concentration, arsenic can either hinder or encourage the growth of immune cells (Ferrario et al., 2016). The main immunotoxin target of inorganic arsenic is human macrophages. Arsenic causes macrophages to round off and lose their capacity to attach quickly (Islam, 2023). Additionally, the expression of macrophage surface markers is also impacted, changing phagocytosis and endocytosis (Fig 1.7). The main mechanism arising from arsenic exposure is a disruption of the unfolded protein response (UPR) signaling system, which affects macrophage activities (Perez & King, 2022). Arsenic exposure affects human lymphocytes, including increased internal free radicals, changes to DNA bases, oxidative damage, Deoxyribonucleic acid (DNA) strand damage, cross-linking of proteins, apoptosis, lipid peroxidation (Krishnamurthy et al., 2024). A study on preschool children in Bangladesh found a relationship between decreased cell-mediated immunity and suppressed concentrations of Th1 cytokines due to persistent arsenic exposure. Th2 cytokines, however, showed no modifications (Islam, 2023).

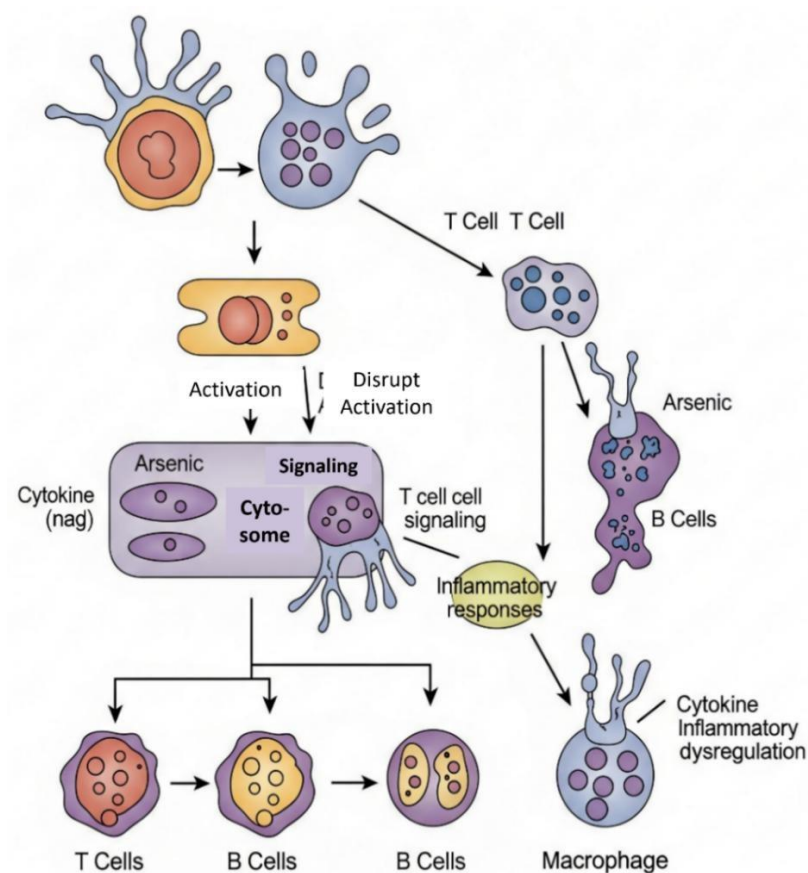


Figure 1.7. Effects of arsenic toxicity in the human immune system

1.6.3 Effect of arsenic on hepatic system: Repeated exposure to arsenic leads to a higher accumulation of the toxic element in the liver, which can lead to serious damage (Fig 1.8). The liver metabolizes inorganic arsenic for detoxification and elimination through urine (Zubair & Martyniuk, 2019). As-induced liver disease may manifest with early clinical signs such as ascites, jaundice, or liver enlargement, and esophageal varices bleeding may occur (Srivastava & Flora, 2020). Severe As poisoning can cause cirrhosis, hepatic lesions, non-cirrhotic portal fibrosis, and hepatic fibrosis, with a high probability of liver failure in later stages (Hatipoğlu et al., 2021). In West Bengal, India, individuals who ingested arsenic (0.05–3.2 mg/L) had larger livers as well as increase levels of liver enzymes, in their blood (Ghosh & Mukhopadhyay, 2018). Chronic exposure to arsenic can significantly impair liver function, resulting in elevated serum levels of alanine transaminase (ALT), bilirubin, alkaline phosphatase (ALP) and aspartate transaminase (AST) (Zhou et al., 2022). The toxic influence of arsenic promotes oxidative stress and overproduction of reactive oxygen species (ROS), which activate key kinase signaling pathways that may lead to hepatocyte apoptosis and cellular injury (Prakash et al., 2022). Moreover, heightened ROS activity can initiate lipid peroxidation, aggravate hepatic cell damage and ultimately contribute to arsenic-induced hepatotoxicity (Hu et al., 2020).

Mechanism of Arsenic in hepatic system

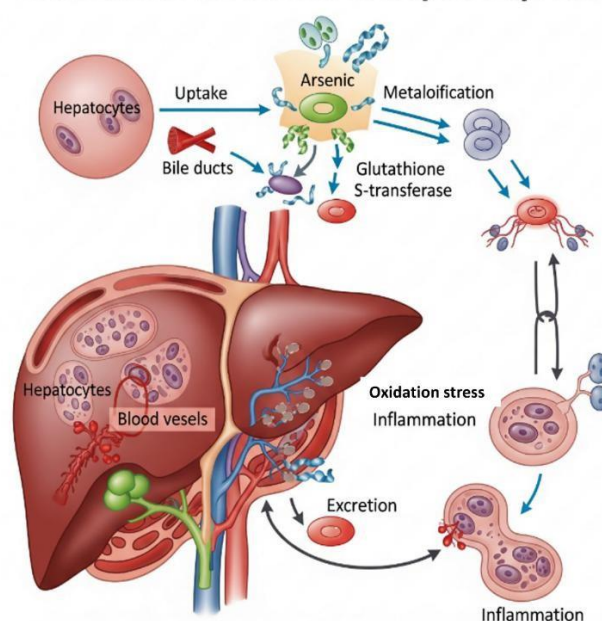


Figure 1.8. Effects of arsenic toxicity in the human hepatic system

1.6.4 Effect of arsenic on endocrine system: Arsenic has been found to disrupt the endocrine system, which includes several important glands such as the thyroid, pancreas, and gonads (Sun et al., 2016). It has been discovered that intake of arsenic during pregnancy reduces thymus function, possibly by inducing apoptosis as well as oxidative stress (Maity et al., 2024). The thyroid gland produces hormones that regulate metabolism in the human body, but the connection with As and thyroid function has not studied properly (Al-Suhaimi & Khan, 2022). However, recently it was suggesting that outdoor workers exposed to arsenic may experience negative effects on thyroid glands and the thyroid hormones (Buha et al., 2018) (Fig. 1.9). Prolonged exposure to inorganic arsenic has been shown to elevate the risk of developing T2DM, highlighting its significant metabolic toxicity (Abolli et al., 2024).

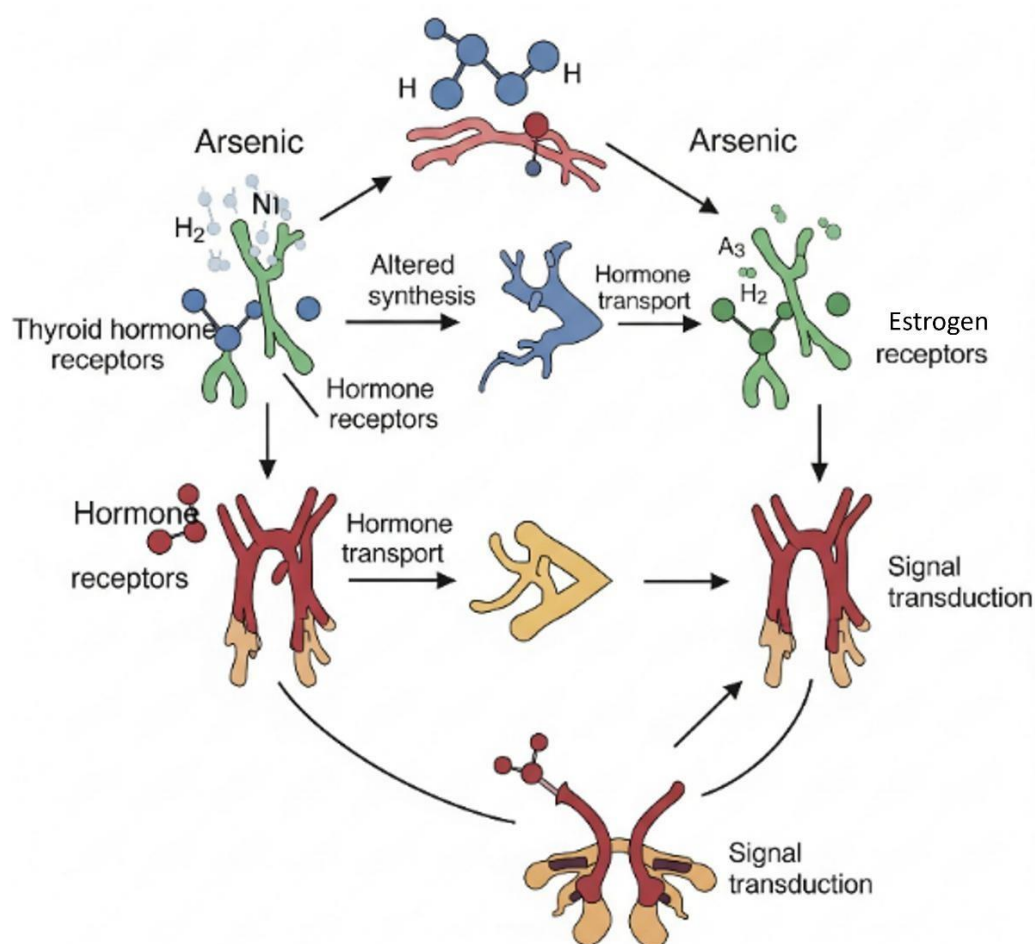


Figure 1.9. Effects of arsenic toxicity in the human endocrine system

1.6.5 Effect of arsenic on renal system: The arsenic accumulation in the renal system shows some clinical symptoms include hypo urea, high serum creatinine levels, proteinuria and blood urea nitrogen (Lin, 2023). A correlation between exposure to As and the development of albuminuria and proteinuria has been noted (Fig 1.10). Epidemiological research also showed a coincidental link between kidney ailments and arsenic exposure (Byber et al., 2016). Renal toxicity is caused by As-induced up regulation of heme oxygenase-1, mitogen-activated protein kinase (MAPK), and other signaling pathways (Hu et al., 2020).

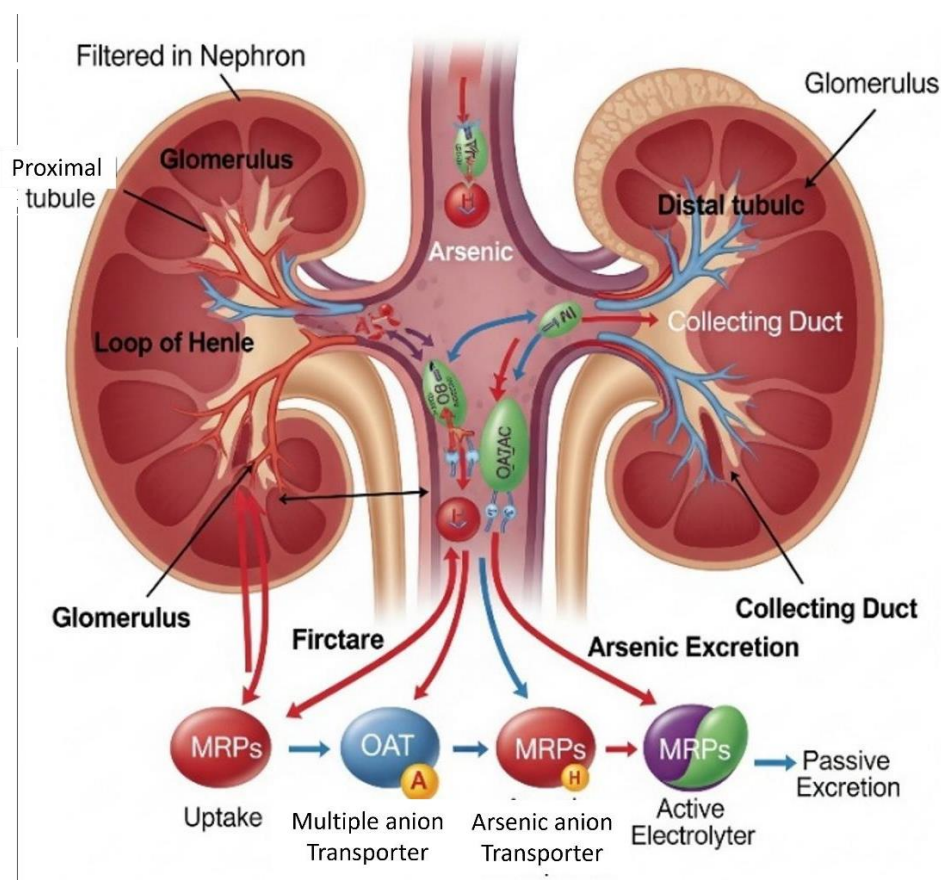


Figure 1.10. Effects of arsenic toxicity in the human renal system

1.6.6 Effect of arsenic on reproductive system: As is a well-known teratogen that has an impact on fetus development. Inorganic As has been proven to be hazardous to the developing brain and reproductive system in animal experiments (Patwa et al., 2022). Growth retardation and embryonic mortality are strongly influenced by both the dose and duration of arsenic exposure (Fig. 1.11). Arsenic adversely affects the

reproductive organs of both males and females, potentially leading to fertility impairments (Udagawa et al., 2019). Exposure to arsenic concentrations exceeding 50 $\mu\text{g/L}$ through drinking water during pregnancy can disrupt uterine and placental development, consequently impacting birth weight and overall fetal growth. Moreover, prenatal arsenic exposure has been closely linked to a heightened risk of birth defects, with evidence suggesting that even low levels of exposure pose significant developmental risks (Milton et al., 2017). Prolonged arsenic exposure further increases the likelihood of late fetal loss and neonatal mortality, underscoring its profound toxic impact on reproductive and developmental health. Arsenic was demonstrated to be actively transferred to fetuses and to cause developmental damage (i.e. malformation, growth retardation and death) (Herring et al., 2018). After arsenic exposure, the dose, method, and gestation day all play a significant role in developmental damage. Arsenic exposure mostly affected development through aberrant DNA methylation patterns, altered cell proliferation, and death (Chakraborty et al., 2022).

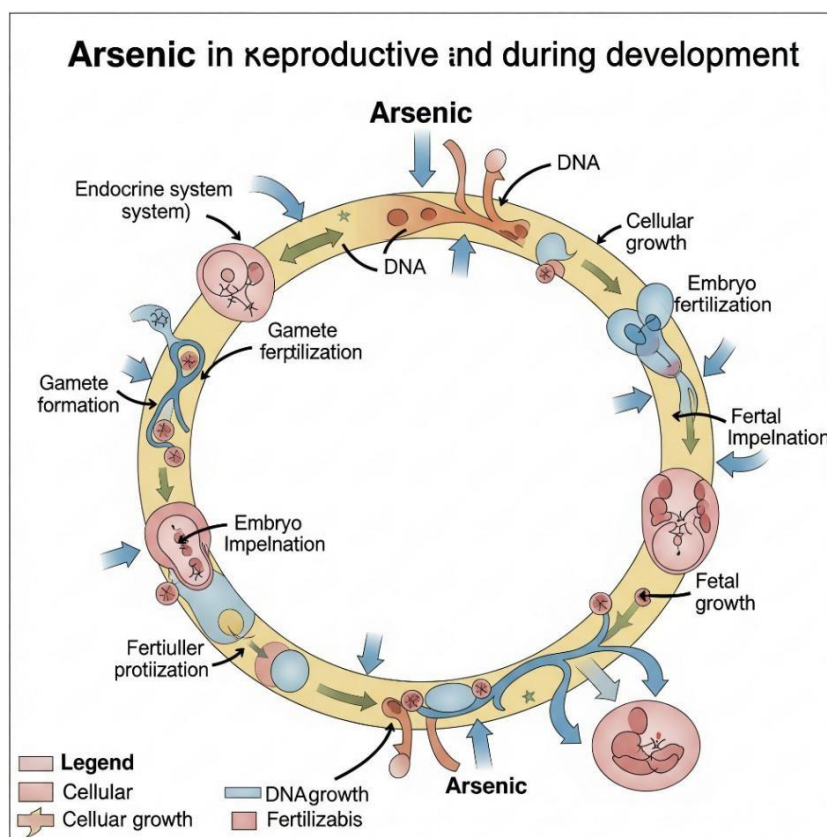


Figure 1.11. Effects of arsenic toxicity in the human reproductive system

1.7 Lead contamination around the globe

Lead (atomic number-82), a naturally occurring heavy metal represents a major public health concern due to its extensive use and pronounced toxicity (Rebelo & Caldas, 2016). It is ranked second on the Agency for Toxic Substances and Disease Registry (ATSDR) National Priorities List, underscoring its prevalence, toxic potency, and high potential for human exposure (ATSDR, 2015). The U.S. Centers for Disease Control and Prevention (CDC, 2020) identify this threshold as necessitating immediate public health intervention, a stance echoed by the World Health Organization (WHO, 2019), which associates such exposure with diminished cognitive ability, behavioral disorders, and learning deficits in children (Lorna et al., 2003). According to the Institute for Health Metrics and Evaluation (IHME), lead exposure accounted for over 1.5 million global deaths in 2021, primarily due to its deleterious cardiovascular effects (Fig. 1.12). In the same year, it was responsible for an estimated 33 million disability-adjusted life years (DALYs) lost worldwide, underscoring its devastating and pervasive influence on human health and life expectancy.

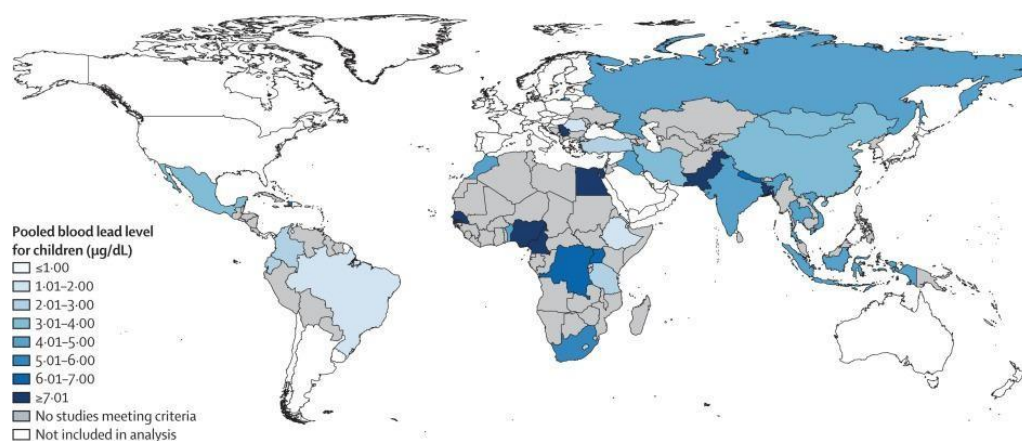


Figure 1.12. Lead contamination distribution worldwide (Image Courtesy-Ericson et al., 2021)

1.7.1 Indian Scenario

Lead remains one of the most dangerous and pervasive environmental toxins in the world, silently endangering millions of lives. Globally, it is estimated that one in three

children around 800 million have elevated blood lead levels. Alarming data show that India alone accounts for about 275 million of these children, meaning that nearly half of all Indian children are affected. According to UNICEF (2020), 23 Indian states report blood lead levels (BLL) exceeding the safe threshold of 5 $\mu\text{g}/\text{dL}$ (Fig 1.13). Particularly concerning are the states of Madhya Pradesh, Bihar, Jharkhand, Uttar Pradesh, Andhra Pradesh and Chhattisgarh which together represent roughly 40% of the nation's population. In these regions, average blood lead levels surpass 7 $\mu\text{g}/\text{dL}$ —an indication of widespread and chronic exposure that poses a grave threat to children's health, development, and futures. Lead's use in India dates back centuries, evidenced by archeological findings of lead artifacts used for tools, decorations, and rituals (Radivojević et al., 2021). Its durability and malleability made it a preferred material for roofing, plumbing, and statuary. Lead compounds were also incorporated into traditional Ayurveda medicine (Marriage & Gjerde, 2024). While showcasing lead's versatility, its historical use raises concerns about long-term environmental contamination and human exposure, contributing to current lead poisoning challenges (Li et al., 2024).

Average BLL prevalence across different states of India

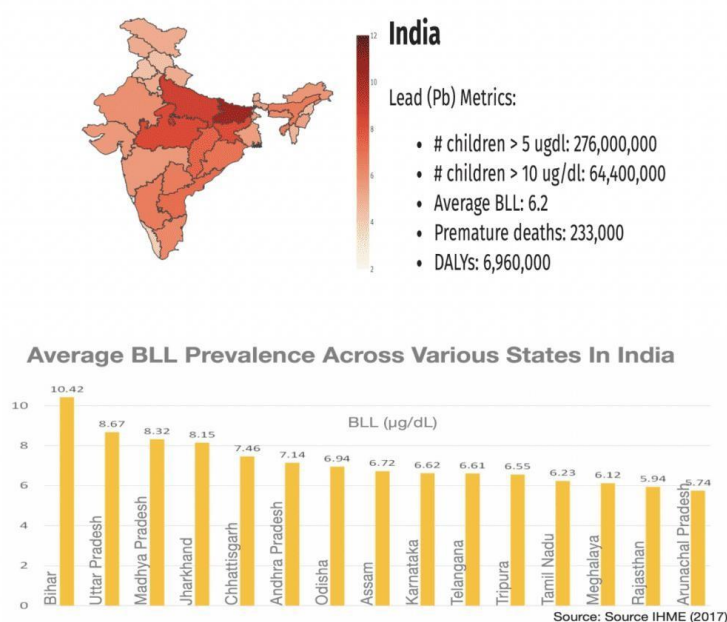


Figure 1.13. Average BLL prevalence across different states of India (Image Courtesy: Institute of Health Metrics & Evaluation, 2017)

1.8 Sources of Lead Exposure in India:

Lead exposure in India stems from various sources, posing a serious threat to public health. Occupational hazards exist in industries like construction, metal processing, and battery manufacturing, where workers are exposed to lead-containing dust and fumes (Odongo, 2019). Lead-based paints, particularly in older buildings, remain a concern. Contaminated water sources, often due to aging plumbing or industrial runoff, contribute to ingestion risks (Kinder, 2019). Unregulated recycling practices in the informal sector, especially electronic waste disposal, contaminate the environment and the food chain (Awere et al., 2020). Lead contamination extends beyond industrial sources and has been detected in various consumer products, including cosmetics, traditional medicines, and certain imported goods (Owusu, 2023). Among occupational settings, battery manufacturing particularly the production and recycling of lead-acid batteries remains one of the most significant contributors to lead exposure in the workplace (Rees & Fuller, 2020). Metal processing industries, such as foundries and smelters, also contribute significantly. Construction workers face risks when handling lead-containing paints and materials in older structures (Apthorpe et al., 2019). The unregulated e-waste recycling sector poses a substantial threat due to inadequate safety protocols. Insufficient regulations and limited awareness exacerbate these risks (Dogbatsey et al., 2024). Effective mitigation requires stricter regulations, robust safety measures, and regular health monitoring for at-risk workers (NASEM, 2018). Environmental lead contamination in India involves air, water, and soil pollution, impacting both human and ecological health (Giorgi, 2020). Industrial emissions, battery manufacturing, and vehicular emissions contribute to airborne lead particles. Lead deposition in soil can persist for extended periods, affecting crops and entering the food chain (Bouida et al., 2022). Water contamination occurs from industrial effluents and improper waste treatment, with older plumbing systems adding to the problem, especially in urban areas (Singh et al., 2022a). The historical use of lead-based paint contributes to soil contamination, particularly in older cities. These combined exposures affect ecosystems and wildlife, such as lead poisoning in birds through ingestion of lead shot or contaminated water (Frank et al., 2019). Addressing environmental exposure requires stricter emission and waste disposal regulations, promotion of lead-free products, infrastructure improvements, and public

awareness campaigns (Rajamani & Iyer, 2023). Lead exposure in India also occurs through consumer products like paint, cosmetics, and traditional medicines (EFSA, 2019). Lead-based paints in homes and infrastructure are a major source, especially as they deteriorate and create lead dust (CDC, 2009). Lead has been detected in cosmetics like lipsticks and eyeliners, raising concerns about chronic exposure. Traditional remedies sometimes contain lead, posing risks to those who use them (Naqvi et al., 2022).

1.9 Vulnerable Population affected due to lead Poisoning

1.9.1 Pregnant Women:

Lead exposure during pregnancy poses significant risks to both mother and fetus. Lead readily crosses the placenta, impacting fetal development (Ortiz-Garcia et al., 2023). Prenatal lead exposure is associated with behavioral problems, increased risk of ADHD and cognitive deficits in offspring (Kumar, 2018). It can also affect the health of mother and child in a long-term. Low birth weight and premature birth are also linked to lead exposure during pregnancy (Manduca et al., 2019). Protecting pregnant women from lead requires addressing occupational and environmental exposures, including contaminated air, water, and consumer products. Prenatal care should include lead exposure testing (Weaver et al., 2024).

1.9.2 Occupational groups: High-risk professions and health implications

Several occupational groups in India face heightened lead exposure risks, resulting in severe health consequences. Workers in battery production, metal processing, and construction are exposed to lead dust and fumes (Odongo, 2019). Battery production, particularly lead-acid battery manufacturing and recycling, releases substantial amounts of lead (Pan et al., 2019). Health impacts range from neurological effects like cognitive impairment and behavioral problems to cardiovascular issues such as hypertension and increased cardiovascular disease risk. Renal consequences, including nephrotoxicity and chronic kidney disease, are also prevalent (WHO, 2015). Reproductive health can be affected, with implications for fertility and development. Lack of awareness, inadequate preventative measures, and infrequent health check-

ups contribute to these risks (WHO, 2017). Mitigation requires collaborative efforts, including stringent occupational safety regulations, regular health screenings for at-risk workers, and educational initiatives to raise awareness about lead exposure hazards (Evans, 2017).

1.10 General health impact

Lead's toxicity affects nearly every organ system. It accumulates in tissues, leading to chronic exposure even at low levels (Fig 1.14). Lead induces oxidative stress, disrupts enzyme function, and displaces essential metals like calcium, impairing physiological processes (Ilieva & Sainova, 2023). Children are particularly vulnerable due to their developing nervous systems. Lead exposure in children can result in behavioral problems, developmental delays, and permanent cognitive deficits (Perlroth & Branco, 2017). Beyond neurological effects, lead also impacts the cardiovascular, renal, and reproductive systems, contributing to nephrotoxicity, hypertension, and reproductive issues (Singh et al., 2018). Chronic lead exposure is related to bigger hazards of cardiovascular disease & certain cancer. Despite awareness of its dangers, lead persists in various sectors, including construction, mining, and battery production, as well as in consumer products like paint and plumbing (Ceballos et al., 2021).

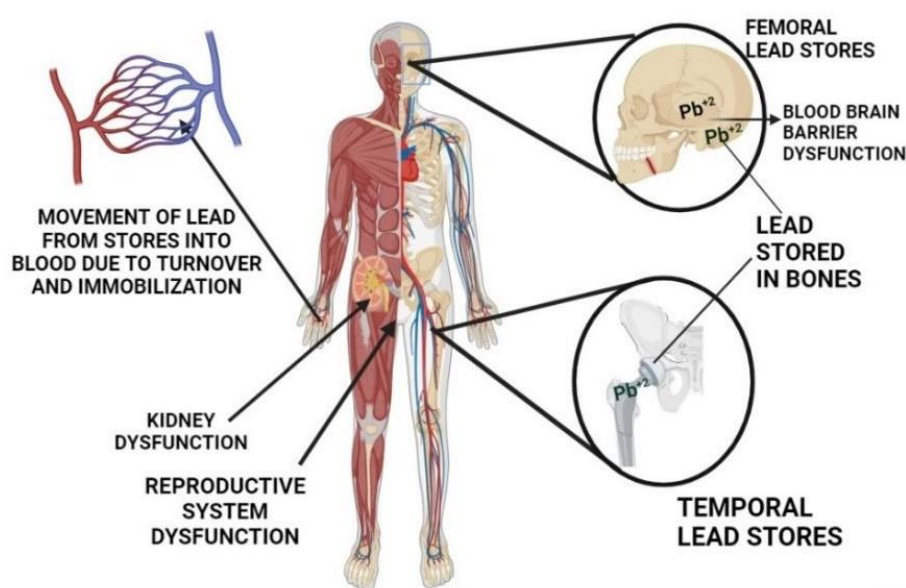


Figure 1.14: Bioaccumulation of lead in different organ system and its dysfunction

1.10.1 Neurological Effects:

Lead poisoning significantly impacts brain function, often accompanied by behavioral and cognitive issues. Even low-level exposure can cause long-term cognitive impairments affecting behavior, academic performance, IQ and diseases like Alzheimer (Fig 1.15). Chronic lead exposure can lead to cognitive decline and behavioral abnormalities in adults, particularly in high-risk occupations (Makhetha, 2021). Lead's mechanisms of neurotoxicity involve oxidative stress, neurotransmitter interference, and disruption of synaptic plasticity (Singh et al., 2023). Behavioral issues associated with lead exposure can resemble ADHD symptoms.

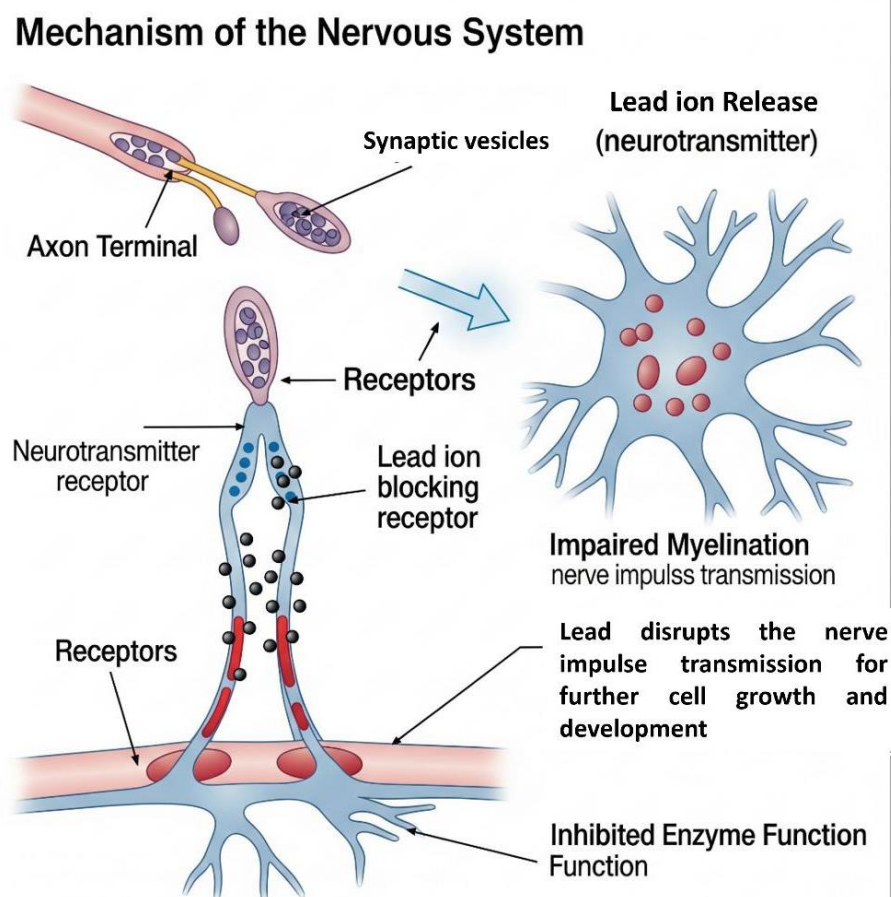


Figure 1.15. Effects of lead toxicity in the human nervous system

1.10.2 Cardiovascular Effects:

Lead contributes to hypertension and is linked to cardiovascular diseases like coronary artery disease and stroke (Wang, 2020). Lead-induced hypertension involves

inflammation, oxidative stress, and altered vascular function (Fig 1.16). Prenatal lead exposure is associated with hypertension later in life (Mitra et al., 2017). Industrial emissions, vehicular pollution, and lead-containing consumer products contribute to widespread exposure (Angrand et al., 2022).

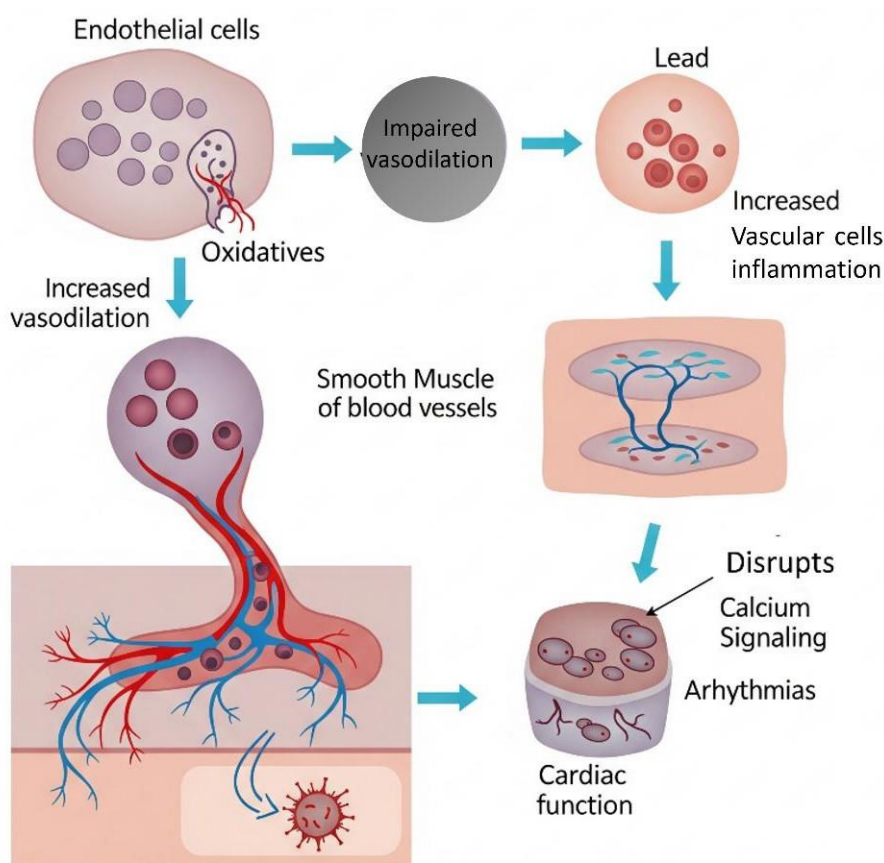


Figure 1.16. Effects of lead toxicity in the human cardiovascular system

1.10.3 Renal effects: Nephrotoxicity and chronic kidney diseases

Lead poisoning in India significantly threatens renal health, with nephrotoxicity and chronic kidney disease as major consequences (WHO, 2015). The kidneys are particularly vulnerable to lead's toxic effects, which include direct renal tubule injury, oxidative stress, and inflammation (Fig 1.17) (Angrand, 2022). Individuals in lead-exposed occupations and contaminated areas face increased kidney disease risk (Johnston, 2019).

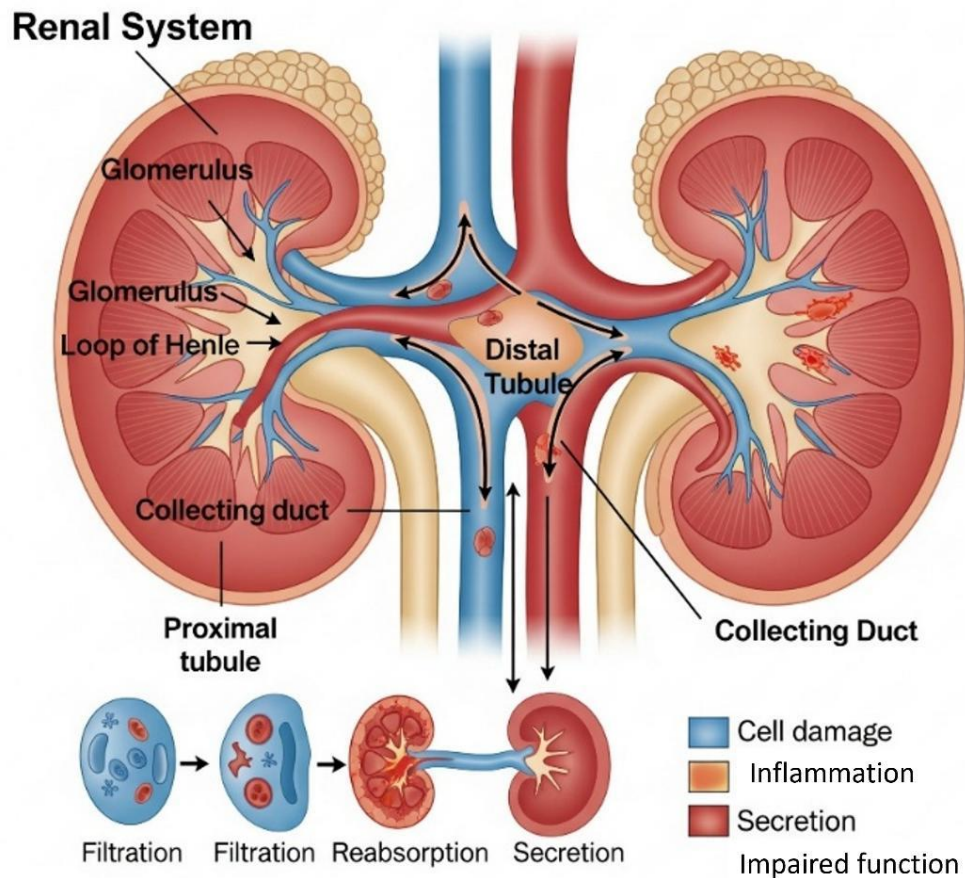


Figure 1.17. Effects of lead toxicity in the human renal system

1.10.4 Reproductive effects: Impacts on fertility and developmental outcomes

Lead also impacts reproductive health, affecting fertility and developmental outcomes. In men, lead exposure is linked to reduced sperm quality, altered motility, and hormonal changes (Kumar, 2018). In women, it can disrupt menstruation and increase spontaneous abortion risk. Prenatal exposure is especially concerning, potentially causing fetal developmental problems (Fig 1.18). Maternal lead exposure is associated with low birth weight and premature delivery (Wai et al., 2017).

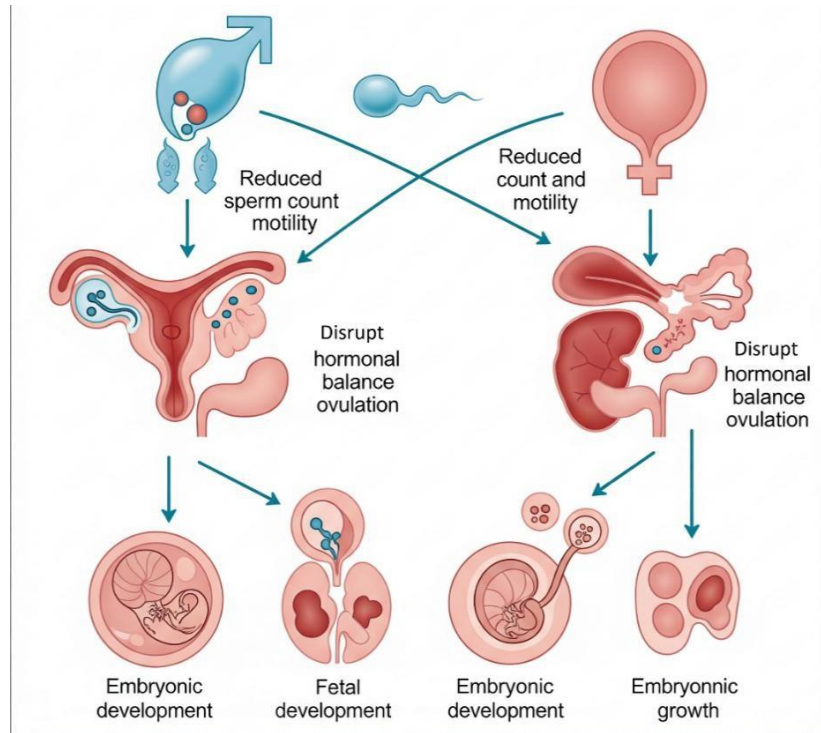


Figure 1.18. Effects of lead toxicity in the human reproductive system

1.10.5 Pediatric health effects

Even low exposure levels can have lasting impacts on development (Perlroth & Branco, 2017). Children in urban areas with high lead levels are at increased risk from contaminated air, dust, food, and water (Luttrell, 2018). Rural children may also be exposed through traditional practices involving lead-containing products (Sarin, 2019). Lead exposure in children is linked to behavioral problems, lower IQ, and cognitive impairments, affecting educational attainment and socioeconomic opportunities (Muller et al., 2018). Interventions should prioritize children, including public awareness campaigns, regular health screenings, and remediation of lead-contaminated environments (Bartrem, 2017). Stronger regulations on lead in children's products are crucial. Parent education on nutrition and hygiene is also essential (Rasesemola et al., 2019).

1.11 Mechanisms of Lead Intake, Distribution, Metabolism, and Excretion:

Lead enters the human body through ingestion, inhalation, or dermal absorption. Absorbed lead enters the bloodstream, where over 95% binds to erythrocyte proteins,

with the remainder linked to plasma before reaching target organs. In adults, roughly 94% of the body's total lead burden is stored in the bones, whereas in children, this proportion is about 73% (Dumková et al., 2017). Red blood cells are the primary lead reservoir in blood (Godswill et al., 2020). Conditions like osteoporosis, menopause, pregnancy, and lactation, which increase bone resorption, can elevate blood lead levels. Lead readily crosses the placental barrier and can also be transmitted through breast milk (Şahin et al., 2022). Once in the body, inorganic lead binds with both protein and non-protein ligands to form stable complexes, while organic lead compounds undergo hepatic metabolism via oxidative de-alkylation mediated by P-450 enzymes (Pretorius, 2021). Lead elimination is a slow and complex process, influenced by its distribution among various body compartments with differing retention times. The biological half-life of lead in blood ranges from about one week to two years, depending on age and exposure history, whereas clearance from bone stores can take much longer typically between 10 and 20 years (Sachdeva et al., 2018).

1.11.1 Absorption:

- **Oral:** Inorganic lead is primarily absorbed through the gastrointestinal tract. The exact mechanisms are not fully understood, but active transport and
- diffusion, both trans cellular and paracellular, are likely involved (Chlubek & Baranowska-Bosiacka, 2023). Absorption of ingested lead in adults is low, generally below 8-10%. In vitro studies suggest that ionized lead concentrations are negligible at low lead levels, with lead phosphate and bile acid complexes being the predominant forms in the intestinal environment (Reeder et al., 2006). These complexes can still release ionized lead for cell membrane passage. Like other divalent cations, lead may utilize saturable transport pathways in intestinal epithelial cells (Sun et al., 2024).
- **Inhalation:** Particle size significantly influences internal dose. Smaller particles ($<0.1\ \mu\text{m}$), common in fumes, have a larger surface area and are more readily absorbed than larger particles via both ingestion and inhalation (Zhang et al., 2021). Particles $<5\ \mu\text{m}$ are absorbed more efficiently in the lungs.

