Environmental Health

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 µg/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 µg/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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Introduction

Arsenic a metalloid has become a major environmental toxicant to human population due to its unwanted accumulation in the hydrosphere especially in the groundwater. It is the 20th most abundant heavy metal naturally occurring in the earth crust and also ubiquitously in nature [1]. Due to various geogenic activities like leaching of earth crust ultimately favors the accumulation of arsenic in the groundwater increasing day by day at a very high rate [2]. Other anthropogenic activities also contribute much amount of arsenic in the aquatic system, which ultimately leads to the high contamination of arsenic in the groundwater [3]. Groundwater arsenic poisoning has become a major environmental and health concern worldwide nowadays. It is estimated that about 300 million people worldwide are affected by arsenic poisoning [4, 5]. As determined in Indian population it is seen that 20 States and 4 Union Territories presently have been reported to be affected by arsenic contamination in groundwater comprising 70 million of the total population [5–7].

Bihar is a state in Eastern India and is located in Ganga-Meghna-Brahmaputra (GMB) basin. Groundwater is the major source of drinking water in this agrarian state which fulfills more than 80 per cent of drinking source in rural Bihar. Unfortunately, 10 million population of the state are exposed to adverse effects of arsenic poisoning. Out of 38 districts, 22 districts are affected by arsenic poisoning that is more than 50% of the state population [5, 8–20].

Various studies have been reported with the arsenic poisoning and caused health hazards in the state of Bihar. But these findings are in the arsenic hotspot regions in the middle Ganga plains [5, 7, 19–21]. The exposed population exhibit the skin manifestations such as hyperkeratosis, melanosis and pigmentations [10]. The long-term arsenic exposure in these exposed population has also led to the reporting of cancer incidences [5, 22, 23].

Breast milk is a dynamic biological fluid for children and newborns because of its nutritional value as it contains many types of biomolecules such as carbohydrates, proteins, fats, and growth factors and most importantly antibodies [24]. Breast milk is known as the "gold standard" of nourishment to infants. It is made up of distinct elements: 87% as water, with the remainder being macro and micronutrients. It consists of 7% carbs (mostly lactose), 4% fats, 1% proteins, and 1% vitamins and minerals. Colostrum is heavy in protein, vitamins A, B12, and K, as well as oligosaccharides. Arsenic exposure causes accumulation in colostrum milk. Colostrum is primarily used to defend a child's immune system against many environmental infections [25–29]. Heavy metals such as arsenic, lead, mercury, and cadmium are toxic and its exposure can be of public health concern. These metals cross the placenta and the blood brain barrier, and are excreted through breast milk [30]. Breast milk is the indicator of exposure to heavy metals during prenatal period, it is also exposed to a high risk in breastfed infants during postnatal period [24, 31]. Exposure to heavy metals disturbs the growth and development in newborn and infants [32]. Many studies have reported that infants are more vulnerable than others when they come in contact with heavy metals due to the lack of development of renal systems and lower tolerance level for these contaminants [31, 33–37]. Moreover, this arsenic contamination is majorly contaminating the staple foods such as wheat, rice and potatoes, which is biomagnified and causing health hazards among the exposed population. Hence, the present study aims to find the exposure caused due to the intake of arsenic contaminated drinking water, staple foods such as wheat, rice and potato by the lactating mothers and infants through their mother's breastmilk intake. Moreover, the study also finds the arsenic contamination in the biological samples of the lactating mothers and to know the carcinogenic risk caused to the infants as well as in their lactating mothers. This exposure study will reveal the results for the first time in the state of Bihar.

Materials and methods

Ethics approval

The Institutional Ethics Committee of the Indian Council of Medical Research Unit- Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar, India, granted ethical clearance with IEC Letter No. RMRI/EC/24/2020 dated September 26, 2020. Before the investigation began, all patients were briefed about the study's aims, and signed informed consent was obtained.

Location

The randomly selected habitations were from the 11 arsenic exposed districts- Buxar, Bhojpur, Patna, Saran, Vaishali, Samastipur, Darbhanga, Begusarai, Khagaria, Munger and Nalanda. The study was carried out from the month of October 2021 to May 2023.

Selection of subjects

The population participated in the study were lactating mothers and their breastfeeding infants. 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. Moreover, $n=184$ infants (with the age between 0.26 and 30 months) from arsenic exposed population. The studied subjects also provided their biological samples for the evaluation. The information regarding their breastfeeding durations were also accounted through a questionnaire.

Collection of the biological samples

The selected women voluntarily provided breastmilk samples (5 ml), blood samples (5 ml) and their urine samples (50 ml) which was collected in respective containers. The collected samples were stored at 2–6 °C in cool box and then transferred to the research laboratory of Mahavir Cancer Sansthan & Research Centre, Patna, Bihar for further storage at -20 degree Centigrade in deep freezer and was analysed thereafter on Graphite Furnace based Atomic Absorption Spectrophotometer (GF-AAS) of Perkin Elmer model number Pinnacle 900T (USA).

Collection of the household water and food samples

The 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 GPS coordinates and their depth related information were recorded. To lower the pH to 2.0, all the water samples were preserved immediately after collection using 1.5 ml/L nitric acid. Moreover, the studied household women also provided their raw food samples such as rice, wheat and potato for the arsenic contamination study. The total arsenic content in the studied institution was evaluated using a Graphite Furnace based Atomic Absorption Spectrophotometer (GF-AAS) of Perkin Elmer model number Pinnacle 900T (USA).

Estimation of breast milk, blood and urine arsenic concentration

For the Breastmilk, blood and urine arsenic estimation, 0·5 ml of samples were taken in 30 ml conical flask (glass) to which, 5 ml of HNO_3 were added and left for overnight reaction. The following day, all the samples were digested on hotplate at 90–120 °C, allowing volume to reduce to 3 ml. Then 5 ml volume of HNO_3:$HClO_4$ (6:1) mixture were added to the pre-digested solution in the conical flask. The samples were then re-digested on the hotplate at 90 –120 °C, until the volume of the solution reduced to about 2 ml. The final volume was adjusted to 10 ml with addition of distilled water after rinsing it with 1% HNO_3 and was then filtered through Whatman filter paper no. 41 for the determination of the final reading on Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS) (Pinnacle 900T, Perkin Elmer, USA) at the wavelength of 197.3 nm [38, 39].

Table 1 Permissible limits of the studied 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

Ground water arsenic determination

The collected water samples were filtered through the 0.45 µm syringe filter and were directly measured on Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS) (Pinnacle 900T, Perkin Elmer, USA) at the wavelength of 197.3 nm for total arsenic content [38, 39].

Food sample arsenic determination

The food such as rice, wheat and potato samples in 0.5 g were taken in 25 ml conical flask and to it added 5 ml Conc. HNO_3 and left for overnight reaction. The following day the samples were kept on water bath at 60 degree centigrade for 2 h. After the water bath digestion, the samples were allowed to cool at room temperature and then 2 ml of $HClO_4$ was added and then after was heated on hotplate at 160^0C for 5 min until the white dense fumes of $HClO_4$ are released. The samples were then cooled at room temperature and the final volume was made by adding 10 ml of demineralized water to the solution. The samples were then filtered with Whatman filter paper No. 41, and were then read through the GF-AAS for arsenic estimation [38, 39].

Quality control

The arsenic standard (1000 mg/L) of PerkinElmer USA (CAS no As7440-38-2; Lot No. 25-127ASY1; PE No N9300180) and standard stock solution was prepared to the point dilution. The calibration correlation coefficient was maintained at 0.999 throughout the analysis period. The detection limit of arsenic in breast milk, blood samples, urine samples and water samples were 0.07 µg/L, 0.09 µg/L, 0.09 µg/L and 10 µg/L respectively. The detection limit of arsenic in food samples such as rice was 0.05 µg/L, wheat as 0.07 µg/L, and potato as 0.05 µg/L respectively. The WHO normal ranges for arsenic contamination in breast milk is 0.64 µg/L, blood −5 µg/L, urine- 50 µg/L and drinking water is 10 µg/L. For the food samples the FAO normal ranges are- for rice 200 µg/Kg, for wheat 100 µg/Kg and for potato is 500 µg/Kg respectively (Table 1).