Approximately 50% of inhaled lead is absorbed into the bloodstream (Guo et al., 2021a).

- **Dermal:** Cutaneous absorption of inorganic lead is considerably lower than inhalation or ingestion. Precise measurements of dermal absorption in humans are limited (Natasha et al., 2020). In vitro studies show varying penetration rates of lead salts through excised skin, with lead oxide showing the least penetration and lead nuolate the most (Niemeier, 2022).

1.11.2 Distribution:

Children and adults have similar distribution patterns, except adults have a higher proportion of lead in their bones (Michaels, 2020). Blood lead levels vary with age, physiological state (pregnancy, lactation, menopause), and exposure. Red blood cells contain about 99% of blood lead (Foster, 2024). Lead transfer across red cell membranes involves multiple channels, including an anion exchanger dependent on bicarbonate (HCO_3^-), a smaller HCO_3^- -independent mechanism, and possibly a calcium (Ca^{2+}) channel (Thornell, 2014). In adults, more than 90% of the body's total lead burden is stored in the bones, where it accumulates over time (Del Rio, 2021).

1.11.3 Metabolism:

Inorganic lead breakdown involves complexation with various protein and non-protein ligands. Extracellular ligands include non-protein sulfhydryl and albumin (Rupa et al., 2023). ALAD is the main intracellular ligand in RBCs. Alkyl lead complexes are broken down in the liver by cytochrome P-450. Lead inhibits ferrochelatase and δ -aminolevulinic acid dehydratase (δ -ALAD), disrupting heme production (Flora, 2009).

1.11.4 Excretion:

Lead is primarily excreted through urine and feces, with inconsiderable routes including saliva, nails, sweat, breast milk, hair and seminal fluid (Michaels, 2020). Fecal excretion accounts for about one-third of absorbed lead. Urinary lead excretion varies with oral lead intake. Blood lead levels decrease after exposure cessation,

reflecting lead removal from blood and bone stores (Nwobi et al., 2022). Blood lead half-life in children varies with age and bone lead levels. Dermal exposure leads to lead excretion in sweat and urine (WHO, 2021b).

1.12 Scope of the study

Chronic heavy metal toxicity has major forensic significance due to its insidious onset, non-specific clinical features, and long-term persistence in the human body. Unlike acute poisoning, chronic exposure often mimics natural diseases, delaying diagnosis and obscuring the true cause of illness or death. Toxic effects involve multiple organ systems and may arise from environmental, occupational, or deliberate sources, requiring intent determination in medico-legal cases. Diagnosis is challenging because symptoms appear late and metals accumulate over time. Forensic toxicologists rely on sensitive analytical techniques to detect metals in blood, hair, nails, and bone, while correlating findings with environmental and occupational evidence. Exposure to heavy metals disrupts normal growth and development in newborns and infants (Szukalska et al., 2021). Numerous studies have shown that infants are particularly vulnerable to these toxic elements due to their immature renal systems and limited capacity to detoxify contaminants (Al-Saleh et al., 2021; Salmani et al., 2018; Mahdavi et al., 2006; Malakootian & Golpayegani, 2013; Hojsak et al., 2015). Heavy metal contamination, in particular, has infiltrated staple foods such as wheat, rice, and potatoes, leading to biomagnification and posing severe health threats to exposed populations. Hence, the present study seeks to assess arsenic and lead exposure resulting from the consumption of contaminated drinking water and food by lactating mothers inhabiting near gangetic plains in rural areas of Bihar, as well as the secondary exposure of infants through breastmilk. Furthermore, it investigates hormones (thyrotropin, estrogen & progesterone) of lactating mothers to evaluate both carcinogenic risk and maternal–infant health implications. The primary objective, therefore, is to elucidate the correlation between heavy metal exposure and reproductive health among women across eight districts of Bihar—Samastipur, Katihar, Darbhanga, Bhagalpur, Begusarai, Khagaria, Munger, and Nalanda.

Chapter 2

Review of Literature:

2.1 Background of Arsenic

The name “arsenic” comes from the Greek word arsenikon, which means “potent” (Jolliffe, 1993; Hoonjan et al., 2018). As early as 2000 BC, arsenic trioxide (ATO, As_2O_3), popularly known as white arsenic, was generated from copper smelting and employed both as a medicinal agent and a toxin (Jolliffe, 1993). Throughout history, arsenic has occupied a dual role as both a medicine and a deadly toxin. During the Middle Ages, it gained infamy as a preferred tool for homicide and suicide, not only because of its effectiveness but also due to its association with numerous high-profile poisonings (Fig. 2.1). Its subtle administration and potent lethality earned arsenic the notorious titles “the king of poisons” and “the poison of kings”, reflecting its frequent use in political intrigues and the elimination of rivals within royal and noble circles during the medieval and Renaissance periods (Vahidnia et al., 2007).

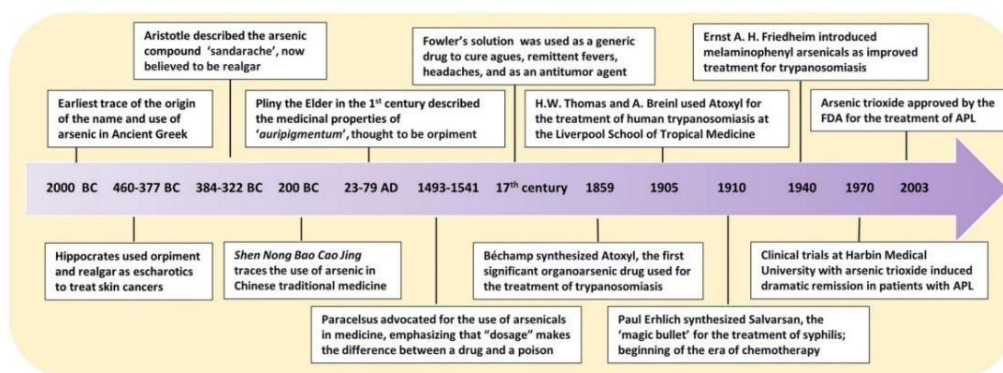


Figure 2.1. Timeline of arsenic usage since 2000 BC (Image courtesy: Paul et al, 2023)

2.2 Historical Context of Arsenic

The first recorded legal case to feature forensic evidence involved a woman sentenced to death after a servant discovered a white powder later “proven” to be arsenic. This identification was based solely on its appearance, texture, solubility in water, and the characteristic garlic-like odor released upon burning (Caudill, 2009; Cullen, 2008). A

major breakthrough in arsenic detection came in 1832, when chemist James Marsh developed an analytical method aimed at providing juries with more reliable and visible proof of arsenic's presence (Cullen, 2008). The Marsh test was famously applied in the 1840 French trial of Marie LaFarge, who was accused of poisoning her husband with arsenic-laced cakes (Cullen, 2008). In this test, the suspect sample was mixed with zinc and acid, then heated over a flame. If arsenic was present, a silvery metallic deposit would form on the glass surface—considered definitive evidence of arsenic contamination. Remarkably, this method could detect quantities as small as 0.02 mg (Hughes et al., 2011; Newton, 2007) (Tables 2.1 & 2.2). This innovation marked a turning point in forensic toxicology, transforming arsenic detection from subjective sensory judgments to a reproducible scientific procedure.

Table 2.1: Arsenic Toxicology: A Chronology of Significant Events

1786	Fowler's solution (1% potassium arsenite)
1836	Marsh Test for detection of arsenic developed
1842	Dimethylarsinic acid detected in the environment
1867	Paris green (copper acetoarsenite) used as insecticide in United States
1887	Hutchison proposes arsenic is a human skin carcinogen
1910	Salvarsan used as a chemotherapeutic agent
1940s	British antilewisite is developed
1942	U.S. arsenic drinking water interim standard set at 50 µg/l
1968	Tseng <i>et al.</i> publish findings on prevalence of skin cancer in an arsenic-exposed Taiwanese population
1975	EPA adopts drinking water interim standard at 50 µg/l
1993	WHO recommends drinking water standard of 10 µg/l
1995	Dimethylarsinic acid a tumor promoter in four rat organs
1998	Dimethylarsinic acid a complete carcinogen in rat urinary bladder
2000	FDA approves arsenic trioxide for leukemia chemotherapy
2001	EPA lowers U.S. arsenic drinking water standard to 10 µg/l (ruling delayed to 2002)
2001	Inorganic arsenic a complete carcinogen in adult mice after transplacental exposure
2002	Arsenic (+3 oxidation state) methyltransferase isolated in rat liver cytosol
2010	Inorganic arsenic a complete carcinogen in adult mice after whole life exposure

Table Courtesy- Hughes et al., 2011

Table 2.2: Electronic and Physical Characteristics of Arsenic as an Element

Property	Value
Classification	A metalloid
Atomic number	33
Atomic mass (weight)	74.9216 g/mol
Melting point	814°C
Boiling point	615°C
Electrons	33
Protons	33
Neutrons in most abundant isotope	42
Electron shells	2,8,18,5
Electron configuration	[Ar] 3d ¹⁰ 4s ² 4p ³
Density	5.7 g/cm ³ at 14°C
Molar volume	13.08 cm ³ /mole
Appearance (Color)	Grey, brittle, nonmetal flakes
Lustre of arsenic	Metallic
State	Solid
Streak of arsenic	Black
Hardness	Between 3 and 4
Specific gravity	Between 5.4 and 5.9
Transparency of arsenic	Crystal and opaque

Courtesy: Hassan, 2018 & ATSDR, 2007.

2.3 International study of arsenic exposure

Aryal, 2025 reported arsenic contamination in Nepal's Terai region posing a major public health risk, affecting millions reliant on groundwater. Chronic exposure leads to cancer, skin lesions, and heart disease, with vulnerable populations most at risk. Arsenic also accumulates in soil and crops, threatening food safety. While portable test kits and iron-based bio-sand filters offer interim solutions, sustainable community treatment systems are urgently needed. This study highlights the need for cost-effective detection, public awareness, and long-term water management to protect health in affected areas.

Nguyet et al., 2025 researched that arsenic, a hazardous element, poses serious health risks due to its mobility and persistence in groundwater. In Vietnam, the Red River and Mekong River Deltas show extreme contamination levels—up to 3050 µg/L and

1351 µg/L respectively—far above the WHO limit of 10 µg/L. Variations in aquifer geochemistry and water flow cause uneven distribution. This review examines arsenic contamination, mobilization, and remediation, highlighting natural laterite adsorption as a potential strategy to reduce groundwater arsenic levels and protect public health.

Khant et al., 2024 studied arsenic (As) contamination in Myanmar's groundwater, particularly in the Ayeyarwady region, poses a serious concern, with levels exceeding 50 µg/L. Other contaminants like fluoride, iron, manganese, and saline water are also present. Gravel-sand filters are commonly used for arsenic removal, but improvements are needed. Despite extensive well testing, health assessments remain limited, and there is no regulatory framework for groundwater management. Sustainable solutions require increased public awareness, better groundwater protection, and support from NGOs and international organizations for improved governance.

Tsai et al., 2021 researched arsenic, a known human carcinogen, poses a global health risk, particularly through contaminated drinking water. This cohort study in Taiwan (1991–2014) followed 788 adults aged ≥ 40 to examine links between arsenic exposure, DNA damage, and bladder/kidney cancer risk. Arsenic levels were measured via well water and urine; DNA damage was assessed using urinary 8-OhdG and N7-MeG. High arsenic exposure and elevated 8-OhdG levels significantly increased bladder cancer risk (HR = 4.60). Findings suggest combined arsenic exposure and DNA damage elevate cancer risk.

Khan et al., 2020 studied a systematic review of 92 studies (2011–2018) on arsenic (As) in Latin America highlights significant health impacts, especially in Chile, Mexico, and other affected countries. Evidence links arsenic exposure—particularly in utero and early life—to bladder, lung, breast, and laryngeal cancers, type 2 diabetes, kidney damage, and developmental issues. Studies also report genetic susceptibility, DNA damage, and altered gene expression. These findings emphasize the need for expanded research and targeted mitigation strategies to address arsenic-related health risks in the region.

Calatayud et al., 2019 observed chronic exposure to inorganic arsenic (iAs) affects children in Argentina, particularly in Santiago del Estero and Chaco. This study evaluated 101 children's As intake through water and food, with higher exposure in Santa Teresa de Carballo and Taco Pozo. Urine and hair As levels exceeded reference values, and increased exposure altered urinary metabolite profiles. Elevated 8-OHdG levels, a marker of DNA damage, were observed in high-exposure areas, indicating significant health consequences linked with arsenic-contaminated water and food in children.

Ratha et al., 2017 found high arsenic levels in Cambodia's groundwater, especially along the Mekong River lowlands, pose a growing public health concern. This review analyses data from seven provinces, showing that 35–38% of tested tube wells exceed the national arsenic limit (50 ppb), with Kandal being the most affected. Contamination is linked to alluvial sediments over bedrock. Addressing the crisis requires detailed area-wise assessment, public education, medical support, improved rural water supply, and regular monitoring to ensure safe drinking water access.

Chakraborti et al., 2015 studied arsenic contamination in Bangladesh's groundwater was first identified by SOES, Jadavpur University in 1992, though not officially acknowledged until after 1998. Despite early warnings, agencies like BGS and the Bangladesh government initially denied the issue. SOES and Dhaka Community Hospital later analyzed over 54,000 tube wells and biological samples, revealing widespread contamination and health impacts. Follow-up studies showed increased public awareness and reduced consumption of contaminated water, though irrigation use persists. Urgent action on surface water and stricter arsenic limits is needed.

In a study from the New Hampshire Birth Cohort, **Carignan et al., 2015** evaluated arsenic exposure in infants by analyzing its levels in tap water, infant urine, and breast milk. The findings revealed that formula-fed infants had 7.5 times higher urinary arsenic concentrations and 5.5 times greater estimated daily arsenic intake compared to breastfed infants. Breast milk contained only minimal arsenic levels, even in regions with contaminated drinking water. These results underscore that formula

powder and water are major sources of early-life arsenic exposure, while breastfeeding serves as a protective factor against such toxic exposure during infancy.

Neamtiu et al., 2015 researched in Timis County, western Romania, arsenic levels in drinking water sources used by pregnant women were generally low (median 3.0 µg/l), though higher in wells than taps. Urine biomarkers showed slightly higher inorganic arsenic exposure in women using contaminated water, but differences were not significant, suggesting other exposure sources. Compared to neighboring Arad and Bihor counties, Timis County's arsenic exposure appears lower, but further investigation is needed to clarify exposure origins and regional risks.

In his **2014** book on the Wakayama arsenic poisoning incident, **Kawai** highlighted the use of advanced analytical methods, including Spring-8, one of the world's largest synchrotron radiation facilities, along with several other scientific techniques. However, it was later revealed that many of the forensic analyses submitted by the prosecution were fabricated, with data manipulated through misleading logarithmic calculations, rendering them scientifically invalid. Furthermore, numerous court testimonies provided by analysts were found to be false, and examples of these falsified analyses are discussed in detail in the book.

2.4 Indian study of arsenic contamination

Tiwari et al., 2025 researched metal toxicity in groundwater near Umaria coalfields poses significant health risks. This study assessed concentrations of As, Al, Cd, Ba, Cr, Co, Fe, Cu, Ni, Mn, Se, Pb, Zn and Sr during post- and pre-monsoon seasons. Al, Fe, and Mn exceeded drinking water standards in many samples, with lower concentrations post-monsoon due to dilution from rainfall. Principal component analysis identified geogenic sources, coal mining, and traffic as contributors. Health risk assessment revealed higher risks for children, with Hazard Index values exceeding safe limits in up to 76% of samples. Ingestion was the primary exposure route; carcinogenic risks were mostly within limits.

Kharkwal et al., 2023 studied health risks from heavy metals in 150 milk samples from rural and urban households in Bathinda and Ludhiana, Punjab. Arsenic,

cadmium, and lead levels were within permissible limits; mercury was undetected. While non-carcinogenic risk was low for all groups, some Bathinda children urban (50% males, 86% females) and rural (25% males) faced carcinogenic risk from arsenic and cadmium. Despite overall low risk, findings highlight the need for regular milk monitoring to prevent heavy metal-related health hazards.

Singh et al., 2022b assesses groundwater quality in Bahraich district, Uttar Pradesh, using Water Quality Index (WQI) and geochemical analysis of 40 groundwater and 5 surface water samples. About 42% of groundwater samples exceeded the WHO arsenic limit (10 µg/L), with high levels of Fe and Mn also detected. Over 80% of samples were classified as poor or unfit for drinking. Hydrogeochemical and isotopic analyses suggest rock–water interaction and local precipitation as key factors, indicating a geogenic origin of arsenic contamination.

Bhardwaj et al., 2020 reported heavy metal contamination in groundwater and surface water in the Singrauli industrial belt using pollution indices (HPI, CD, HEI). Analysis of 48 samples showed widespread contamination with arsenic, mercury, lead, and cadmium—88% of groundwater and 90% of surface water exceeded safe mercury levels, and all samples exceeded WHO arsenic limits. Spatial mapping linked contamination to industrial zones. Land-use changes from 2000–2016 revealed declining water bodies and rising mining activities. Findings urge urgent action to manage and reduce water pollution.

Sen & Sarkar, 2019 found water pollution, especially from industrial and domestic waste, severely affects underground water quality in India, notably in West Bengal. Around 50 million people in the Ganga–Brahmaputra basin face high arsenic contamination, causing health issues like hyperpigmentation, black foot disease, and cancer. A study of 59 tube well samples across six West Bengal districts found arsenic most abundant in Burdwan, with the highest levels in Dumdum and Kolkata areas. Iron, phosphate, and microbial contaminants were also present. Despite awareness, many continue using contaminated water, highlighting the urgent need for effective arsenic removal technologies to ensure safe drinking water.

Mahanta et al., 2016's study estimates the health costs of arsenic contamination in Assam, India, affecting nearly one million people. Using survey data from 355 households and a 3SLS model, it finds that each 1 µg/L increase in arsenic raises annual health costs by INR 4 per household. Reducing arsenic to the safe limit (50 µg/L) yields an average welfare gain of INR 862 per household. For Assam's population, this translates to INR 153 million in welfare gains, highlighting the urgent need for safe drinking water policies.

2.5 Bihar study on arsenic toxicity

Chayal et al., 2025 studied about arsenic contamination in groundwater and food poses severe health risks worldwide. In this study, 286 household handpump water samples, food grains (wheat and rice), and biological samples (hair and nails) were analyzed from six arsenic-affected districts of Bihar. Arsenic levels exceeded permissible limits in 14% of water, 44% of wheat, and 3% of rice samples. Elevated concentrations were also found in 69% of hair and 11% of nail samples, showing strong correlations with water and food arsenic levels. Hazard quotient and cancer risk assessments indicated greater exposure through wheat than rice, highlighting an urgent need for effective mitigation strategies.

Kumar et al., 2024a reported that 77% of household hand pump water samples contained arsenic concentrations exceeding the WHO's recommended limit. 60% of urine samples from individuals showed elevated arsenic levels, reaching up to 374 µg/L, while 64% of hair samples exceeded the permissible limit, with concentrations as high as 11,398 µg/kg. The hazard quotient (HQ) analysis indicated an arsenic risk of 87.11% in children, 83.15% in females, and 82.27% in males through groundwater exposure—values that surpass the threshold for carcinogenic risk (CR) across all population groups studied. These findings highlight that residents of Kali Prasad village face a severe health burden and are at high risk of arsenic-related diseases.

Kumar et al., 2024b found that groundwater arsenic contamination in Bihar presents severe health risks, particularly for children exposed through breastmilk. A study involving 378 lactating women, 58 non-lactating women with diseases, and 77 women in a health study revealed concerning high arsenic levels in breastmilk (55%

exceeding WHO limits), and infant urine (67% exceeding permissible limits). Alarming, infants eliminated only 50% of ingested arsenic, highlighting significant exposure risks. Contaminated staple foods like rice, wheat, and potato contribute to this problem, necessitating urgent intervention and strict regulations to safeguard future generations.

Sakamoto et al., 2024 found that for every 1% increase in well- and canal-irrigated areas, the incidence rate of gallbladder cancer (GBC) rose by up to 3.0% and 5.2%, respectively—an effect likely linked to carcinogenic exposure through irrigation water. Furthermore, improved access to electricity may have facilitated greater use of groundwater pumps, inadvertently increasing exposure to these contaminants. The study emphasizes the urgent need for targeted public health policies, fine-scale spatial analyses, and continuous environmental monitoring and research to unravel the complex interplay of factors driving GBC risk.

Kumar et al., 2023a reveals that groundwater arsenic contamination poses severe health risks, including cancer. A study of 203 women with breast cancer revealed significantly elevated blood arsenic levels in 72% of them, with a maximum concentration of 1620µg/L, compared to minimal levels in controls. In Bihar, chronic arsenic exposure leads to hormonal imbalances, particularly elevated estrogen and prolactin. Prolonged high prolactin induces breast lumps, which, if untreated, can become malignant after 10-15 years, establishing a clear link between arsenic and breast carcinogenesis.

Kumar et al., 2023b reported gallbladder cancer (GBC) has alarmingly risen in India, particularly in Bihar's arsenic hotspots. This study, involving 28 GBC patients and 11 controls, revealed significantly elevated arsenic levels ($p < 0.05$) in blood, tissue, stones, bile, and hair of GBC cases. Further analysis of 512 GBC patient blood samples confirmed high arsenic concentrations. These findings strongly suggest a correlation between chronic arsenic exposure and GBC incidence in the Indo-Gangetic plains, highlighting arsenic as a potential key factor in the disease's development.

Kumar et al., 2022 researched the rapid spread of arsenic contamination in Bihar, India, imperils millions, often unknowingly, compounding severe health risks. A study in Chapar village, Samastipur, revealed alarming levels of arsenic in groundwater, hair, and urine samples of 113 residents, linking it to widespread health issues like skin lesions, organ dysfunction, and even cancer. With over half the groundwater exceeding WHO limits and carcinogenic risks nearing 100%, the crisis demanded urgent action. The study's findings prompted the government to install arsenic filter units, now supplying safe water to 250 households, demonstrating a vital intervention for this afflicted community.

Kumar et al., 2021c published a study about arsenic contamination in groundwater, a global health crisis, severely impacts India, particularly the Ganga-Brahmaputra plains. In Bihar's Saran district, Sabalpur village reveals alarming arsenic exposure, evidenced by high levels in groundwater (61% exceeding WHO limits, peak 244.20 µg/L), hair (88% above 0.2 mg/Kg), and nails (92% above 0.5 mg/Kg). A village survey linked this to prevalent health issues: asthma, anemia, and even cancer (0.94%). To mitigate this, an arsenic filter plant was installed in March 2020. Though progress is expected, sustained awareness and motivation are crucial for long-term health improvement.

Kumar et al., 2021d studied severe arsenic contamination in Buxar, Bihar, revealing alarmingly high levels in groundwater (up to 1908 µg/L) and human blood (up to 462.1 µg/L), with 12 cancer cases observed within two years. To mitigate this, an arsenic filter plant was installed. After one year, a significant reduction in disease symptoms was noted, demonstrating the intervention's effectiveness. The research highlights the critical need for remediation in arsenic-affected regions.

Kumar et al., 2020 found that arsenic contamination poses a grave global health crisis, affecting over 300 million people worldwide, with 180 million in the Ganga-Meghna-Brahmaputra plains alone. In Bihar, 18 out of 38 districts suffer severely. A study in Gyaspur Mahaji, Patna, revealed alarmingly high arsenic levels in groundwater, correlating with poor health: arsenicosis, gastrointestinal issues, anemia,

and even cancer. Urgent intervention is crucial to provide safe drinking water and mitigate the devastating impact on this highly vulnerable population.

Kumar & Ghosh, 2019a reveals that globally, 200 million face arsenic poisoning & Bihar alone impacting the population of 50 million. Consuming contaminated groundwater, used for drinking and irrigation, exposes populations to high inorganic arsenic levels. This triggers skin diseases, anemia, and cancers. A study links daily intake of 2 liters of 50 µg/L arsenic water to a 1% cancer risk, confirming arsenic's role in various cancers, including skin, bladder, and lung, highlighting a severe public health crisis.

Global arsenic poisoning has surged, with Bihar, India, facing a severe crisis, particularly in Buxar district. **Kumar et al., 2019b's** study investigated the impact of arsenic-contaminated groundwater on Simri village schoolchildren. Water and hair samples revealed alarmingly high arsenic levels, reaching 857 µg/L and 12.609 mg/Kg respectively, indicating prolonged exposure. Neuro-behavioral tests (CPT, SDT, PMT, SAB) demonstrated significant impairments in intelligence, memory, attention, and concentration. The direct correlation between water and hair arsenic levels and these cognitive deficits underlines the urgent requirement for aimed intrusions to alleviate this devastating health issue in the region.

In the **Kumar et al., 2019c** study, arsenic contamination plagues Bihar's groundwater, impacting over five million people with levels exceeding WHO limits, particularly in Buxar's Simri village, displaying 1929 µg/L. Beyond arsenicosis, residents suffer weakness, anemia, and mental stress. Studies confirm high arsenic levels in both water and biological samples, linking contamination to rising cancer cases. This necessitates urgent strategic policies to mitigate cancer risks in these arsenic-affected regions.

Kumar et al., 2016 researched that arsenic contamination gravely threatens the Middle Gangetic Plain, with Bihar's 16 districts, including Buxar's Simri village, facing severe groundwater pollution. A study of 322 water samples revealed alarming arsenic levels, particularly in Halwa Patti and Doodhi Patti strips. Statistical analyses, including ANOVA and Dunnett's test ($p < 0.05$), showed significant correlations

between arsenic concentration, hand pump depth, and distance from the Ganga. This novel research highlights the village's widespread contamination, placing a large population at high health risk. Urgent intervention is crucial to mitigate this looming health crisis.

2.6 Background of Lead

Lead was one of the first metals used by humans, valued for its simplicity of extraction and amazing ductility. As a result, lead poisoning has been documented throughout history (Waldron, 1973). The first precise records of lead toxicity date back to the second century BC, when Hellenistic physician Nicander of Colophon documented the acute symptoms of high-dose exposure, such as paralysis and saturnine colic (Riva, 2011; Gochfeld, 2005; Bertazzi, 2010).

2.7 History of Lead

The toxic nature of lead was recognized as early as 2000 BC, and its extensive use throughout history led to widespread chronic plumbism in several civilizations. Hippocrates (450–380 BC) observed a link between gout and diet, particularly the consumption of food and wine, although the connection between gout and lead poisoning was not yet understood. During the Roman era, however, gout became increasingly common among the elite, a condition now believed to have stemmed from their high levels of lead exposure (Needleman, 1999).

Lead poisoning among children was historically linked to lead-based paints, leading to their widespread prohibition. Nonetheless, older buildings may still contain residual lead paint, posing a risk of accidental exposure. In many industrialized nations, white lead paint has been banned, though yellow lead chromate continues to see limited use. It is strongly advised that old paint not be removed by sanding, as this process releases inhalable lead dust (Marino et al., 1990). Beyond paints, traditional medicines—particularly some Ayurvedic preparations—have been found to contain hazardous levels of lead and other heavy metals, leading to reported cases of metal toxicity (Karri et al., 2008). Similarly, fruits and vegetables grown in lead-contaminated soils may accumulate dangerous concentrations of the metal. Such contamination often originates from old water pipes, lead-based paints, and residual

emissions from leaded gasoline used prior to environmental regulations introduced by the U.S. Environmental Protection Agency (EPA) around 1980. To mitigate domestic lead exposure, public awareness of major contamination sources is crucial. Lead plumbing systems, in particular, remain a significant contributor to household exposure (Moore, 1977). Once ingested, the body absorbs 20–70% of lead, with children exhibiting even higher absorption rates, whereas dermal absorption of inorganic lead remains comparatively low (Table 2.3).

Table 2.3: Physical and Chemical Properties of Lead and Compounds

Property	Lead
Classification	A metal
Atomic number	82
Molecular weight	207.2
Color	Bluish-white, silvery, gray metal
Physical state	Solid
Melting point	327.4°C
Boiling point	1,740°C
Density	11.34 g/cm ³ at 20°C
Solubility:	
Water	Insoluble
Acids	Soluble in dilute nitric acid ^d ; reacts with sulfuric acid
Bases	No data

Courtesy- ATSDR, 2007

2.8 Lead Literature Review

In **1656 (16th century)**, German physician Samuel Stockhausen identified lead dust and fumes as the cause of a disease long known to miners and metalworkers as *morbi metallici*—an ailment described since Roman times that afflicted miners, smelters, and potters regularly exposed to the metal (Eisinger, 1982; Gochfeld, 2005). The Italian painter Caravaggio is believed to have been a victim of lead poisoning; analyses of bones from a grave thought to be his revealed high lead concentrations. Given that paints of the era contained large amounts of lead salts, this finding is consistent with his known erratic and violent behavior, a classic symptom of lead toxicity (Kington, 2010).

In **17th-century** Germany, physician Eberhard Gockel traced an epidemic of colic to lead-contaminated wine, observing that monks who abstained from wine remained healthy, while drinkers suffered severe abdominal pain (Pearce, 2007). He discovered that the culprit was “sugar of lead,” a sweetener produced by simmering litharge with vinegar (Eisinger, 1982). This led Eberhard Ludwig, Duke of Württemberg, to issue a 1696 law banning the adulteration of wines with litharge (Eisinger, 1982).

During the **18th century**, lead poisoning became widespread due to the use of lead components in rum stills, particularly the *worm*—a coiled condenser. This practice caused significant mortality among slaves and sailors in the colonial West Indies (Varney et al., 2012; Curtin, 1989), and similar cases were reported in Boston (Brands, 2002). Around the same period, “Devonshire colic” was diagnosed among cider drinkers in Devon, England, where lead-lined presses contaminated the beverage (Pearce, 2007). Lead was also illegally added to cheap wines as a sweetener during the 18th and early 19th centuries (Mai, 2006). The composer Ludwig van Beethoven, a heavy wine drinker, was later found to have higher lead levels in his hair, suggesting lead toxicity as a possible cause in his hearing impairment and death (Mai, 2006; Weiss, 2005).

With the advent of the Industrial Revolution in the **19th century**, (Goldfrank & Hoffman, 2006), the introduction of lead-based paints for residential use during this period further increased childhood exposure, marking a shift from primarily occupational to domestic lead toxicity (Bellinger, 2004). Reform efforts soon followed. William James Furnival (1853–1928) presented extensive evidence to the British Parliament in 1901, advocating for the removal of lead from the ceramic industry. In the United States, Alice Hamilton (1869–1970) conducted pioneering investigations into industrial lead exposure, presenting her findings in 1911 at the 23rd Annual Meeting of the American Economic Association (Hamilton, 1911). A major advancement in public health awareness occurred in 1897, when lead toxicity in children from household paint was first identified in Australia (Goldfrank & Hoffman, 2006). However, the United States did not enact similar legislation until 1971, and lead-based paint was not fully banned until 1978 (Gilbert & Weiss, 2006). From the 1920s onward, tetraethyl lead was added to gasoline to improve combustion

efficiency, resulting in widespread environmental contamination. Although the use of leaded gasoline was gradually phased out beginning in the 1980s, its residues persist in soil and household dust. Encouragingly, global blood lead levels have declined sharply since that phase-out (Fig. 2.2) (Rossi, 2008).

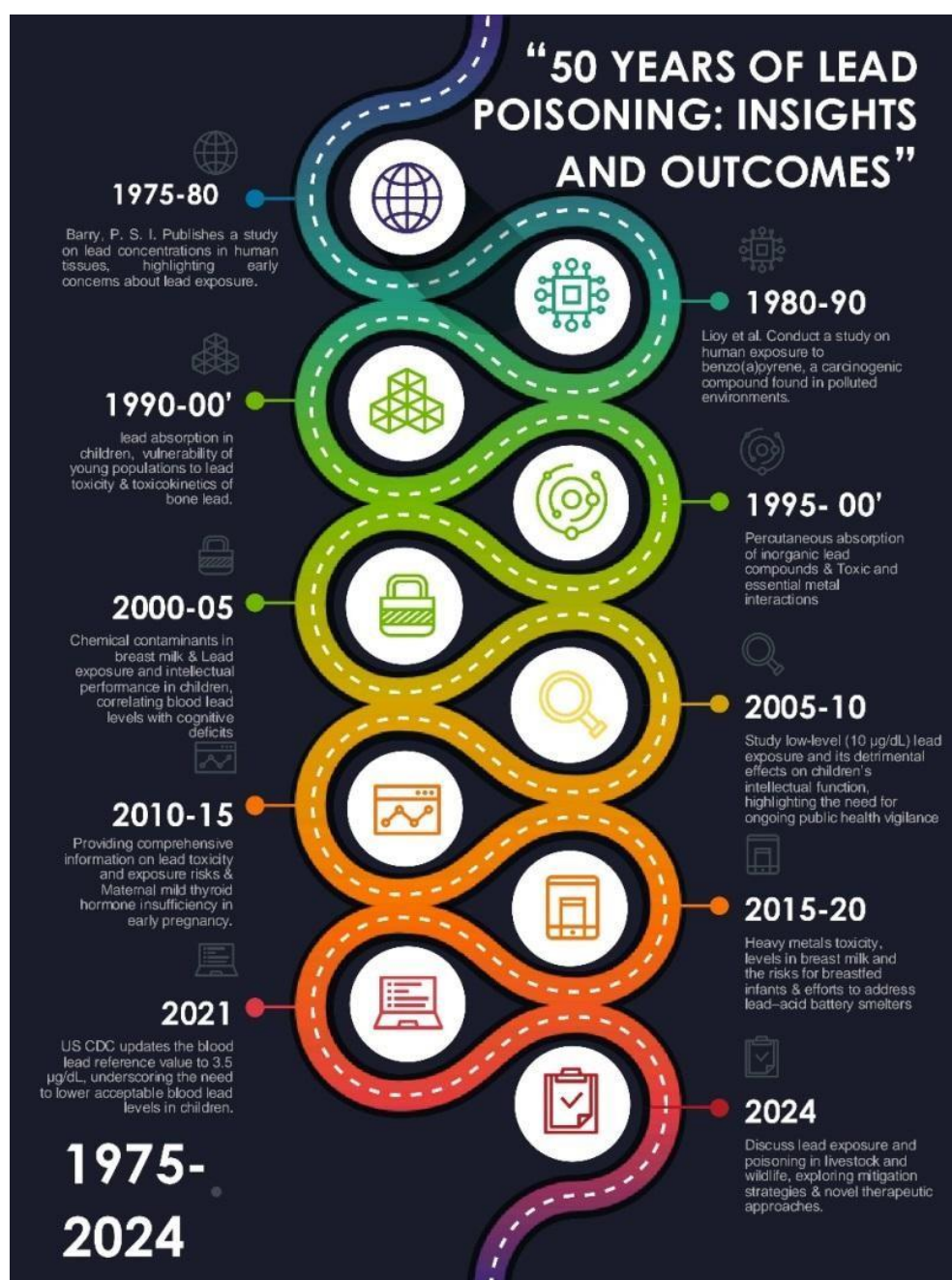


Figure 2.2: Timeline of 50 years of lead poisoning: insights & outcomes

DiMaio et al. (1983) reported that lead poisoning resulting from a retained bullet or missile is an uncommon occurrence, typically arising when the projectile is embedded within bone or situated near a joint, where lead can be gradually absorbed into the bloodstream. In such cases, systemic toxicity may develop, and in rare instances, the condition can prove fatal.

Najari et al., 2021 studied the escalating industrialization and addiction rates have propelled lead toxicity into a critical public health crisis, demanding robust data for effective management. This study, analyzing 30 cadavers suspected of lead poisoning, revealed significant correlations: pulmonary autopsy findings with drug use duration ($P = 0.01$), cardiac pathology with age ($P = 0.006$) and blood lead levels ($P = 0.03$), liver and kidney pathology with age ($P = 0.00$), and brain pathology with drug administration route ($P = 0.01$). While lead doesn't induce unique organ-specific changes, the observed non-specific pathologies, particularly in the brain and kidneys, collectively contribute to fatal outcomes.

Bhardwaj et al., 2021 reported that exposure to heavy metals such as cadmium (Cd), mercury (Hg), arsenic (As), lead (Pb), zinc (Zn), and copper (Cu) can profoundly disrupt reproductive function in both males and females. These metals have been shown to cause a decline in sperm count, motility, and viability, impair spermatogenesis, and induce hormonal imbalances, follicular atresia, and delayed oocyte maturation, among other effects. Collectively, these findings highlight the critical role of heavy metals in reproductive toxicology.

Collin et al., 2022 provided a comprehensive review of lead poisoning, its prevention, and therapeutic approaches. The study emphasized that micronutrients and antioxidants play a significant role in mitigating the toxic effects of lead exposure. For clinical management, chelation therapy is recommended for adults with blood lead levels (BPb) exceeding 45 µg/dL, while succimer is the preferred treatment option for affected children.

Dutta et al., 2022 reported that metal exposure adversely affects female reproductive health at every level of its regulation and function—including development,

maturation, and endocrine activity—and is strongly associated with a rising incidence of infertility among women.

Fry & Taylor, 2024 revealed that even with lead product phase-outs, hidden contamination remains a public health concern. When standard sources fail to explain elevated blood lead levels, a forensic approach is vital. In a notable case from Sydney, a pewter pepper grinder containing exceptionally high lead levels (>6000 mg/kg, with 70% bioavailability) was identified as the source of poisoning through pXRF, ICP-MS, PbIC, and hematological analyses. Following the removal of this contaminated source, the couple's blood lead concentrations declined rapidly, falling below the Australian intervention threshold of 5 µg/dL within a year.

Kumar et al., 2025 concluded heavy metals, including lead, arsenic, cadmium, and mercury, pose significant health risks due to their high atomic weight and reactivity. Exposure, through inhalation, ingestion, or skin contact, leads to accumulation and potential irreversible neurotoxicity. This damage to the nervous system, whether acute or chronic, is particularly concerning in children, impairing cognitive and behavioral development. Heavy metals induce neurotoxicity by generating oxidative stress, disrupting antioxidant balance, and binding to macromolecules. While affecting all demographics, the central nervous system is most vulnerable. Overcoming the blood-brain barrier for treatment is a challenge, but nano formulations like solid lipid nanoparticles offer targeted drug delivery, enhancing efficacy and minimizing toxicity.

Moradnia et al., 2025 conducted this study in Isfahan city examined the relationship between urinary heavy metal levels, thyroid hormones, and birth outcomes in pregnant women. Results showed significant regional differences in arsenic, chromium, nickel, lead, selenium, and T4 levels. Lead exposure was significantly associated with reduced infant birth weight. All metals correlated positively with maternal TSH and T3/T4 ratio (except selenium). Higher TSH and T3/T4 ratios were linked to lower birth weight and head circumference, especially in female infants. Monitoring heavy metal exposure and thyroid function during pregnancy is vital for infant health.

2.9 Hypothesis

Hypothesis formulation represents a critical component of any research, particularly when statistical analysis forms the foundation of the study. In this work, emphasis is placed on the development of the null hypothesis (H_0)- a formal statement regarding a population parameter that serves as the basis for statistical testing. The null hypothesis assumes no significant difference or association within the population, and its acceptance or rejection depends on the comparative outcomes derived from the analysis. The hypotheses proposed in this study are grounded in logical assumptions established prior to the commencement of the research. The primary objective, therefore, is to conduct a rigorous evaluation to determine the validity acceptance or rejection of these hypotheses. The null hypotheses considered for the study are presented below:

1. There are hazardous effects on the environment due to the heavy metals such as soil, water, food, etc.
2. The effects after consumption of the natural resources i. e. soil for growing food, water for the purpose of drinking is significantly harmful to the human body.
3. The after effects on the human body when exposed to heavy metals are significantly dangerous.
5. The existing laws to prevent environmental degradation due to heavy metals are not very much efficient.

The above mention points are fundamentally null hypothesis of the study, which will be evaluated with respect to the case studies being considered for the study. The techniques adopted to analyze the hypothesis are briefly presented in next section.

2.10 Aims & Objectives

The key aim of the recommended study is to carry out an epidemiological study to understand the environmental and health issues developed due to the heavy metal exposure near Gangetic Plains of Bihar. In order to accomplish this aim, following are the research objectives:

- 1) To collect the data of real time samples from heavy metal affected areas.
- 2) Assessment of presence of heavy metals in the water, blood, breast milk and urine in the exposed female subjects.
- 3) Assessment of hormones – estrogen, progesterone and thyroid hormone levels in female subjects.
- 4) To submit the data to government to make policies based on GIS mapping regarding this menace.

Chapter 3

Materials & Methods

3.1 Research design

The primary objective of this study is to conduct an in-depth investigation into the effects of arsenic and lead on the human body and the environment in the rural regions of Bihar with low-income groups, particularly those situated along the Gangetic Plains and exposed to heavy metal contamination. A random sampling method was employed to ensure unbiased data representation. This sampling approach was deemed most suitable for populations that are difficult to access or where certain individuals may possess greater knowledge, willingness, or availability to participate. Participant selection was further refined through community input and respondent recommendations. The in-depth interview method was chosen as the core research tool, as it facilitates the development of comprehensive narratives and yields detailed, context-rich insights into the subject matter. This approach enables the collection of robust evidence to substantiate the forensic and analytical objectives of the study.

3.2 Description of the study area

The assignment of data collection and processing was carried out from July 2022 to December 2024 in laboratory of Mahavir Cancer Sansthan & Research Centre, Patna, Bihar. The districts for study were selected randomly as they were near Gangetic plains including Samastipur, Darbhanga, Begusarai, Khagaria, Munger, Katihar, Bhagalpur & Nalanda (Table 3.1). The total area is about 94,163 km². The quality of groundwater due to heavy metal exposure is under constant threat of contamination directly or indirectly. Therefore, it becomes necessary to evaluate the female reproductive health risks in the exposed population of state of Bihar so that the immediate strategies can be made to control the problem.

Table 3.1: Studied districts and their GPS Co-ordinates-

S.No.	Studied Districts	Latitude	Longitude
1.	Samastipur	25° 51' 43.99" N	85° 46' 46.31" E

2.	Darbhanga	26° 9' 10.7028'' N	85° 54' 5.0868'' E
3.	Begusarai	26° 9' 10.7028'' N	85° 54' 5.0868'' E
4.	Khagaria	25° 30' 23.9724'' N	86° 28' 21.0972'' E
5.	Munger	25° 22' 32.5560'' N	86° 28' 27.7428'' E
6.	Katihar	25° 32' 0.6288'' N	87° 35' 1.4928'' E
7.	Bhagalpur	25° 15' 12.2076'' N	86° 59' 20.6124'' E
8.	Nalanda	25° 7' 20.1540'' N	85° 27' 22.2372'' E

3.3 Sample Size

The participants in the present study comprised lactating mothers, with a total of n= 250 females (age between 18-30 years) through the sample size formula used in previous studies done by the MCSRC institute with the help of Govt. funded projects. Women who voluntarily provided informed consent took part in the research. Among them, n= 200 participants were recruited from the exposed area, while n= 50 were selected from Nalanda, serving as the control group. Each participant contributed biological samples including blood, breast milk, and urine for analytical evaluation. The sample size was determined using a standard statistical formula and aligned with methodologies adopted in previous investigations conducted by the Mahavir Cancer Sansthan & Research Centre, Bihar.

3.4 Ethics approval

Ethics approval was granted with IEC Letter No. RMRI/EC/24/2020 dated September 26, 2020, by the Institutional Ethics Committee of the Indian Council of Medical Research Unit—Rajendra Memorial Research Institute of Medical Sciences (MoU for Ethics approval), Patna, Bihar, India. This approval applies to all aspects of the research activity. The objectives of the research were explained to all the subjects, and their written and signed informed consent was acquired before the inquiry started. All procedures used in the study were compliant with the ethical norms outlined in the Declaration of Helsinki (WMA, 2025) as well as those established by the Indian Council of Medical Research and the Government of India regarding the use of human subjects in research.

3.5 Survey technique

The descriptive part of the study was done utilizing a study look into technique which includes random field visits to different villages. Study techniques were arranged by personally meeting the respondents and collecting the information as well as the samples of ground water, blood, breast milk & urine. All the samples were collected by the researcher herself. The questions were asked verbally and then noted in the questionnaire. While collecting the biological samples from the females, most difficult challenge was to take the blood & breast milk sample because they did not volunteer easily for the sample as they were not aware of the heavy metal exposure which they were dealing with. So, that's why the team have to search a lot for the participants in the village but somehow managed to collect all the samples from different districts. The research survey & experimental parameters are as shown in Table 3.2.

Table 3.2: Research survey parameters

Research Methodology	Qualitative, Quantitative and Experimental techniques
Questionnaire Design	Structured questionnaire
Location	Samastipur, Darbhanga, Begusarai, Khagaria, Munger, Katihar, Bhagalpur & Nalanda (Bihar, India)
Total No. of Respondents	250 females
Tools Used	Graph Prism 8.0, Statistical Package for Social Sciences (SPSS)20.0, Arc GiS
Participant Sampling	Ground Water samples- 250 Blood samples- 250 Breast milk samples -250 Urine Samples -250
Analytical Instrument	Graphite-Furnace Atomic Absorption Spectrophotometer (Pinnacle 900T, Perkin Elmer, USA), Enzyme Linked Immunosorbent Assay (ALTA)

Table 3.3 Criteria for selection of subjects

Inclusion Criteria	Women who are lactating & having severe health problems Age Bar Should be at locality for 6 months
Exclusion criteria	Women at older age. Male subjects.

3.6 Collection of the household water samples

The household groundwater samples were collected in 30mL high density polyethylene bottles with a narrow opening. The sample bottles were rinsed and pre-treated with 2% HCl before sampling. The hand pump water source's GPS coordinates and depth were recorded. All samples of water were preserved immediately after collection using 1.5ml/L nitric acid to lower the pH to 2.0. The total concentrations of lead and arsenic in the samples were determined using a Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS), specifically the Perkin Elmer Pinnacle 900T model (USA), ensuring high precision and analytical reliability in the quantification process.

3.7 Collection of the biological samples

The chosen women willingly provided 5mL of breastmilk samples in sterile falcon tubes (15mL), blood samples 0.5 mL in EDTA vial and 1.5 mL in plain vial. The blood samples collected in plain vacutainer were transferred in micro centrifuge tube for serum separation through mini centrifuge in the field and stored in Tropicool portable fridge at -20 degree centigrade and their urine samples in a sterile plastic container (50ml) which were collected in respective containers (Fig 3.1). Then all the samples were transported to the research laboratory of Mahavir Cancer Sansthan & Research Centre, Patna, Bihar for further storage at -20 degree centigrade in deep freezer. Then the samples were analyzed on Graphite Furnace based Atomic Absorption Spectrophotometer (GF-AAS) and ELISA.





Figure 3.1. Collection of real time samples while doing field visits

3.8 Instruments used for the study

3.8.1 Atomic absorption spectrophotometer

In the current investigation, the Perkin Elmer PinAAcle 900T (Model No. AAN3160080) was employed for all liquid sample analysis in Graphite mode. Atomic absorption spectrophotometer with graphite furnace device, hollow-cathode lamp with appropriate temperature and time controls, and a strip chart recorder for furnace work were employed, as well as wavelengths of 193.7 nm for arsenic and 283.3 nm for lead (Fig 3.2).



Figure 3.2: PerkinElmer PinAAcle 900T Atomic Absorption Spectrometer instrument for heavy metal analysis (Bionex Scientific)

3.8.2 Principle of AAS

Atomic Absorption Spectroscopy (AAS) is based on the principle that free ground-state atoms absorb light at element-specific wavelengths, and the absorbed intensity is proportional to analyte concentration (Yeung et al., 2017). Element-specific radiation from a hollow cathode lamp passes through an atomized sample, where target atoms selectively absorb the light (Aggarwal et al., 2022). Absorbance follows the Beer–Lambert law, enabling quantitative analysis using calibration curves prepared from known standards (Boldyrev, 2018; Donati & Amais, 2019). An AAS system consists of a light source, atomizer (flame or graphite furnace), monochromator, and detector (Yeung et al., 2017). For arsenic and lead, samples are analyzed in dissolved form or after acid digestion using HNO_3 and HClO_4 (Price & Whiteside, 2017) (Fig. 3.3).

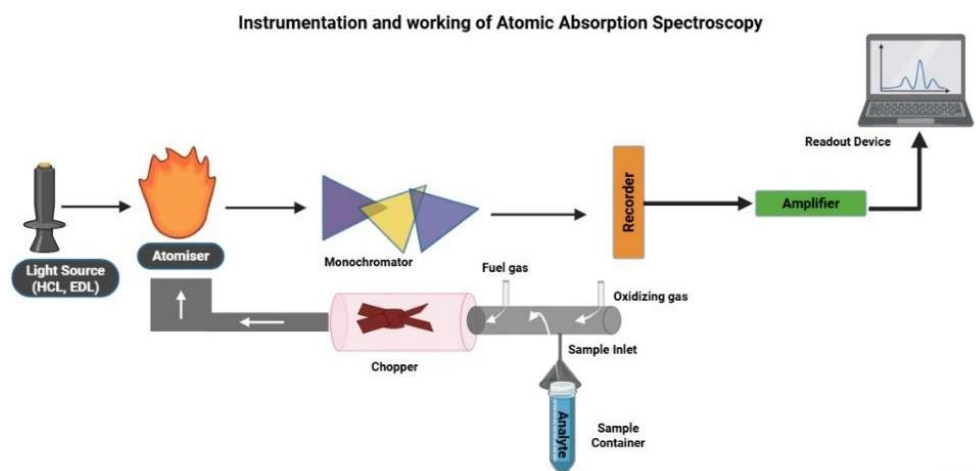


Figure 3.3: Basic working principle of Atomic Absorption Spectroscopy

3.9 Quality control

Arsenic and lead standards, each with a concentration of 1000 mg/L, were procured from Perkin Elmer-Arsenic from the USA (CAS No. As7440-38-2, Lot No. 25-127ASY1, PE No. N9300180) and lead from Singapore (CAS No. Pb7439-92-1, Lot No. 25170PBY1, PE No. N9300175). Standard stock solutions were prepared through precise point dilutions and optimum working condition (Table 3.5). Throughout the analytical process, the calibration correlation coefficient was consistently maintained at 0.999, ensuring high analytical accuracy and reliability. The detection limits for arsenic were determined as 0.07 µg/L in breast milk, 0.09 µg/L in blood, 0.09 µg/L in urine, and 10 µg/L in water samples. Correspondingly, the detection limits for lead were 0.05 µg/L in breast milk, 0.08 µg/L in blood, and 0.06 µg/L in urine samples, demonstrating the method's high sensitivity across diverse biological and environmental matrices.

Table 3.4: Instruments & Chemicals used

Products	Company Name
HNO ₃	Merck Life Sciences Private Limited
HClO ₄	Merck Life Sciences Private Limited
Distilled Water	Nice Chemicals Private Limited
Whatman 41 Filter paper	Cytiva
Water Bath	Rescholar
Hotplate	Superteck
AAS	Perkin Elmer

ELISA	ALTA
Matrix Modifiers	Perkin Elmer

Table 3.5: Working Conditions of the instrument

Setup Data	For Lead (Pb)	For Arsenic (As)
Wavelength	283.3 nm	193.7 nm
Slit	0.7 nm	0.7 nm
Rollover	1.50 abs	1.30 abs
Temperatures		
Pyrolysis	850 °C	1200 °C
Atomization	1600 °C	2000 °C
Atomization site	Pyro/Platform	Pyro/Platform
Chemical Modifier	0.050 mg NH ₄ H ₂ PO ₄ + 0.003 mg Mg(NO ₃) ₂	0.005 mg Pd + 0.003 mg Mg(NO ₃) ₂
Performance Checks		
Characteristic mass	30 pg/0.0044 A-s	40 pg/0.0044 A-s
(typical)	50 µg/L for 0.15 A-s	50 µg/L for 0.11 A-s
Sensitivity check		

3.9.1 Calibration for Groundwater

Prepare a blank with 1% HNO₃ and sufficient standards reagent containing 0 to 100 µg/L of respective metal As & Pb by diluting suitable volumes of standard arsenic and lead solution with 1% HNO₃ and standard solution. Aspirate the reagent blank and carry out zero adjustment. Extract consecutively the standard solutions (25 µg/L, 50 µg/L, 75 µg/L, 100 µg/L) and measure the absorbance at 193.7 for As and similarly for Pb at 283.3 nm (Fig 3.4).

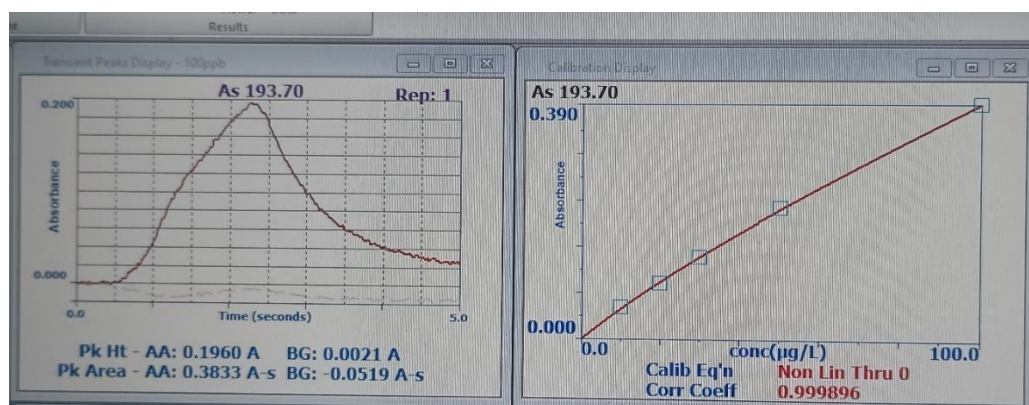


Figure 3.4: Calibration curve of arsenic standard

3.9.2 Calibration for biological samples

A series of standard solutions ranging from 0 to 50 µg/L for As and Pb were prepared by appropriately diluting the respective standard stock solutions with 1% nitric acid in volumetric flasks. A reagent blank was first aspirated to perform zero adjustment. Subsequently, the prepared standard solutions- 10 µg/L, 20 µg/L, 30 µg/L, and 50 µg/L were aspirated sequentially (Fig 3.4).

3.9.3 Ground water heavy metal estimation

The collected and acidified household samples of water were filtered through the 0.45µm syringe filter and were directly measured on Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS) (Pinnacle 900T, Perkin Elmer, Singapore) at the wavelength of 193.7 nm for arsenic and 283.3 nm for total lead content (NIOSH, 1975 & 1977).

3.9.4 Estimation of breast milk, blood and urine contamination of heavy metal

For the heavy metal estimation, 0.5 ml volume of breast milk or blood or urine was taken in 25ml conical flask (glass) to which 5mL of HNO₃ was added and left it for overnight reaction. The next day, all the samples were digested on hotplate at 90°C-120°C, then the volume was reduced to 3 ml. Then 5 ml volume of HNO₃:HClO₄ (6:1) mixture was added to the pre-digested solution in the conical flask. The samples were re-digested on the hotplate at 90°C-120°C, until the volume of the solution reduced to about 2 mL. Furthermore, 1 ml of 1% HNO₃ was added and final volume was adjusted to 10 mL with distilled water. Finally, the samples were analyzed through GF-AAS for heavy metal estimation through GF-AAS (NIOSH, 1975 & 1977).

Result calculation for readings of heavy metal assessment

After digesting the samples, the volume was diluted upto 10 ml. So, the final observed reading has to be calculated through formula:

$$\text{Dilution factor} = \frac{\text{Volume make up}}{\text{amount of sample taken}} = \frac{10}{0.5} = 20$$

$$\text{Final AAS Reading} = \text{Dilution factor} \times \text{AAS Observed Reading}$$

3.10 Spatial analysis

Spatial analysis was done using the Arc-GIS Software (10.3.1) by superimposing the GPS coordinates of samples, on shape file of Bihar district and Google map was used as base map.

3.11 Statistical analysis

Graph Pad Prism 8.0 and SPSS -25.0 statistical software were used for the statistical analysis. As and Pb concentrations in groundwater, water, blood, breastmilk and urine were measured and graphed.

Table 3.6: Permissible limit of Arsenic in different types of samples

Sample type	Permissible limit	Reference
Water	10 µg/L	WHO, 2017
Blood	10 µg/L	ATSDR, 2007
Breastmilk	<1 µg/L	Bartmess, 1990
Urine	50 µg/L	CDC, 2004
Rice	200 µg/Kg	FAO/WHO, 2011
Wheat	100 µg/Kg	FAO/WHO, 2011
Potato	500 µg/Kg	FAO/WHO, 2011

Table 3.7: Permissible limit of Lead in different types of samples

Sample type	Permissible limit	Reference
Water	10 µg/L	WHO, 1995
Blood	50 µg/L	CDC, 2021
Breastmilk	<5 µg/L	Cherkani-Hassani et al, 2021
Urine	50 µg/L	CDC, 1994
Rice	200 µg/Kg	FAO/WHO, 2011
Wheat	200 µg/Kg	FAO/WHO, 2011
Potato	100 µg/Kg	FAO/WHO, 2011

3.12 Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme-Linked Immunosorbent Assay (ELISA) is a highly sensitive and specific biochemical technique used for the detection and quantification of proteins, hormones, antibodies, and antigens based on antigen–antibody interactions (Konstantinou, 2017; Mehraj et al., 2020). The binding of an enzyme-labeled antibody to the target analyte produces a measurable color change after substrate addition, enabling quantitative analysis (Tripathi & Chung, 2023). Owing to its accuracy, high throughput, and versatility, ELISA is widely applied in clinical diagnostics, environmental analysis, and forensic science (Zhang et al., 2024). In forensic investigations, ELISA is extensively used for identifying body fluids, detecting drugs of abuse, confirming biological evidence in sexual assault cases, and diagnosing infectious diseases relevant to medico-legal evaluations (Rana et al., 2024; Anzar et al., 2024; Mousawi et al., 2025; Sharma et al., 2025; Yadav & Sharma, 2024).

3.12.1 Principle of ELISA

The principle of ELISA is based on the specific binding between an antigen and an antibody coupled with enzyme-mediated signal amplification (Clark & Engvall, 2018). Either an antigen or antibody is immobilized on a microtiter plate, followed by the addition of a sample containing the target analyte, allowing specific binding (O’Kennedy & Murphy, 2017). An enzyme-linked secondary antibody—commonly conjugated with horseradish peroxidase or alkaline phosphatase—binds to the target molecule (Davis, 2019). Upon addition of a suitable substrate, the enzyme catalyzes a colorimetric reaction, the intensity of which is directly proportional to the analyte concentration (Pala et al., 2020; Aydin et al., 2025). In Sandwich ELISA, the antigen is captured between two antibodies, providing high sensitivity and specificity for detecting trace analytes in complex biological samples (Lengfeld et al., 2021). In this study, the ELISA instrument used was ALTA (Model No.- EDX-150) for different hormonal analysis (estrogen, thyrotropin & progesterone) of blood serum samples. The AccuBind kits were used to estimate the hormone level and it was observed at primary wavelength of 450 nm.

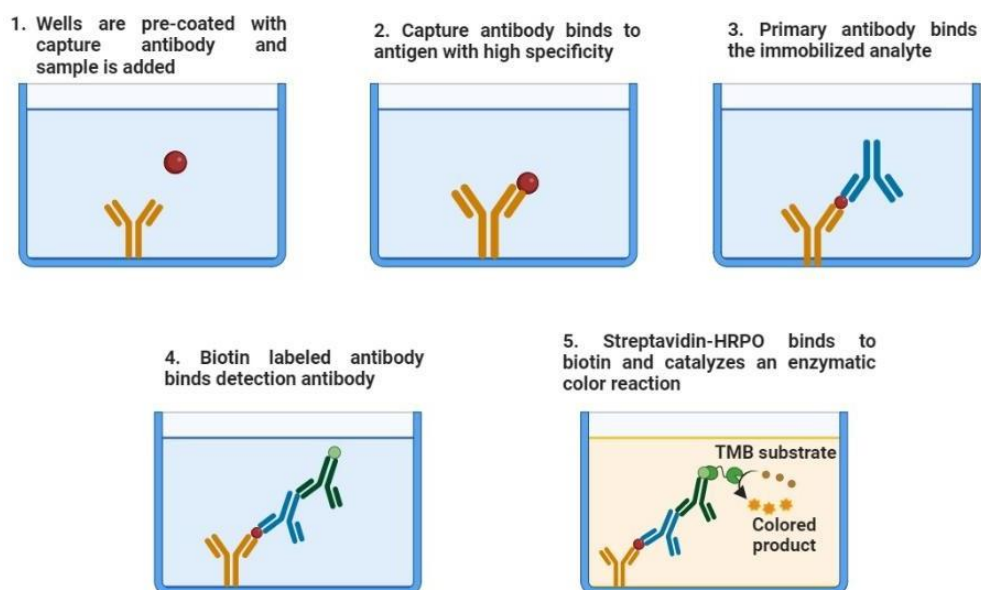


Figure 3.5: Working principle of sandwich ELISA

3.12.2 Calibration & Quality Control

For hormone assessment, AccuBind kits were used of selected female hormones- Thyrotropin (TSH), Estrogen & Progesterone. There were seven standards of different concentration in each hormone kit. The machine was standardized with each hormone and maintained a calibration curve which was shown in protocol. Each calibration curve for hormones (TSH, Estrogen & Progesterone) is depicted below in following figures (Fig 3.6, 3.7 & 3.8).

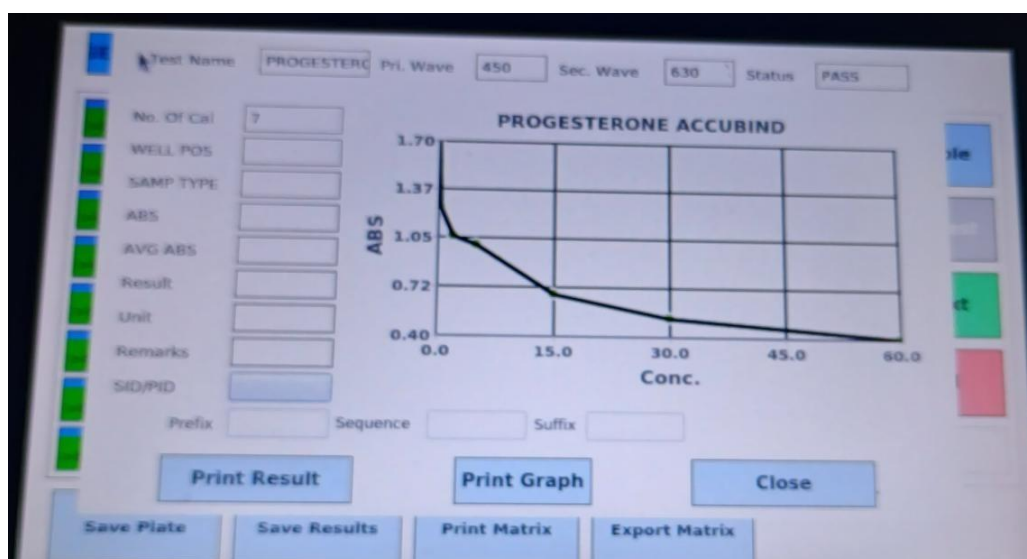


Figure 3.6: Calibration Curve obtained for Progesterone Hormone

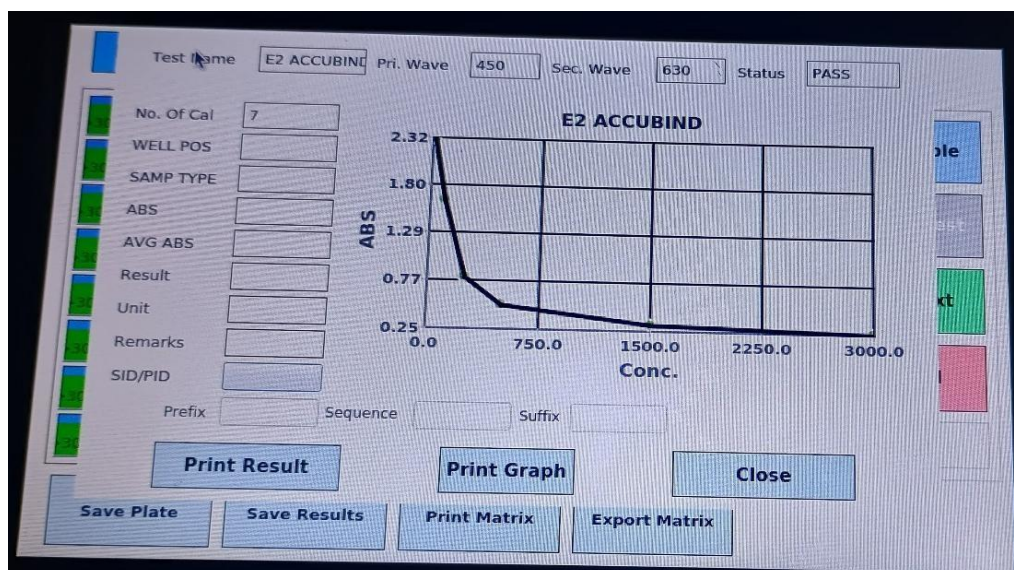


Fig 3.7: Calibration Curve obtained for Estrogen Hormone

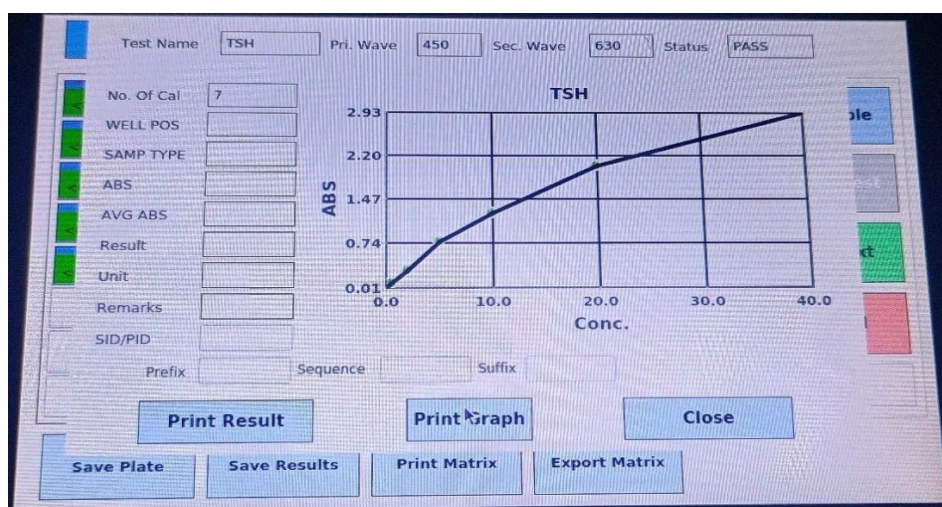


Fig 3.8: Calibration Curve obtained for Thyroid Hormone

3.13 Hormonal Analysis

The collected blood samples were preserved at 4°C until analysis using the respective hormonal assay kits. Following storage, the EDTA vials were centrifuged at 6000 rpm for 10 minutes to separate the serum, which was then carefully aliquoted and stored at -20°C for subsequent ELISA-based hormonal analysis.

3.13.1 Thyrotropin (TSH) Analysis (AccuBind & Lot No. EIA-3K2H1)

The assay was initiated by pipetting 0.050 ml (50 µl) of the appropriate serum sample into each designated well. Subsequently, 0.100 ml (100 µl) of TSH enzyme reagent was added to all wells. The microplate was gently swirled for 20–30 seconds to ensure homogeneous mixing and then covered. It was incubated at room temperature for 60 minutes. Following incubation, the well contents were removed by decantation or aspiration, and the plate was blotted dry with absorbent paper. A wash buffer (0.350 ml or 350 µl) was added to each well, followed by decantation or aspiration; this washing step was repeated twice more, totaling three washes. Thereafter, 0.100 ml (100 µl) of the working substrate solution was added to each well, and the plate was incubated again at room temperature for 15 minutes. The reaction was terminated by adding 0.050 ml (50 µl) of stop solution to each well and gently mixing for 15–20 seconds & the absorbance was then measured at 450 nm.

3.13.2 Estradiol (E2) Analysis (AccuBind & Lot No. EIA-49K3C1)

The assay commenced with the pipetting of 0.025 ml (25 µl) of the appropriate serum sample into each designated well. Subsequently, 0.050 ml (50 µl) of Estradiol Biotin Reagent was added to all wells, and the microplate was gently swirled for 20–30 seconds to ensure complete mixing. The plate was then covered and incubated for 30 minutes at room temperature. Following this, 0.050 ml (50 µl) of Estradiol Enzyme Reagent was added to each well, and the plate was again swirled gently for 20–30 seconds to achieve uniform mixing. It was then covered and incubated for 90 minutes at room temperature. After incubation, the contents of the wells were removed by decantation or aspiration, and the plate was blotted dry with absorbent paper. A wash buffer (0.350 ml) was added to each well, followed by decantation or aspiration; this step was repeated twice more, totaling three washes. Subsequently, 0.100 ml of the working substrate solution was added to all wells, and the plate was incubated for 20 minutes at room temperature. The reaction was terminated by adding 0.050 ml of stop solution to each well, followed by gentle mixing for 15–20 seconds & the absorbance was measured at 450 nm.

3.13.3 Progesterone Analysis (AccuBind & Lot No. EIA-48K2J1)

The procedure began by pipetting 0.025 ml (25 μ l) of the appropriate serum sample into each designated well. Subsequently, 0.050 ml (50 μ l) of Progesterone Enzyme Reagent was added to all wells, and the microplate was gently swirled for 10–20 seconds to ensure uniform mixing. Following this, 0.050 ml (50 μ l) of Progesterone Biotin Reagent was added, and the plate was again swirled gently for 10–20 seconds to achieve complete homogenization. The plate was then covered and incubated for 60 minutes at room temperature. After incubation, the contents of the wells were removed by decantation or aspiration, and the plate was blotted dry using absorbent paper. A wash buffer 0.350 ml was added to each well, followed by decantation or aspiration; this step was repeated twice more for a total of three washes. Subsequently, 0.100 ml of the working substrate solution was added to each well, and the plate was incubated at room temperature for 20 minutes. The reaction was terminated by adding 0.050 ml of stop solution to all wells, followed by gentle mixing for 15–20 seconds & the absorbance was recorded at 450 nm.

Overall, the study employed a robust and systematic methodology integrating environmental and biological sampling to assess chronic arsenic and lead exposure among lactating women in Bihar. Standardized collection procedures, validated analytical techniques, and strict quality control ensured data accuracy and reproducibility. The inclusion of health risk assessment and GIS-based spatial analysis strengthened exposure characterization and forensic relevance, providing a reliable foundation for result interpretation and subsequent conclusions.

Chapter- 4

Results & Discussions

The current study is focused on evaluation of heavy metal concentration in water and biological fluids (blood, breast milk and urine) through AAS & hormone concentration through ELISA in serum of females inhabiting near Gangetic plains of Bihar. The results obtained are discussed further through graphical representation and descriptive statistical analysis and spatial map analysis for submitting the data to government.

4.1 Spatial map analysis of study sites

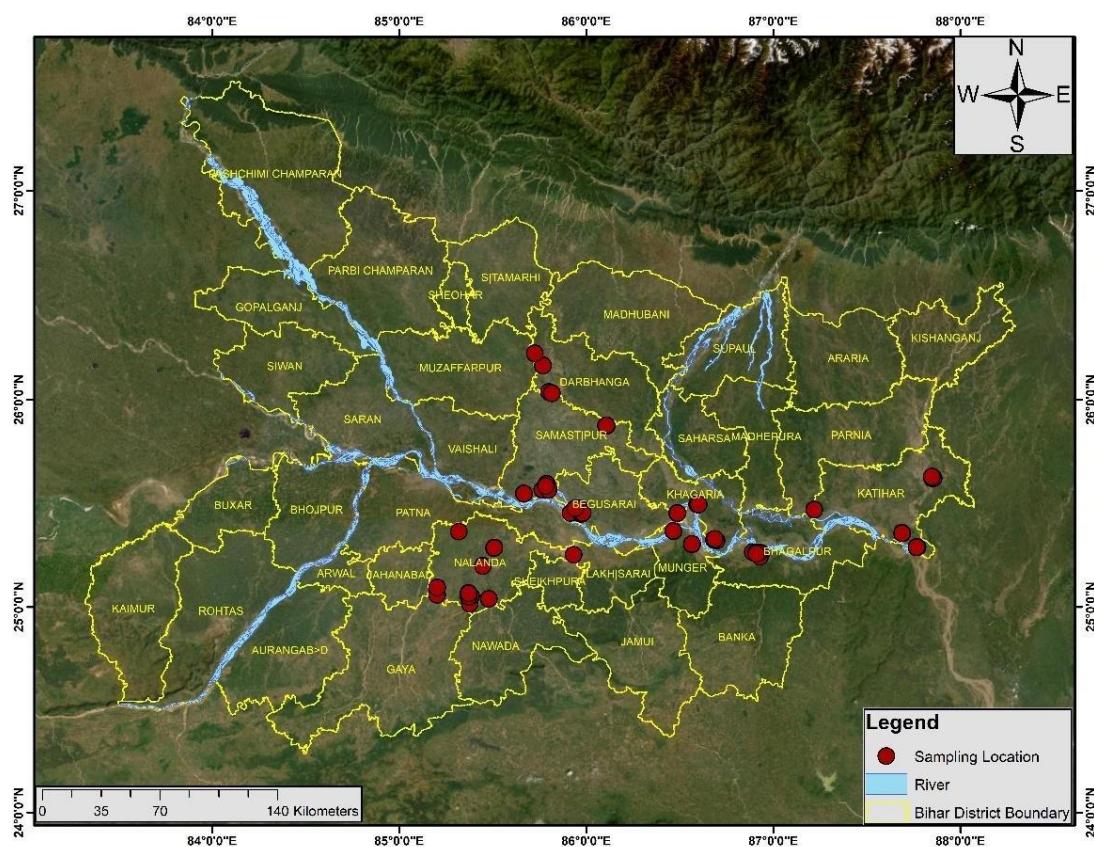


Figure 4.1: GIS location map of collected sample sites

4.2 Graphical Representation of Arsenic Results

Age wise distribution-

The following Figure 4.2A represents the age wise distribution of the female subjects inhabiting in the heavy metal exposed area. It clearly shows that in exposed area, out of total of $n=200$ subjects, $n=181$ subjects were in the age group between 17-30 years. In heavy metal non-exposed area, the figure 4.2B shows that out of $n=50$ subjects, $n=43$ subjects were in the age group between 17-30 years. Hence, the study interprets that the young age group subjects are at very high risk of heavy metal exposure. Hence, the study interprets that the young age group subjects are at very high risk of heavy metal exposure.

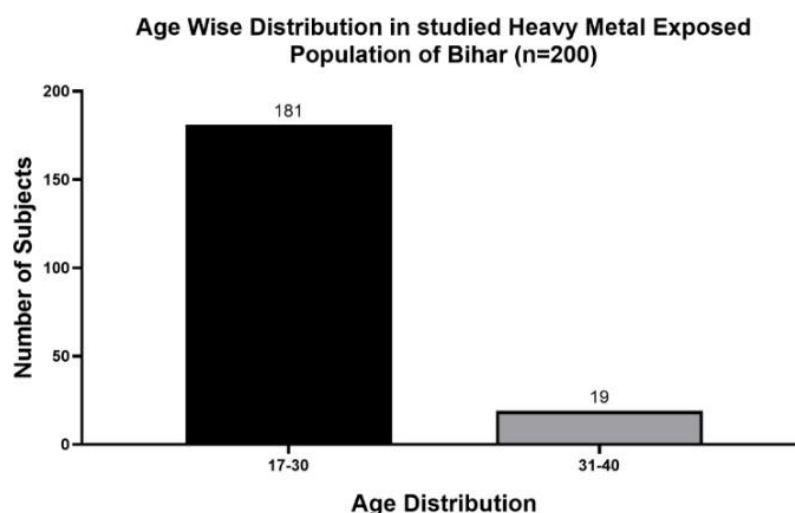


Figure 4.2A. Age wise distribution in heavy metal exposed area of Bihar

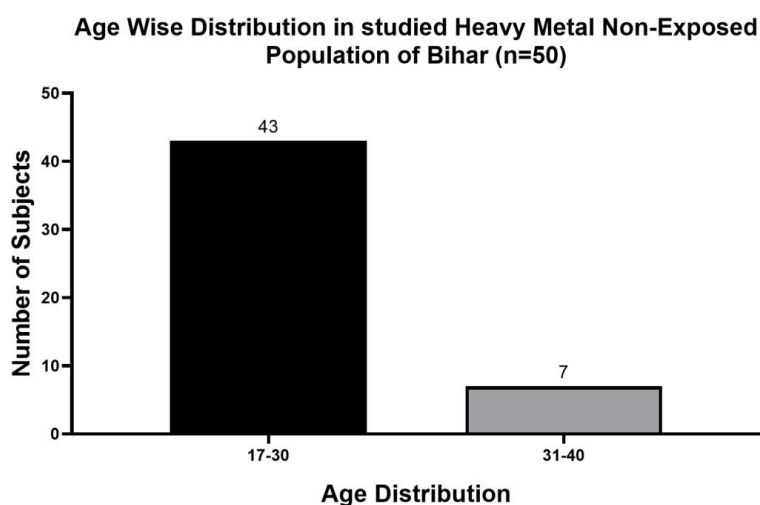


Figure 4.2B. Age wise distribution in heavy metal non-exposed area of Bihar

Water arsenic concentration- The household water samples were collected from handpumps installed at depths ranging from 60 to 130 feet. In the heavy metal–exposed area, out of a total of n=200 households, n=175 had groundwater arsenic concentrations below the permissible limit of 10 µg/L, while n=25 households exceeded this threshold (Fig. 4.3A). In contrast, in the non-exposed area, all 50 households exhibited arsenic concentrations below the permissible limit (Fig. 4.3B). These findings indicate that most groundwater samples were safe for drinking; however, the maximum arsenic concentration recorded reached 257.5 µg/L, highlighting localized contamination concerns.

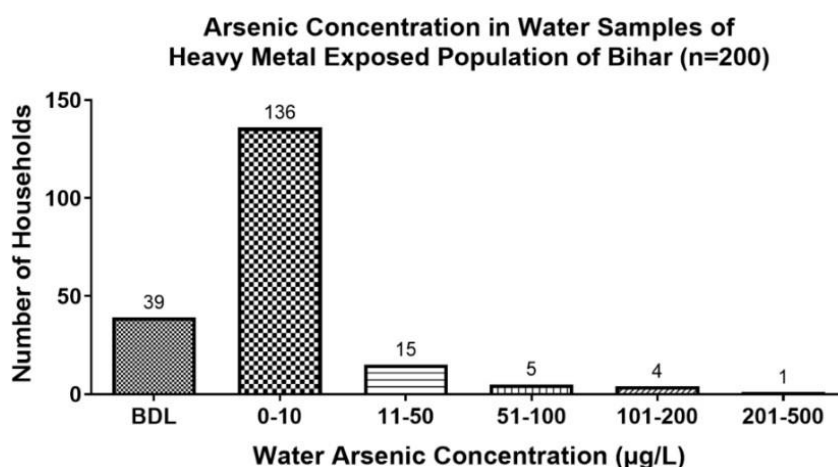


Figure 4.3A. Graph with the levels of arsenic concentration in the drinking water samples of exposed districts.

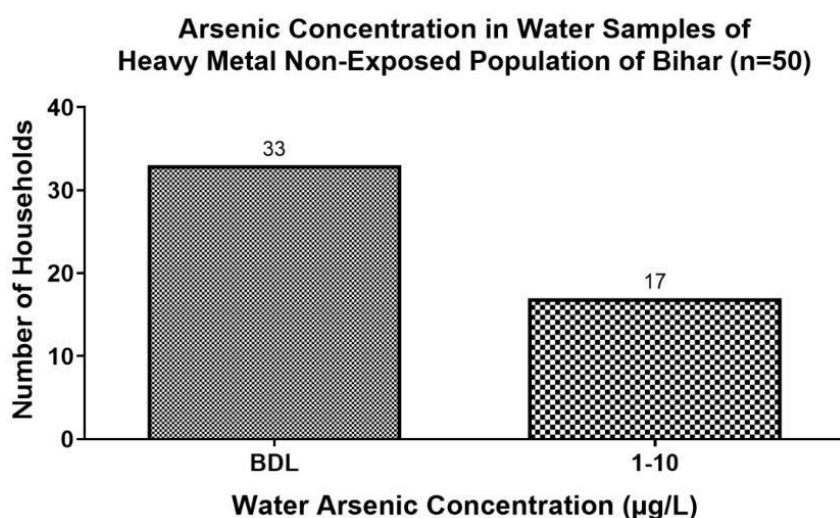


Figure 4.3B. Graph with the levels of arsenic concentration in the drinking water samples of non-exposed districts.

Blood arsenic concentration- In the heavy metal–exposed area, out of a total of n=200 female subjects, n=47 exhibited blood arsenic concentrations below 10 µg/L, while n=153 showed levels above the permissible limit, with the highest concentration reaching 379.6 µg/L [Figure 4.4A]. This indicates that a major proportion of the studied subjects had arsenic levels in their blood exceeding the acceptable threshold. In contrast, in the non-exposed are, n=44 subjects had blood arsenic concentrations below 10 µg/L, whereas only n=6 subjects exceeded this limit [Figure 4.4B].

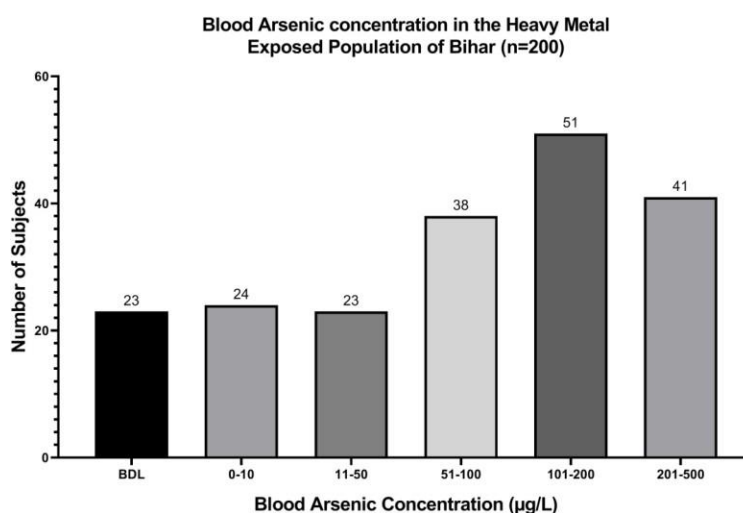


Figure 4.4A. Graph with the levels of arsenic concentration in the blood samples of subjects of exposed districts

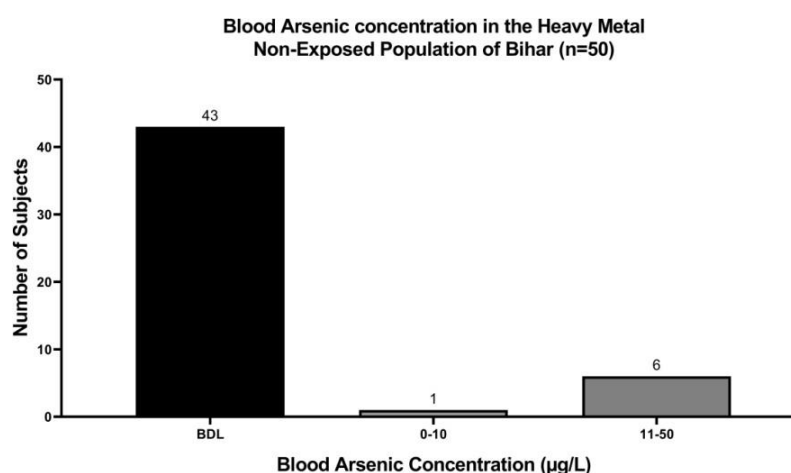


Figure 4.4B. Graph with the levels of arsenic concentration in the blood samples of subjects of non-exposed districts

Breastmilk arsenic concentration- In the heavy metal–exposed area, out of a total of n=200 female subjects, n=11 had breast milk arsenic concentrations below 1 µg/L, while n=189 showed levels exceeding the permissible limit, with the highest concentration reaching 458.8 µg/L [Figure 4.5A]. This finding suggests a potential pathway of arsenic exposure to infants through maternal breast milk. Meanwhile, in the non-exposed area, all n=50 subjects had breast milk arsenic concentrations below the permissible limit [Figure 4.5B].

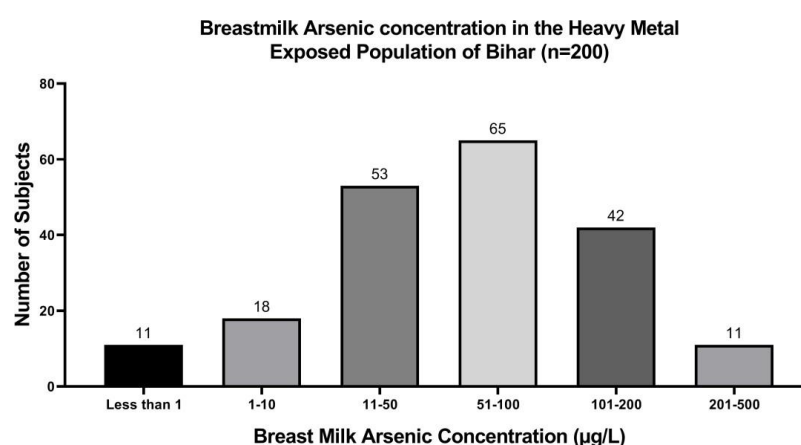


Figure 4.5A. Graph with the levels of arsenic concentration in the breastmilk samples of subjects of exposed districts.

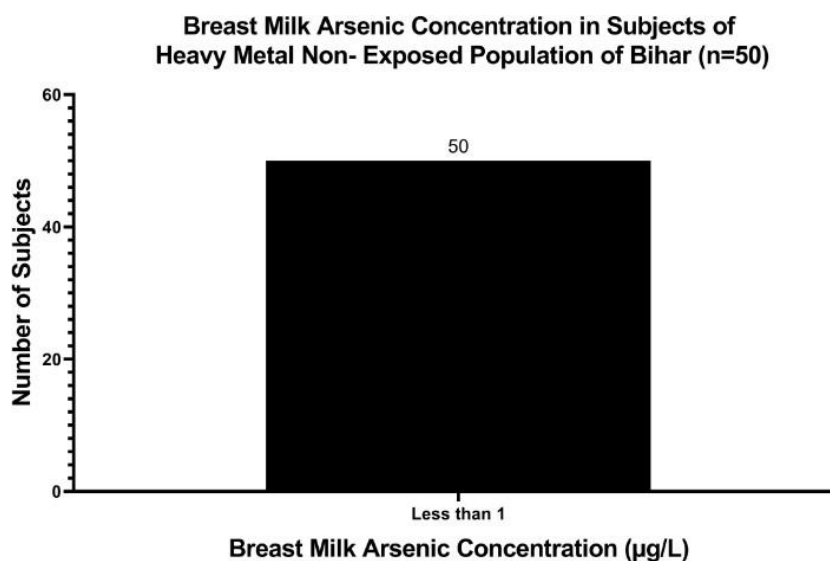


Figure 4.5B. Graph with the levels of arsenic concentration in the breastmilk samples of subjects of non-exposed districts.