The arsenic determination was done with accuracy and precision method. Accuracy is denoted as recovery percentage and precision is denoted as repeatability which is expressed in % relative standard deviation (%RSD). A known amount of arsenic standard was added in the spike samples of water, breast milk, blood, mother's urine, child's urine, potato, rice and wheat to determine spike recovery percentage. The recovery percentage of water, breast milk, blood, mother's urine, child's urine, potato, rice and wheat were 68%, 96.89%, 96%, 84.71%, 85.94%, 95%, 98%, and 97.43% respectively. Repeatability of samples were also calculated by analyzing 5 replicates of each spike samples. The percentage of relative standard deviation of water, breast milk, blood, mother's urine, child urine, potato, rice and wheat were 0.9%, 1.09%, 0.85%, 2.72%, 0.37%, 2.66%, 0.50%, and 0.92% respectively. All the spike samples were within the limit of accuracy and precision (ISO 17025:2017; ISO 725-2:1994; EPA SW-846) [40–42].

Hazard quotient

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) [43].

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).

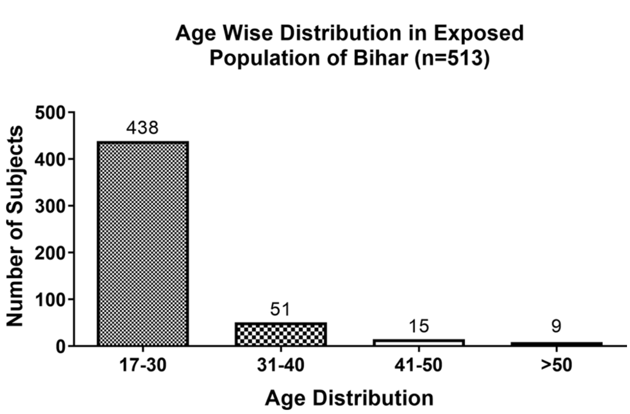

Fig. 1 Showing the graph of age distribution in arsenic exposed districts of Bihar

If CR value is $\leq 1\times10^{-4}$ then, it is tolerable menace levels.

Spatial analysis

A shape file was constructed by superimposing the GPS coordinates of samples using Arc-GIS Software (10.1). Google map (Google Earth) was utilized as the foundation map. Arsenic concentrations in groundwater, breast milk, blood and urine were classified into three groups. It is expressed in groundwater as BDL (Below Detection Limit), 0–10 μg/L, 11–50 μg/L, 51–100 μg/L, 101–200 μg/L, 201–500 μg/L, >500 μg/L. It was expressed in blood as BDL (Below Detection Limit), 0–10 μg/L, 11–50 μg/L, 51–100 μg/L, 101–200 μg/L, 201–500 μg/L, >500 μg/L. It was expressed in breast milk as <1 μg/L, 1–10 μg/L, 11–50 μg/L, 51–100 μg/L, >200 μg/L. It was expressed in urine as 0–10 μg/L, 11–50 μg/L, 51–100 μg/L, 101–200 μg/L, 201–500 μg/L, >500 μg/L. It was expressed in food samples in rice as <200 μg/Kg, 201–500 μg/Kg, >500 μg/Kg, in wheat as <100 μg/Kg, 101–200 μg/Kg, in potato as <500 μg/Kg, 501–1000 μg/Kg, >500 μg/Kg.

Statistical analysis

Graph Pad Prism 8.0 and SPSS −25.0 statistical software were used for the statistical analysis. Arsenic concentrations in groundwater, urine, blood and food samples such as rice, wheat and potato were measured and graphed. With 5 variables, an 8-correlation analysis was performed. The variation across groups was examined using one-way analysis of variance.

Results

Age-wise distribution

The age wise distribution of the subjects inhabiting in the arsenic exposed area depicts that out of total of $n=513$ subjects, $n=489$ subjects were in the age group between 17 and 40 years, $n=24$ subjects were above 40 years (Fig. 1).

Water arsenic concentration

The geospatial maps show significant arsenic contamination in the groundwater in arsenic hotspot 11 districts of Bihar (Fig. 2A). The household water samples of the studied handpumps were in 60–130 feet of depth range. Out of total $n=513$ households, $n=450$ households had their groundwater arsenic concentration below the permissible limit (10 μg/L) [44, 45]. Hence, the study interprets that most of the groundwater samples was safe for drinking. However, the highest arsenic concentration in the groundwater found was 550.7 μg/L (Fig. 2B).

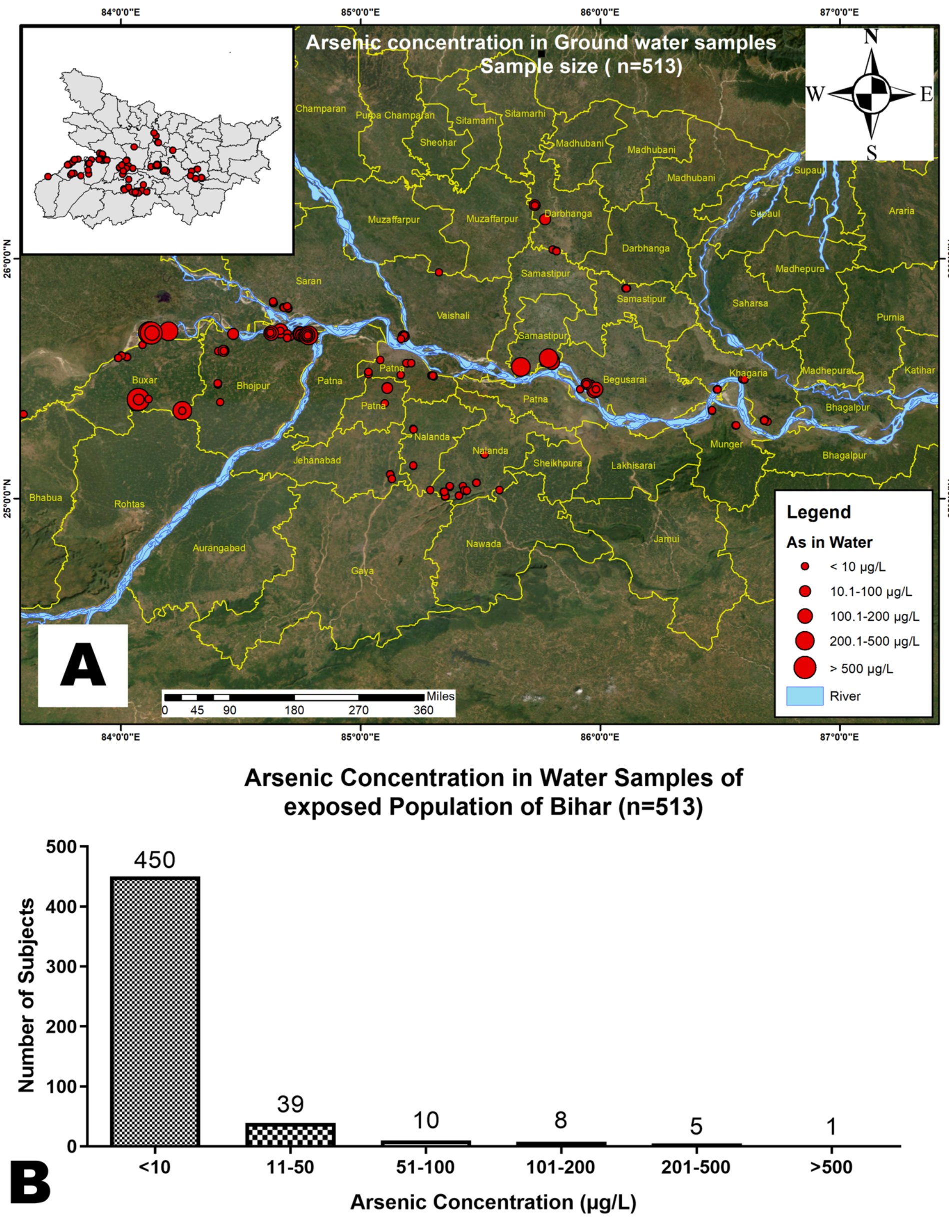

Fig. 2 **A** Showing geospatial maps of studied arsenic 11 hotspot districts with groundwater poisoning. **B** Showing the graph with the levels of arsenic concentration in the drinking water samples of exposed districts

Blood arsenic concentration

The geospatial maps show significant arsenic content in the blood samples of the subjects in arsenic hotspot 11 districts of Bihar (Fig. 3A). There was significant blood arsenic contamination observed in $n=205$ subjects. The study showed that $n=84$ subjects had their blood arsenic concentration below 10 µg/L. About $n=121$ subjects had arsenic contamination in their blood above the permissible limit of 10 µg/L with the highest content as 732 µg/L (Fig. 3B). This denotes that major chunk of studied subjects had their arsenic content in blood above the permissible limit of 10 µg/L [46].

Breastmilk arsenic concentration

The geospatial maps show significant arsenic content in the breast milk samples of the subjects in arsenic hotspot 11 districts of Bihar (Fig. 4A). The arsenic contamination study in breastmilk was carried out in $n=378$ subjects. The study showed that $n=168$ subjects had their breastmilk arsenic content below the permissible level, while $n=210$ subjects had arsenic content in their breastmilk more than the permissible limit < 1 µg/L [47]. The highest arsenic content in the breastmilk was 458 µg/L (Fig. 4B). The correlation coefficient between mother's breast milk with mother's urine ($r^2=0.562$) and its comparison with breastmilk and child's urine ($r^2=0.287$) shows mild