Urine arsenic concentration- In the heavy metal–exposed region, among n=200 subjects, only n=18 exhibited urinary arsenic levels below 50 µg/L, while n=182 showed concentrations exceeding this threshold, with the maximum value reaching 671.8 µg/L [Figur4.6A]. Such elevated levels indicate a significant long-term risk of carcinogenic outcomes due to chronic arsenic exposure. Conversely, in the non-exposed group, all n=50 participants had urinary arsenic concentrations within the safe limit [Figure 4.6B].

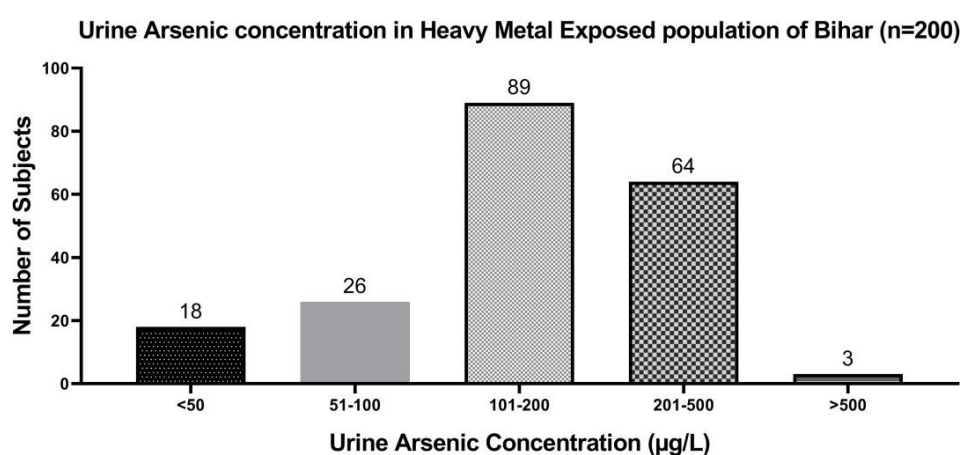


Figure 4.6A. Graph with the levels of arsenic concentration in the mother’s urine samples of subjects of exposed districts.

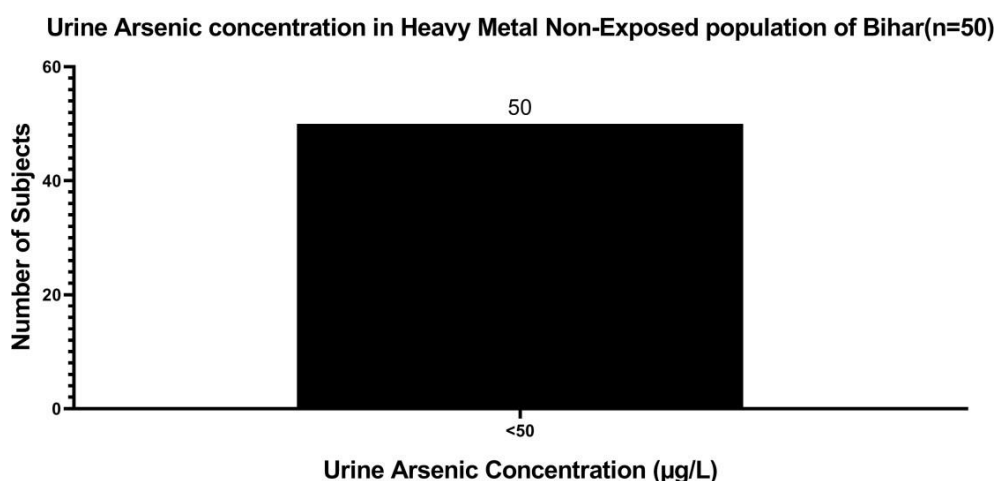


Figure 4.6B. Graph with the levels of arsenic concentration in the urine samples of subjects of non-exposed districts.

Table 4.1: Descriptive statistics of arsenic datasets

	Water	Blood	Breastmilk	Urine
Number of values	200	200	200	200
Minimum	0.000	0.000	0.000	0.000
Maximum	257.5	379.6	458.8	671.8
Range	257.5	379.6	458.8	671.8
Mean	10.42	120.2	86.89	166.2
Std. Deviation	32.24	85.91	54.75	107.1
Std. Error of Mean	2.280	8.154	3.871	7.571
Coefficient of variation	309.4%	71.47%	63.01%	64.41%

4.3 Health risk assessment (Arsenic)

The hazard quotient represents the non-carcinogenic health risk. There are no negative consequences to the potential or level.

$$HQ = ADD/RfD$$

Where,

HQ = Health Quotient.

ADD = Average Daily Dose.

RfD = Oral Reference Dose (0.3 µg/Kg/day) (USEPA, 2014, Kumar et al., 2024).

If the derived HQ value is more than one, a non-carcinogenic impact may be projected; if the determined HQ is one or less than one, no health effect from exposure can be predicted.

Carcinogenic risk (CR)

Carcinogenic risk can be calculated by using this equation.

$$CR = ADD \times CSF$$

Where,

CR = Carcinogenic Risk.

ADD = Average Daily Dose.

CSF = Cancer Slope Factor (1.5 mg/Kg/day).

Table 4.2: References of the health risk assessment studies for arsenic

Parameters	Female	References
IR (Rice)	0.389 kg	Yu et al., 2022; Rai et al., 2019
IR (Wheat)	0.159 kg	Liu et al., 2020
IR (Water)	2.5 kg	Kumar et al., 2024a
IR (Potato)	0.1 kg	Luo et al., 2021
ED	67	Narsimha and Rajith, 2018
EF	365	Tyagi et al., 2020; USEPA, 2014
BW	55	USEPA, 2008 & ICMR, 2009
AT	24455	USEPA, 2014
RfD	0.0003	USEPA, 2014.
C(mg/L)		Present study

IR = Ingestion rate ED = Exposure duration EF = Exposure frequency BW = Body weight AT = Average time

Human health risk assessment is a significant study to evaluate the potential harmful effects on humans due to exposure to any chemical or biological substance. Groundwater and breast milk arsenic concentrations were utilized to assess the potential health risks among the studied mothers. The findings indicated that 13.82% of groundwater samples exceeded the hazard quotient threshold, suggesting a potential risk of non-carcinogenic health effects for the same proportion of the population. When evaluating carcinogenic risk, it was observed that 42.19% of groundwater samples surpassed the Incremental Lifetime Cancer Risk (ILCR) limit, signifying that nearly two-fifths of the mothers are at considerable risk of developing cancer in the future [Figure 4.7]. Also, the health risk assessment was also calculated through the intake of arsenic-contaminated wheat, rice and potato caused high arsenic contamination in breastmilk of mothers is calculated in the form of hazard quotient and cancer risk in the present study. The risk of excessive consumption of arsenic was calculated by comparing the guideline of arsenic concentration in wheat given by WHO/FAO i.e. 100 µg/Kg, rice as 200 µg/Kg in and potato as 500 µg/Kg. In the present study, toxicological reference value of 0.003 mg/Kg/day was taken as the

reference dose given by USEPA, 2006. A total of $n = 200$ wheat samples, $n = 200$ rice samples, $n = 162$ potato samples were taken for the calculation of health risk assessment for lactating mothers. Out of $n = 200$ samples of wheat for the mother's population, 37.73% samples were found higher than the threshold limit i.e. 1 (Fig. 4.8), while out of $n = 200$ rice samples, 58.86% of the samples were found higher than threshold limit i.e. 1 (Fig. 4.9), and out of $n = 162$ potato samples, 34.73% of the samples were found higher than threshold limit i.e. 1 (Fig. 4.10). It means average 36% of mother population have the potential risk of non-carcinogenic health effects. About the carcinogenic risk assessment, it was found that in wheat samples 43.61%, in rice samples 64.89% and in potato samples 62.1% of the mother's population (Fig.4.7, 4.8, 4.9) have higher than the Incremental lifetime cancer risk (ILCR) i.e. 0.0001, which shows the population which has higher than the ILCR have the potential risk of causing cancer.

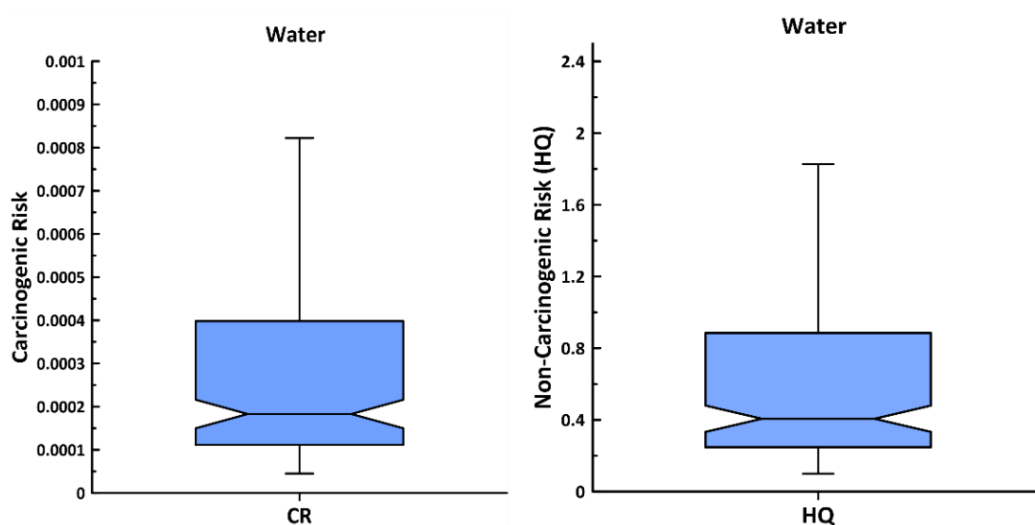


Figure 4.7. Boxplot illustrating the population's cancer risk vs non-carcinogenic arsenic risk results for mothers in water samples

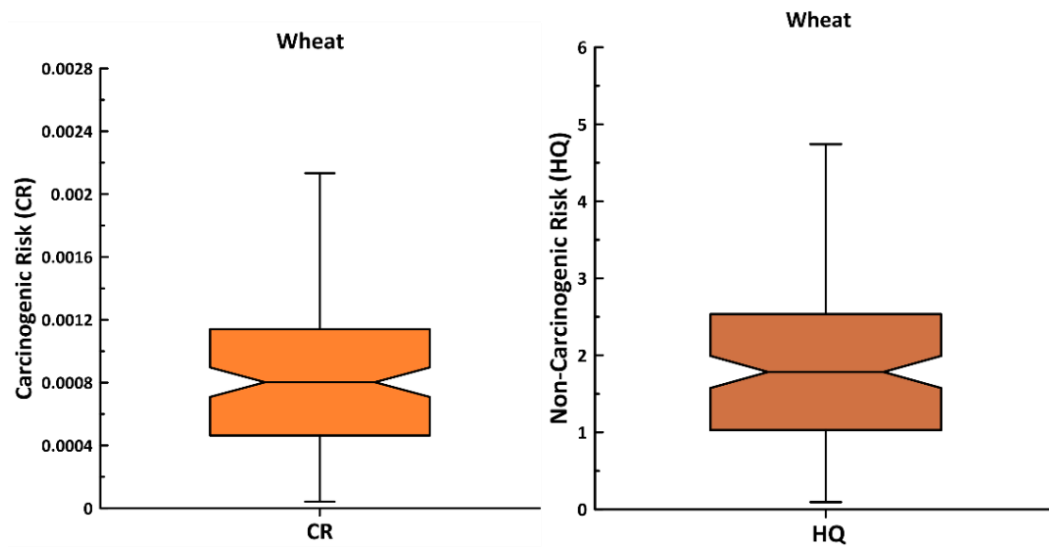


Figure 4.8. Boxplot illustrating the population's cancer risk vs non-carcinogenic arsenic risk results for mothers in wheat samples

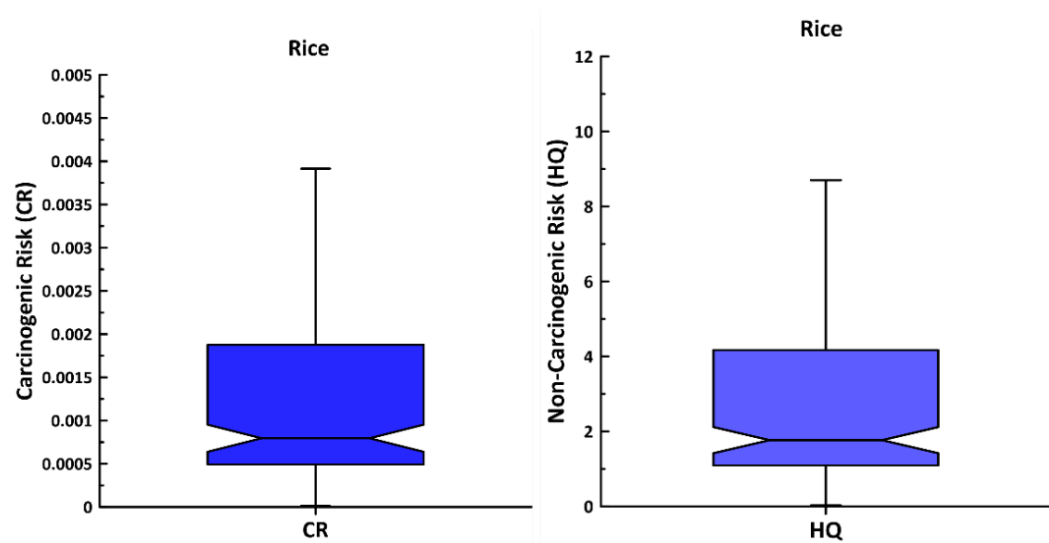


Figure 4.9: Boxplot illustrating the population's cancer risk vs non-carcinogenic arsenic risk results for mothers in rice samples

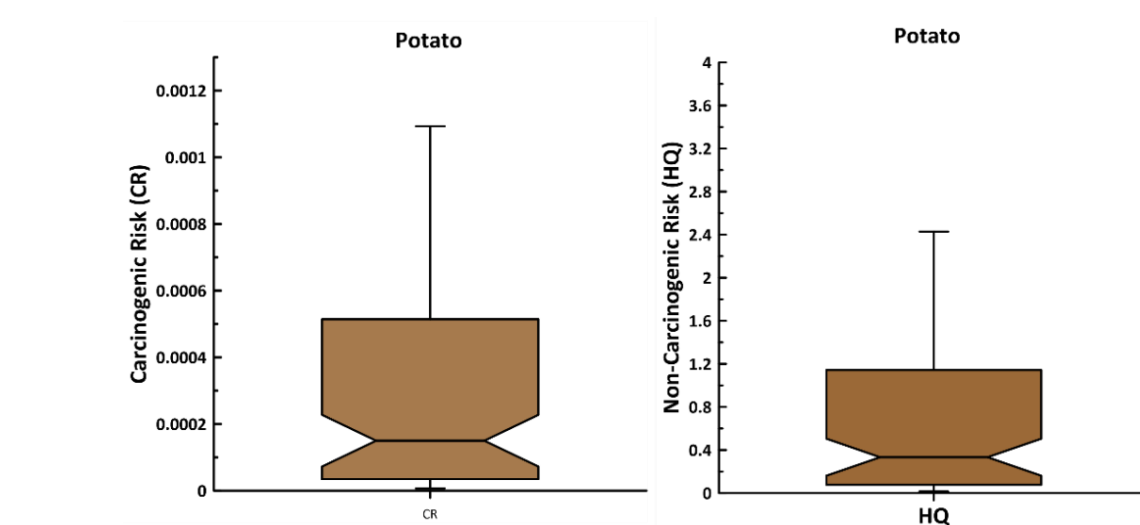


Figure 4.10: Boxplot illustrating the population's cancer risk vs non-carcinogenic arsenic risk results for mothers in potato samples

4.4 Graphical Representation of Lead Results

Water lead concentration

The following figure 4.11A shows that in lead exposed area, out of total n=200 households, all households had their groundwater lead concentration with in the permissible limit (10 μ g/L). In heavy metal non-exposed area, the figure 4.11B shows that all n= 50 households had their groundwater arsenic concentration below the permissible limit. Hence, the study interprets that all the groundwater samples were safe for drinking.

Water Lead concentration in House the Studied Population of Bihar (n=200)

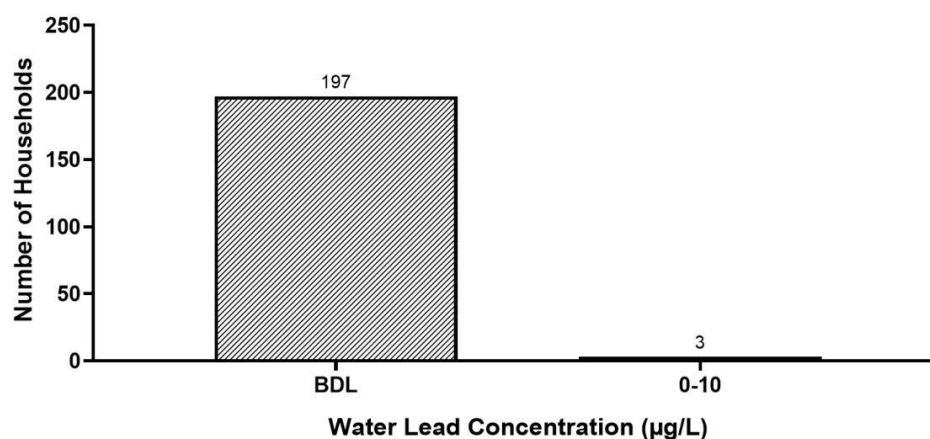


Figure 4.11A. Water lead concentration in exposed area of Bihar

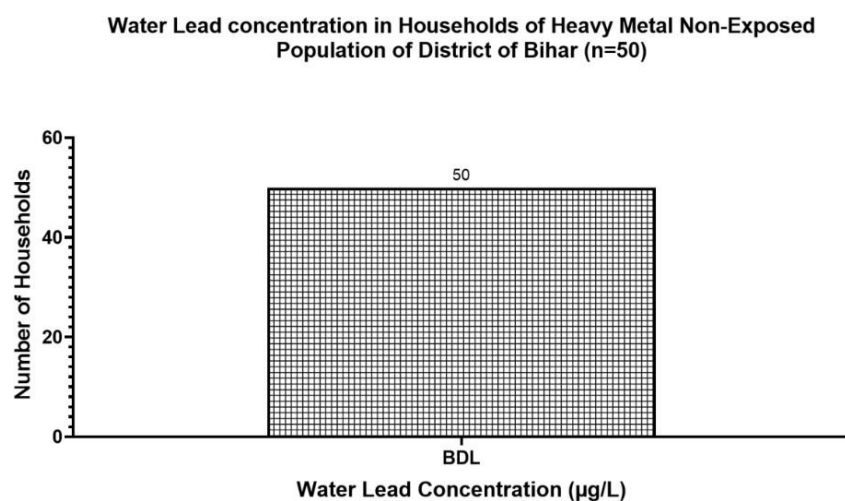


Figure 4.11B. Water lead concentration in non-exposed area of Bihar

Blood lead concentration

In the study, the blood lead content n=200 was observed. Only n=29 subjects had the blood lead content <50 µg/L (the permissible limit), while the rest, n= 171 subjects had lead concentration in their blood above the permissible limit (>50 µg/L) with the highest content as 1724µg/L (figure 4.12A). In heavy metal non-exposed area, the figure 4.12B shows that n= 33 subjects had their blood lead concentration below the permissible limit and n= 17 subjects had their blood lead concentration above the permissible limit. This denotes that major chunk of studied subjects had their lead content in blood above the permissible limit of 50µg/L.

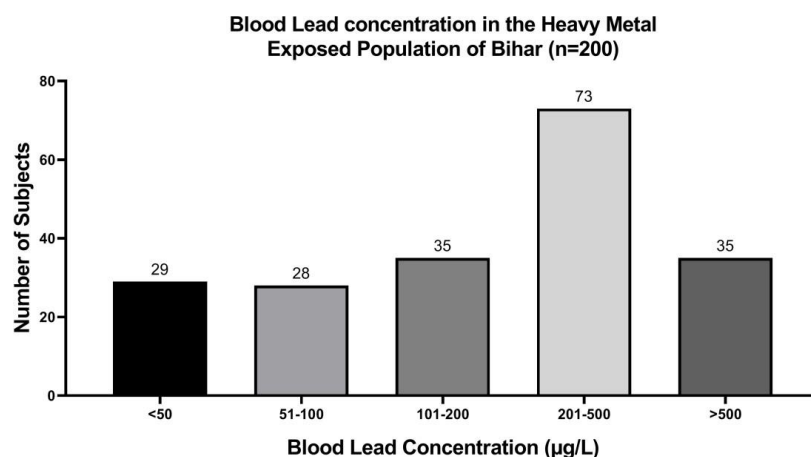


Figure 4.12A. Blood lead concentration in exposed area of Bihar

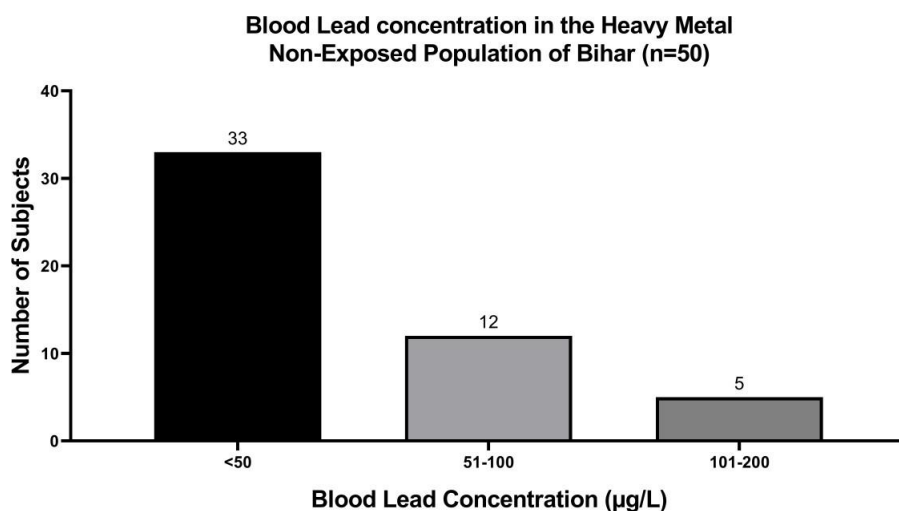


Figure 4.12B. Blood lead concentration in non-exposed area of Bihar

Breastmilk lead concentration

In the heavy metal–exposed region, analysis of n=200 subjects revealed that only n=12 mothers had breast milk lead levels within the safe limit of <5 µg/L, while the remaining n=188 exhibited concentrations exceeding this threshold, with the maximum recorded value reaching 1309 µg/L [Figure 4.13A]. Conversely, in the non-exposed area, n=16 participants had lead levels within the permissible range, whereas n=34 showed elevated concentrations, indicating notable exposure even in comparatively safer zones (Figure 4.13B).

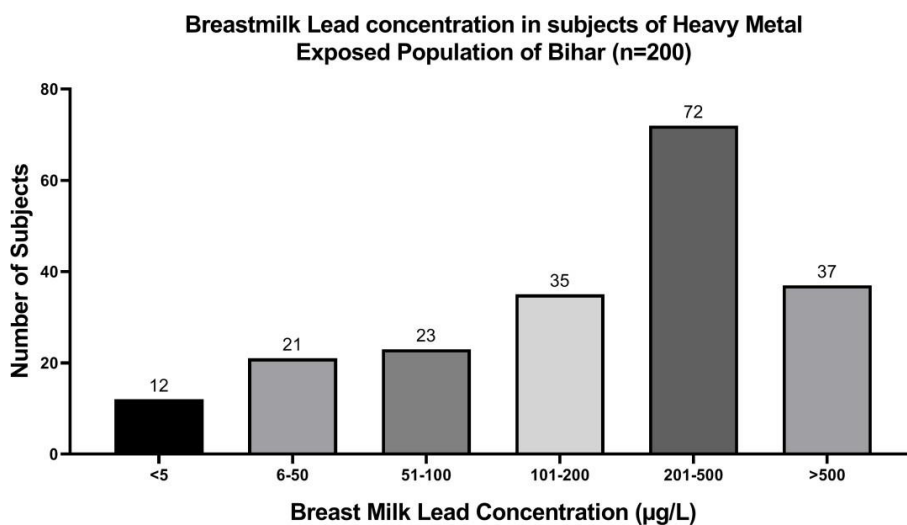


Figure 4.13A. Breastmilk lead concentration in exposed area of Bihar

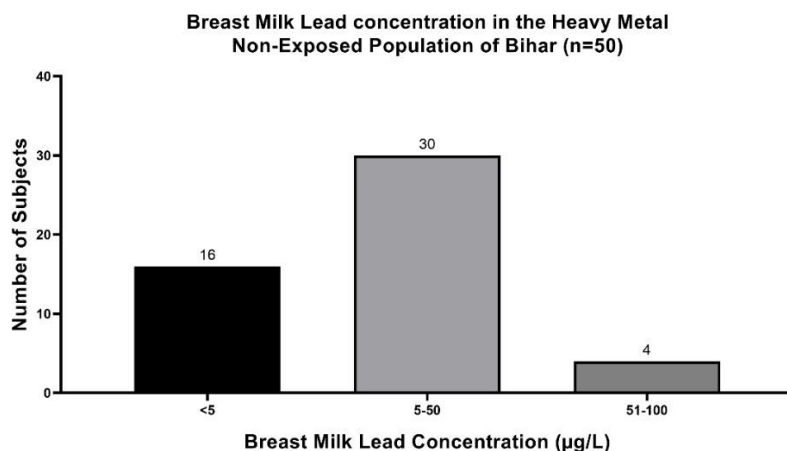


Figure 4.13B. Breastmilk lead concentration in non-exposed area of Bihar

Urine lead concentration

In heavy metal exposed area, out of total $n=200$ subjects, the study showed that $n=57$ subjects had their urine lead concentration below $50\mu\text{g/L}$. About $n=143$ subjects had lead contamination in their urine above the permissible limit of $<50\text{ }\mu\text{g/L}$ with the highest content as $4168\text{ }\mu\text{g/L}$ [Figure 4.14A]. In heavy metal non-exposed area, the figure 4.14B shows that that $n=31$ subjects had their urine lead concentration below the permissible limit and $n=19$ subjects are above the permissible limit. This denotes that the subjects in exposed area are at very high risk of cancer in future due to long term lead exposure.

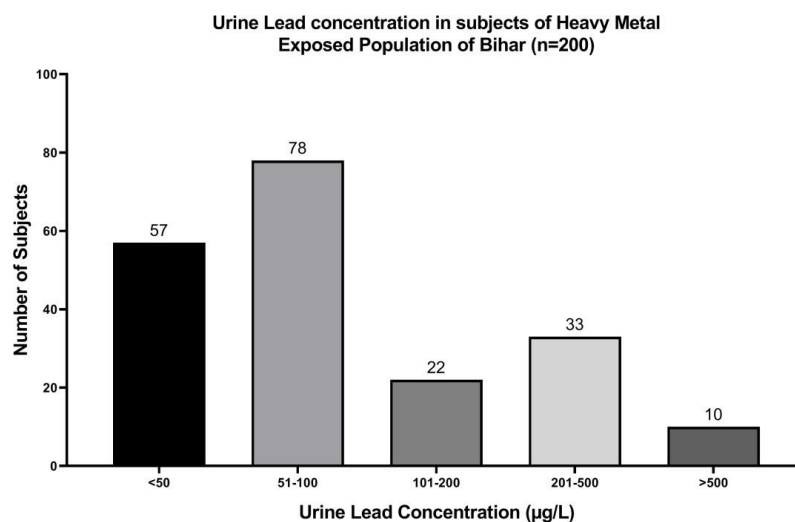


Figure 4.14A. Urine lead concentration in exposed area of Bihar

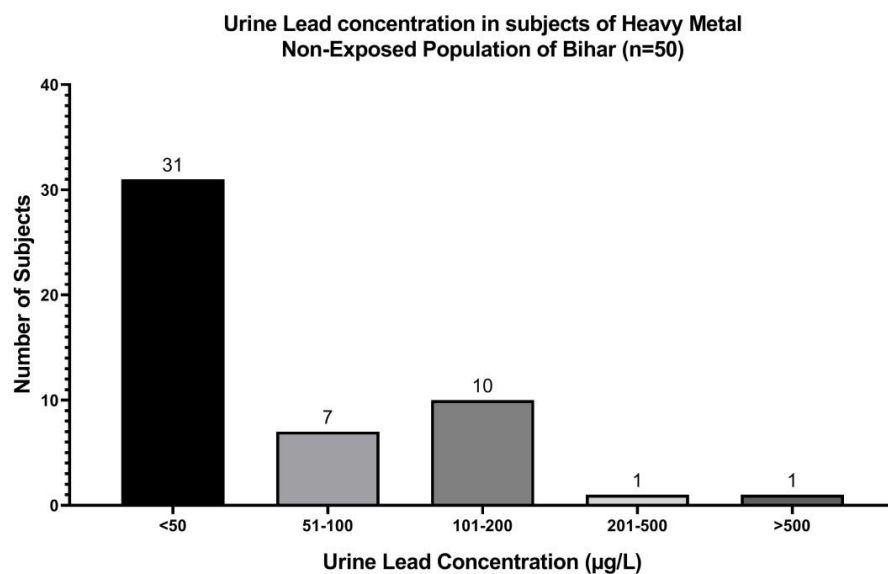


Figure 4.14B. Urine lead concentration in non-exposed area of Bihar

Table 4.3 Descriptive statistics for lead results

	Water	Blood	Breastmilk	Urine
Number of values	200	200	200	200
Minimum	0.000	34.04	0.000	15.26
Maximum	1.748	1724	1309	4168
Range	1.748	1690	1309	4153
Mean	0.02622	304.6	337.6	160.2
Std. Deviation	0.2130	296.0	274.6	325.6
Std. Error of Mean	0.01506	24.93	19.42	23.02
Coefficient of variation	812.4%	97.18%	81.33%	203.3%

4.5 Health risk assessment (Lead)

The non-carcinogenic health risk is represented by hazard quotient. The potential or level show no adverse effects.

$$HQ = ADD/RfD$$

Where,

HQ = Health Quotient.

ADD = Average Daily Dose.

RfD = Oral Reference Dose (4 µg/Kg/day) (USEPA, 2006),

If the obtained HQ value is more than 1 then non-carcinogenic effect can be predicted and if determined HQ is 1 or less than 1 then, no health effect can be predicted due to exposure.

Carcinogenic risk (CR)

Carcinogenic risk can be calculated by using this equation.

$$CR = ADD \times CSF$$

Where,

CR = Carcinogenic Risk.

ADD = Average Daily Dose.

CSF = Cancer Slope Factor, (0.0085 mg/Kg/day).

If CR value is $\leq 1 \times 10^{-4}$ then, it is tolerable menace levels.

Table 4.4: References of the health risk assessment studies for lead

Parameters	Female	References
IR (Wheat)	.159 kg	Liu et al., 2020
IR (Rice)	.199 kg	Giri & Singh, 2017
IR (Potato)	.100 kg	Luo et al., 2021
ED	67	Narsimha and Rajith, 2018
EF	365	Tyagi et al., 2020; USEPA,2014
BW	55 kg	USEPA, 2008 & ICMR, 2009
AT	24455	USEPA,2014
C(mg/L)		Present study

Human health risk assessment is a significant study to evaluate the potential harmful effects on humans due to exposure to any chemical or biological substance. The health risk assessment due to the intake of lead-contaminated wheat, rice and potato caused high lead contamination in breastmilk of mothers is calculated in the form of hazard quotient and cancer risk in the present study. The risk of excessive consumption of lead was calculated by comparing the guideline of lead concentration in wheat and rice given by WHO/FAO i.e. 200 $\mu\text{g/Kg}$ and in potato as 100 $\mu\text{g/Kg}$. In the present study, toxicological reference value of 0.004 mg/Kg/day was taken as the reference dose given by USEPA 2006. A total of n = 200 wheat samples, n = 200 rice samples, n = 162 potato samples were taken for the calculation of health risk assessment for lactating mothers and their children respectively. Out of n = 200 samples of wheat for the mother's population, 100% samples were found higher than the threshold limit i.e. 1 (Fig. 4.15), while out of n = 200 rice samples, 98.79% of the samples were found higher than threshold limit i.e. 1 (Fig. 4.16), and out of n = 162 potato samples, 61.3% of the samples were found higher than threshold limit i.e. 1 (Fig. 4.17). It means on an average 87% of the mother population have the potential risk of non-carcinogenic health effects. About the carcinogenic risk assessment, it was found that in wheat samples 100%, in rice samples 98.79% and in potato samples 61.3% of the mother's population (Fig. 4.15, 4.16, 4.17) have higher than the Incremental lifetime cancer risk (ILCR) i.e. 0.0001, which shows the population which has higher than the ILCR have the potential risk of causing cancer. For the calculation of carcinogenic risk, the cancer slope factor 0.0085 was taken (Liu et al., 2020).

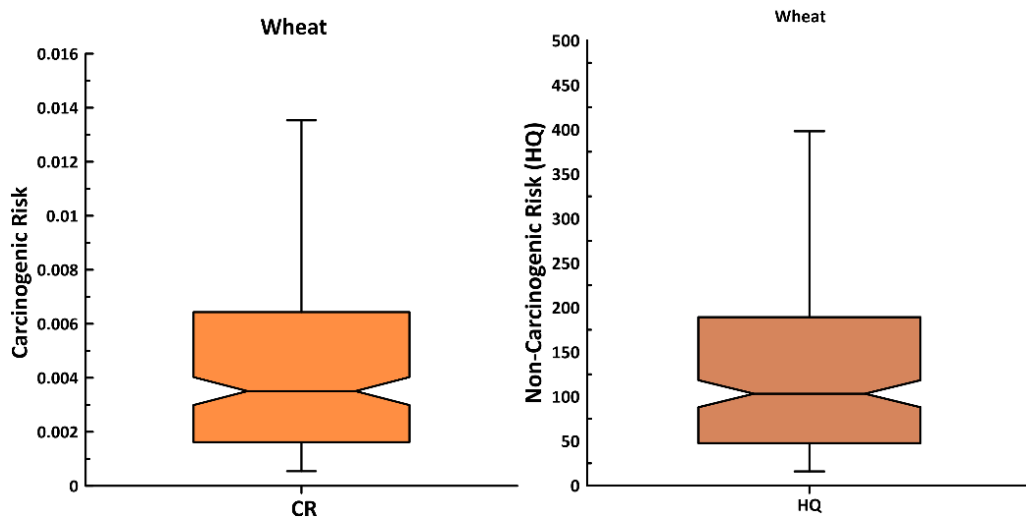


Figure 4.15: Boxplot illustrating the population's cancer risk vs non-carcinogenic risk of lead results for mothers in wheat samples

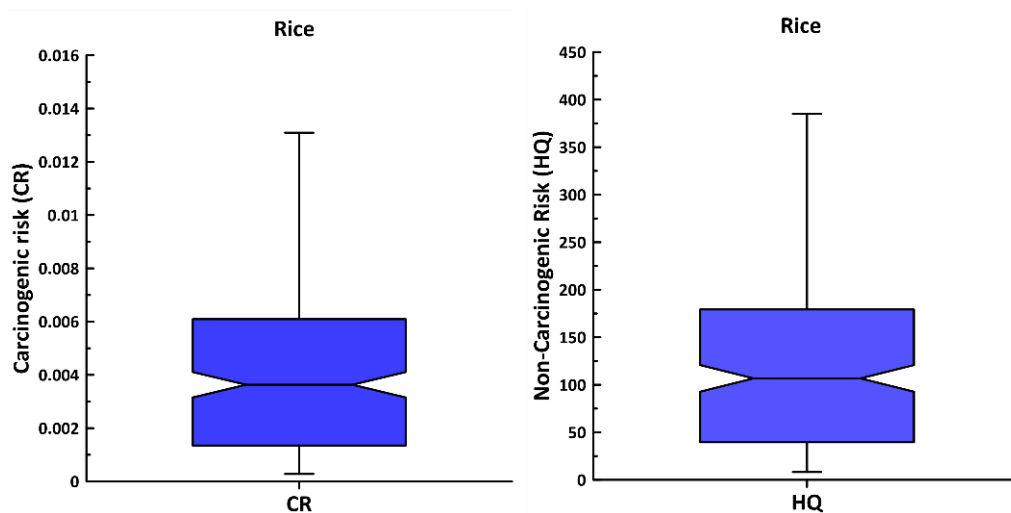


Figure 4.16: Boxplot illustrating the population's cancer risk vs non-carcinogenic risk of lead results for mothers in rice samples

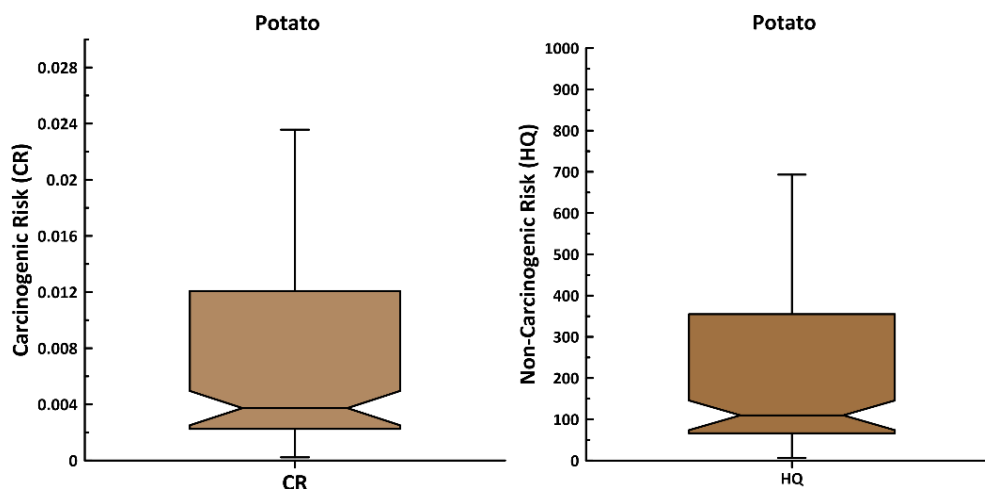


Figure 4.17 Boxplot illustrating the population's cancer risk vs non-carcinogenic risk of lead results for mothers in potato samples

4.6 Graphical Representation of Hormone Results

Blood Estrogen Level

In Heavy metal exposed area, out of total $n=200$ subjects, the study showed that $n= 19$ subjects had their estrogen level below the permissible range (9-289 pg/ml). About $n=114$ subjects had estrogen level lies in normal range and $n= 67$ subjects had their levels above the permissible range (Figure 4.18A). In heavy metal non-exposed area, the figure 4.18B shows that that $n= 16$ subjects had their estrogen level below the permissible range (9-289 pg/ml). About $n=25$ subjects had estrogen level lies in normal range and $n= 9$ subjects had their levels above the permissible range.

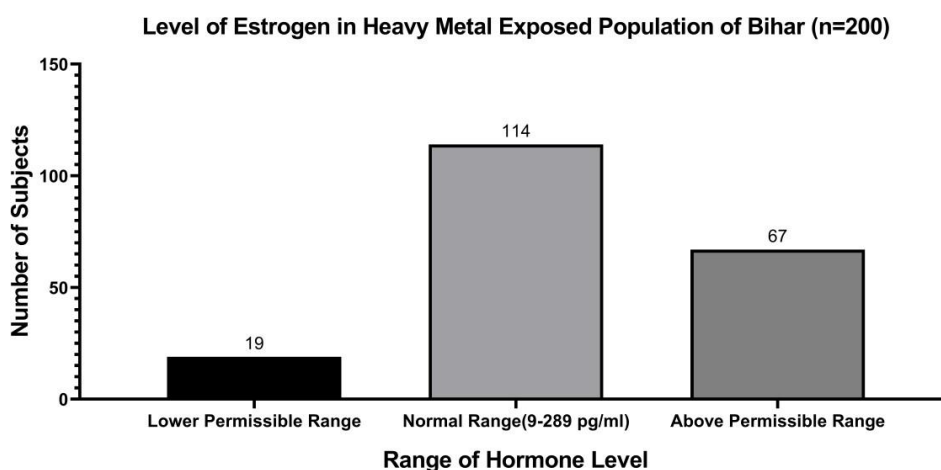


Figure 4.18A. Blood estrogen levels in exposed area of Bihar

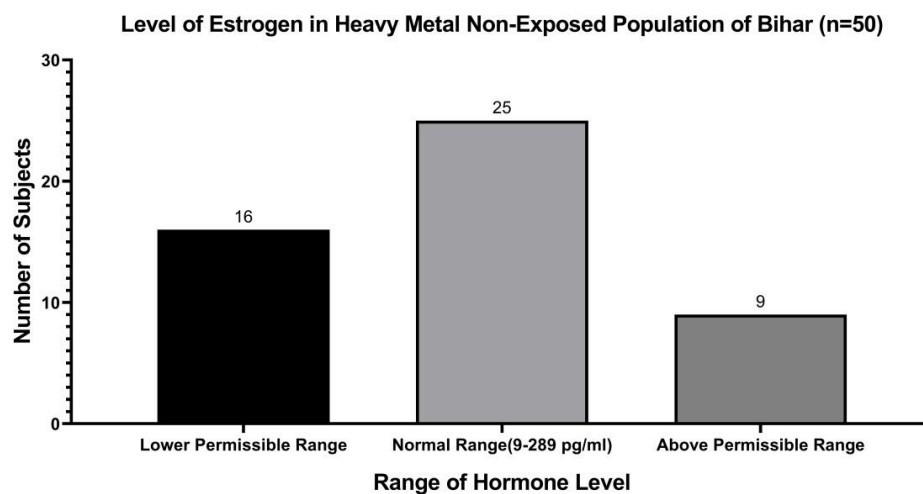


Figure 4.18B. Blood estrogen levels in non-exposed area of Bihar

Blood Progesterone Level

In heavy metal exposed area, out of total n=200 subjects, the study showed that n= 25 subjects had their progesterone level below the permissible range (0.15-422 ng/ml). About n=169 subjects had progesterone level lies in normal range and n= 31 subjects had their levels below the permissible range (Figure 4.19A). In heavy metal non-exposed area, the figure 4.19B shows that that n= 13 subjects had their progesterone level below the permissible range. About n=37 subjects had progesterone level lies in normal range.

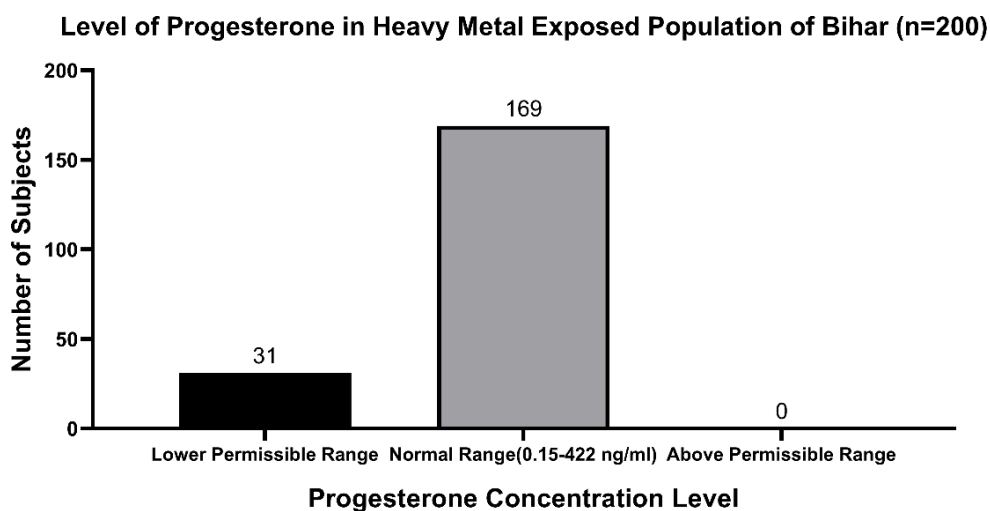


Figure 4.19A. Blood progesterone levels in exposed area of Bihar

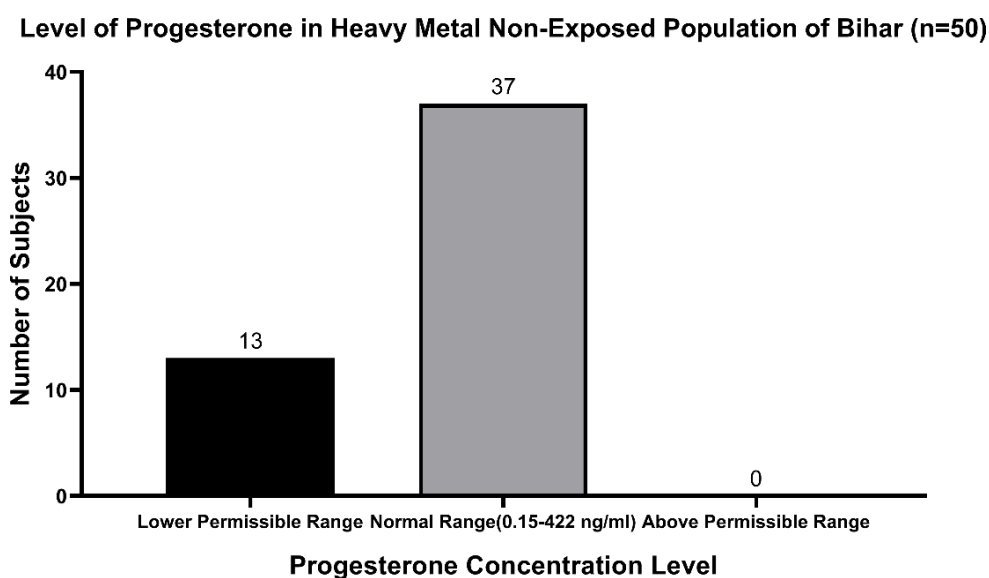


Figure 4.19B. Blood progesterone levels in non-exposed area of Bihar

Blood Thyrotropin (TSH) Level

In Heavy metal exposed area, out of total n=200 subjects, the study showed that n= 7 subjects had their thyrotropin level below the permissible range (0.28-6.28 μ IU/ml). About n=180 subjects had thyrotropin level lies in normal range and n= 13 subjects had their levels above the permissible range (Figure 4.20A). In heavy metal non-exposed area, the figure 4.20B shows that that n= 9 subjects had their thyrotropin level below the permissible range. About n=29 subjects had thyrotropin level lies in normal range and n= 12 subjects had their levels above the permissible range.

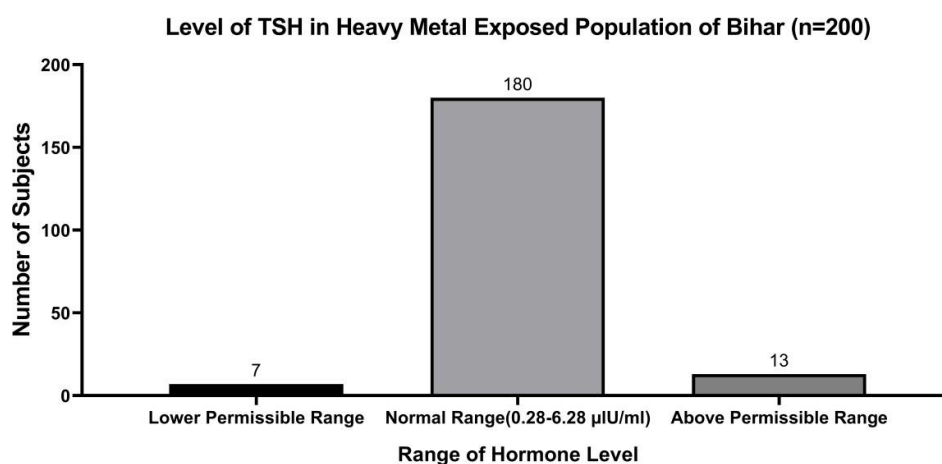


Figure 4.20A. Blood Thyrotropin levels in exposed area of Bihar

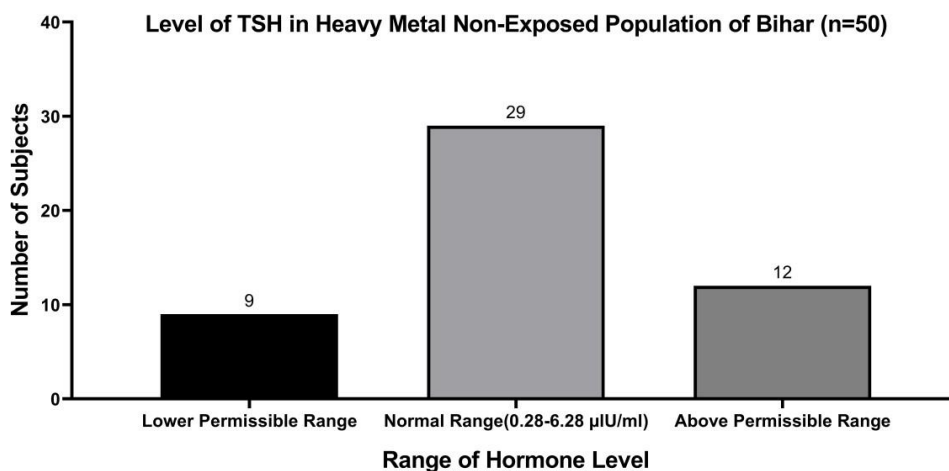


Figure 4.20B. Blood Thyrotropin levels in non-exposed area of Bihar

Table 4.5 Descriptive statistics of Hormone results

	TSH	Progesterone	Estrogen
Number of values	200	200	200
Minimum	0.019	0	0.5
Maximum	46.4	59.88	5116
Range	46.38	59.88	5116
Mean	2.477	9.068	350.9
Std. Deviation	3.747	13.41	723.4
Std. Error of Mean	0.2272	0.9024	56.15
Coefficient of variation	151.20%	147.90%	206.20%

4.7 Discussion

Heavy metal poisoning has caused a lot of health hazards in the world. It has been observed that in the Gangetic plains of Bihar the heavy metal exposure is very high in several districts of Bihar. Moreover, the women inhabiting in this area having the contamination of arsenic, lead & mercury which are causing severe health problems to their infants. Moreover, other studies have also reported the arsenic poisoning in the groundwater causing the exposure in the population. Forensically, chronic heavy metal exposure is significant because it can lead to a variety of health issues, including organ damage, neurological problems, and increased cancer risk, which can

be crucial in determining the cause and manner of death, or in investigations involving poisoning or environmental contamination (Verma, 2018). Heavy metals are highly toxic, even in small amounts, and can accumulate in the body over time. Chronic exposure can lead to damage to vital organs like the kidneys, liver, and brain. Heavy metal poisoning can cause neurological problems like memory loss, concentration difficulties, and even dementia (Koyama, 2024). Some heavy metals are known carcinogens, meaning they can increase the risk of developing cancer. Chronic exposure can also lead to gastrointestinal problems, skin lesions, and cardiovascular disease (Balali-Mood, 2021). The presence of heavy metals in a deceased individual's tissues or organs can help determine the cause of death, especially in cases of suspected poisoning or exposure to toxic substances. The level and type of heavy metals found can help differentiate between intentional poisoning (homicidal or suicidal) and accidental exposure (Verma, 2018; Kumar et al., 2024b). Forensic analysis of heavy metals can be used to investigate environmental contamination, such as in cases of industrial pollution or contaminated food sources. By analyzing the types and levels of heavy metals in the body, forensic toxicologists can help identify the source of exposure, whether it's occupational, environmental, or through ingestion of contaminated substances (Fisher & Gupta, 2024). Forensic analysis can help assess the health risks associated with chronic heavy metal exposure, both for individuals and for populations exposed to contaminated environments (Filipoiu et al., 2022).

In the present study, for the first time it has been reported with arsenic and lead poisoning in the infants through their breastmilk of their mothers. heavy metal exposure study was carried out in 8 districts of Bihar (India)- Samastipur, Darbhanga, Begusarai, Khagaria, Munger, Katihar, Bhagalpur and Nalanda. Among the arsenic-exposed participants, 90.5% belonged to the 17–30 years age group, while 9.5% were aged 31–40 years. In the non-exposed population, 86% fell within the 17–30 years bracket and 14% within 31–40 years. Analysis of $n=200$ groundwater samples from exposed areas revealed that 88% contained arsenic concentrations below the WHO guideline value of 10 $\mu\text{g/L}$, whereas 12% exceeded this limit, ranging between 11 and 500 $\mu\text{g/L}$, with the highest value recorded at 330.7 $\mu\text{g/L}$. Meanwhile, all 50 samples from the non-exposed region had arsenic levels within the permissible range. The blood arsenic concentration in the studied arsenic exposed subjects in $n = 200$ sam-

ples, 23.5% had the blood arsenic content in the normal range that is below 10 µg/L, while 76.5% blood samples had arsenic content above the permissible limit with highest content as 379.6 µg/L. In the non-exposed area, 88% of the samples had blood arsenic concentrations below the allowed range, while 12% exceeded this limit. The elevated arsenic levels in their blood may be attributed to the use of staple foods including rice, wheat, and potatoes that were polluted with arsenic (Kumar et al, 2024b). In a study of 200 people from an arsenic-exposed population, 5.5% exhibited breastmilk arsenic levels below the acceptable limit of < 1 µg/L, whereas 94.5% had arsenic concentrations exceeding this limit, with the highest recorded level at 458.8 µg/L. In the non-exposed area, all samples had breast milk arsenic concentrations below the allowed level. In the analysed maternal urine of an arsenic-exposed population of 200 subjects, 9% exhibited arsenic concentrations below the permitted limit of 50 µg/L. Nonetheless, 91% of patients exhibited urinary arsenic levels exceeding the permitted threshold, with a maximum value of 671.8 µg/L. In the non-exposed area, all samples exhibited breast milk arsenic concentrations below the acceptable limit (Kumar et al., 2024b; Agarwal et al., 2025).

The biomagnification of arsenic contamination in the studied human populations via the consumption of food and water samples represents a novel finding in these six districts of Bihar. The majority of the population is at significant risk due to the consumption of arsenic-contaminated food, which is transmitted to infants through breast milk. The results indicate that the population exposed to arsenic in the districts along the river Ganges- Samastipur, Begusarai, Khagaria, Darbhanga, Munger, Katihar, Bhagalpur, and Nalanda faces a significant risk of disease burden. The infants face a significant risk of future disease burden due to their mothers' lactation of breast milk containing high levels of arsenic. This may result in mental disorders, reduced intelligence, and impaired memory in these infants. This study indicates that elevated arsenic levels in breast milk and urine suggest biomagnification of arsenic in mothers, resulting in the secretion of arsenic-contaminated breast milk that is inadvertently consumed by infants. Evidence indicates that the transmission of arsenic to the mammary glands is diminished, thereby protecting neonates from arsenic exposure (Concha, 1998; Quansah, 2015). It has been asserted that fetuses and infants are shielded from arsenic exposure due to arsenic methylation during maternal

pregnancy and lactation (GSI, 2022; Vahter, 2009). In contrast to existing studies, there is a lack of research documenting real-time arsenic poisoning in children residing in the Gangetic plains of Bihar, India. Furthermore, the child's daily activities will be severely impacted, necessitating medical interventions. Geological analyses indicate that arsenic poisoning among subjects in the Gangetic plains of Bihar is attributable to the oscillatory movement of the Ganga River along its course. The districts examined included Samastipur, Begusarai, Khagaria, Munger, Darbhanga, Katihar, Bhagalpur, and Nalanda. The current study identified the high arsenic levels in the breastmilk of the exposed population in Bihar, arranged in the following increasing order: Khagaria > Begusarai > Samastipur > Darbhanga > Munger > Katihar > Bhagalpur > Nalanda. The hazard quotient study indicates that lactating mothers are at risk of exposure to carcinogens. This risk study indicates a direct correlation between arsenic poisoning and the child's exposure to arsenic (USEPA, 2014; GSI, 2022; USEPA, 2008; Narsimha & Rajitha, 2018; ICMR, 2009). This may further deteriorate the fundamental activities, development, and cognitive growth of the child as they age. Therefore, immediate intervention is necessary to address the issue within the vulnerable population, particularly lactating mothers and their infants.

Women can accumulate harmful heavy metals in their systems for the duration of their lives if they are exposed to them through skin exposure or absorption through the digestive system, even during pregnancy (Budi et al., 2022). Depending on the exact moment, amount, and length of exposure, toxic metals that build up before or during pregnancy may become mobilized at that time and pass through the placental barrier, impairing fetal development (Flora and Agrawal, 2017). Presence of heavy metals in the mother's body, cord blood, breast milk, and other sources were detected to evaluate the exposure of fetuses and newborns (Dursun et al., 2016). One effective method of gathering information regarding the level and scope of pollution is to monitor environmental contaminants in human milk (Malisch et al., 2023).

In the household water sources for lead study, all the studied water samples were within the permissible limit ($<10 \mu\text{g/L}$). In the blood lead content in the studied exposed area $n=200$ subjects, 14.5% subjects had the lead content in their blood within the permissible limit ($<50 \mu\text{g/L}$), while 85.5% subjects had the lead content above

the permissible limit with highest content as 677.2 µg/L. In non-exposed area, 66% of the samples had their blood lead concentration below permissible limit (<50 µg/L) and 34% samples were above the permissible limit. In the breastmilk lead content study, 6% of the mother's had their breastmilk within the permissible limit (<5 µg/L), while 94% of the mother's had their breastmilk above the permissible limit with maximum content as 1309 µg/L. In non-exposed area, 32% of the samples had their blood lead concentration below permissible limit (<50 µg/L) and 68% samples were above the permissible limit. In the mother's urine lead content study, 28.5% of the urine samples had lead content below permissible limit (<50 µg/L), while 71.5% of the subjects had lead content more than the permissible limit with maximum content as 4168 µg/L. In non-exposed area, 62% of the samples had their urine lead concentration below permissible limit (<50 µg/L) and 38% samples were above the permissible limit. The study denotes that the lead exposure in the exposed population is maybe through the food samples rather than the water sources (Agarwal et al., 2024). The GIS mapping shows the pattern of the lead toxicity in the different habitations of the studied districts of Bihar. The exposure route can be deciphered from the present study denotes that the staple food (wheat, rice and potato) could be highly contaminated with the lead which is the prime source of lead exposure. However, the household water sources have lead contamination within the permissible limit. This denotes that the exposure to the food crops is through the soil and it could be geogenic effect responsible for the exposure. Moreover, the urine samples of subjects contained high lead content denotes that their metabolic organs are completely involved with this lead contamination.

However, it has been reported that adulteration in turmeric powder, ayurvedic medicines, cosmetics such as whitening creams, and moreover the indiscriminate usage of pesticides in the agricultural practices could attribute more lead exposure in the exposed population. Cosmetics are a leading source of lead poisoning, according to (Borowska and Brzóska, 2015) findings, which may cause health problems when applied regularly to the skin, mucous membranes, hair, and nails. In a study conducted in Bangladesh in n = 152 households and their n = 524 samples showed high lead content in their turmeric samples with highest concentration as 1152 µg/g denoting the level of adulteration (Forsyth et al., 2019). In a study carried out in

Indonesia reported high lead content in the cosmetic products used by the individuals such as face whitening creams containing lead content as high as 1.215 ppm (Agustina et al., 2019). The recent studies show that the lead exposure can cause damage to the vital organs such as brain, heart, bones, intestines, kidneys reproductive and nervous systems. In India, study conducted in the Jodhpur city of Rajasthan in n = 41 adult population shows high blood lead content (Chambial et al., 2015). A national level study (in India) on n = 2247 children, also showed lead contamination in 82.5% subjects (Kumar et al., 2023c). In Bihar, previous two studies shows that in n = 41 children blood lead levels were as high as 450 µg/L in Karmalichak region of Patna district where informal recycling of lead batteries workshops existed (Ansari et al., 2020). In the second study carried out on n = 135 children subjects in Patna district showed 87.5% of the children exposed with elevated blood lead levels (Brown et al., 2022). This increased lead exposure has caused neurological damage leading to deteriorating academic performance, delirium, abdominal pain, loss of appetite and energy, headache, irritability etc. in some exposed children (Samarghandian et al., 2021). Similar other studies have been carried out worldwide, which shows similar type of impact in the exposed population, in Southern China study on n = 530 children showed decline in the intelligence quotient (IQ) levels due to lead exposure (Pan et al., 2018). In India, similar study was carried out in n = 43 children showed cognitive impairment with significant correlation between blood lead levels and IQ levels. Moreover, there was significant impact on the children with loss of memory and low intelligence quotient (Rawat et al., 2022). Various other studies also demonstrate the lead exposure caused health effects in the exposed population (Dike et al., 2020; Axelrad et al., 2022; Shen et al., 2021; Wu et al., 2018; Nwobi et al., 2019). Similar study in Turkey, reported the lead exposure in the children by their mother's breastmilk (Rebelo and Caldas, 2016). The lead exposure in turn in children can damage the vital organs of their body causing brain and nerve damage, speech and language disorder, decreased mental disabilities, hearing problem and low IQ levels (World Health Organization, 2019; Al Osman et al., 2019; Bunch and Pyle-Eilola, 2023; Shefa and H´eroux, 2017; Ouyang et al., 2023; Aktürk et al., 2022). In the present study, the health risk assessment study was carried out in the mothers and their children shows that the lead exposed mothers are at high risk in comparison to

their children as their HQ levels were very high. All the mother population were found with higher HQ which is more than the threshold limit i.e. 1. It means on average 87% of the mother's population have the potential risk of non-carcinogenic health effects. About the carcinogenic risk assessment, it was found that the mother's population have higher than the Incremental lifetime cancer risk (ILCR) i.e. 0.0001, which shows the population which has higher ILCR than the threshold has the potential risk of causing cancer (Liu et al., 2020). This could cause serious health impact in them in long term lead exposure leading to carcinogenic risk in them (Macho'n-Grecka et al., 2022; Silbergeld, 2003; de Almeida et al., 2022; Ahmed et al., 2023). In a study conducted in Hamadan city of Iran carried out study on lead exposure in breastmilk in 94% individuals and high HQ levels in 61% of the exposed mothers (Samiee et al., 2019). In research carried out in Iran, the study assessed the potential toxic elements (PTEs) in wheat, Sangak, and Lavash flour samples, examining the impact of milling process depreciation. PTE levels in 270 samples from 10 Iranian factories were measured using ICP-OES. The human health risk from consuming these flours was estimated, considering 95% hazard quotient (HQ), Hazard Index (HI), and Total Hazard Index (THI). Significant differences in Ni concentration were found between wheat and the flours. PTE concentrations were within safe limits, but at the 95th percentile, children showed a higher non-carcinogenic and carcinogenic health risk (Pirhadi et al., 2022). Similar study was carried out in China, where children were exposed to lead and their cancer risks were 5–200 times higher than the maximum acceptable level of 1.0×10^{-4} (Cao et al., 2015). Similar other studies were also conducted by other researchers (Alidadi et al., 2019; Parker et al., 2022; Mohammadi et al., 2019; de Almeida et al., 2022). It was indicated by this study that the lead exposed population inhabiting in the districts along the river Ganges and its tributaries – Samastipur, Beugusarai, Khagaria, Darbhanga, Munger and Nalanda are at the verge of high risk of disease burden due to lead contamination. The lead exposure through the breastmilk can cause serious health risks in their children as the lead HQ is very high. Moreover, the exposed mother and children are at very high risk for the disease burden.

The present study also focuses on hormonal analysis of the female subjects such as- Estrogen, Progesterone & Thyrotropin because heavy metal poisoning can cause

imbalance in hormone secretion and their respective normal level in the body. In exposed area, n=200 serum samples were analyzed for estrogen and it showed that 9.5% of the serum samples were below the permissible range (9-289 pg/ml) & 33.5% of the samples were above the permissible range. Similarly, in non-exposed area out of n=50 subjects, 32% of the samples were below the permissible range and 18% of the serum sample were above the permissible range. For progesterone study, in exposed area 15.5% of the serum samples were below the permissible range (0.15-422 ng/ml) while 84.5% of the samples lies in the permissible range and in the non-exposed area, 26% of the samples were below the permissible range. Lastly, for thyrotropin study, in exposed area 3.5% of the serum samples were below the permissible range (0.28-6.28 μ U/ml) while 6.5% of the samples were above the permissible range. Wang et al, 2023 studied the exposure to heavy metals may alter sex hormone levels, but evidence is limited and inconsistent. In 1,355 midlife women from the SWAN Multi-Pollutant Study, urinary arsenic, cadmium, lead, and mercury were measured at baseline, with serum hormones tracked through 2017. Adjusted models showed that doubling mercury and lead levels was linked to lower estradiol; lead increased FSH, and cadmium increased SHBG. No link was found with testosterone. Overall, heavy metal mixtures were inversely associated with estradiol and positively with FSH. Ding et al, 2024 researched experimental and epidemiological studies suggest metals may affect women's reproductive aging, potentially by disrupting ovarian folliculogenesis and reducing ovarian reserve. This study explored the relationship between heavy metal exposure and anti-Müllerian hormone (AMH)—a key indicator of ovarian reserve—in 549 women, encompassing 2,252 AMH measurements taken over a 10-year period leading up to the final menstrual period (FMP). The analysis revealed that elevated urinary arsenic and mercury levels were associated with lower AMH concentrations at the FMP, while higher cadmium and mercury exposure correlated with a more rapid decline in AMH over time. These findings suggest that arsenic, cadmium, and mercury may function as ovarian toxicants, potentially accelerating reproductive aging among women approaching menopause. Igharo et al, 2018 found electronic waste (e-waste) contains toxic, endocrine-disrupting metals. This cross-sectional study in Benin City, Nigeria, compared 63 male e-waste workers with 41 unexposed men. Blood levels of

chromium, cadmium, arsenic, and mercury and serum gonadal hormones were measured. E-waste workers had significantly lower levels of testosterone, progesterone, LH, FSH, prolactin, and estrogen, but higher inhibin. Chromium and other metals negatively correlated with key hormones and positively with inhibin. Findings suggest occupational e-waste exposure may disrupt male reproductive hormones via toxic metal exposure. Chikezie et al, 2017 investigated the effects of endocrine-disrupting metals on hormones in 99 normal-weight males in Ibadan, including 50 auto-mechanics and 49 controls. Auto-mechanics had higher blood levels of lead, cadmium, and mercury (not arsenic), lower libido, and reduced total antioxidant capacity (TAC) compared to controls. Only 90% were eu-gonadic. In auto-mechanics, lead inversely correlated with testosterone and positively with FSH; mercury was positively associated with LH, while LH inversely correlated with TAC. These findings suggest occupational exposure to metals may induce oxidative stress and testicular dysfunction, even in normal-weight men without metabolic syndrome. Guo et al, 2021b examined essential trace elements and thyroid function in 87 residents from an e-waste site and 81 from a reference site. The exposed group had significantly lower iron and manganese, and higher TSH levels. In females, lower iron was significantly associated with elevated TSH, which in turn was linked to increased oxidative stress markers (MDA, 8-iso-PG). Copper and zinc were also lower, though not significantly. Findings suggest e-waste exposure may disrupt the hypothalamic-pituitary-thyroid axis by depleting essential elements, particularly iron, with greater effects in women, highlighting the need for targeted health protections in these populations. Zhang et al, 2022 investigated the link between serum heavy metals and polycystic ovary syndrome (PCOS) in 40 PCOS patients and 40 controls. PCOS patients had significantly higher serum chromium (Cr), mercury (Hg), and copper (Cu) levels. Cr was positively associated with LH and negatively with prolactin; Hg correlated with LH, testosterone, androstenedione, IL-6, and TNF- α ; Cu was linked to higher LDL, IL-6, TNF- α , and WBC count. Cu levels were higher in nonpregnant women undergoing IVF-ET. These findings suggest elevated Cr, Hg, and Cu may contribute to hormonal, metabolic, and inflammatory disturbances in PCOS and be linked to poorer pregnancy outcomes. Zhang et al, 2023 analyzed 614 postmenopausal women from the 2013–2016 NHANES to examine associations

between serum heavy metals (Cd, Pb, Hg, Mn, Se) and sex hormones. Cadmium was positively associated with total testosterone (TT), bioavailable TT, and TT/E2 ratio, especially in naturally menopausal and obese women. Lead was positively linked to SHBG and TT/E2, notably in most subgroups. Manganese showed non-linear associations with SHBG and TT/E2, while selenium had U-shaped associations with TT and SHBG, varying by BMI and hysterectomy status. Results suggest environmental heavy metal exposure may disrupt sex hormone levels in postmenopausal women. The whole study proves that there is a need of urgent attention for the state government to intervene and make policies regarding this problem. It can also be correlated that heavy metals can be one of the many factors that can cause cancer, hinderance in child's growth development & intelligence.

Chapter 5

Summary & Conclusions

The present study provides powerful evidence that chronic arsenic and lead toxicity constitutes a pervasive and under-recognized public health crisis across the Gangetic plains of Bihar in the districts of Samastipur, Begusarai, Khagaria, Munger, Darbhanga, Katihar, Bhagalpur, and Nalanda. By analyzing environmental sources and biological samples, this research establishes the persistent presence of arsenic and lead in the blood, urine, and breast milk of lactating mothers, as well as the subsequent transfer of these toxic metals to breastfeeding infants. These findings reveal an alarming exposure scenario with profound implications for maternal health, infant development, and the health of future generations. The study demonstrates that heavy metal contamination in this region arises from a complex interplay of geogenic factors such as naturally occurring arsenic and lead-rich alluvial sediments and anthropogenic activities, including industrial emissions, agricultural runoff, improper waste disposal, cosmetic adulteration, and informal recycling practices. Most important, the investigation establishes that contaminated drinking water alone does not fully explain the observed body burden of metals. Instead, staple foods such as wheat, rice, and potatoes play a critical role in the food-chain mediated biomagnification of arsenic and lead, thereby creating continuous and unavoidable exposure for the local population. This environmental pervasiveness significantly complicates mitigation efforts and underscores the need for integrated environmental and public health interventions. One of the most significant contributions of this study is the clear demonstration of breast milk as a critical exposure pathway for infants. Elevated concentrations of arsenic and lead detected in breast milk showed a strong correlation with maternal body burden, confirming direct maternal–infant transfer during lactation. The findings raise grave concerns, as early-life exposure to arsenic and lead is associated with irreversible neurodevelopmental deficits, including reduced IQ, impaired memory, delayed cognitive development, and compromised mental growth in children. These effects not only impair individual health outcomes but also have long-term societal consequences by adversely affecting educational attainment, productivity, and quality of life. For mothers, chronic exposure to arsenic and lead was associated with significant systemic toxicity. Elevated levels of these

metals were linked to renal, hepatic, and cardiovascular stress, as well as pronounced endocrine disruption. Hormonal imbalances manifested clinically as dysmenorrhea, irregular menstrual cycles, subfertility, and infertility, highlighting the detrimental impact of heavy metals on female reproductive health. Such findings are particularly concerning in a population already burdened by limited access to healthcare, delayed diagnosis, and socioeconomic vulnerability. The nonspecific and delayed onset of symptoms further complicates clinical recognition, allowing chronic poisoning to progress silently until significant organ damage has occurred. Health risk assessment revealed an unacceptably high non-carcinogenic and carcinogenic risk in the exposed population, with infants exhibiting the highest vulnerability, followed by lactating mothers. The elevated cancer risk associated with chronic arsenic and lead exposure is of particular concern, as early-life exposure to carcinogens significantly increases the probability of malignancy later in life. These results emphasize the urgent need for continuous biomonitoring, early detection, and long-term surveillance in affected regions. From a forensic science perspective, the findings of this study hold substantial medico-legal significance. Chronic arsenic and lead poisoning presents a unique forensic challenge due to its insidious nature, prolonged latency, and symptom overlap with common diseases. The persistent detection of these metals in blood, urine, and breast milk provides reliable biomarkers for reconstructing chronic exposure histories. Such biological evidence can assist forensic toxicologists in differentiating environmental and occupational exposure from intentional or negligent poisoning. Moreover, the integration of toxicological findings with GIS-based spatial mapping strengthens source attribution, enabling linkage between contaminated environments, affected populations, and responsible entities. This approach has direct applicability in legal investigations, compensation claims, public interest litigation, and policy enforcement. Also, the study highlights both possibilities and limitations in treatment. While effective chelation therapies such as dimercaptosuccinic acid (DMSA) and dimercapto-propanesulfonic acid (DMPS) are available for lead poisoning and show promise in reducing body burden, prevention remains the most effective strategy. The absence of specific antidotes for certain heavy metals further underscores the necessity of minimizing exposure rather than relying solely on therapeutic intervention. The findings strongly advocate for urgent and coordinated

action at multiple levels. Public health education targeting lactating mothers and families is essential, with emphasis on the use of arsenic- and lead-free drinking water, safe food practices, and avoidance of contaminated consumer products. At the policy level, sustained involvement of State and Central Governments is necessary. This includes the implementation of regular screening programs, regulation of agricultural and industrial practices, provision of safe water through community-level filtration systems, monitoring of food commodities, and strengthening of environmental regulations. Nutritional interventions to reduce metal absorption and institutionalization of long-term biomonitoring programs are equally critical. In conclusion, this study brings to light a silent yet severe public health emergency in the Gangetic plains of Bihar. The neurological, reproductive, endocrine, and carcinogenic risks identified demand immediate, evidence-based, and multisectoral interventions. Beyond its public health relevance, the study provides a strong forensic framework for understanding, detecting, and legally addressing chronic heavy metal poisoning. Future research should build upon these findings through longitudinal mother-child cohort studies, advanced metal speciation, isotopic fingerprinting and omics-based approaches to further elucidate exposure pathways, biological mechanisms, and long-term outcomes. Only through sustained scientific, medical, forensic, and policy-driven efforts can this complex and deeply rooted health crisis be effectively mitigated and the well-being of vulnerable populations safeguarded.

Bibliography

Abd Elnabi, M. K., Elkaliny, N. E., Elyazied, M. M., Azab, S. H., Elkhalfifa, S. A., Elmasry, S., Mouhamed, M. S., Shalamesh, E. M., Alhorieny, N. A., Abd Elaty, A. E., Elgendy, I. M., Etman, A. E., Saad, K. E., Tsigkou, K., Ali, S. S., Kornaros, M., & Mahmoud, Y. A. (2023). Toxicity of Heavy Metals and Recent Advances in Their Removal: A Review. *Toxics*, 11(7), 580. <https://doi.org/10.3390/toxics11070580>

Abolli, Samaneh & Dehghani, Samaneh & Atlasi, Rasha & Maleki, Zahra & Yunesian, Masoud & Tabatabaei-Malazy, Ozra & Saraei, Maryam & Khosravifar, Mina & Soleimani, Zahra. (2024). Arsenic and type 2 diabetes: Revealing the environmental exposure relationship through effective factors - A systematic review. *Results in Engineering*. 22. 102054. 10.1016/j.rineng.2024.102054.

Aggarwal, Ankur & Verma, Dr. Tarun & Ghosh, Sumangal. (2022). Heavy Metal Residues in Milk and Milk Products and Their Detection Method. 10.5772/intechopen.105004.

Agarwal, R., Kumar, A., Pandey, T., Chayal, N. K., Verma, K. S., Kumar, D., Kumar, K., Aryal, S., Singh, P., Ali, M., Srivastava, A., Singh, M., & Ghosh, A. K. (2024). High lead contamination in Mother's breastmilk in Bihar (India): Health risk assessment of the feeding children. *Chemosphere*, 364, 143064. <https://doi.org/10.1016/j.chemosphere.2024.143064>

Agarwal, R., Kumar, A., Pandey, T., Verma, D., Kumar, K., Chayal, N. K., Kumar, P., Kumar, N., Ali, M., Srivastava, A., Verma, K. S., Kumar, D., Sharma, A., & Ghosh, A. K. (2025). Environmental Arsenic Contamination and Human Health Risk Assessment in the Kosi Plain Region of Bihar, India. *Biological trace element research*, 10.1007/s12011-025-04886-6. Advance online publication. <https://doi.org/10.1007/s12011-025-04886-6>

Agustina, A. & Primadi, M. & Randhanugraha, H. & Khotimah, K. & Luriyani, I.. (2021). Analysis of Lead (Pb) and Cadmium (Cd) in Whitening Creams using Atomic Absorption Spectroscopy. *Indonesian Journal of Chemistry and Environment*. 2. 1-6. 10.21831/ijce.v2i2.38187.

Ahmed, R. S., Abuarab, M. E., Ibrahim, M. M., Baioumy, M., & Mokhtar, A. (2023). Assessment of environmental and toxicity impacts and potential health hazards of heavy metals pollution of agricultural drainage adjacent to industrial zones in Egypt. *Chemosphere*, 318, 137872. <https://doi.org/10.1016/j.chemosphere.2023.137872>

Aktürk, T., Çeliker, G., & Saçmacı, H. (2022). Impact of occupational lead exposure on nerve conduction study data. *The International journal of neuroscience*, 132(3), 306–312. <https://doi.org/10.1080/00207454.2021.1873784>

Ali, Hazrat & Khan, Ezzat & Ilahi, Ikram. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *Journal of Chemistry*. 2019. 1-14. 10.1155/2019/6730305.

Alidadi, H., Tavakoly Sany, S. B., Zarif Garaati Oftadeh, B., Mohamad, T., Shamszade, H., & Fakhari, M. (2019). Health risk assessments of arsenic and toxic heavy metal exposure in drinking water in northeast Iran. *Environmental health and preventive medicine*, 24(1), 59. <https://doi.org/10.1186/s12199-019-0812-x>

Al Osman, M., Yang, F., & Massey, I. Y. (2019). Exposure routes and health effects of heavy metals on children. *Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine*, 32(4), 563–573. <https://doi.org/10.1007/s10534-019-00193-5>

Al-Saleh I. (2021). Health Risk Assessment of Trace Metals Through Breast Milk Consumption in Saudi Arabia. *Biological trace element research*, 199(12), 4535–4545. <https://doi.org/10.1007/s12011-021-02607-3>

Al-Suhaimi, E.A., Khan, F.A. (2022). Thyroid Glands: Physiology and Structure. In: Al-Suhaimi, E.A. (eds) *Emerging Concepts in Endocrine Structure and Functions*. Springer, Singapore. https://doi.org/10.1007/978-981-16-9016-7_5

Angrand, R. C., Collins, G., Landrigan, P. J., & Thomas, V. M. (2022). Relation of blood lead levels and lead in gasoline: an updated systematic review. *Environmental*

health : a global access science source, 21(1), 138. <https://doi.org/10.1186/s12940-022-00936-x>

Ansari, J. A., Mahdi, A. A., Malik, P. S., & Jafar, T. (2020). Blood Lead Levels in Children Living Near an Informal Lead Battery Recycling Workshop in Patna, Bihar. *Journal of health & pollution*, 10(25), 200308. <https://doi.org/10.5696/2156-9614-10.25.200308>

Anushree, Ahsan, J. (2021). Effects of Arsenic: Neurological and Cellular Perspective. In: Kumar, N. (eds) *Arsenic Toxicity: Challenges and Solutions*. Springer, Singapore. https://doi.org/10.1007/978-981-33-6068-6_5

Anzar, N., Suleman, S., Singh, Y., Kumari, S., Parvez, S., Pilloton, R., & Narang, J. (2024). The Evolution of Illicit-Drug Detection: From Conventional Approaches to Cutting-Edge Immunosensors-A Comprehensive Review. *Biosensors*, 14(10), 477. <https://doi.org/10.3390/bios14100477>

Ariaifar, S., Makhdoomi, S., & Mohammadi, M. (2023). Arsenic and Tau Phosphorylation: a Mechanistic Review. *Biological trace element research*, 201(12), 5708–5720. <https://doi.org/10.1007/s12011-023-03634-y>

Aryal, M. (2025). “Groundwater Arsenic Contamination in Nepal.” *World Water Policy* 11, no. 3: 871–888. <https://doi.org/10.1002/wwp2.70008>.

ATSDR. (2007). *Toxicological Profile for Arsenic*. Atlanta: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services; Flora, S. J. S. 2015. *Handbook of Arsenic Toxicity*, ed. S. J. S. Flora, 1–49. Amsterdam: Elsevier and Academic Press. <http://www.atsdr.cdc.gov/toxprofiles/tp2.pdf>

TOXICOLOGICAL PROFILE FOR ARSENIC. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2007 Aug. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK591633/>

Awere, E., Obeng, P. A., Bonoli, A., & Obeng, P. A. (2020). E-waste recycling and public exposure to organic compounds in developing countries: a review of recycling

practices and toxicity levels in Ghana. *Environmental Technology Reviews*, 9(1), 1–19. <https://doi.org/10.1080/21622515.2020.1714749>

Axelrad, D. A., Coffman, E., Kirrane, E. F., & Klemick, H. (2022). The relationship between childhood blood lead levels below 5 µg/dL and childhood intelligence quotient (IQ): Protocol for a systematic review and meta-analysis. *Environment international*, 169, 107475. <https://doi.org/10.1016/j.envint.2022.107475>

Aydin, S., Emre, E., Ugur, K., Aydin, M. A., Sahin, İ., Cinar, V., & Akbulut, T. (2025). An overview of ELISA: a review and update on best laboratory practices for quantifying peptides and proteins in biological fluids. *The Journal of international medical research*, 53(2), 3000605251315913. <https://doi.org/10.1177/03000605251315913>

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Frontiers in pharmacology*, 12, 643972. <https://doi.org/10.3389/fphar.2021.643972>

Bartrem, C. (2017). Environmental Health and Risk Assessment: Lead Poisoning, Remediation, and Project Sustainability in Zamfara, Nigeria and Lead-Arsenate Contamination in Washington, USA (Doctoral dissertation, University of Idaho) https://verso.uidaho.edu/esploro/outputs/doctoral/Environmental-Health-and-Risk-Assessment-Lead/996637920001851/filesAndLinks?institution=01ALLIANCE_UID&skipUsageReporting=true&recordUsage=false&index=0.

Bartmess JE. Minor and Trace elements in breast milk: report of a Joint WHO/IAEA Collaborative Study. *J Hum Lactation*. 1990;6(1):28–9. <https://doi.org/10.1177/089033449000600125>.

David C. Bellinger; Lead. *Pediatrics* April (2004); 113 (Supplement_3): 1016–1022. 10.1542/peds.113.S3.1016

Bertazzi P. A. (2010). Il lavoro come bisogno umano e fattore di salute [Work as a basic human need and health promoting factor]. *La Medicina del lavoro*, 101 Suppl 2, 28–43.

Bhardwaj, S., Soni, R., Gupta, S. K., & Shukla, D. P. (2020). Mercury, arsenic, lead and cadmium in waters of the Singrauli coal mining and power plants industrial zone, Central East India. *Environmental monitoring and assessment*, 192(4), 251. <https://doi.org/10.1007/s10661-020-8225-2>

Bhardwaj, J. K., Paliwal, A., & Saraf, P. (2021). Effects of heavy metals on reproduction owing to infertility. *Journal of biochemical and molecular toxicology*, 35(8), e22823. <https://doi.org/10.1002/jbt.22823>

Binkowski, Ł.J. (2019). Arsenic, As. In: Kalisińska, E. (eds) *Mammals and Birds as Bioindicators of Trace Element Contaminations in Terrestrial Environments*. Springer, Cham. https://doi.org/10.1007/978-3-030-00121-6_13

Boldyrev, M. (2018). Lead: properties, history, and applications. *WikiJournal of Science*, 1(2), 1-23. <https://doi.org/10.15347/wjs/2018.007>

Börner, A., Zeidler, J. (2023). The Periodic Table of Elements and Basic Consequences for the Structure of Natural Substances and the Course of Biochemical Processes. In: *The Chemistry of Biology*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-66521-3_1

Borowska, S., & Brzóska, M. M. (2015). Metals in cosmetics: implications for human health. *Journal of applied toxicology : JAT*, 35(6), 551–572. <https://doi.org/10.1002/jat.3129>

Bouida, L., Rafatullah, M., Kerrouche, A., Qutob, M., Alosaimi, A. M., Alorfi, H. S., & Hussein, M. A. (2022). A Review on Cadmium and Lead Contamination: Sources, Fate, Mechanism, Health Effects and Remediation Methods. *Water*, 14(21), 3432. <https://doi.org/10.3390/w14213432>

Brands, H. W. (2002). *The first American: The life and times of Benjamin Franklin*. Anchor Books.

Brown, M. J., Patel, P., Nash, E., Dikid, T., Blanton, C., Forsyth, J. E., Fontaine, R., Sharma, P., Keith, J., Babu, B., Vaisakh, T. P., Azarudeen, M. J., Riram, B., & Shrivastava, A. (2022). Prevalence of elevated blood lead levels and risk factors among children living in Patna, Bihar, India 2020. *PLOS global public health*, 2(10), e0000743. <https://doi.org/10.1371/journal.pgph.0000743>

Budi, H. S., Catalan Opulencia, M. J., Afra, A., Abdelbasset, W. K., Abdullaev, D., Majdi, A., Taherian, M., Ekrami, H. A., & Mohammadi, M. J. (2022). Source, toxicity and carcinogenic health risk assessment of heavy metals. *Reviews on environmental health*, 39(1), 77–90. <https://doi.org/10.1515/reveh-2022-0096>

Buha, A., Matovic, V., Antonijevic, B., Bulat, Z., Curcic, M., Renieri, E. A., Tsatsakis, A. M., Schweitzer, A., & Wallace, D. (2018). Overview of Cadmium Thyroid Disrupting Effects and Mechanisms. *International journal of molecular sciences*, 19(5), 1501. <https://doi.org/10.3390/ijms19051501>

Bunch, D., & Pyle-Eilola, A. L. (2023). Lead poisoning: Clinical and laboratory considerations. *Advances in clinical chemistry*, 117, 209–222. <https://doi.org/10.1016/bs.acc.2023.08.001>

Byber, K., Lison, D., Verougstraete, V., Dressel, H., & Hotz, P. (2016). Cadmium or cadmium compounds and chronic kidney disease in workers and the general population: a systematic review. *Critical reviews in toxicology*, 46(3), 191–240. <https://doi.org/10.3109/10408444.2015.1076375>

Calatayud, M., Farias, S. S., de Paredes, G. S., Olivera, M., Carreras, N. Á., Giménez, M. C., Devesa, V., & Vélez, D. (2019). Arsenic exposure of child populations in Northern Argentina. *The Science of the total environment*, 669, 1–6. <https://doi.org/10.1016/j.scitotenv.2019.02.415>

Cao, S., Duan, X., Zhao, X., Wang, B., Ma, J., Fan, D., Sun, C., He, B., Wei, F., & Jiang, G. (2015). Health risk assessment of various metal(loid)s via multiple exposure pathways on children living near a typical lead-acid battery plant, China. *Environmental pollution (Barking, Essex : 1987)*, 200, 16–23. <https://doi.org/10.1016/j.envpol.2015.02.010>

Carignan, C. C., Cottingham, K. L., Jackson, B. P., Farzan, S. F., Gandolfi, A. J., Punshon, T., Folt, C. L., & Karagas, M. R. (2015). Estimated exposure to arsenic in breastfed and formula-fed infants in a United States cohort. *Environmental health perspectives*, 123(5), 500–506. <https://doi.org/10.1289/ehp.1408789>

Caudill, D. S. (2009). Arsenic and old chemistry: images of mad alchemists, experts attacking experts, and the crisis in forensic science. Boston Univ. J. Sci. Tech. Law 15, Villanova Law/Public Policy Research Paper no.2009-13. Available at: <http://ssrn.com/abstract/4140275>. Accessed on December 3, 2010.