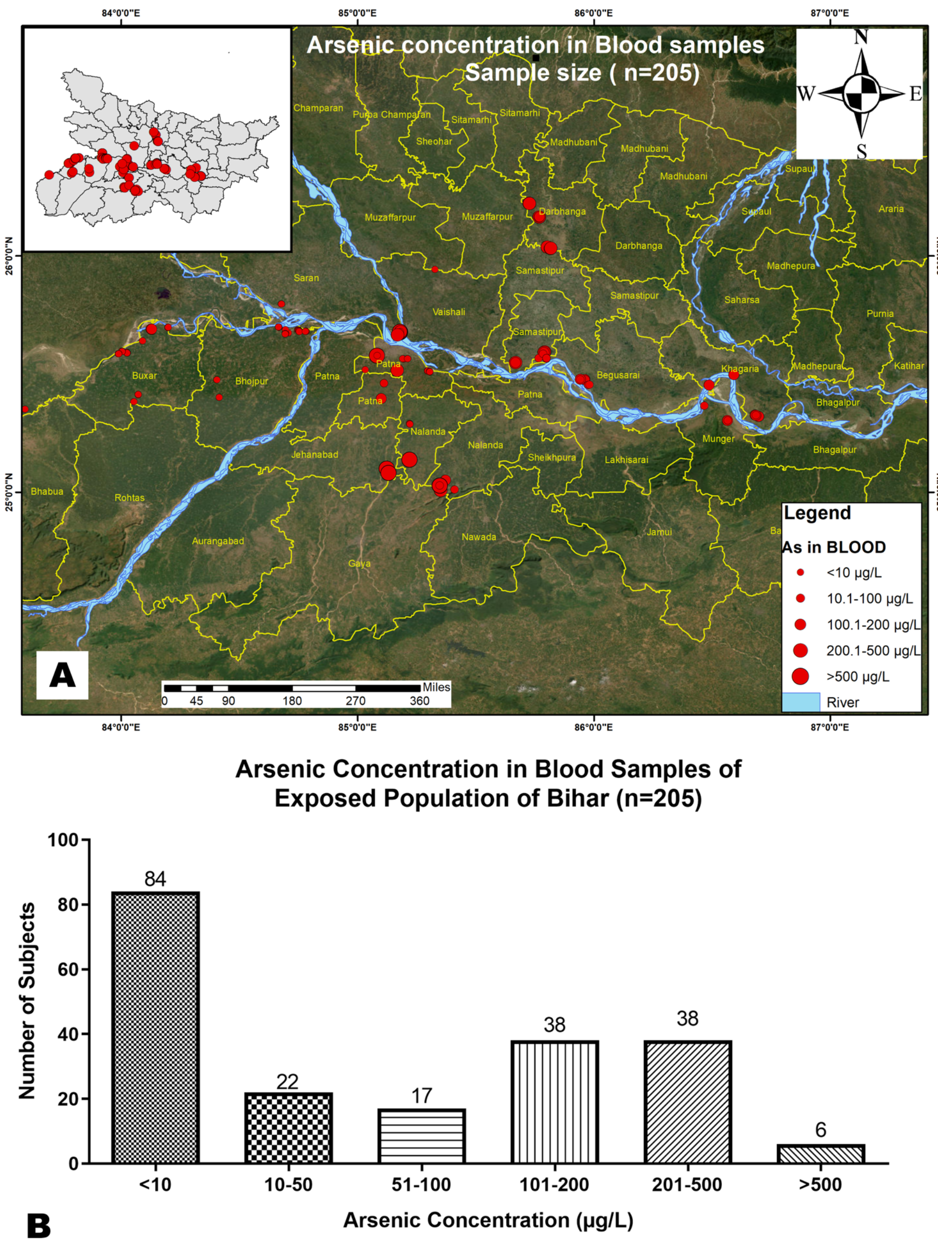

Fig. 3 **A** Showing geospatial maps of studied arsenic 11 hotspot districts with arsenic poisoning in the blood samples. **B** Showing the graph with the levels of arsenic concentration in the blood samples of subjects of exposed districts

correlation (Fig. 4C). This denotes that arsenic exposure is caused to the infants is through their mother's breast milk.

Mother's urine arsenic concentration

The geospatial maps show significant arsenic contamination in the mother's urine samples of the subjects in arsenic hotspot 11 districts of Bihar (Fig. 5A). The estimation of urine arsenic content was carried out in $n=461$ in the arsenic exposed subjects. The study showed that $n=92$ subjects had their urine arsenic content below the permissible level (50 µg/L) [48], while $n=369$ subjects had arsenic content in their urine more than the permissible limit. The highest arsenic content in the urine was 1039 µg/L (Fig. 5B). The Hazard Quotient study shows significant arsenic exposure association with a higher non-carcinogenic risk in the lactating mothers (Fig. 5C), (Table 1, Supplement-1; table 2, Supplement-2). This denotes that the subjects are at very high risk of cancer in future due to long term arsenic exposure.