CDC (1994), Lead in blood and urine 8003, <https://www.cdc.gov/niosh/docs/2003-154/pdfs/8003.pdf>

Ceballos, D. M., Herrick, R. F., Dong, Z., Kalweit, A., Miller, M., Quinn, J., & Spengler, J. D. (2021). Factors affecting lead dust in construction workers' homes in the Greater Boston Area. *Environmental research*, 195, 110510. <https://doi.org/10.1016/j.envres.2020.110510>

Centers for Disease Control and Prevention. "Blood Lead Levels in Children." (Page last reviewed: May 28, 2020). <https://www.cdc.gov/nceh/lead/prevention/blood-leadlevels.htm> (Accessed June 23rd, 2020)

Centers for Disease Control and Prevention (CDC). National Health and Nutrition Examination Survey. 2004. <http://www.cdc.gov/nchs/nhanes.htm> accessed on April 2 2008.

Centers for Disease Control and Prevention (CDC) (2009). Children with elevated blood lead levels related to home renovation, repair, and painting activities--New York State, 2006-2007. *MMWR. Morbidity and mortality weekly report*, 58(3), 55–58.

Chakraborti, D., Rahman, M.M., Das, B. *et al.* (2017) Groundwater arsenic contamination and its health effects in India. *Hydrogeol J* 25, 1165–1181. <https://doi.org/10.1007/s10040-017-1556-6>

Chakraborti, D., Singh, S. K., Rahman, M. M., Dutta, R. N., Mukherjee, S. C., Pati, S., & Kar, P. B. (2018). Groundwater Arsenic Contamination in the Ganga River

Basin: A Future Health Danger. *International journal of environmental research and public health*, 15(2), 180. <https://doi.org/10.3390/ijerph15020180>

Chakraborti, D., Mukherjee, S. C., Pati, S., Sengupta, M. K., Rahman, M. M., Chowdhury, U. K., Lodh, D., Chanda, C. R., Chakraborti, A. K., & Basu, G. K. (2003). Arsenic groundwater contamination in Middle Ganga Plain, Bihar, India: a future danger?. *Environmental health perspectives*, 111(9), 1194–1201. <https://doi.org/10.1289/ehp.5966>

Chakraborti, D., Rahman, M. M., Paul, K., Chowdhury, U. K., Sengupta, M. K., Lodh, D., Chanda, C. R., Saha, K. C., & Mukherjee, S. C. (2002). Arsenic calamity in the Indian subcontinent What lessons have been learned?. *Talanta*, 58(1), 3–22. [https://doi.org/10.1016/s0039-9140\(02\)00270-9](https://doi.org/10.1016/s0039-9140(02)00270-9)

Chakraborti, D., Rahman, M. M., Mukherjee, A., Alauddin, M., Hassan, M., Dutta, R. N., Pati, S., Mukherjee, S. C., Roy, S., Quamruzzman, Q., Rahman, M., Morshed, S., Islam, T., Sorif, S., Selim, M., Islam, M. R., & Hossain, M. M. (2015). Groundwater arsenic contamination in Bangladesh-21 Years of research. *Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS)*, 31, 237–248. <https://doi.org/10.1016/j.jtemb.2015.01.003>

Chakraborty, A., Ghosh, S., Biswas, B., Pramanik, S., Nriagu, J., & Bhowmick, S. (2022). Epigenetic modifications from arsenic exposure: A comprehensive review. *The Science of the total environment*, 810, 151218. <https://doi.org/10.1016/j.scitotenv.2021.151218>

Chambial, S., Shukla, K. K., Dwivedi, S., Bhardwaj, P., & Sharma, P. (2015). Blood Lead Level (BLL) in the Adult Population of Jodhpur: A Pilot Study. *Indian journal of clinical biochemistry : IJCB*, 30(3), 357–359. <https://doi.org/10.1007/s12291-015-0496-y>

Chatterjee, Debashis & Kundu, Arindam & Saha, D. & Barman, Sushanta & Mandal, U.. (2017). Groundwater Arsenic in the Bengal Delta Plain: Geochemical and Geomorphological Perspectives. *Procedia Earth and Planetary Science*. 17. 622-625. [10.1016/j.proeps.2016.12.166](https://doi.org/10.1016/j.proeps.2016.12.166).

- Chaudhary, M. M., Hussain, S., Du, C., Conway, B. R., & Ghori, M. U. (2024). Arsenic in Water: Understanding the Chemistry, Health Implications, Quantification and Removal Strategies. *ChemEngineering*, 8(4), 78. <https://doi.org/10.3390/chemengineering8040078>
- Chayal, N. K., Kumar, A., Verma, K. S., Agarwal, R., Kumar, K., Kumar, S., Kumar, D., Pandey, T., Ali, M., Srivastava, A., Bishwapriya, A., Sharma, A., Singh, M., & Ghosh, A. K. (2025). Arsenic Poisoning in Bihar: A Comparative Health Risk Assessment Through Water and Food Sources. *Biological trace element research*, 10.1007/s12011-025-04824-6. Advance online publication. <https://doi.org/10.1007/s12011-025-04824-6>
- Chen, Q. Y., & Costa, M. (2021). Arsenic: A Global Environmental Challenge. *Annual review of pharmacology and toxicology*, 61, 47–63. <https://doi.org/10.1146/annurev-pharmtox-030220-013418>
- Chen, C. J., Kuo, T. L., & Wu, M. M. (1988). Arsenic and cancers. *Lancet (London, England)*, 1(8582), 414–415. [https://doi.org/10.1016/s0140-6736\(88\)91207-x](https://doi.org/10.1016/s0140-6736(88)91207-x)
- Cherkani-Hassani, A., Slaoui, M., Ghanname, I., Mojemmi, B., Eljaoudi, R., Belhaj, A., Kers, B., Flayou, M., & Mouane, N. (2021). Lead concentrations in breast milk of Moroccan nursing mothers and associated factors of exposure: CONTAMILK STUDY. *Environmental toxicology and pharmacology*, 85, 103629. <https://doi.org/10.1016/j.etap.2021.103629>
- Effects of Endocrine Disrupting Heavy Metals on Pituitary and Gonadal Hormones in Normal Weight Automechanics in Ibadan, Nigeria. (2024). *African Journal of Biomedical Research*, 20(1), 25-35. <https://doi.org/10.4314/>
- Chlubek, Mikołaj & Baranowska-Bosiacka, Irena. (2023). Selected biochemical mechanisms of lead neurotoxicity. *Pomeranian Journal of Life Sciences*. 69. 10.21164/pomjlifesci.939.
- Clark, Brian & Engvall, Eva. (2018). Enzyme-Linked Immunosorbent Assay (Elisa): Theoretical and Practical Aspects. 10.1201/9781351071840-8.

Collin, M. S., Venkatraman, S. K., Vijayakumar, N., Kanimozhi, V., Arbaaz, S. M., Stacey, R. S., ... & Swamiappan, S. (2022). Bioaccumulation of lead (Pb) and its effects on human: A review. *Journal of Hazardous Materials Advances*, 7, 100094 <https://doi.org/10.1016/j.hazadv.2022.100094>.

Concha G, Vogler G, Nermell B, Vahter M. (1998) Low-level arsenic excretion in breast milk of native andean women exposed to high levels of arsenic in the drinking water. *Int Arch Occup Environ Health*;71(1):42–6. <https://doi.org/10.1007/s004200050248>.

Criteria for a Recommended Standard... Occupational Exposure to Inorganic Arsenic, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 75–149 (1975).

Cullen, W. R. (2008). *Is Arsenic an Aphrodisiac? The Sociochemistry of an Element*. Royal Society of Chemistry, Cambridge, U.K. <https://doi.org/10.1039/9781847558602>

Curtin, P. D. (1989). *Death by migration: Europe's encounter with the tropical world in the nineteenth century*. Cambridge University Press. <https://doi.org/10.1017/CBO9780511665240>

J. M. Davis (2019), in *Genomics and Clinical Diagnostics*, ed. D. Whitehouse and R. Rapley, The Royal Society of Chemistry, pp. 405–439. <https://doi.org/10.1039/9781839168840>

de Almeida, C. C., Baião, D. D. S., Rodrigues, P. A., Saint'Pierre, T. D., Hauser-Davis, R. A., Leandro, K. C., Paschoalin, V. M. F., da Costa, M. P., & Conte-Junior, C. A. (2022). Toxic Metals and Metalloids in Infant Formulas Marketed in Brazil, and Child Health Risks According to the Target Hazard Quotients and Target Cancer Risk. *International journal of environmental research and public health*, 19(18), 11178. <https://doi.org/10.3390/ijerph191811178>

de Carvalho Machado, C., & Dinis-Oliveira, R. J. (2023). Clinical and Forensic Signs Resulting from Exposure to Heavy Metals and Other Chemical Elements of the

Periodic Table. *Journal of clinical medicine*, 12(7), 2591. <https://doi.org/10.3390/jcm12072591>

Del Rio, M., Rodriguez, C., Alvarado Navarro, E., Wekumbura, C., Galkaduwa, M. B., Hettiarachchi, G. M., & Sobin, C. (2023). Stability of blood lead levels in children with low-level lead absorption. *PloS one*, 18(6), e0287406. <https://doi.org/10.1371/journal.pone.0287406>

DiMaio, V. J., DiMaio, S. M., Garriott, J. C., & Simpson, P. (1983). A fatal case of lead poisoning due to a retained bullet. *The American journal of forensic medicine and pathology*, 4(2), 165–169. <https://doi.org/10.1097/00000433-198306000-00013>

Dike, I. C., Onwurah, C. N., Uzodinma, U., & Onwurah, I. N. (2020). Evaluation of Pb concentrations in selected vegetables and portable drinking water, and intelligent quotients of school children in Ishiagu-a Pb mining community: health risk assessment using predictive modelling. *Environmental monitoring and assessment*, 192(2), 126. <https://doi.org/10.1007/s10661-020-8071-2>

Ding, N., Wang, X., Harlow, S. D., Randolph, J. F., Jr, Gold, E. B., & Park, S. K. (2024). Heavy Metals and Trajectories of Anti-Müllerian Hormone During the Menopausal Transition. *The Journal of clinical endocrinology and metabolism*, 109(11), e2057–e2064. <https://doi.org/10.1210/clinem/dgad756>

Dogbatsey, L., Barima, A. O., Yandam, R., & Schandorf, C. (2024). Assessment of the Health and Safety Culture at the E-Waste Facilities at the Greater Accra Metropolitan Area of Ghana. *International Journal of Research and Innovation in Social Science*, 8(5), 530-542. <https://dx.doi.org/10.47772/IJRISS.2024.805040>

Donati, G. L., & Amais, R. S. (2019). Fundamentals and new approaches to calibration in atomic spectrometry. *Journal of Analytical Atomic Spectrometry*, 34(12), 2353-2369. <https://doi.org/10.1039/C9JA00273A>

Dueñas-Laita, A., Pérez-Miranda, M., González-López, M. A., Martín-Escudero, J. C., Ruiz-Mambrilla, M., & Blanco-Varela, J. (2005). Acute arsenic poisoning. *Lancet*

(London, England), 365(9475), 1982. [https://doi.org/10.1016/S0140-6736\(05\)66670-6](https://doi.org/10.1016/S0140-6736(05)66670-6)

Dumková, J., Smutná, T., Vrlíková, L., Le Coustumer, P., Večeřa, Z., Dočekal, B., Mikuška, P., Čapka, L., Fictum, P., Hampl, A., & Buchtová, M. (2017). Sub-chronic inhalation of lead oxide nanoparticles revealed their broad distribution and tissue-specific subcellular localization in target organs. *Particle and fibre toxicology*, 14(1), 55. <https://doi.org/10.1186/s12989-017-0236-y>

Dursun, A., Yurdakok, K., Yalcin, S. S., Tekinalp, G., Aykut, O., Orhan, G., & Morgil, G. K. (2016). Maternal risk factors associated with lead, mercury and cadmium levels in umbilical cord blood, breast milk and newborn hair. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, 29(6), 954–961. <https://doi.org/10.3109/14767058.2015.1026255>

Dutta, S., Gorain, B., Choudhury, H., Roychoudhury, S., & Sengupta, P. (2022). Environmental and occupational exposure of metals and female reproductive health. *Environmental science and pollution research international*, 29(41), 62067–62092. <https://doi.org/10.1007/s11356-021-16581-9>

EFSA Scientific Committee, More, S. J., Bampidis, V., Benford, D., Bennekou, S. H., Bragard, C., Halldorsson, T. I., Hernández-Jerez, A. F., Koutsoumanis, K., Naegeli, H., Schlatter, J. R., Silano, V., Nielsen, S. S., Schrenk, D., Turck, D., Younes, M., Benfenati, E., Castle, L., Cedergreen, N., Hardy, A., ... Hogstrand, C. (2019). Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. *EFSA journal. European Food Safety Authority*, 17(3), e05634. <https://doi.org/10.2903/j.efsa.2019.5634>

Eid, M. H., Eissa, M., Mohamed, E. A., Ramadan, H. S., Tamás, M., Kovács, A., & Szűcs, P. (2024). New approach into human health risk assessment associated with

heavy metals in surface water and groundwater using Monte Carlo Method. *Scientific reports*, 14(1), 1008. <https://doi.org/10.1038/s41598-023-50000-y>

Eisinger J. (1982). Lead and wine. Eberhard Gockel and the colica Pictonum. *Medical history*, 26(3), 279–302. <https://doi.org/10.1017/s0025727300041508>

Ericson, B., Hu, H., Nash, E., Ferraro, G., Sinitsky, J., & Taylor, M. P. (2021). Blood lead levels in low-income and middle-income countries: a systematic review. *The Lancet. Planetary health*, 5(3), e145–e153. [https://doi.org/10.1016/S2542-5196\(20\)30278-3](https://doi.org/10.1016/S2542-5196(20)30278-3)

Evans, M., Discacciati, A., Quershi, A. R., Åkesson, A., & Elinder, C. G. (2017). End-stage renal disease after occupational lead exposure: 20 years of follow-up. *Occupational and environmental medicine*, 74(6), 396–401. <https://doi.org/10.1136/oemed-2016-103876>

Exposure to Lead Pollution Undermines a Generation of Future Potential. Available at: <https://www.pureearth.org/unicef-and-pure-earth-call-for-urgentaction-to-protect-800-million-children-affected-by-lead/>

FAO; WHO. (2011) Working document for information and use in discussions related to contaminants and toxins in the GSCTFF. In: Codex Committee on Contaminants in Foods. Fifth Session. The Hague: FAO; 2011. 89p.

Ferrario, D., Gribaldo, L., & Hartung, T. (2016). Arsenic Exposure and Immunotoxicity: a Review Including the Possible Influence of Age and Sex. *Current environmental health reports*, 3(1), 1–12. <https://doi.org/10.1007/s40572-016-0082-3>

Filipoiu, D. C., Bungau, S. G., Endres, L., Negru, P. A., Bungau, A. F., Pasca, B., Radu, A. F., Tarce, A. G., Bogdan, M. A., Behl, T., Nechifor, A. C., Hassan, S. S. U., & Tit, D. M. (2022). Characterization of the Toxicological Impact of Heavy Metals on Human Health in Conjunction with Modern Analytical Methods. *Toxics*, 10(12), 716. <https://doi.org/10.3390/toxics10120716>

Fisher RM, Gupta V. Heavy Metals. [Updated 2024 Feb 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557806/>

Forsyth, J.E., Nurunnahar, S., Islam, S.S., Baker, M., Yeasmin, D., Islam, M.S., Rahman, M., Fendorf, S., Ardoin, N.M., Winch, P.J., Luby, S.P., (2019). Turmeric means "yellow" in Bengali: lead chromate pigments added to turmeric threaten public health across Bangladesh. *Environ. Res.* 179 (Pt A), 108722 <https://doi.org/10.1016/j.envres.2019.108722>.

Foster, A. V. E. (2024). *Oral Contraceptive Use and Blood Lead Concentrations in US Premenopausal Individuals*. (Doctoral thesis) (Michigan State University) https://d.lib.msu.edu/etd/51731/downloads?_goaway_challenge=header-refresh&_goaway_id=6afc13d298d03c79a71bd31ffc443535&_goaway_referer=https%3A%2F%2Fd.lib.msu.edu%2F.

Flora, S.J.S (2009). Metal poisoning: threat and management. *Journal of the Medical Sciences*, 2, 4-26. <https://ajms.alameenmedical.org/articlepdfs/AJMS4S.4-26.pdf>

Flora, S.J.S. (2020). Preventive and Therapeutic Strategies for Acute and Chronic Human Arsenic Exposure. In: Srivastava, S. (eds) *Arsenic in Drinking Water and Food*. Springer, Singapore. https://doi.org/10.1007/978-981-13-8587-2_13

Flora, S.J., Agrawal, S., (2017). Arsenic, cadmium, and lead. In: *Reproductive and Developmental Toxicology*. Academic Press, pp. 537–566. <https://doi.org/10.1016/B978-0-12-804239-7.00031-7>

Frank, J. J., Poulakos, A. G., Tornero-Velez, R., & Xue, J. (2019). Systematic review and meta-analyses of lead (Pb) concentrations in environmental media (soil, dust, water, food, and air) reported in the United States from 1996 to 2016. *The Science of the total environment*, 694, 133489. <https://doi.org/10.1016/j.scitotenv.2019.07.295>

Frisbie, S. H., & Mitchell, E. J. (2022). Arsenic in drinking water: An analysis of global drinking water regulations and recommendations for updates to protect public health. *PloS one*, 17(4), e0263505. <https://doi.org/10.1371/journal.pone.0263505>

- Fry, K. L., & Taylor, M. P. (2024). Peppered with lead: An environmental forensics approach to identify the source of rising blood lead levels. *Environmental research*, 252(Pt 3), 118832. <https://doi.org/10.1016/j.envres.2024.118832>
- Ghosh, G., Mukhopadhyay, D.K. (2019). Human Health Hazards Due to Arsenic and Fluoride Contamination in Drinking Water and Food Chain. In: Sikdar, P. (eds) *Groundwater Development and Management*. Springer, Cham. https://doi.org/10.1007/978-3-319-75115-3_15
- Gilbert, S. G., & Weiss, B. (2006). A rationale for lowering the blood lead action level from 10 to 2 microg/dL. *Neurotoxicology*, 27(5), 693–701. <https://doi.org/10.1016/j.neuro.2006.06.008>
- Giorgi, G., Lecca, L. I., Alessio, F., Finstad, G. L., Bondanini, G., Lulli, L. G., Arcangeli, G., & Mucci, N. (2020). COVID-19-Related Mental Health Effects in the Workplace: A Narrative Review. *International journal of environmental research and public health*, 17(21), 7857. <https://doi.org/10.3390/ijerph17217857>
- Giri, S., & Singh, A. K. (2017). Human health risk assessment due to dietary intake of heavy metals through rice in the mining areas of Singhbhum Copper Belt, India. *Environmental science and pollution research international*, 24(17), 14945–14956. <https://doi.org/10.1007/s11356-017-9039-9>
- Gochfeld M. (2005). Chronologic history of occupational medicine. *Journal of occupational and environmental medicine*, 47(2), 96–114. <https://doi.org/10.1097/01.jom.0000152917.03649.0e>
- Godswill, A. G., Somtochukwu, I. V., Ikechukwu, A. O., & Kate, E. C. (2020). Health Benefits of Micronutrients (Vitamins and Minerals) and their Associated Deficiency Diseases: A Systematic Review. *International Journal of Food Sciences*, 3(1), 1–32. <https://doi.org/10.47604/ijf.1024>
- Goldfrank, L. R., & Hoffman, R. S. (2006). *Goldfrank's toxicologic emergencies* (Vol. 831). McGraw-Hill.

[https://books.google.co.in/books/about/Goldfrank's Toxicologic Emergencies Eighth Edition.html?id=cvJuLqBxGUcC&redir_esc=y](https://books.google.co.in/books/about/Goldfrank's%20Toxicologic%20Emergencies%20Eigh.html?id=cvJuLqBxGUcC&redir_esc=y)

GSI. (2022). Geological assessment of Incidence of Arsenic in groundwater around Harnatar, Nautanwa, Bagaha, Bhairoganj and adjoining areas West Champaran district, Bihar. *Geological Survey of India report, unpublished report (FS:2021-22)*.

Guo, Y., Bera, H., Shi, C., Zhang, L., Cun, D., & Yang, M. (2021a). Pharmaceutical strategies to extend pulmonary exposure of inhaled medicines. *Acta Pharmaceutica Sinica B*, 11(8), 2565-2584. <https://doi.org/10.1016/j.apsb.2021.05.015>

Guo, C., Qian, Y., Yan, L., Li, Z., Liu, H., Li, X., Wang, Z., Zhu, X., Wang, Z., Wang, J., & Wei, Y. (2021b). The changes of essential trace elements in residents from an e-waste site and the relationships between elements and hormones of the hypothalamic-pituitary-thyroid (HPT) axis. *Ecotoxicology and environmental safety*, 222, 112513. <https://doi.org/10.1016/j.ecoenv.2021.112513>

Hamilton, A. (1911). Lead-poisoning in Illinois. *Journal of the American Medical Association*, 56(17), 1240-1244. <https://doi.org/10.1001/JAMA.1911.02560170004003>

Hassan, M. M. (2018). *Arsenic in groundwater: poisoning and risk assessment*. Crc Press. <https://doi.org/10.1201/9781315117034>

Hassan, A. S. (2019). Inorganic-based pesticides: A review article. *Egypt Sci J Pestic*, 5, 39-52. <http://esjpesticides.org.eg/JornalWebsite/Publications/2019/5/4/5.pdf>

Hassan, Z., & Westerhoff, H. V. (2024). Arsenic Contamination of Groundwater Is Determined by Complex Interactions between Various Chemical and Biological Processes. *Toxics*, 12(1), 0. <https://doi.org/10.3390/toxics12010089>

Hatipoğlu, S., Dirican, A., Ateş, M., Çevik, M. Ö., Yumrutaş, Ö., Işık, M. A., & Yılmaz, S. (2021). Differences of Accumulated Heavy Metal Levels in End-Stage Liver Disease, Wilson's Disease, and Other Etiologies. *Medical Journal of Istanbul Kanuni Sultan Süleyman/Istanbul Kanuni Sultan Süleyman Tıp Dergisi*, 13(3). <https://doi.org/10.4274/iksstd.2021.82712>

Herring, C. M., Bazer, F. W., Johnson, G. A., & Wu, G. (2018). Impacts of maternal dietary protein intake on fetal survival, growth, and development. *Experimental biology and medicine* (Maywood, N.J.), 243(6), 525–533. <https://doi.org/10.1177/1535370218758275>

Hojsak, I., Braegger, C., Bronsky, J., Campoy, C., Colomb, V., Decsi, T., Domellöf, M., Fewtrell, M., Mis, N. F., Mihatsch, W., Molgaard, C., van Goudoever, J., & ESPGHAN Committee on Nutrition (2015). Arsenic in rice: a cause for concern. *Journal of pediatric gastroenterology and nutrition*, 60(1), 142–145. <https://doi.org/10.1097/MPG.0000000000000502>

Hoonjan, M., Jadhav, V., & Bhatt, P. (2018). Arsenic trioxide: insights into its evolution to an anticancer agent. *Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry*, 23(3), 313–329. <https://doi.org/10.1007/s00775-018-1537-9>

Hu, Y., Li, J., Lou, B., Wu, R., Wang, G., Lu, C., Wang, H., Pi, J., & Xu, Y. (2020). The Role of Reactive Oxygen Species in Arsenic Toxicity. *Biomolecules*, 10(2), 240. <https://doi.org/10.3390/biom10020240>

Hughes, M. F., Beck, B. D., Chen, Y., Lewis, A. S., & Thomas, D. J. (2011). Arsenic exposure and toxicology: a historical perspective. *Toxicological sciences: an official journal of the Society of Toxicology*, 123(2), 305–332. <https://doi.org/10.1093/toxsci/kfr184>

ICMR (Indian Council of Medical Research). Nutrient 566 requirements and recommended 567 dietary allowances for indians. Hyderabad: Indians National Institute of Nutrition; 2009. p. 334.

Endocrine disrupting metals lead to alteration in the gonadal hormone levels in Nigerian e-waste workers. (2018). *Universa Medicina*, 37(1), 65-74. <https://doi.org/10.18051/UnivMed.2018.v37.65-74>

Ilieva, I., & Sainova, I. Mechanisms of Action of Heavy Metals, Related with Abnormal Protein and Enzyme Activity in Male Infertility Aspect (2023). *Acta*

Morphologica et Anthropologica, 30, 1-2. <https://doi.org/10.7546/AMA.30.1-2.2023.09>

Institute for Health Metrics and Evaluation. Lead exposure-Level 3 risk. Seattle: University of Washington; 2024 (<https://www.healthdata.org/research-analysis/diseases-injuries-risks/factsheets/2021-lead-exposure-level-3-risk>)

Ishaq, S., Liaqat, A., Hameed, A., & Ahmed, T. (2021). Biochemical mechanisms of aluminium and other metals exposure, their brain entry mechanisms, effects on blood brain barrier and important pharmacokinetic parameters in neurological disorders. In *Biochemical Mechanisms of Aluminium Induced Neurological Disorders* (pp. 1-32). Bentham Science Publishers. <https://doi.org/10.2174/97816810888391210101>

Islam, L. N. (2023). An update on the immunotoxic effects of arsenic exposure. In *Handbook of arsenic toxicology* (pp. 551-592). Academic Press. <https://doi.org/10.1016/B978-0-323-89847-8.00034-1>

Izah, S. C., Chakrabarty, N., & Srivastav, A. L. (2016). A review on heavy metal concentration in potable water sources in Nigeria: human health effects and mitigating measures. *Exposure and health*, 8, 285-304. <https://doi.org/10.1007/s12403-016-0195-9>

Jha, S. K., Mishra, V. K., Damodaran, T., Sharma, D. K., & Kumar, P. (2017). Arsenic in the groundwater: Occurrence, toxicological activities, and remedies. *Journal of environmental science and health. Part C, Environmental carcinogenesis & ecotoxicology reviews*, 35(2), 84–103. <https://doi.org/10.1080/10590501.2017.1298359>

Johnston, J. E., Lopez, M., Gribble, M. O., Gutschow, W., Austin, C., & Arora, M. (2019). A Collaborative Approach to Assess Legacy Pollution in Communities Near a Lead-Acid Battery Smelter: The "Truth Fairy" Project. *Health education & behavior : the official publication of the Society for Public Health Education*, 46(1_suppl), 71S–80S. <https://doi.org/10.1177/1090198119859406>

- Jolliffe D. M. (1993). A history of the use of arsenicals in man. *Journal of the Royal Society of Medicine*, 86(5), 287–289. <https://doi.org/10.1177/014107689308600515>
- Karagas, M. R., Stukel, T. A., Morris, J. S., Tosteson, T. D., Weiss, J. E., Spencer, S. K., & Greenberg, E. R. (2001). Skin cancer risk in relation to toenail arsenic concentrations in a US population-based case-control study. *American journal of epidemiology*, 153(6), 559–565. <https://doi.org/10.1093/aje/153.6.559>
- Karri, S. K., Saper, R. B., & Kales, S. N. (2008). Lead encephalopathy due to traditional medicines. *Current drug safety*, 3(1), 54–59. <https://doi.org/10.2174/157488608783333907>
- Khan, K. M., Chakraborty, R., Bundschuh, J., Bhattacharya, P., & Parvez, F. (2020). Health effects of arsenic exposure in Latin America: An overview of the past eight years of research. *The Science of the total environment*, 710, 136071. <https://doi.org/10.1016/j.scitotenv.2019.136071>
- Khant, N. A. (2024). Groundwater contamination by arsenic in Myanmar and their sustainability: a review. *Episodes*. <https://doi.org/10.18814/EPIUGS/2024/024009>
- Kharkwal, V., Bains, K., Bishnoi, M., & Devi, K. (2023). Health risk assessment of arsenic, lead and cadmium from milk consumption in Punjab, India. *Environmental monitoring and assessment*, 195(6), 723. <https://doi.org/10.1007/s10661-023-11256-z>
- Kawai, J. (2016). Forensic Analysis of the Wakayama Arsenic Murder Case. In *Forensic Analysis - From Death to Justice*. InTech. <https://doi.org/10.5772/63531>
- Kinder, C. (2019). Lead Contamination In Our Environment. <https://teachersinstitute.yale.edu/curriculum/units/files/97.07.05.pdf>
- Kington, T. (2010). The mystery of Caravaggio's death solved at last—painting killed him. *The Guardian*, 16. <https://www.theguardian.com/artanddesign/2010/jun/16/caravaggio-italy-remains-ravenna-art>

Kliegman, R. M., Behrman, R. E., Jenson, H. B., & Stanton, B. M. (2007). Nelson textbook of pediatrics e-book. Elsevier Health Sciences. https://books.google.co.in/books?id=mseNCgAAQBAJ&printsec=copyright&source=gbp_info_r#v=onepage&q&f=false

Konstantinou G. N. (2017). Enzyme-Linked Immunosorbent Assay (ELISA). *Methods in molecular biology (Clifton, N.J.)*, 1592, 79–94. https://doi.org/10.1007/978-1-4939-6925-8_7

Koyama, H., Kamogashira, T., & Yamasoba, T. (2024). Heavy Metal Exposure: Molecular Pathways, Clinical Implications, and Protective Strategies. *Antioxidants*, 13(1), 76. <https://doi.org/10.3390/antiox13010076>

Krishnamurthy, H. K., Pereira, M., Rajavelu, I., Jayaraman, V., Krishna, K., Wang, T., Bei, K., & Rajasekaran, J. J. (2024). Oxidative stress: fundamentals and advances in quantification techniques. *Frontiers in chemistry*, 12, 1470458. <https://doi.org/10.3389/fchem.2024.1470458>

Kulkarni, H. V., N. Mladenov, K. H. Johannesson et al. (2017). Contrasting dissolved organic matter quality in groundwater in Holocene and Pleistocene aquifers and implications for influencing arsenic mobility. *Applied Geochemistry* 77:194–205. <https://doi.org/10.1016/j.apgeochem.2016.06.002>

Kumar S. (2018). Occupational and Environmental Exposure to Lead and Reproductive Health Impairment: An Overview. *Indian journal of occupational and environmental medicine*, 22(3), 128–137. https://doi.org/10.4103/ijoem.IJOEM_126_18

Kumar, A., Rahman, M. S., Iqbal, M. A., Ali, M., Niraj, P. K., Anand, G., Kumar, P., Abhinav, & Ghosh, A. K. (2016). Ground Water Arsenic Contamination: A Local Survey in India. *International journal of preventive medicine*, 7, 100. <https://doi.org/10.4103/2008-7802.188085>

Kumar, A., & Ghosh, A. K. (2019a). Arsenic and cancer. In *Environmental Exposures and Human Health Challenges* (pp. 106-132). IGI Global. <https://doi.org/10.4018/978-1-5225-7635-8.ch005>

Kumar, Arun; Rahman, Md. Samiur¹; Kumar, Ranjit; Ali, Mohammad; Niraj, Pintoo Kumar; Srivastava, Abhinav; Singh, Sushil Kumar¹; Ghosh, Ashok Kumar (2019b). Arsenic Contamination in Groundwater Causing Impaired Memory and Intelligence in School Children of Simri Village of Buxar District of Bihar. *Journal of Mental Health and Human Behaviour* 24(2):p 132-138, Jul–Dec. | DOI: 10.4103/jmhbb.jmhbb_31_18

Kumar, A., Kumar, R., Ali, M., & Ghosh, A. K. (2019c). Prevalence of cancer incidences in Bihar, India due to poisoning in groundwater. In *Environmental Arsenic in a Changing World* (pp. 328-330). CRC Press. <https://www.taylorfrancis.com/chapters/oa-edit/10.1201/9781351046633-130/prevalence-cancer-incidences-bihar-india-due-poisoning-groundwater-kumar-kumar-ali-ghosh>

Kumar, A., Ali, M., Kumar, R., Rahman, M. S., Srivastava, A., Chayal, N. K., ... & Ghosh, A. K. (2020). High arsenic concentration in blood samples of people of village Gyaspur Mahaji, Patna, Bihar drinking arsenic-contaminated water. *Exposure and Health*, 12, 131-140. <https://doi.org/10.1007/s12403-018-00294-5>

Kumar A, Ghosh AK. (2021a). Assessment of arsenic contamination in groundwater and affected population of Bihar. N. Kumar, editor, *Arsenic Toxicity: Challenges and Solutions*. [ISBN 978-981-33-6067-9; ISBN 978-981-33-6068-6 (eBook)] https://doi.org/10.1007/978-981-33-6068-6_7

Kumar A, Ali M, Kumar R, Kumar M, Sagar P, Pandey RK, Akhouri V, Kumar V, Anand G, Niraj PK, Rani R, Kumar S, Kumar D, Bishwapriya A, Ghosh AK. (2021b). Arsenic exposure in Indo Gangetic plains of Bihar causing increased cancer risk. *Sci Rep*, 11(1), 2376. <https://doi.org/10.1038/s41598-021-81579-9>.

Kumar, A., Kumar, R., Rahman, M. S., Ali, M., Kumar, R., Nupur, N., Gaurav, A., Raj, V., Anand, G., Niraj, P. K., Kumar, N., Srivastava, A., Biswapriya, A., Chand, G.

B., Kumar, D., Rashmi, T., Kumar, S., Sakamoto, M., & Ghosh, A. K. (2021c). Assessment of arsenic exposure in the population of Sabalpur village of Saran District of Bihar with mitigation approach. *Environmental science and pollution research international*, 28(32), 43923–43934. <https://doi.org/10.1007/s11356-021-13521-5>.

Kumar, Dr Arun & Rahman, Md & Ali, Mohammad & Kumar, Ranjit & Niraj, Kumar & Akhouri, Vivek & Singh, Sushil & Kumar, Dhruv & Rashmi, Tuhin & Bishwapriya, Akhouri & Chand, Gyanendra & Sakamoto, Maiko & Ghosh, Ashok. (2021d). Assessment of arsenic exposure and its mitigation intervention in severely exposed population of Buxar district of Bihar, India. *Toxicology and Environmental Health Sciences*. 13, 287-297. 10.1007/s13530-021-00086-6.

Kumar, A., Rahman, M. S., Ali, M., Salaun, P., Gourain, A., Kumar, S., Kumar, R., Niraj, P. K., Kumar, M., Kumar, D., Bishwapriya, A., Singh, S., Murti, K., Dhingra, S., Sakamoto, M., & Ghosh, A. K. (2022). Assessment of disease burden in the arsenic exposed population of Chapar village of Samastipur district, Bihar, India, and related mitigation initiative. *Environmental science and pollution research international*, 29(18), 27443–27459. <https://doi.org/10.1007/s11356-021-18207-6>

Kumar, A., Kumar, C., & Krishna, Y. V. (2023a). Blood Arsenic Contamination Causing Breast Cancer Risk in Exposed Population of Bihar. *Health Science Journal*, 17(5), 1-6. DOI:10.36648/1791-809X.17.5.1017

Kumar, A., Ali, M., Raj, V., Kumari, A., Rachamalla, M., Niyogi, S., Kumar, D., Sharma, A., Saxena, A., Panjawani, G., Jain, P., Vidyarthi, A., Kumar, N., Kumar, M., Niraj, P. K., Rahman, M. S., Bishwapriya, A., Kumar, R., Sakamoto, M., Kumar, S., ... Ghosh, A. K. (2023b). Arsenic causing gallbladder cancer disease in Bihar. *Scientific reports*, 13(1), 4259. <https://doi.org/10.1038/s41598-023-30898-0>.

Kumar, A., Kumar, K., Ali, M., Raj, V., Srivastava, A., Kumar, M., Niraj, P. K., Kumar, M., Kumar, R., Kumar, D., Bishwapriya, A., Kumar, R., Kumar, S., Anand, G., Kumar, S., Sakamoto, M., & Ghosh, A. K. (2024a). Severe Disease Burden and the Mitigation Strategy in the Arsenic-Exposed Population of Kaliprasad Village in

Bhagalpur District of Bihar, India. *Biological trace element research*, 202(5), 1948–1964. <https://doi.org/10.1007/s12011-023-03822-w>

Kumar, A., Agarwal, R., Kumar, K., Chayal, N. K., Ali, M., Srivastava, A., Kumar, M., Niraj, P. K., Aryal, S., Kumar, D., Bishwapriya, A., Singh, S., Pandey, T., Verma, K. S., Kumar, S., Singh, M., & Ghosh, A. K. (2024b). High arsenic contamination in the breast milk of mothers inhabiting the Gangetic plains of Bihar: a major health risk to infants. *Environmental health: a global access science source*, 23(1), 77. <https://doi.org/10.1186/s12940-024-01115-w>

Kumar, A., Abhinav, S., Rachamalla, M., Ali, M., Navin, S., Kumar, D., & Ghosh, A. K. (2025). Treatment of heavy metal induced neurotoxicity via advanced drug delivery systems. In *Novel Drug Delivery Systems in the management of CNS Disorders* (pp. 393-407). Academic Press. <https://doi.org/10.1016/B978-0-443-13474-6.00025-1>

Kumar, D., Awasthi, S., Mahdi, A. A., Singh, S., Pandey, A. K., Agarwal, G. G., Anish, T. S., A R, S., Kar, S., Nair, S., Mathew, J. L., Bhat, M. A., Mahanta, B. N., Singh, K., & Singh, C. M. (2025). Assessment of Blood Lead Level of School Children in 10 Cities of India: A Cross-Sectional Study. *Indian journal of pediatrics*, 92(2), 131–137. <https://doi.org/10.1007/s12098-023-04864-7>

B. Suresh Lal, (2019), "Water for Life: Issues and Challenges", *International Journal of Science and Research (IJSR)*, 8(6), 1949-1957. <https://dx.doi.org/10.21275/ART20199011>, <https://www.ijsr.net/getabstract.php?paperid=ART20199011>

Lengfeld, J., Zhang, H., Stoesz, S., Murali, R., Pass, F., Greene, M. I., Goel, P. N., & Grover, P. (2021). Challenges in Detection of Serum Oncoprotein: Relevance to Breast Cancer Diagnostics. *Breast cancer (Dove Medical Press)*, 13, 575–593. <https://doi.org/10.2147/BCTT.S331844>

Levin, R., Brown, M. J., Kashtock, M. E., Jacobs, D. E., Whelan, E. A., Rodman, J., Schock, M. R., Padilla, A., & Sinks, T. (2008). Lead exposures in U.S. Children,

2008: implications for prevention. *Environmental health perspectives*, 116(10), 1285–1293. <https://doi.org/10.1289/ehp.11241>

Li, X., Shen, X., Jiang, W., Xi, Y., & Li, S. (2024). Comprehensive review of emerging contaminants: Detection technologies, environmental impact, and management strategies. *Ecotoxicology and environmental safety*, 278, 116420. <https://doi.org/10.1016/j.ecoenv.2024.116420>

Li, C., Wang, J., Yan, B., Miao, A. J., Zhong, H., Zhang, W., & Ma, L. Q. (2021). Progresses and emerging trends of arsenic research in the past 120 years. *Critical Reviews in Environmental Science and Technology*, 51(13), 1306–1353. <https://doi.org/10.1080/10643389.2020.1752611>

Lin, W. (2023). Clinical Significance of Serum Creatinine, Urea Nitrogen and Uric Acid Levels in Patients with Chronic Renal Failure. *International Journal of Biology and Life Sciences*, 3(3), 19-26. <https://doi.org/10.54097/ijbls.v3i3.04>

Liu, Y. M., Liu, D. Y., Zhang, W., Chen, X. X., Zhao, Q. Y., Chen, X. P., & Zou, C. Q. (2020). Health risk assessment of heavy metals (Zn, Cu, Cd, Pb, As and Cr) in wheat grain receiving repeated Zn fertilizers. *Environmental pollution (Barking, Essex : 1987)*, 257, 113581. <https://doi.org/10.1016/j.envpol.2019.113581>

Lorna Fewtrell, Rachel Kaufmann, Annette Prüss-Üstün. "Lead: Assessing the Environmental Burden of Disease at National and Local Levels." (The World Health Organization, Environmental Burden of Disease Series, No. 2, 2003). https://www.who.int/quantifying_ehimpacts/publications/en/leadebd2.pdf?ua=1

Luo, Y., Duan, Z., & Wu, Y. (2021). Risk Assessment for Oral Bioaccessibility of Lead and Cadmium in the Potato Growing in Smelter-Impacted Soil. *Bulletin of environmental contamination and toxicology*, 106(2), 363–369. <https://doi.org/10.1007/s00128-020-03099-y>

Luttrell, R. (2018). *Social media: How to engage, share, and connect*. Rowman & Littlefield. <https://books.google.co.in/books?hl=en&lr=&id=P7ecEQAAQBAJ&oi=fnd&pg=PP1>

<https://doi.org/10.1080/01480545.2020.1734607>

<https://doi.org/10.1080/01480545.2020.1734607>

Machoń-Grecka, A., Dobrakowski, M., Kasperczyk, A., Birkner, E., & Kasperczyk, S. (2022). Angiogenesis and lead (Pb): is there a connection?. *Drug and chemical toxicology*, 45(2), 589–593. <https://doi.org/10.1080/01480545.2020.1734607>

Mahanta, R., Chowdhury, J., & Nath, H. K. (2016). Health costs of arsenic contamination of drinking water in Assam, India. *Economic Analysis and Policy*, 49, 30-42. <https://doi.org/10.1016/j.eap.2015.11.013>

Mahdavi, R., Nikniaz, L., Arefhosseini, S. R., & Vahed Jabbari, M. (2010). Determination of aflatoxin M(1) in breast milk samples in Tabriz-Iran. *Maternal and child health journal*, 14(1), 141–145. <https://doi.org/10.1007/s10995-008-0439-9>

Mai F. M. (2006). Beethoven's terminal illness and death. *The journal of the Royal College of Physicians of Edinburgh*, 36(3), 258–263. <https://pubmed.ncbi.nlm.nih.gov/17214130/>

Maity, J., Pal, P., Pal, R., & Mukhopadhyay, P. K. (2024). Co-administration of L-Ascorbic Acid and α -Tocopherol Alleviates Arsenic-Induced Immunotoxicities in the Thymus and Spleen by Dwindling Oxidative Stress-Induced Inflammation. *Biological trace element research*, 202(5), 2199–2227. <https://doi.org/10.1007/s12011-023-03841-7>

Makhetha, M. M. (2021). *The Effects of Childhood Lead Exposure on the Cognitive Abilities of Adolescents and Young Adults: A Systematic Review and Meta-Analysis*. University of Johannesburg (South Africa). <https://ujcontent.uj.ac.za/esploro/outputs/graduate/The-effects-of-childhood-lead-exposure/9911479307691#:~:text=The%20effects%20of%20childhood%20lead%20exposure%20on,adults%20:%20a%20systematic%20review%20and%20meta%20analysis.>

Malakootian M, Golpayegani A. Determination of Pb, Cd, Al, Zn and ca in infant formula and baby foods in Iran and estimation of daily infant intake of these metals. Iran J Nut Sci Food Technol. 2013;8:251–9. <http://nsft.sbmu.ac.ir/article-1-1443-en.html>

Malisch, R., Malisch, K., van Leeuwen, F.R., Moy, G., Tritscher, A., Witt, A., Alvarez, J., (2023). Overview of WHO- and UNEP-Coordinated Human Milk Studies and Their Link to the Stockholm Convention on Persistent Organic Pollutants. In: Malisch, R., Fürst, P., Šebková, K. (eds) Persistent Organic Pollutants in Human Milk. Springer, Cham. https://doi.org/10.1007/978-3-031-34087-1_2

Manduca, P., Al Barauni, N., Al Barauni, L., Abu Abadi, D., Abdallah, H., Hamad, G. A., Mosa, T. A., Balousha, S., Miqdad, H., Mohammed, W., Salah, M., & El Shawwa, R. (2019). Hospital centered surveillance of births in Gaza, Palestine, 2011-2017 and heavy metal contamination of the mothers reveals long-term impact of wars. *Reproductive toxicology* (Elmsford, N.Y.), 86, 23–32. <https://doi.org/10.1016/j.reprotox.2019.02.003>

Marino, P. E., Landrigan, P. J., Graef, J., Nussbaum, A., Bayan, G., Boch, K., & Boch, S. (1990). A case report of lead paint poisoning during renovation of a Victorian farmhouse. *American journal of public health*, 80(10), 1183–1185. <https://doi.org/10.2105/ajph.80.10.1183>

Marriage, G., & Gjerde, M. (2024). Metals: issues with everyday use and toxicity within standard construction metals. In *Sustainability and Toxicity of Building Materials* (pp. 221-246). Woodhead Publishing. https://books.google.co.in/books/about/Sustainability_and_Toxicity_of_Building.html?id=6-XzEAAAQBAJ&redir_esc=y

Martínez-Castillo, M., García-Montalvo, E. A., Arellano-Mendoza, M. G., Sánchez-Peña, L. D. C., Soria Jasso, L. E., Izquierdo-Vega, J. A., Valenzuela, O. L., & Hernández-Zavala, A. (2021). Arsenic exposure and non-carcinogenic health effects. *Human & experimental toxicology*, 40(12_suppl), S826–S850. <https://doi.org/10.1177/09603271211045955>

Medunić, G., Fiket, Ž., & Ivanić, M. (2020). Arsenic contamination status in Europe, Australia, and other parts of the world. *Arsenic in drinking water and food*, 183-233. 10.1007/978-981-13-8587-2_6.

Mehraj, U., Nisar, S., Qayoom, H., & Mir, M. A. (2020). Antigen-antibody interaction. *Immunoglobulins, magic bullets and therapeutic antibodies*, 69. <https://doi.org/10.52305/FGDD2367>

Michaels, Robert. (2019). Legacy Contaminants of Emerging Concern: Lead (Pb), Flint (MI), and Human Health (published online 12 September 2019; in print January 2020). *Environmental Claims Journal*. 32. 6-45. 10.1080/10406026.2019.1661947.

Milton, A. H., Hussain, S., Akter, S., Rahman, M., Mouly, T. A., & Mitchell, K. (2017). A Review of the Effects of Chronic Arsenic Exposure on Adverse Pregnancy Outcomes. *International journal of environmental research and public health*, 14(6), 556. <https://doi.org/10.3390/ijerph14060556>

Ministry of Jal Shakti, Database, 2022, <https://www.pib.gov.in/PressReleasePage.aspx?PRID=1882785>, Accessed on 12 December 2022

Mishra, R.K. (2023) Fresh Water Availability and Its Global Challenge. *British Journal of Multidisciplinary and Advanced Studies*, 4, 1-78. <https://doi.org/10.37745/bjmas.2022.0208>

Mishra, S., Dwivedi, S., Kumar, A., Chauhan, R., Awasthi, S., Mattusch, J., & Tripathi, R. D. (2016). Current status of ground water arsenic contamination in India and recent advancements in removal techniques from drinking water. *International Journal of Plant and Environment*, 2(1 and 2), 01-15. <https://doi.org/10.18811/ijpen.v2i1-2.6613>

Mitra, P., Sharma, S., Purohit, P., & Sharma, P. (2017). Clinical and molecular aspects of lead toxicity: An update. *Critical reviews in clinical laboratory sciences*, 54(7-8), 506–528. <https://doi.org/10.1080/10408363.2017.1408562>

Mohammadi, A. A., Zarei, A., Majidi, S., Ghaderpoury, A., Hashempour, Y., Saghi, M. H., Alinejad, A., Yousefi, M., Hosseingholizadeh, N., & Ghaderpoori, M. (2019). Carcinogenic and non-carcinogenic health risk assessment of heavy metals in drinking water of Khorramabad, Iran. *MethodsX*, 6, 1642–1651. <https://doi.org/10.1016/j.mex.2019.07.017>

Moore M. R. (1977). Lead in drinking water in soft water areas--health hazards. *The Science of the total environment*, 7(2), 109–115. [https://doi.org/10.1016/0048-9697\(77\)90002-x](https://doi.org/10.1016/0048-9697(77)90002-x)

Moradnia, M., Broberg, K., Lozano, M., Chavoshani, A., Salari, M., Attar, H. M., Kelishadi, R., & Darvishmotevalli, M. (2025). Association between urinary heavy metal concentrations, thyroid hormones, and birth outcomes among pregnant women in Isfahan City. *Scientific reports*, 15(1), 24264. <https://doi.org/10.1038/s41598-025-10137-4>

Mousawi, F., Haidar, M., & Al Otaibi, D. (2025). Conventional Serological and Microscopic Techniques for Human Body Fluid Detection. In *Advances in Forensic Biology and Genetics* (pp. 23-45). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-96-4585-5_2

Muller, C., Sampson, R., & Winter, A. (2018). Environmental Inequality: The Social Causes and Consequences of Lead Exposure. *Annual Review of Sociology*, 44(1), 1-20. <http://dx.doi.org/10.1146/annurev-soc-073117-041222> Retrieved from <https://escholarship.org/uc/item/7z15t63g>

Mueller, B. (2017). Arsenic in groundwater in the southern lowlands of Nepal and its mitigation options: A review. *Environmental Reviews*, 25(3), 296-305. <https://doi.org/10.1139/er-2016-0068>

Mukherjee, A., Sengupta, M. K., Hossain, M. A., Ahamed, S., Das, B., Nayak, B., Lodh, D., Rahman, M. M., & Chakraborti, D. (2006). Arsenic contamination in groundwater: a global perspective with emphasis on the Asian scenario. *Journal of health, population, and nutrition*, 24(2), 142–163.

Najari, F. , Abolbagaei, S. M. , Mostafazadeh, B. and Najari, D. (2021). Investigation of autopsy results in cadavers with lead poisoning in Tehran Legal Medicine Center over 10 years. *Journal of Emergency Practice and Trauma*, 7(1), 32-35. doi: 10.34172/jept.2020.26

Naqvi, S. A. R., Idrees, F., Sherazi, T. A., Hassan, S. U., & Ishfaq, N. (2022). TOXICOLOGY ASSOCIATED WITH HEAVY METALS FOUND IN COSMETICS. *Journal of the Chilean Chemical Society*, 67(3), 5615-5622. Retrieved from <https://jcchems.com/index.php/JCCHEMS/article/view/1964>

Narsimha, A., & Rajitha, S. (2018). Spatial distribution and seasonal variation in fluoride enrichment in groundwater and its associated human health risk assessment in Telangana State, South India. *Human and Ecological Risk Assessment: An International Journal*, 24(8), 2119–2132. <https://doi.org/10.1080/10807039.2018.1438176>

National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Health Sciences Policy; Division of Behavioral and Social Sciences and Education; Committee on National Statistics; Division on Earth and Life Studies; Board on Agriculture and Natural Resources; Committee on Developing a Smarter National Surveillance System for Occupational Safety and Health in the 21st Century. A Smarter National Surveillance System for Occupational Safety and Health in the 21st Century. Washington (DC): National Academies Press (US); 2018 Oct 9. 1, Introduction. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK493431/>

Natasha, Dumat, C., Shahid, M., Khalid, S., Murtaza, B. (2020). Lead Pollution and Human Exposure: Forewarned is Forearmed, and the Question Now Becomes How to Respond to the Threat!. In: Gupta, D., Chatterjee, S., Walther, C. (eds) Lead in Plants and the Environment. Radionuclides and Heavy Metals in the Environment. Springer, Cham. https://doi.org/10.1007/978-3-030-21638-2_3

Nayek, U., Shenoy, T. N., & Abdul Salam, A. A. (2024). Data mining of arsenic-based small molecules geometrics present in Cambridge structural

database. *Chemosphere*, 360, 142349.

<https://doi.org/10.1016/j.chemosphere.2024.142349>

Neamtii, I., Bloom, M. S., Gati, G., Goessler, W., Surdu, S., Pop, C., Braeuer, S., Fitzgerald, E. F., Baci, C., Lupsa, I. R., Anastasiu, D., & Gurzau, E. (2015). Pregnant women in Timis County, Romania are exposed primarily to low-level (<10µg/l) arsenic through residential drinking water consumption. *International journal of hygiene and environmental health*, 218(4), 371–379. <https://doi.org/10.1016/j.ijheh.2015.01.004>

Needleman, H.L. (1999) History of Lead Poisoning in the World. In: George, A.M., Ed., Lead Poisoning Prevention and Treatment: Implementing a National Program in Developing Countries, The George Foundation, Bangalore, 17-25. https://www.biologicaldiversity.org/campaigns/get_the_lead_out/pdfs/health/Needleman_1999.pdf

Newton, D. E. (2007). In Forensic Chemistry. Infobase Publishing, New York, NY. https://books.google.com.af/books?id=BDMvLwEACAAJ&source=gbs_book_other_versions_r&cad=1

Nguyet, P. N., Le Luu, T., Le, N. A., Ngan, N. T. K., & Trang, N. T. H. (2025). Groundwater arsenic pollution in Vietnam: Current opinion on the mobilization and remediation. *Current Opinion in Environmental Science & Health*, 100596. doi:10.1016/j.coesh.2025.100596.

Nickson, R., Sengupta, C., Mitra, P., Dave, S. N., Banerjee, A. K., Bhattacharya, A., Basu, S., Kakoti, N., Moorthy, N. S., Wasuja, M., Kumar, M., Mishra, D. S., Ghosh, A., Vaish, D. P., Srivastava, A. K., Tripathi, R. M., Singh, S. N., Prasad, R., Bhattacharya, S., & Deverill, P. (2007). Current knowledge on the distribution of arsenic in groundwater in five states of India. *Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering*, 42(12), 1707–1718. <https://doi.org/10.1080/10934520701564194>

Niemeier, R. (2022). *Examination of Factors Associated with the Dermal Penetration and Absorption of Inorganic Lead (Pb) Compounds for Occupational Risk*

Assessment [Doctoral dissertation, University of Cincinnati]. OhioLINK Electronic Theses and Dissertations Center.
http://rave.ohiolink.edu/etdc/view?acc_num=ucin1649771501283059

NIOSH Manual of Analytical Methods, 2nd. ed., V. 1, P&CAM 139, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-157-A. (1977).
<https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB274845.xhtml>

Nwobi, N. L., Adedapo, S. K., Olukolade, O., Oyinlade, O. A., Lagunju, I. A., Atulomah, N. O., Nwazuoke, I. A., & Anetor, J. I. (2019). Positive and inverse correlation of blood lead level with erythrocyte acetylcholinesterase and intelligence quotient in children: implications for neurotoxicity. *Interdisciplinary toxicology*, 12(3), 136–142. <https://doi.org/10.2478/intox-2019-0016>

Nwobi, N.L., Nwobi, J.C., Ogunbona, R.A., Adetunji, A.O., Anetor, J.I. (2023). Erythrocyte Acetylcholinesterase as a Biomarker of Environmental Lead Exposure. In: Patel, V.B., Preedy, V.R., Rajendram, R. (eds) Biomarkers in Toxicology. Biomarkers in Disease: Methods, Discoveries and Applications. Springer, Cham. https://doi.org/10.1007/978-3-031-07392-2_4

Odongo, A. O. (2019). *Assessment of occupational exposures to lead among artisans working in the informal automobile sector in Nakuru town, Kenya* (Doctoral dissertation, Egerton University). <http://172.16.31.117:4000/handle/123456789/2243>

O'Kennedy, R., & Murphy, C. (Eds.). (2017). Immunoassays: Development, Applications and Future Trends (1st ed.). Jenny Stanford Publishing. <https://doi.org/10.1201/9781315206547>

Olson, K. R., & Chau, K. M. (2022). Natural and Anthropic sources of Arsenic in the Groundwater and Soils of the Mekong Delta. *Open Journal of Soil Science*, 12(11), 541-570. <https://doi.org/10.4236/ojss.2022.1211023>

Ortiz-Garcia, N. Y., Cipriano Ramírez, A. I., Juarez, K., Brand Galindo, J., Briceño, G., & Calderon Martinez, E. (2023). Maternal Exposure to Arsenic and Its Impact on

Maternal and Fetal Health: A Review. *Cureus*, 15(11), e49177. <https://doi.org/10.7759/cureus.49177>

Ouyang, L., Li, Q., Rao, S., Su, R., Zhu, Y., Du, G., Xie, J., Zhou, F., Feng, C., & Fan, G. (2023). Cognitive outcomes caused by low-level lead, cadmium, and mercury mixture exposure at distinct phases of brain development. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 175, 113707. <https://doi.org/10.1016/j.fct.2023.113707>

Owusu-Sekyere, K., & Aladago, D. A. (2023). Material flow analysis and risk evaluation of informal E-waste recycling processes in Ghana: Towards sustainable management strategies. *Journal of Cleaner Production*, 430, 139706. <https://doi.org/10.1016/j.jclepro.2023.139706>

Pal, A., & Chowdhury, D. K., & Ahmed, S. (2024). Murshidabad- The Arsenic Affected District. *International Journal of Innovative Research in Technology (IJIRT)*, 10(11), 680–688. https://ijirt.org/publishedpaper/IJIRT163100_PAPER.pdf

Pala, L., Sirec, T., & Spitz, U. (2020). Modified Enzyme Substrates for the Detection of Bacteria: A Review. *Molecules (Basel, Switzerland)*, 25(16), 3690. <https://doi.org/10.3390/molecules25163690>

Pan, S., Lin, L., Zeng, F., Zhang, J., Dong, G., Yang, B., Jing, Y., Chen, S., Zhang, G., Yu, Z., Sheng, G., & Ma, H. (2018). Effects of lead, cadmium, arsenic, and mercury co-exposure on children's intelligence quotient in an industrialized area of southern China. *Environmental pollution (Barking, Essex : 1987)*, 235, 47–54. <https://doi.org/10.1016/j.envpol.2017.12.044>

Pan, H., Geng, Y., Dong, H., Ali, M., & Xiao, S. (2019). Sustainability evaluation of secondary lead production from spent lead acid batteries recycling. *Resources, Conservation and Recycling*, 140, 13–22. <https://doi.org/10.1016/j.resconrec.2018.09.012>

Parker, G. H., Gillie, C. E., Miller, J. V., Badger, D. E., & Kreider, M. L. (2022). Human health risk assessment of arsenic, cadmium, lead, and mercury ingestion from

baby foods. *Toxicology reports*, 9, 238–249.

<https://doi.org/10.1016/j.toxrep.2022.02.001>

Parvez, F., Akhtar, E., Khan, L., Haq, M. A., Islam, T., Ahmed, D., Eunos, H. M., Hasan, A. R., Ahsan, H., Graziano, J. H., & Raqib, R. (2019). Exposure to low-dose arsenic in early life alters innate immune function in children. *Journal of immunotoxicology*, 16(1), 201–209. <https://doi.org/10.1080/1547691X.2019.1657993>

Parvin, F., Haque, M. M., & Tareq, S. M. (2022). Recent status of water quality in Bangladesh: a systematic review, meta-analysis and health risk assessment. *Environmental Challenges*, 6, 100416. <https://doi.org/10.1016/j.envc.2021.100416>

Paul, N. P., Galván, A. E., Yoshinaga-Sakurai, K., Rosen, B. P., & Yoshinaga, M. (2023). Arsenic in medicine: past, present and future. *Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine*, 36(2), 283–301. <https://doi.org/10.1007/s10534-022-00371-y>

Pearce J. M. (2007). Burton's line in lead poisoning. *European neurology*, 57(2), 118–119. <https://doi.org/10.1159/000098100>

Perez, L.S. and King, B.L. (2022). Role of Non-coding RNA s in Innate Immune Responses Perturbed by Environmental Arsenic Exposure . In *Genomic and Epigenomic Biomarkers of Toxicology and Disease*, S.C. Sahu (Ed.). <https://doi.org/10.1002/9781119807704.ch6>

Perlroth, N. H., & Castelo Branco, C. W. (2017). Current knowledge of environmental exposure in children during the sensitive developmental periods. *Jornal de pediatria*, 93(1), 17–27. <https://doi.org/10.1016/j.jped.2016.07.002>

Apthorpe, L., Hines, J., (2019). Aerosols. In: Reed, S., Pisaniello, D., & Benke, G. (Eds.) *Principles of Occupational Health and Hygiene: An introduction* (3rd ed.). Routledge. <https://doi.org/10.4324/9781003116820>

Pirhadi, M., Alikord, M., Tajdar-Oranj, B., Khaniki, G.J., Nazmara, S., Fathabad, A.E., Ghalhari, M.R., Sadighara, P., 2022. Potential toxic elements (PTEs) concentrati

Pirhadi, M., Alikord, M., Tajdar-Oranj, B., Khaniki, G. J., Nazmara, S., Fathabad, A. E., Ghalhari, M. R., & Sadighara, P. (2022). Potential toxic elements (PTEs) concentration in wheat and flour products in Iran: A probabilistic risk assessment. *Heliyon*, 8(11), e11803. <https://doi.org/10.1016/j.heliyon.2022.e11803>

Prakash, C., Chhikara, S., & Kumar, V. (2022). Mitochondrial Dysfunction in Arsenic-Induced Hepatotoxicity: Pathogenic and Therapeutic Implications. *Biological trace element research*, 200(1), 261–270. <https://doi.org/10.1007/s12011-021-02624-2>

Pretorius, L (2021), Investigating the effect of the heavy metals cadmium, lead and chromium, alone and in combination on the components of the coagulation system, MSc dissertation, University of Pretoria, Pretoria, viewed yymdd <http://hdl.handle.net/2263/83625>

Price, W. J., & Whiteside, P. J. (2017). Atomic absorption. In *Food Analysis* (pp. 387-433). Routledge. <https://www.taylorfrancis.com/chapters/edit/10.1201/9780203755686-9/atomic-absorption-john-price-peter-whiteside?context=ubx>

Pye, V. I., & Patrick, R. (1983). Ground water contamination in the United States. *Science* (New York, N.Y.), 221(4612), 713–718. <https://doi.org/10.1126/science.6879171>

Quansah, R., Armah, F. A., Essumang, D. K., Luginaah, I., Clarke, E., Marfoh, K., Cobbina, S. J., Nketiah-Amponsah, E., Namujju, P. B., Obiri, S., & Dzodzomenyo, M. (2015). Association of arsenic with adverse pregnancy outcomes/infant mortality: a systematic review and meta-analysis. *Environmental health perspectives*, 123(5), 412–421. <https://doi.org/10.1289/ehp.1307894>

Radivojević, M., Roberts, B., Marić, M., Kuzmanović-Cvetković, J., & Rehren, T. (Eds.). (2021). *The rise of metallurgy in Eurasia: Evolution, organisation and consumption of early metal in the Balkans*. Archaeopress Publishing Ltd. <https://doi.org/10.32028/9781803270425>

Rahman, Z., & Singh, V. P. (2019). The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview. *Environmental monitoring and assessment*, 191(7), 419. <https://doi.org/10.1007/s10661-019-7528-7>

Rai, A., Maharjan, M. R., Harris Fry, H. A., Chhetri, P. K., Wasti, P. C., & Saville, N. M. (2019). Consumption of rice, acceptability and sensory qualities of fortified rice amongst consumers of social safety net rice in Nepal. *PloS one*, 14(10), e0222903. <https://doi.org/10.1371/journal.pone.0222903>

Rajamani, S. K., & Iyer, R. S. . (2025). Waste Electrical and Electronic Equipment (WEEE) disposal problem in India, routes of disposal, toxicology, and impact on human health- a narrative review: Waste Electrical and Electronic Equipment (WEEE) disposal problem in India. *MMJ - A Journal by MIMER Medical College, Pune, India*, 7(2). Retrieved from <https://portal.themmj.in/index.php/mmj/article/view/42>

Rajan, M., Karunanidhi, D., Jaya, J., Preethi, B., Subramani, T., & Aravinthasamy, P. (2024). A comprehensive review of human health hazards exposure due to groundwater contamination: A global perspective. *Physics and Chemistry of the Earth, Parts A/B/C*, 103637. <https://doi.org/10.1016/j.pce.2024.103637>

Rasesemola, R. M., Matshoge, G. P., & Ramukumba, T. S. (2019). Compliance to the Integrated School Health Policy: Intersectoral and multisectoral collaboration. *Curationis*, 42(1), e1–e8. <https://doi.org/10.4102/curationis.v42i1.1912>

Rana, M., Shrivastava, P., Srivastava, A., Kushwaha, P., & Kumawat, R. K. (2023). Forensic Serology. In *Textbook of Forensic Science* (pp. 545-579). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-1377-0_18

Ranjan, A. (2019). Spatial analysis of arsenic contamination of groundwater around the world and India. *International Journal of Innovative Studies in Sociology and Humanities* (IJISSH). https://www.academia.edu/53993227/Spatial_analysis_of_arsenic_contamination_of_groundwater_around_the_world_and_India

Ratha, P., Nandalal, K. D. W., Pitawala, H. M. T. G. A., Dharmagunawardhane, H. A., & Weerakoon, S. B. (2017, August). Arsenic contamination in cambodia: a status review. In *Proceedings of the 2nd International Symposium on Conservation and Management of Tropical Lakes, Siem Reap, Cambodia* (pp. 24-26).

Rawat, P. S., Singh, S., Mahdi, A. A., & Mehrotra, S. (2022). Environmental lead exposure and its correlation with intelligence quotient level in children. *Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS)*, 72, 126981. <https://doi.org/10.1016/j.jtemb.2022.126981>

Rebelo, F. M., & Caldas, E. D. (2016). Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants. *Environmental research*, 151, 671–688. <https://doi.org/10.1016/j.envres.2016.08.027>

Reeder, R. J., Schoonen, M. A., & Lanzirotti, A. (2006). Metal speciation and its role in bioaccessibility and bioavailability. *Reviews in Mineralogy and Geochemistry*, 64(1), 59-113. <https://doi.org/10.1515/9781501509421-004>

Rees, N., & Fuller, R. (2020). The toxic truth: children’s exposure to lead pollution undermines a generation of future potential. UNICEF. <https://www.unicef.org/media/73246/file/The-toxic-truth-children%E2%80%99s-exposure-to-lead-pollution-2020.pdf>

Reitsema, R. E., Meire, P., & Schoelynck, J. (2018). The Future of Freshwater Macrophytes in a Changing World: Dissolved Organic Carbon Quantity and Quality and Its Interactions With Macrophytes. *Frontiers in plant science*, 9, 629. <https://doi.org/10.3389/fpls.2018.00629>

Riva, M. A., Sironi, V. A., Fano, D., & Cesana, G. (2011). Workers’ Health Conditions in the Greco-Roman World: The Contribution of Non-Medical Sources. *Archives of Environmental & Occupational Health*, 66(1), 54–55. <https://doi.org/10.1080/19338244.2011.538656>

Rossi E. (2008). Low level environmental lead exposure--a continuing challenge. *The Clinical biochemist. Reviews*, 29(2), 63–70.

- Rupa, S. A., Patwary, M. A. M., Matin, M. M., Ghann, W. E., Uddin, J., & Kazi, M. (2023). Interaction of mercury species with proteins: towards possible mechanism of mercurial toxicology. *Toxicology research*, 12(3), 355–368. <https://doi.org/10.1093/toxres/tfad039>
- Sachdeva, C., Thakur, K., Sharma, A., & Sharma, K. K. (2018). Lead: Tiny but Mighty Poison. *Indian journal of clinical biochemistry : IJCB*, 33(2), 132–146. <https://doi.org/10.1007/s12291-017-0680-3>
- Şahin, B., Topaloğlu, Ö. C., & Hao, S. P. (2022). Management of parathyroid disorders in pregnancy and postpartum period. In *ENT Diseases: Diagnosis and Treatment during Pregnancy and Lactation* (pp. 745-760). Springer International Publishing. https://doi.org/10.1007/978-3-031-05303-0_57
- Sakamoto, M., Kumar, A., Choudhary, D. K., Bishwapriya, A., & Ghosh, A. (2024). Geo-spatial epidemiology of gallbladder cancer in Bihar, India. *The Science of the total environment*, 928, 172460. <https://doi.org/10.1016/j.scitotenv.2024.172460>
- Salmani, M. H., Rezaie, Z., Mozaffari-Khosravi, H., & Ehrampoush, M. H. (2018). Arsenic exposure to breast-fed infants: contaminated breastfeeding in the first month of birth. *Environmental science and pollution research international*, 25(7), 6680–6684. <https://doi.org/10.1007/s11356-017-0985-z>
- Samarghandian, S., Shirazi, F. M., Saeedi, F., Roshanravan, B., Pourbagher-Shahri, A. M., Khorasani, E. Y., Farkhondeh, T., Aaseth, J. O., Abdollahi, M., & Mehrpour, O. (2021). A systematic review of clinical and laboratory findings of lead poisoning: lessons from case reports. *Toxicology and applied pharmacology*, 429, 115681. <https://doi.org/10.1016/j.taap.2021.115681>
- Samiee, F., Vahidinia, A., Taravati Javad, M., & Leili, M. (2019). Exposure to heavy metals released to the environment through breastfeeding: A probabilistic risk estimation. *The Science of the total environment*, 650(Pt 2), 3075–3083. <https://doi.org/10.1016/j.scitotenv.2018.10.059>

- Sarin, M. (1982). *Urban Planning in the Third World: The Chandigarh Experience* (1st ed.). Routledge. <https://doi.org/10.4324/9780429343254>
- Sati, V. P. (2021). *Ganges*. Springer International Publishing. <https://doi.org/10.1007/978-3-030-79117-9>
- Sen, R., Sarkar, S. (2019). Arsenic Contamination of Groundwater in West Bengal: A Report. In: Ghosh, S. (eds) *Waste Management and Resource Efficiency*. Springer, Singapore. https://doi.org/10.1007/978-981-10-7290-1_22
- Shaji, E., Santosh, M., Sarath, K. V., Prakash, P., Deepchand, V., & Divya, B. V. (2021). Arsenic contamination of groundwater: A global synopsis with focus on the Indian Peninsula. *Geoscience frontiers*, 12(3), 101079. <https://doi.org/10.1016/j.gsf.2020.08.015>
- Sharma, A., Parmar, K., Ganju, S. A., Sharma, M., & Rattan, S. (2025). Postmortem Testing for HIV, HBV, and HCV: Insights from Autopsy Findings. *International journal of legal medicine*, 139(6), 3033–3038. <https://doi.org/10.1007/s00414-025-03525-y>
- Shefa, S. T., & Héroux, P. (2017). Both physiology and epidemiology support zero tolerable blood lead levels. *Toxicology letters*, 280, 232–237. <https://doi.org/10.1016/j.toxlet.2017.08.015>
- Shen, M., Zhang, C., Yi, X., Guo, J., Xu, S., Huang, Z., He, M., Chen, X., Luo, D., & Yang, F. (2021). Association of multi-metals exposure with intelligence quotient score of children: A prospective cohort study. *Environment international*, 155, 106692. <https://doi.org/10.1016/j.envint.2021.106692>
- Silbergeld E. K. (2003). Facilitative mechanisms of lead as a carcinogen. *Mutation research*, 533(1-2), 121–133. <https://doi.org/10.1016/j.mrfmmm.2003.07.010>
- Singh, N. (2023). Identifying the true cost of nitrate contamination in the mid-gangetic plain, bihar (india): who's paying for those costs and who's receiving the benefits. "The Deccan Geographer" Journal, 61(3), 1-11. <https://www.scribd.com/document/859883833/CROPREGIONS>

Singh, R., Andaluri, G., & Pandey, V. C. (2022a). Cities' water pollution—Challenges and controls. In *Algae and aquatic Macrophytes in cities* (pp. 3-22). Elsevier. <https://doi.org/10.1016/B978-0-12-824270-4.00015-8>

Singh, N., Kumar, A., Gupta, V. K., & Sharma, B. (2018). Biochemical and Molecular Bases of Lead-Induced Toxicity in Mammalian Systems and Possible Mitigations. *Chemical research in toxicology*, 31(10), 1009–1021. <https://doi.org/10.1021/acs.chemrestox.8b00193>

Singh, C., Shekhar, A., & Singh, R. (2023). Neurotoxic effect of Lead: a review. *Lead Toxicity: Challenges and Solution*, 33-50. <https://www.springerprofessional.de/en/neurotoxic-effect-of-lead-a-review/25901166>

Singh, S., Sharma, P., Mudhulkar, R., Chakravorty, B., Singh, A., & Sharma, S. D. (2022b). Assessment of hydrogeochemistry and arsenic contamination in groundwater of Bahraich District, Uttar Pradesh, India. *Arabian Journal of Geosciences*, 15, 9. <https://doi.org/10.1007/s12517-021-09222-5>

Smith, A. H., Goycolea, M., Haque, R., & Biggs, M. L. (1998). Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. *American journal of epidemiology*, 147(7), 660–669. <https://doi.org/10.1093/oxfordjournals.aje.a009507>

Smith, A. H., Marshall, G., Yuan, Y., Ferreccio, C., Liaw, J., von Ehrenstein, O., Steinmaus, C., Bates, M. N., & Selvin, S. (2006). Increased mortality from lung cancer and bronchiectasis in young adults after exposure to arsenic in utero and in early childhood. *Environmental health perspectives*, 114(8), 1293–1296. <https://doi.org/10.1289/ehp.8832>

Solgi, E., & Jalili, M. (2021). Zoning and human health risk assessment of arsenic and nitrate contamination in groundwater of agricultural areas of the twenty two village with geostatistics (Case study: Chahardoli Plain of Qorveh, Kurdistan Province, Iran). *Agricultural Water Management*, 255, 107023. <https://doi.org/10.1016/j.agwat.2021.107023>

Soni, P., Pandey, R. K., Gupta, S., Singh, A., & Singh, L. (2024). Molecular Basis of Neurodegenerative Changes and Neurotoxicity Linked with Arsenic Exposure: A Review. *Frontiers in Health Informatics*, 13(8) 720-732. <https://healthinformaticsjournal.com/index.php/IJMI/article/view/1676/1743>

Srivastava, S., & Flora, S. J. (2020). Arsenicals: toxicity, their use as chemical warfare agents, and possible remedial measures. In *Handbook of toxicology of chemical warfare agents* (pp. 303-319). Academic Press. <https://doi.org/10.1016/B978-0-12-374484-5.00009-2>

Sun, H. J., Xiang, P., Luo, J., Hong, H., Lin, H., Li, H. B., & Ma, L. Q. (2016). Mechanisms of arsenic disruption on gonadal, adrenal and thyroid endocrine systems in humans: A review. *Environment international*, 95, 61–68. <https://doi.org/10.1016/j.envint.2016.07.020>

Sun, S., Zhuang, P., Li, Z., & Mo, H. (2024). Transmembrane and transcriptome analysis reveals the mechanism of dietary supplements in reducing cadmium absorption and toxicity. *Environmental Technology & Innovation*, 36, 103771. <https://doi.org/10.1016/j.eti.2024.103771>

Szukalska, M., Merritt, T. A., Lorenc, W., Sroczyńska, K., Miechowicz, I., Komorowicz, I., Mazela, J., Barańkiewicz, D., & Florek, E. (2021). Toxic metals in human milk in relation to tobacco smoke exposure. *Environmental research*, 197, 111090. <https://doi.org/10.1016/j.envres.2021.111090>

Taiwo, A. M., Ogunsola, D. O., Babawale, M. K., Isichei, O. T., Olayinka, S. O., Adeoye, I. A., Adekoya, G. A., & Tayo, O. E. (2023). Assessment of Water Quality Index and the Probable Human Health Implications of Consuming Packaged Groundwater from Abeokuta and Sagamu, Southwestern Nigeria. *Sustainability*, 15(4), 3566. <https://doi.org/10.3390/su15043566>

Thornell, I. M. (2014). *Characterization of Phosphatidylinositol 4, 5-Bisphosphate Regulation of the Electrogenic Na/bicarbonate Cotransporter NBCe1*. The University of Alabama at Birmingham. <https://digitalcommons.library.uab.edu/etd-collection/3141>

Tiwari, A. K., Giri, S., Mahato, M. K., & Singh, A. K. (2025). Anthropogenic influence on groundwater metal toxicity and risk to human health assessment in Umaria coalfield of Madhya Pradesh, India. *Scientific reports*, 15(1), 12007. <https://doi.org/10.1038/s41598-025-88783-x>

Tripathi, R. M., & Chung, S. J. (2023). Ultrasensitive and selective colorimetric detection of uric acid using peroxidase mimetic activity of biogenic palladium nanoparticles. *Luminescence : the journal of biological and chemical luminescence*, 38(7), 1330–1338. <https://doi.org/10.1002/bio.4425>

Tsai, T. L., Kuo, C. C., Hsu, L. I., Tsai, S. F., Chiou, H. Y., Chen, C. J., Hsu, K. H., & Wang, S. L. (2021). Association between arsenic exposure, DNA damage, and urological cancers incidence: A long-term follow-up study of residents in an arseniasis endemic area of northeastern Taiwan. *Chemosphere*, 266, 129094. <https://doi.org/10.1016/j.chemosphere.2020.129094>

Tseng, W. P., Chu, H. M., How, S. W., Fong, J. M., Lin, C. S., & Yeh, S. (1968). Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. *Journal of the National Cancer Institute*, 40(3), 453–463. <https://doi.org/10.1093/jnci/40.3.453>

Tyagi, N., Raghuvanshi, R., Upadhyay, M. K., Srivastava, A. K., Suprasanna, P., & Srivastava, S. (2020). Elemental (As, Zn, Fe and Cu) analysis and health risk assessment of rice grains and rice based food products collected from markets from different cities of Gangetic basin, India. *Journal of Food Composition and Analysis*, 93, 103612. <https://doi.org/10.1016/j.jfca.2020.103612>

Udagawa, O., Okamura, K., Suzuki, T., Nohara, K. (2019). Arsenic Exposure and Reproductive Toxicity. In: Yamauchi, H., Sun, G. (eds) *Arsenic Contamination in Asia. Current Topics in Environmental Health and Preventive Medicine*. Springer, Singapore. https://doi.org/10.1007/978-981-13-2565-6_3

US CDC Advisory Committee on Childhood Lead Poisoning Prevention, 2021. CDC Updates Blood Lead Reference Value to 3.5µg/dL. US Centres for Disease Control

and Prevention, Atlanta. <https://www.cdc.gov/nceh/lead/news/cdc-updates-blood-lead-reference-value.html>.

US Department of Health and Human Services. (2007). Toxicological Profile for Lead. Atlanta: Agency for Toxic Substances and Disease Registry. <http://www.atsdr.cdc.gov/toxprofiles/tp13.pdf>.

USEPA, 2006. Environmental Protection Agency, Region 3, Risk-Based Concentration Table: Technical Background Information. United States Environmental Protection Agency, Washington, DC. <https://semspub.epa.gov/work/05/229825.pdf>

U.S. EPA. Child-Specific Exposure Factors Handbook. (2008, Final Report). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-06/096F, 2008. <https://cfpub.epa.gov/ncea/efp/recordisplay.cfm?deid=199243>

USEPA (United States Environmental Protection Agency). 2014. Human Health Evaluation Manual, Supplemental Guidance: Update of Standard Default Exposure Factors-OSWER Directive 9200.1-120. PP.6. https://www.epa.gov/sites/default/files/2015-11/documents/oswer_directive_9200.1-120_exposurefactors_corrected2.pdf

Vahidnia, A., van der Voet, G. B., & de Wolff, F. A. (2007). Arsenic neurotoxicity--a review. *Human & experimental toxicology*, 26(10), 823-832. <https://doi.org/10.1177/0960327107084539>

Valskys, V., Hassan, H. R., Wołkowicz, S., Satkūnas, J., Kibirkštis, G., & Ignatavičius, G. (2022). A Review on Detection Techniques, Health Hazards and Human Health Risk Assessment of Arsenic Pollution in Soil and Groundwater. *Minerals*, 12(10), 1326. <https://doi.org/10.3390/min12101326>

Varney, T. L., Swanston, T., Coulthard, I., Cooper, D. M., George, G. N., Pickering, I. J., & Murphy, A. R. (2012). A preliminary investigation of lead poisoning in a Napoleonic era naval cemetery in Antigua, WI. <https://hdl.handle.net/20.500.14078/2083>

- Vahter M. (2009). Effects of arsenic on maternal and fetal health. *Annual review of nutrition*, 29, 381–399. <https://doi.org/10.1146/annurev-nutr-080508-141102>
- Verma, A. (2018). Forensic Aspect of Metal Poisoning: A Review. *International Journal for Research in Applied Science and Engineering Technology*. <https://doi.org/10.22214/IJRASET.2018.1165>
- Verma, N., Kanojia, N., Kalra, S. and Dua, K. (2023). Chemical Speciation of Chromium and Arsenic and Biogeochemical Cycle in the Aquatic System. In *Hydrogeochemistry of Aquatic Ecosystems* (eds S. Madhav, V.B. Singh, M. Kumar and S. Singh). <https://doi.org/10.1002/9781119870562.ch7>
- Wai, K. M., Mar, O., Kosaka, S., Umemura, M., & Watanabe, C. (2017). Prenatal Heavy Metal Exposure and Adverse Birth Outcomes in Myanmar: A Birth-Cohort Study. *International journal of environmental research and public health*, 14(11), 1339. <https://doi.org/10.3390/ijerph14111339>
- Waldron H. A. (1973). Lead poisoning in the ancient world. *Medical history*, 17(4), 391–399. <https://doi.org/10.1017/s0025727300019013>
- Wang, T., Zhang, J., & Xu, Y. (2020). Epigenetic Basis of Lead-Induced Neurological Disorders. *International journal of environmental research and public health*, 17(13), 4878. <https://doi.org/10.3390/ijerph17134878>
- Wang, X., Ding, N., Harlow, S. D., Randolph, J. F., Jr, Mukherjee, B., Gold, E. B., & Park, S. K. (2023). Exposure to heavy metals and hormone levels in midlife women: The Study of Women's Health Across the Nation (SWAN). *Environmental pollution (Barking, Essex : 1987)*, 317, 120740. <https://doi.org/10.1016/j.envpol.2022.120740>
- Weaver, B., Babich, R., Edwards, G., Fletcher, A., Ruckart, P., Hu, H., ... & Tsai, R. (2024). Prevention of Lead Exposure in Adults. <https://www.cdc.gov/lead-prevention/media/pdfs/2024/12/Draft-Copy-PLEA-Workgroup-Report-to-LEPAC-25NOV2024.pdf>
- Weiss, R. (2005). Study concludes Beethoven died from lead poisoning. *Washington Post*, Dec, 6. <https://www.washingtonpost.com/archive/politics/2005/12/06/study->

[concludes-beethoven-died-from-lead-poisoning/042021b3-d515-4d9b-aadd-e42310e1f466/](https://www.who.int/publications/i/item/9241544600)

WHO (1993) Guidelines for Drinking-Water Quality. Second Edition, Volume 1 Recommendations. World Health Organization, Geneva, 11 p. <https://www.who.int/publications/i/item/9241544600>

WHO, 1995. Inorganic Lead. Geneva. World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 165. <http://www.inchem.org/documents/ehc/ehc/ehc165.htm>.

WHO. (2017). Guidelines for drinking-water quality, 4th edition incorporating the first addendum. Geneva, World Health Organization, pp. 315–318 (<https://apps.who.int/iris/bitstream/handle/10665/254637/9789241549950-eng.pdf>).

World Health Organization, ‘Lead Poisoning and Health’ Fact Sheet, 23 August 2019, (<https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health>) [Accessed on July 24 2020] World Health Organization ‘Childhood Lead Poisoning’ 2010. Geneva, Switzerland. (<https://www.who.int/ceh/publications/leadguidance.pdf>)

World Health Organization. (2015). Lead exposure in African children: contemporary sources and concerns. <https://iris.who.int/bitstream/handle/10665/200168/9780869707876.pdf?sequence=1>

World Health Organization. (2017). Recycling used lead-acid batteries: health considerations. https://iris.who.int/bitstream/handle/10665/259447/9789241512855-eng.pdf?utm_source=miragenews&utm_medium=miragenews&utm_campaign=news

World Health Organization, 2019. International Lead Poisoning Prevention Week 2019 Campaign Resource Package. World Health Organization. No. WHO/CED/PHE/EPE/19.5). <https://iris.who.int/server/api/core/bitstreams/a8cb226b-eca4-40c6-928a-4d76c679ff32/content>

WHO guideline for clinical management of exposure to lead [Internet]. Geneva: World Health Organization; 2021b. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK575284/>

World Medical Association (2025). World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Participants. *JAMA*, 333(1), 71–74. <https://doi.org/10.1001/jama.2024.21972>

Wu, Y., Sun, J., Wang, M., Yu, G., Yu, L., & Wang, C. (2018). The Relationship of Children's Intelligence Quotient and Blood Lead and Zinc Levels: a Meta-analysis and System Review. *Biological trace element research*, 182(2), 185–195. <https://doi.org/10.1007/s12011-017-1093-0>

Xiang, Y., Hou, J., Ren, L., Xiong, J., Wan, B., Wang, M., ... & Kappler, A. (2025). Spontaneous Abiotic Reduction of Arsenate to Arsenite Mediated by Structural Fe (II) Resulting from Abundant Oxygen Vacancy Clusters in Poorly Crystalline Ferrihydrite in Drought Environments. *Environmental Science & Technology* 59 (10), 5191-5201. DOI: 10.1021/acs.est.4c10674

Yadav, P. K., & Sharma, S. (2024). Application of forensic techniques to blood analysis. In *Advancements in Body Fluid Analysis in Forensics: Current and Emerging Methods* (pp. 17-90). Cham: Springer Nature Switzerland. <https://doi.org/10.1007/978-3-031-65804-4>

Yeung, V., Miller, D.D., Rutzke, M.A. (2017). Atomic Absorption Spectroscopy, Atomic Emission Spectroscopy, and Inductively Coupled Plasma-Mass Spectrometry. In: Nielsen, S.S. (eds) *Food Analysis*. Food Science Text Series. Springer, Cham. https://doi.org/10.1007/978-3-319-45776-5_9

Yu, H., Ma, T., Du, Y., Shen, S., & Han, Z. (2022). Distribution, bioavailability, and human health risk assessment of arsenic in groundwater-soil-rice system in the Jiangnan Plain, Central China. *Environmental science and pollution research international*, 29(11), 16193–16202. <https://doi.org/10.1007/s11356-021-16497-4>

Zhang, L., Gao, Y., Wu, S., Zhang, S., Smith, K. R., Yao, X., & Gao, H. (2020). Global impact of atmospheric arsenic on health risk: 2005 to 2015. *Proceedings of the National Academy of Sciences of the United States of America*, 117(25), 13975–13982. <https://doi.org/10.1073/pnas.2002580117>

- Zhang, L., Ou, C., Magana-Arachchi, D., Vithanage, M., Vanka, K. S., Palanisami, T., Masakorala, K., Wijesekara, H., Yan, Y., Bolan, N., & Kirkham, M. B. (2021). Indoor Particulate Matter in Urban Households: Sources, Pathways, Characteristics, Health Effects, and Exposure Mitigation. *International journal of environmental research and public health*, 18(21), 11055. <https://doi.org/10.3390/ijerph182111055>
- Zhang, C., Xu, L., Zhao, Y., & Wang, Y. (2022). Changes in serum heavy metals in polycystic ovary syndrome and their association with endocrine, lipid-metabolism, inflammatory characteristics and pregnancy outcomes. *Reproductive toxicology (Elmsford, N.Y.)*, 111, 20–26. <https://doi.org/10.1016/j.reprotox.2022.05.002>
- Zhang, W., Cui, Y., & Liu, J. (2023). The association between blood heavy metals level and sex hormones among postmenopausal women in the US. *Frontiers in endocrinology*, 14, 1175011. <https://doi.org/10.3389/fendo.2023.1175011>
- Zhang, Y., Li, Q., & Zhang, B. (2026). Application of ELISA in Cultural Heritage: Recent Advances and Challenges. *Critical reviews in analytical chemistry*, 56(1), 26–36. <https://doi.org/10.1080/10408347.2024.2379853>
- Zhou, X., Feng, Y., & Gong, Z. (2022). Associations between lead, cadmium, mercury, and arsenic exposure and alanine aminotransferase elevation in the general adult population: an exposure-response analysis. *Environmental science and pollution research international*, 29(35), 53633–53641. <https://doi.org/10.1007/s11356-022-19698-7>
- Zubair, M., & Martyniuk, C. J. (2019). A review on hemato-biochemical, accumulation and patho-morphological responses of arsenic toxicity in ruminants. *Toxin Reviews*, 38(3), 176–186. <https://doi.org/10.1080/15569543.2018.1442347>

Appendices

Permission Letter from MCSRC, Patna

Ref. No MCS/RC/2022/163 Dated: 30/08/2022


MAHAVIR
CANCER SANSTHAN
Run by Mahavir Mandir Trust
Phulwari Sharif,
Patna - 801 505 (INDIA)
Phones : (0612) 2250127, 2253956
Fax : (0612) 2253957
email : mcspatna98@gmail.com
website : www.mahavircancersansthan.com

No-Objection Certificate

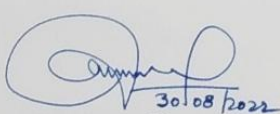
To
Lovely Professional University,
Phagwara, Punjab

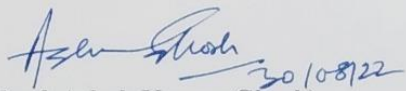
Sub: Regarding No-Objection Certificate

Dear Sir/Madam,

We have no objection if Ms. Radhika Agarwal (Provisional Reg. No. 12208293), who is Junior Research Fellow in Indian Council of Medical Research (ICMR) Project entitled "Assessment of Female Reproductive Health And Child Health Risks In Arsenic Exposed Population Of Bihar" in our organization Mahavir Cancer Sansthan and Research Centre, Patna, Bihar since March 2022 is admitted to the Ph.D programme in Forensic Science at your Institute - Lovely Professional University as a REGULAR student.

It is certified that, we shall grant her leave of absence to attend classes/research works at the Lovely Professional University during her Ph.D programme.


30/08/2022
(Dr. Arun Kumar)
PI, ICMR Project
Research Centre
Mahavir Cancer Sansthan


30/08/2022
(Prof. Ashok Kumar Ghosh)
HoD, Research Centre &
Co-P.I ICMR Project
Mahavir Cancer Sansthan



Ethics Approval Letter



ETHICS COMMITTEE



ICMR- Rajendra Memorial Research Institute of Medical Sciences
Agamkuan, Patna 800 007

DCGI Registration No. ECR/480/Inst/RR/2014/RR-20
NABH Accreditation Certificate No. - EC- CT- 2018- 0059
Provisionally- Registered by DHR (File No.EC/NEW/INST/2020/906)

Letter No. RMR/EC/24/2020 Date 26-09-2020

Venue: RMRIMS, Patna Date of the Meeting: 25th Sep 2020 Time: 11:30 to 13:20

Protocol Title: Assessment of Female reproductive health and child health risks in Arsenic Exposed population of Bihar.

Principal Investigator : Dr. Arun Kumar (MCS,Patna)

Co Investigator : Prof. Ashok Kumar Ghosh (MCS,Patna)

Documents Reviewed

S. No	Title
1.	Study Protocol
2.	Informed Consent English, Version 1.0
3.	Informed Consent Hindi, Version 1.0
4.	Curriculum Vitae of Principal Investigator
5.	Field survey form cum Questionnaire

Due to COVID-19 pandemic, meeting of the Ethics Committee was held on 25th Sep 2020 through video conferencing using Zoom App. The opinion of the members participating in video conferencing was as follows:-

Sr. No.	Name of EC member	EC Designation	Opinion
1	Dr. Sanjay Kumar	Chairman	Agree
2	Dr. Vidya Nand Rabi Das	Member Secretary	Agree
3	Dr. Ajay Kumar Sinha	Clinician	Agree
4	Dr. Satish Kumar	Clinician	Agree
5	Dr. Md Anwar Hussain	Basic Medical Scientist	Agree

Approved by
Ethics Committee,
ICMR-RMRIMS
Agamkuan, Patna

Tel: +91-612-2631565 | Fax: +91-612-2634379 | www.rmrim.org.in | rmiec2014@gmail.com

6	Dr. Asha Singh	Basic Medical Scientist	Agree
7	Dr. Dipendra Bhushan	Social Scientist	Agree
8	Mr. Manoranjan	Legal Expert	Agree
9	Mr. Abhisek Kumar	Legal Expert	Agree
10	Dr. Pravin kumar	Lay Person	Agree
11	Dr. Pranay Kumar Gupta	Lay Person	Agree

The Ethics Committee **approved** to undertake this study as per the study procedures and concerned PI supposed to initiate their study after approval only.

The EC Committee expects to be informed about the progress of the study, any changes in the protocol and asks to be provided a copy of the final report at the end of study.

It is hereby certify that this committee is organised and operates according to ICH GCP guidelines and Indian regulatory requirements; that at the time of the voting the required quorum was present; and any member of the committee in case involved in the study was present only during the presentation and discussion but absent at the time of voting.



EC Chairman's Signature

26/SEP/ 2020

DD/MMM / YYYY

Chairman
Ethics Committee,
ICMR-RMRIMS,
Agamkuan, Patna-7

Approved by
Ethics Committee,
ICMR-RMRIMS
Agamkuan, Patna

Questionnaire



Mahavir Cancer Sansthan & Research Centre Phulwarisharif, Patna

Project: Assessment of Female reproductive health and child health risks in Arsenic Exposed population of Bihar

Village:-

Name:

Age:

Sex:

House hold Name:

Mobile No.

Members in House hold:

Female Member Details|

Children Details:

GPS Location:

Source of Drinking Water:

Depth of HP:

Age H.P

Disease History:

Cancer Patients in Family History:

Water Sample Collection Code:

Food Sample Collection Code:

Blood Sample Collection Code:

Urine Sample Collection Code:

Breast milk Sample Collection Code:

Hair / Nail Sample Collection Code:

Child: Saliva collection Code:

Child: Urine collection Code:

Data Collected by:

(PI Signature)



FEMALE - Clinical Details

1. Appearance:

Normal ☐ Marks of ~~Arsenicosis~~ ☐ Weight ☐ Age:

2. Any Skin Lesion

Palm ☐ Sole ☐ Trunk ☐ Limbs ☐

Chest ☐ Back ☐ Any Others ☐

3. Any Nodular Change

Area ☐

4. Any other Problem

Gastritis ☐ Anaemia ☐ Constipation ☐ Diarrhoea ☐ Loss of Appetite ☐

5. Any Systemic Disease

Heart ☐ Lungs ☐ Abdomen ☐ Diabetes ☐

BP ☐ Others ☐ _____

6. On Any Medication

Duration ☐

7. For how much time they are using a water:

8. Menstrual status:

9. Any other Information:

Data Collected by:

(PI Signature)



CHILD - Clinical Details

1. Appearance:

Normal ☐

Marks of Arsenicosis ☐

Age:

Gender: M / F

Weight ☐

2. Any Skin Lesion

Palm ☐

Sole ☐

Trunk ☐

Limbs ☐

Chest ☐

Back ☐

Any Others ☐

3. Any Nodular Change

Area ☐

4. Any other Problem

Gastritis ☐

Anaemia ☐

Constipation ☐

Diarrhoea ☐

Loss of Appetite ☐

5. Any Systemic Disease

Heart ☐

Lungs ☐

Abdomen ☐

Diabetes ☐

BP ☐

Others ☐

6. On Any Medication

Duration

7. Child Details (Neurological or other problems) :

8. Any Development Issue:

Vision ☐

Hearing ☐

Speaking ☐

Movement ☐

Other ☐

Consent form

सूचित सहमति

परियोजना का शीर्षक: बिहार की आर्सेनिक एक्सपोज्ड जनसंख्या में महिला प्रजनन स्वास्थ्य और बाल स्वास्थ्य जोखिमों का आकलन

(Assessment of Female reproductive health and child health risks in Arsenic Exposed population of Bihar)

हाल के दिनों में, आर्सेनिक के संपर्क में आने वाले बच्चों में स्वास्थ्य संबंधी गंभीर समस्याएँ, विशेषकर मानसिक विकलांगता के मामले सामने आए हैं। कई अध्ययनों से यह स्पष्ट है कि आर्सेनिक के संपर्क में आने से जनसंख्या में मानसिक स्वास्थ्य जोखिम हो सकता है लेकिन बच्चों पर इसका प्रभाव केवल रिपोर्ट किया गया है, यह महिला आबादी के स्वास्थ्य की स्थिति और मानसिक स्वास्थ्य के मूल्यांकन के लिए बहुत आवश्यक हो जाता है बिहार के आर्सेनिक के संपर्क वाले क्षेत्र में रहने वाले बच्चों की स्थिति।

दूसरे, बिहार राज्य में आज तक ऐसा कोई अध्ययन नहीं किया गया है, जिसमें महिलाओं में आर्सेनिक के संपर्क का आकलन किया गया हो, जिससे आर्सेनिक दूषित स्तन दूध का साव हुआ हो और जब यह दूध शिशुओं द्वारा लिया गया हो तो उन पर क्या प्रभाव पड़ता है। यह अध्ययन स्तन दूध के माध्यम से आर्सेनिक जोखिम दर को जानने के लिए एक आख खोलने वाला होगा। इसके अलावा, सीसा या पारा जैसी अन्य भारी धातुएं विषाक्तता को बढ़ा रही हैं, वर्तमान अध्ययन पर भी प्रकाश डाला जाएगा।

बिहार में आर्सेनिक से पीड़ित बच्चों में मानसिक विकलांगता के मामलों की घटनाओं में कई गुना वृद्धि हुई है। यह बहुत संभव है कि शिशुओं या बच्चों को उनकी माँ के दूध के माध्यम से आर्सेनिक के संपर्क में और अधिक वृद्धि हो सकती है, जिसके कारण उनमें विक्षिप्तता पैदा होती है जिससे वे मानसिक स्वास्थ्य को बाधित करते हैं।

अनुसंधान के उद्देश्य और तरीके :

- 1) भारी धातुओं का आकलन - रक्त में आर्सेनिक, सीसा और पारा, प्रभाव का परिक्षण, महिला विषयों में रक्त, बाल, नाखून और स्तन के दूध में सीसा और पारा।
- 2) भारी धातुओं का आकलन - शिशुओं या बच्चों में आर्सेनिक, सीसा और पारा उनके बाल या नाखून के नमूने में 5 साल से कम है।
- 3) हार्मोन का आकलन - महिला विषयों में एस्ट्रोजन, प्रोजेस्टेरोन, एफएसएच, एलएच, प्रोलैक्टिन, ऑक्सीटोसिन और थायराइड हार्मोन का स्तर।
- 4) बच्चों में न्यूरोटॉक्सिसिटी का आकलन - एसिटाइलकोलिनैस्टरेज गतिविधि और डोपामाइन हार्मोन का स्तर।
- 5) बच्चों और महिलाओं के स्वास्थ्य में न्यूरोटॉक्सिसिटी के लिए नैदानिक और चिकित्सीय मार्करों की पहचान करने के लिए जीनोमिक अध्ययन।
- 6) आर्क-जीआईएस मैपिंग सिस्टम का उपयोग करके प्रस्तावित अध्ययन क्षेत्र की जियो मैपिंग।

अध्ययन के लाभ :

यह देश के इस हिस्से में किया गया पहला अध्ययन होगा जहाँ आर्सेनिक के खतरे के कारण आबादी में गंभीर स्वास्थ्य खतरे पैदा हुए हैं। गंगा का मैदानी क्षेत्र अन्य क्षेत्र की तुलना में अधिक प्रभावित है। इस अध्ययन के माध्यम से, पहली बार बाल मानसिक स्वास्थ्य जोखिमों के साथ-साथ महिलाओं के प्रजनन स्वास्थ्य का मूल्यांकन किया जाएगा। स्तन के दूध में मौजूद लेड या मरकरी के साथ आर्सेनिक जैसी भारी धातुएँ इस पूरे क्षेत्र में किए गए नया अध्ययन होगा। उत्पन्न पूरा डेटा आख खोलने वाला होगा और उपयुक्त रणनीतिक नियंत्रण उपायों के माध्यम से वर्तमान समस्या का मुकाबला करने का मार्ग प्रशस्त करेगा।

रक्त नमूना संग्रह:

- मरीज़ की बांह में सुई (माँ और बच्चे) लगाकर लगभग 5ML रक्त खींचा जाएगा। यह परीक्षण के लिए रक्त प्राप्त करने के लिए उपयोग की जाने वाली मानक विधि है।
- प्रशिक्षित कर्मी खून निकालेंगे। रक्त खींचने के लिए उपयोग की जाने वाली आपूर्ति सुरक्षित और निष्फल होगी और इसका उपयोग केवल एक बार किया जाएगा और उपयोग के बाद आपूर्ति नष्ट हो जाएगी। रोगी द्वारा जो रक्त दिया जाएगा उसका उपयोग केवल अनुसंधान के उद्देश्य से किया जाएगा।
- तैयार किए गए रक्त को अछूता वाहक बक्से में संग्रहीत किया जाएगा। एकत्र रक्त को संसाधित और फ्रीजर (-20°C / -80°C) में विश्लेषण और दीर्घकालिक भंडारण तक संग्रहीत किया जाएगा।

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- भारी धातुओं के अध्ययन के लिए बाल और नाखून के नमूने भी एकत्र किए जाएंगे।
 - भारी धातु के एनालिसिस के लिए स्तन का दूध (5 ML) एकत्र किया जाएगा।
 - हम रक्त भारी धातु विश्लेषण, जैव रासायनिक परीक्षण, हार्मोनल परीक्षण और विभिन्न जीन म्यूटेशन अध्ययनों के लिए रक्त का परीक्षण करेंगे। नैदानिक मूल्यांकन (जैसा कि उपचार ऑन्कोलॉजिस्ट द्वारा सलाह दी गई है)
- जोखिम :** इस अध्ययन से मरीज़ कोई को नुकसान या बहुत असुविधा नहीं होगी। हालांकि, जब रक्त एक रक्त परीक्षण के लिए लिया जाता है, तो चोट लगने की संभावना है, सुई पंचर से असुविधा होती है, और कुछ बहुत ही दुर्लभ मामलों में संक्रमण होता है। कोई अन्य प्रत्याशित प्रमुख नैदानिक दुष्प्रभाव नहीं हैं। यदि पंचर साइट पर स्थानीय संक्रमण का सामना करना पड़ता है, तो एक बहुत ही दुर्लभ दुष्प्रभाव, एंटीसेप्टिक के आवेदन के साथ स्थानीय साइट की पर्याप्त सफाई की जाएगी और बाद में चिकित्सक द्वारा समीक्षा की जाएगी।
- लाभ :** इस अध्ययन में भाग लेने से, रोगी को कोई प्रत्यक्ष लाभ नहीं होगा लेकिन इस अध्ययन से निश्चित रूप से शोधकर्ताओं को आर्सेनिक की स्पष्ट समझ हासिल करने में मदद मिलेगी और यह महिलाओं और बाल स्वास्थ्य पर प्रभाव डालता है। भागीदारी से आर्सेनिक के संपर्क में आने वाली आबादी में आर्सेनिक और बीमारी के बोझ के बीच संबंध जानने में मदद मिलेगी।
- वित्तीय लागत:** इस अध्ययन में भाग लेने के परिणामस्वरूप रोगी को कोई भी लागत नहीं लगेगी। अध्ययन में भाग लेने के लिए रोगी को क्षतिपूर्ति नहीं दी जाएगी क्योंकि अस्पताल में आपकी नियमित यात्राओं के दौरान अध्ययन की योजना बनाई गई है।
- अभिलेखों की गोपनीयता:** विवरण के बारे में जानकारी दी जाएगी, जिसे गुप्त रखा जाएगा और केवल अध्ययन कर्मचारियों के पास डेटा तक पहुंच होगी। डेटा को विभिन्न साइटों पर और महावीर कैंसर संस्थान में पासवर्ड संरक्षित कंप्यूटरों पर संग्रहीत किया जाएगा।
- भाग लेने और अध्ययन से हटने के लिए व्यक्ति की स्वतंत्रता:** रोगी किसी भी समय अध्ययन को छोड़ने का विकल्प चुन सकता है और आपके डेटा का उपयोग नहीं किया जाएगा

सहमति

मैं स्वेच्छा से उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूँ और आपको कोई प्रतिपूर्ति नहीं दी जाती है।

विषय के हस्ताक्षर (या अंगूठे का निशान)

दिनांक: ____/____/____

हस्ताक्षरकर्ता का नाम: _____

प्रतिपक्ष गवाह / कानूनी रूप से स्वीकार्य प्रतिनिधि हस्ताक्षर _____

दिनांक: ____/____/____

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दिनांक: ____/____/____

प्राधिकरण का नाम: _____

संपर्क जानकारी

यदि आपके पास इस अध्ययन के बारे में किसी भी समय प्रश्न हैं, तो आप उस शोधकर्ता से संपर्क कर सकते हैं जिसकी संपर्क जानकारी नीचे दी गई है।

डॉ० अरुण कुमार (P.I)

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Version.1.0

List of Publications

1. Agarwal, R., Kumar, A., Pandey, T., Chayal, N. K., Verma, K. S., Kumar, D., Kumar, K., Aryal, S., Singh, P., Ali, M., Srivastava, A., Singh, M., & Ghosh, A. K. (2024). High lead contamination in Mother's breastmilk in Bihar (India): Health risk assessment of the feeding children. *Chemosphere*, 364, 143064. <https://doi.org/10.1016/j.chemosphere.2024.143064>
2. Agarwal, R., Kumar, A., Pandey, T., Verma, D., Kumar, K., Chayal, N. K., Kumar, P., Kumar, N., Ali, M., Srivastava, A., Verma, K. S., Kumar, D., Sharma, A., & Ghosh, A. K. (2025). Environmental Arsenic Contamination and Human Health Risk Assessment in the Kosi Plain Region of Bihar, India. *Biological trace element research*, 10.1007/s12011-025-04886-6. Advance online publication. <https://doi.org/10.1007/s12011-025-04886-6>
3. Agarwal, R., Pandey, T., Verma, D., & Kumar, A. (2026). Forensic significance of ELISA technique for hormone assessment: a comprehensive review. *Journal of immunoassay & immunochemistry*, 47(1), 1–17. <https://doi.org/10.1080/15321819.2025.2603249>
4. Kumar, A., Agarwal, R., Kumar, K., Chayal, N. K., Ali, M., Srivastava, A., Kumar, M., Niraj, P. K., Aryal, S., Kumar, D., Bishwapriya, A., Singh, S., Pandey, T., Verma, K. S., Kumar, S., Singh, M., & Ghosh, A. K. (2024). High arsenic contamination in the breast milk of mothers inhabiting the Gangetic plains of Bihar: a major health risk to infants. *Environmental health : a global access science source*, 23(1), 77. <https://doi.org/10.1186/s12940-024-01115-w>
5. Kumar, A., Agarwal, R., Kumar, K., Chayal, N. K., Kumar, G., Kumar, R., Ali, M., Srivastava, A., Aryal, S., Pandey, T., Verma, K. S., Kumar, D., Gajbhiye, R. L., Dhingra, S., Pothuraju, N., Peraman, R., Bishwapriya, A., Nandan, R., Sharma, A., Singh, M., ... Ghosh, A. K. (2025). Mercury poisoning in women and infants inhabiting the Gangetic plains of Bihar: risk assessment. *BMC public health*, 25(1), 1275. <https://doi.org/10.1186/s12889-025-22336-9>
6. Chayal, N. K., Kumar, A., Verma, K. S., Kumar, D., Agarwal, R., Kumar, K., Pandey, T., Ali, M., Srivastava, A., & Ghosh, A. K. (2025). Lead exposure in

- Bihar, Eastern India: a risk assessment study. *Environmental geochemistry and health*, 47(9), 368. <https://doi.org/10.1007/s10653-025-02687-3>
7. Chayal, N. K., Kumar, A., Verma, K. S., Agarwal, R., Kumar, K., Kumar, S., Kumar, D., Pandey, T., Ali, M., Srivastava, A., Bishwapriya, A., Sharma, A., Singh, M., & Ghosh, A. K. (2025). Arsenic Poisoning in Bihar: A Comparative Health Risk Assessment Through Water and Food Sources. *Biological trace element research*, 10.1007/s12011-025-04824-6. Advance online publication. <https://doi.org/10.1007/s12011-025-04824-6>
 8. Kumar, A., Agarwal, R., Kumar, K., Kumar, G., Kumar, S., Sharma, M., Pandey, T., Ali, M., Srivastava, A., Kumar, R., Kumar, D., Gajbhiye, R. L., Murti, K., Dhingra, S., Pothuraju, N., Peraman, R., Bishwapriya, A., Sharma, A., Khandelwal, C., Singh, M., ... Ghosh, A. K. (2025). Discovery of uranium content in breastmilk and assessment of associated health risks for mothers and infants in Bihar, India. *Scientific reports*, 15(1), 41389. <https://doi.org/10.1038/s41598-025-25307-7>
 9. Verma, D., Kumar, A., Pandey, T. *et al.* High Cadmium Contamination in the Biological Samples of the Exposed Subjects in the Kosi Plain Region of Bihar. *Expo Health* **18**, 6 (2026). <https://doi.org/10.1007/s12403-025-00744-x>



High lead contamination in Mother's breastmilk in Bihar (India): Health risk assessment of the feeding children

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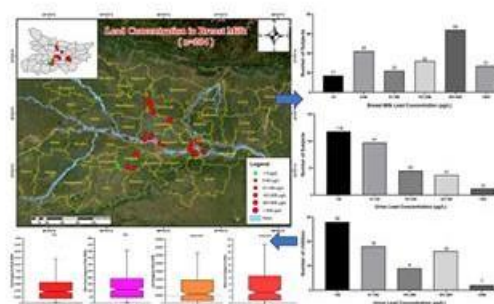
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HIGHLIGHTS

- Study of lead poisoning in breastmilk of women inhabiting in Bihar (India).
- Biological & food samples collected from households in 6 districts of state.
- High lead concentration in 327 biological samples and food samples.
- Study indicates lead exposure through food reached mother's breastmilk & to their infant's body.
- The HQ and CR are significantly high in mother's followed by their infants.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Jian-Ying Hu

Keywords:

Lead poisoning
Breastmilk lead exposure
Health assessment
Food lead contamination
Hazard quotient (HQ)
Children

ABSTRACT

Lead poisoning in the recent times has caused serious health threats in the exposed human population. It is estimated that about 815 million people are exposed to lead poisoning worldwide and in India total 275 million children are exposed to blood lead contamination. The present study was carried out in 6 districts of Bihar to know the extent of lead exposure in the children through their mother's breastmilk. The biological samples such as breastmilk, mother's urine, child's urine, and mother's blood samples were collected for quantitative lead estimation. Moreover, the selected household water sources (handpump) and the food consumed by the individuals-wheat, rice and potato samples were also collected for lead quantification. The study reveals that the breastmilk had high lead content in 92% of the samples (highest value 1309 µg/L), in blood presence of lead was

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<https://doi.org/10.1016/j.chemosphere.2024.143064>

Received 17 May 2024; Received in revised form 23 July 2024; Accepted 8 August 2024

Available online 12 August 2024

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Environmental Arsenic Contamination and Human Health Risk Assessment in the Kosi Plain Region of Bihar, India

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Received: 26 August 2025 / Accepted: 20 October 2025

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Abstract

Groundwater arsenic poisoning in the recent times has caused serious health hazards to the exposed population of Bihar. The arsenic causing disease burden in the Gangetic plains of Bihar has been well documented. But the present study aims to find out the arsenic exposure in the Kosi River plain region of Bihar for the first time. This study investigates arsenic exposure through breastfeeding among children in Bihar's Kosi region -Madhepura, Saharsa, Supaul, Katihar districts while Bhagalpur and Nalanda in the Gangetic plain region where groundwater arsenic poisoning poses severe health risks. From a cohort of $n=312$ lactating women, breast milk, blood, and urine samples, along with urine samples from $n=101$ breastfed infants, were analyzed. Results show 25.3% of breast milk samples exceeded the WHO limit of $0.64 \mu\text{g/L}$, with elevated levels also in maternal blood and urine samples. Infant's urine indicated 2% above the permissible limit ($<50 \mu\text{g/L}$). The multivariate study reveals a moderate to strong correlation between water arsenic levels and arsenic accumulation in rice. As water arsenic contamination increases, rice arsenic content also increases, indicating a significant uptake from irrigation. This underscores the role of rice as a major dietary source of arsenic in the studied area. The Monte Carlo simulation study shows the exceeding carcinogenic risk assessment in the rice samples as 79.94% in comparison to the breastmilk samples 1.73%. In conclusion, additional research in the Kosi region is imperative to thoroughly tackle this health challenge, with far-reaching implications for the well-being of future generations.

Keywords Arsenic exposure · Breastmilk · Child exposure · Health risk

Introduction

Arsenic, a naturally occurring element found in the Earth's crust, often contaminates groundwater through natural geological processes [1]. In some areas, human activities like industrial and agricultural practices worsen the problem [2]. In India, arsenic in drinking water has become a serious health challenge, particularly in Bihar's Kosi plains [3]. This region's unique geological conditions have made it especially vulnerable to arsenic leaching into the groundwater, where millions of rural families rely on for drinking, cooking, and farming [4]. The Kosi river basin, which includes the districts of Madhepura, Saharsa & Supaul is known for its fertile soil and agricultural productivity and Katihar and Bhagalpur are also situated near Kosi-Gangetic plain region. Unfortunately, it has also gained notoriety for dangerously high arsenic levels in its groundwater. Over the years, the problem has only intensified [5]. Long-term

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Forensic significance of ELISA technique for hormone assessment: a comprehensive review

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ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) is a rapid, sensitive, and economical tool for detecting hormones in diverse biological matrices, making it valuable in forensic endocrinology. This review summarizes ELISA's principles, applications, advantages, and limitations in medico-legal practice. ELISA enables quantification of hormones such as cortisol, testosterone, estrogen, melatonin, thyroid hormones, progesterone, and human chorionic gonadotropin (hCG). It supports postmortem toxicology, sexual assault investigations, doping control, and forensic psychiatry. Cortisol may help reconstruct perimortem stress, while hCG detection assists in pregnancy confirmation in assault or maternal death cases. In sports, ELISA screens for anabolic steroids, erythropoietin, and growth hormone, with LC-MS/MS required for confirmation. Its compatibility with blood, saliva, urine, and hair enhances versatility. Key challenges include antibody cross-reactivity, matrix interference, degradation, variability among commercial kits, and limited multiplexing. False positives, hook effects, and inconsistent validation affect admissibility, necessitating strict quality assurance, ISO/IEC 17025 compliance, and confirmatory testing. ELISA is unsuitable for paternity determination; DNA profiling remains the legal standard. Emerging advances – digital ELISA, nanotechnology, AI, and biosensors – promise greater sensitivity and automation but face regulatory, cost, and training barriers. ELISA should be regarded as a complementary, high-throughput screening method integrated into validated, multimodal forensic workflows.

KEYWORDS

Forensic endocrinology;
ELISA; hormone biomarkers;
Post-mortem analysis;
doping control

RESEARCH

Open Access



High arsenic contamination in the breast milk of mothers inhabiting the Gangetic plains of Bihar: a major health risk to infants

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Abstract

Groundwater arsenic poisoning has posed serious health hazards in the exposed population. The objective of the study is to evaluate the arsenic ingestion from breastmilk among pediatric population in Bihar. In the present study, the total women selected were $n=513$. Out of which $n=378$ women after consent provided their breastmilk for the study, $n=58$ subjects were non-lactating but had some type of disease in them and $n=77$ subjects denied for the breastmilk sample. Hence, they were selected for the women health study. In addition, urine samples from $n=184$ infants' urine were collected for human arsenic exposure study. The study reveals that the arsenic content in the exposed women (in 55%) was significantly high in the breast milk against the WHO permissible limit $0.64 \mu\text{g/L}$ followed by their urine and blood samples as biological marker. Moreover, the child's urine also had arsenic content greater than the permissible limit ($< 50 \mu\text{g/L}$) in 67% of the studied children from the arsenic exposed regions. Concerningly, the rate at which arsenic is eliminated from an infant's body via urine in real time was only 50%. This arsenic exposure to young infants has caused potential risks and future health implications. Moreover, the arsenic content was also very high in the analyzed staple food samples such as rice, wheat and potato which is the major cause for arsenic contamination in breastmilk. The study advocates for prompt action to address the issue and implement stringent legislative measures in order to mitigate and eradicate this pressing problem that has implications for future generations.

Keywords Groundwater arsenic contamination, Lactating women, Arsenic contamination in breast milk, Infant risk assessment, Gangetic plains, Cancer risk assessment

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RESEARCH

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Mercury poisoning in women and infants inhabiting the Gangetic plains of Bihar: risk assessment

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Abstract

Mercury is a persistent, bio-accumulative, and hazardous contaminant. When released into the environment, it accumulates in water sediments, converting it to poisonous methylmercury that enters the food chain. The present study was carried out in habitations from the 11 districts of Bihar (India). In the study, $n = 224$ lactating women and their infants $n = 172$ infants participated. After obtaining the written informed consent, their breast milk, urine, and blood were collected for mercury estimation. The breastmilk content was measured in $n = 181$ subjects, in which 74% women had their breastmilk higher than the WHO permissible limit ($< 1.7 \mu\text{g/L}$), while 26% of the women had their breast milk below the permissible limit. The blood mercury content showed that 19% subjects had mercury content above the permissible limit ($20 \mu\text{g/L}$). In urine mercury estimation, 49% women had mercury content above the permissible limit ($10 \mu\text{g/L}$), while, 51% women had the mercury content below the permissible limit. In the child's urine, 54% infants had their mercury content in urine above the permissible limit ($10 \mu\text{g/L}$) while 46% infants had content below the permissible limit. The study indicates that 20% of infants had the complete accumulation of mercury in their body which is highly toxic for them. However, the mercury content in the food (wheat) had the contamination with in the permissible limit ($100 \mu\text{g/kg}$). There was a significant correlation found between the breastmilk and child's urine and mother's urine. The HQ study also correlates the mercury poisoning effect with 100% of the mother's and 66% of the infants exceeding the limit of non-carcinogenic risk. The Monte Carlo and multivariate study correlates the high health risk in the studied population due to mercury poisoning. The entire study concludes that population inhabitation in the Gangetic plains of Bihar are exposed to mercury poisoning which may be due to geogenic or anthropogenic sources. But, the levels of mercury contamination above the permissible limit could lead to neuro-generative changes in the lactating mothers and their infants. To control the present problem medical intervention is immediately required.

Keywords Mercury poisoning, Breastmilk, Lactating mothers, Infants, Geospatial mapping

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Arsenic Poisoning in Bihar: A Comparative Health Risk Assessment Through Water and Food Sources

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Received: 1 July 2025 / Accepted: 3 September 2025
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Abstract

Arsenic poisoning from groundwater has caused serious health hazards to the exposed population globally. The human body is contaminated by arsenic through drinking water and food, causing skin manifestations, neurological disorders, gastrointestinal disorders, cancer, etc. In the present study, a total of $n = 286$ household handpump water samples and food consumed (wheat and rice) by the studied subjects and each household member's biological samples (hair and nail) were collected for arsenic quantification. The hazard quotient (HQ) and cancer risk (CR) study was evaluated in the exposed studied population from the consumed wheat and rice. Arsenic content in 14% of water samples, 44% of wheat samples, and 3% of rice samples have exceeded their respective permissible limits of 10 $\mu\text{g/L}$, 100 $\mu\text{g/kg}$, and 200 $\mu\text{g/kg}$. In the studied subjects, 69% had arsenic contamination in their hair samples more than the permissible limit (200 $\mu\text{g/kg}$), while 11% had arsenic contamination in their nail samples more than the permissible limit (500 $\mu\text{g/kg}$). Arsenic contamination in the participants' household water, hair, and nail samples was positively correlated with one another by a correlation coefficient study. The food samples showed a significant correlation between the subjects' hair and nail samples and rice and wheat. In the six districts of Bihar, the geospatial analysis of all the variables revealed a considerable level of arsenic contamination. The health risk assessment also showed a high risk of arsenic contamination through wheat rather than rice. The risk assessment revealed elevated hazard quotient (HQ) and cancer risk (CR) in the six districts of Bihar's arsenic-exposed population. The present study thus concludes that arsenic contamination in water and food grains is causing health hazards in the studied exposed population of Bihar. Therefore, there is an urgent need to initiate an effective mitigation plan to safeguard the health of the exposed population.

Keywords Arsenic poisoning · Health risk assessment · Groundwater arsenic contamination · Hazard quotient · Cancer risk · Gangetic plains of Bihar

Introduction

The human health is seriously threatened by environmental toxins [37, 52]. A large number of population is facing the health risks due to exposure by various pollutants. One such problem is the poisoning caused by groundwater arsenic [46]. Arsenic contamination has impacted an estimated 300 million people worldwide and is linked to various health concerns [22–24, 28, 29]. Majorly geogenic and partially human activities are the major causes of arsenic contamination in groundwater [1, 31, 34]. The anthropogenic sources are metal mining, pesticides, wood and coal combustion, waste incineration, etc. [3, 13, 18, 44, 45].

Various studies have reported the arsenic contamination in groundwater and have shown that Taiwan and Chile have the highest arsenic poisoning. The other countries reported to

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Lead exposure in Bihar, Eastern India: a risk assessment study

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Received: 1 September 2024 / Accepted: 22 July 2025
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Abstract An estimated 800 million people globally are reported to be suffering from lead poisoning. In India, lead exposure has affected many states such as Andhra Pradesh, Uttar Pradesh, Jharkhand, Madhya Pradesh, and Chhattisgarh. The current research study has been conducted in various districts for Bihar such as Patna, Vaishali, Saran, Nalanda, Buxar and Bhojpur for the first time to estimate the lead exposure levels in subjects. The study was conducted on n = 282 lactating mothers and their breastfed infants. In the studied n = 282 lactating mother's blood samples, 94% had lead contamination higher than the WHO permissible limit of 50 µg/L. In the lactating mother's urine samples, 90% had lead contamination higher than the WHO permissible limit of 50 µg/L.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10653-025-02687-3>.

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Similarly, in the child's urine study, 41% had lead contamination higher than the WHO permissible limit of 50 µg/L. However, the analysed household handpump water also had lead contamination in 6% of the water sources more than the WHO permissible limit of 10 µg/L, while 94% of water sources had safer levels. This indicates that the source of lead contamination in biological samples of the studied subjects was mildly due to lead contaminated water. Hence, food, vegetables, animal milk or animal meat could be the source of lead contamination in the exposed subjects. Moreover, this could also be due to the use of local market turmeric, use of ayurvedic medicines, cosmetic – whitening creams by the exposed subjects. The long-term exposure to lead has caused non-carcinogenic HQ higher values i.e. > 1 in child and their mothers. Hence, the current study concludes that significant amount of lead contamination was observed in the exposed population's biological samples. The exposed lactating mother's breastfed infants are at high risk of developing neurological disorders which could cause severe health risk to them. The study recommends the state Government to take preventive measures to control the lead poisoning within the exposed community of Bihar (India).

Keywords Lead poisoning · Lactating mothers · Child health risk assessment · HQ · Gangetic plains of Bihar

Published online: 10 August 2025



OPEN

Discovery of uranium content in breastmilk and assessment of associated health risks for mothers and infants in Bihar, India

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In recent years, groundwater uranium (U^{238}) poisoning has posed serious health hazards in the exposed population. In India, an estimated 151 districts and 18 states are reported with groundwater uranium contamination, and about 1.7% of groundwater sources are affected in the state of Bihar (India). The objective of the study is to evaluate the uranium contamination in the breastmilk of lactating mothers and their breastfed infants. To evaluate the uranium exposure in the infants exposed through their mother's breastmilk, $n = 40$ lactating women were selected randomly from different districts of Bihar. After obtaining the written informed consent, their breast milk was collected and analysed for quantification of U^{238} . The infants and their mother's carcinogenic risk (CR) and hazard quotient (HQ) were also studied to know the potential health hazard effects of uranium. The uranium exposure to the infants through their mother's breastmilk is at a hazardous level. All the analysed breastmilk samples had U^{238} contents, which could pose health impacts to infants. The infants are highly vulnerable to potential non-carcinogenic risk in comparison to their mothers due to the real-time uranium elimination from their bodies. The study reveals that the uranium content in the breast milk was significantly high. The 70% of the infant population had the potential to cause non-carcinogenic health effects. From the studied districts, Katihar breastmilk samples had the highest U^{238} content. The exposure of uranium to the infant's body through breast milk is significantly high, which may impact their health. Furthermore, there is also a need for biomonitoring of U^{238} in these regions at a broader level.

Keywords U^{238} breastmilk contamination, Lactating mothers, Infants, Risk assessment

Uranium (U) is a naturally occurring radioactive element found widely in granite and other rocks. It can enter groundwater through natural processes, especially in oxygen-rich environments, and also due to human activities like mining, burning coal and fuel, emissions from the nuclear industry, and the use of phosphate fertilizers which contains uranium¹⁻². The average concentration of uranium in the Earth's crust is about 2.8 parts per million (ppm) to 4 ppm. Exposure to uranium can pose significant health risks, including kidney damage, bone damage, an increased risk of cancer, and also developmental issues³⁻⁵. The World Health Organization (WHO) has set a provisional guideline limit of 30 $\mu\text{g/L}$ in drinking water. Germany, for instance, has adopted a stricter limit of 10 $\mu\text{g/L}$ in 2011⁶. Higher levels of uranium in groundwater have been also observed globally, including in North America - Canada⁷, United States^{8,9}, Europe - Finland³, Sweden¹⁰, Switzerland¹¹, United Kingdom¹², and in Asia, Bangladesh¹³, China^{14,15}, Korea¹⁶, Mongolia¹⁷, Pakistan¹⁸, and the lower Mekong delta¹⁹. Furthermore, several health studies have been also carried out in Finland. It was reported that the drilled wells for drinking water had uranium concentrations in the range of 5.6–3410 $\mu\text{g/L}$ ²⁰. However, no clear clinical symptoms have been observed among the exposed population.

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High Cadmium Contamination in the Biological Samples of the Exposed Subjects in the Kosi Plain Region of Bihar

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Received: 15 March 2025 / Revised: 19 September 2025 / Accepted: 16 November 2025
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Abstract

The persistent nature of cadmium in the environment and its extensive industrial usage makes it a major threat to human health across the world. Cadmium is a human carcinogen because of its extreme toxicity and the fact that it affects many different organ systems. The present study was carried out in three districts of Kosi plain region of Bihar - Madhepura, Saharsa, and Supaul. A total of $n=60$ lactating women voluntarily participated in the study after obtaining the written informed consent. Their biological samples such as blood, breastmilk, urine samples were collected for cadmium contamination study. Moreover, their household water and food samples such as rice, wheat and pulse were also collected for cadmium contamination study. Analysis revealed that 16.66% of the water samples exceeded permissible cadmium levels, whereas food samples, particularly rice, wheat, and pulses, exhibited significant cadmium contamination, highlighting dietary sources as a major route of exposure. In the analyzed biological samples, 100% of blood samples exhibited cadmium concentrations exceeding the WHO permissible limit ($<1.2 \mu\text{g/L}$). Likewise, all lactating mothers had breast milk cadmium levels above the WHO permissible limit ($<1.0 \mu\text{g/L}$), while 83% of urine samples also surpassed the WHO threshold ($<5.0 \mu\text{g/L}$). These findings indicate that mothers exposed to cadmium in the Kosi plain region are transferring this toxic metal through breast milk to their infants, posing potential health hazards. Prolonged exposure in early life may result in gastrointestinal disturbances and impaired renal function, underscoring the critical risk to infant health. The potential health risks average daily dose (ADD) for the infants are higher than their lactating mothers. These findings highlight the urgent need for medical interventions in the Kosi plain region to address the prevailing cadmium poisoning problem. Moreover, a more comprehensive and systematic assessment of cadmium exposure across all districts of Bihar is required for better understanding the magnitude of the risk and to guide targeted public health strategies.

Keywords Cadmium poisoning · Lactating mothers · Infants · Breastmilk · Health risk assessment (HQ) · Geospatial mapping

Introduction

Cadmium (Cd) is a heavy metal that poses significant environmental and occupational hazards due to its high toxicity (Genchi et al. 2020). It contributes environmental pollution through a variety of pathways, including air, water, and food contamination (Suhani et al. 2021) due to its widespread use in industrial processes, especially in the production of batteries, and electroplating (World Health Organization 2019). Furthermore, the persistent nature of cadmium exacerbates its risk factors, making it a critical contaminant of concern for environmental health and public safety (Shahid et al. 2021).

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List of Conferences

1. Presented Oral Paper titled “Female health risk estimation due to arsenic poisoning” at PAFMATCON-2023, 21st Annual National Conference of Punjab Academy of Forensic Medicine & Toxicology held on 23rd December 2023 organized by Department of Forensic Medicine & Toxicology, GGSMC, Faridkot, Punjab.



2. Presented Poster titled “Assessment of female reproductive health risks due to heavy metal toxicity at the 2nd International Conference on Advances in Forensic Science (ICAFS) held on 20th & 21st April, 2023 organized by Department of Forensic Science, Kristu Jayanti College, Autonomous, Bengaluru, Karnatka.



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Toxicological analysis of biological fluids of females to prevent heavy metal toxicity