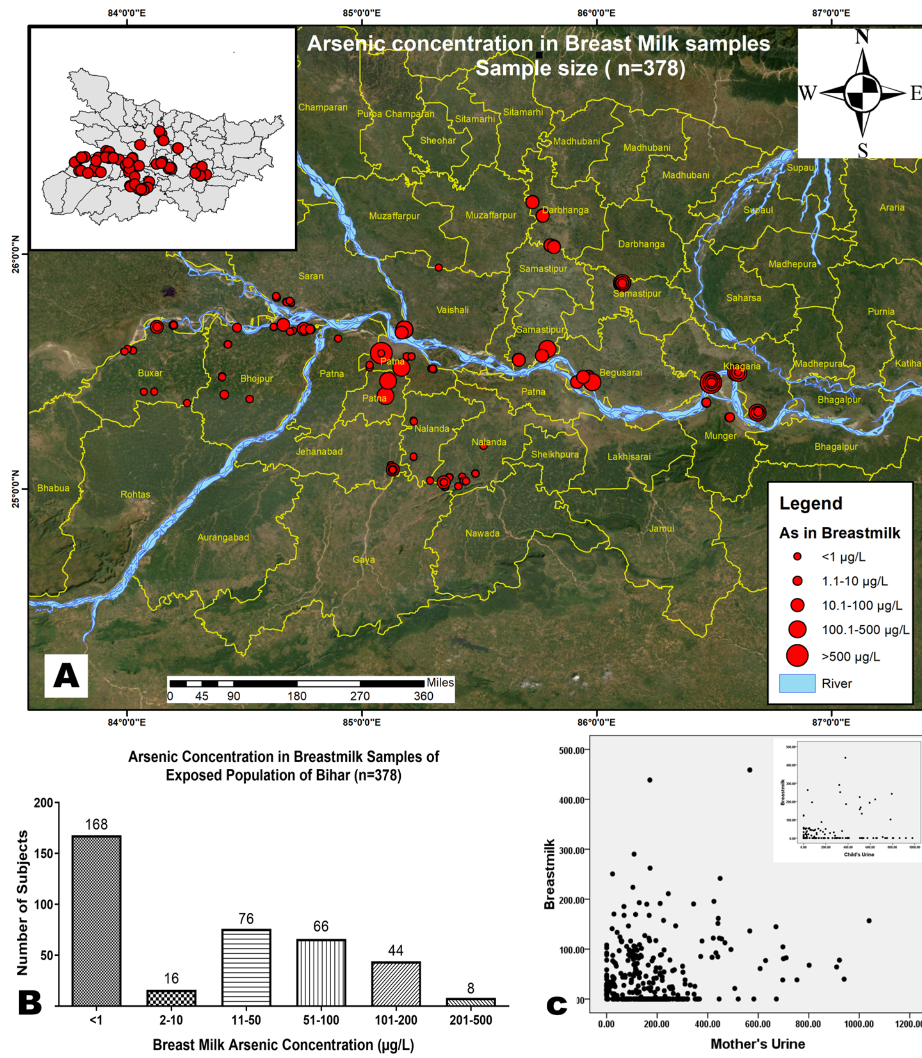

Fig. 4 **A** Showing geospatial maps of studied arsenic 11 hotspot districts with arsenic poisoning in the breastmilk samples of the subjects. **B** Showing the graph with the levels of arsenic concentration in the breastmilk samples of subjects of exposed districts. **C** Correlation coefficient of Mother's breast milk with mother's urine ($r^2=0.562$) and its comparison with breastmilk and Child's urine ($r^2=0.287$)

Child's urine arsenic concentration

The geospatial maps show significant arsenic content in the child's (infants) urine samples in the arsenic hotspot 11 districts of Bihar (Fig. 6A) with illustration showing the reason behind the arsenic poisoning in this particular region (Fig. 6B). The estimation of child's urine arsenic content was carried out in $n=184$ in the arsenic exposed subjects. The study showed that $n=60$ child subjects had their urine arsenic concentration below the permissible level (50 µg/L) [48], while $n=124$ child subjects had arsenic content in their urine more than the permissible limit. The highest arsenic content in the urine was 1031 µg/L (Fig. 6C). The Hazard Quotient study shows significant arsenic exposure association with a higher non-carcinogenic risk in the infants (Fig. 6D). It is possible that children's smaller body weight contributes to the higher risk they face as a group. On the other hand, each sample had a Carcinogenic Risk (CR) that was higher than the threshold value of 1×10^{-6} in the children population group, whereas the threshold value was found to be lower in the population group of nursing mothers (Table 1, Supplement-1; Table 2, Supplement-2). The possible reason of high arsenic content in the child's urine is their mother's contaminated breast milk.

Rice arsenic concentration

The geospatial maps show significant arsenic contamination in the rice samples of the subjects in arsenic hotspot 11 districts of Bihar (Fig. 7A). The arsenic content study in rice samples were carried out in $n=369$ in the arsenic exposed population. The study showed that $n=319$ households had their rice arsenic content below the permissible level, while $n=50$ households had arsenic content in their rice samples more than the permissible limit

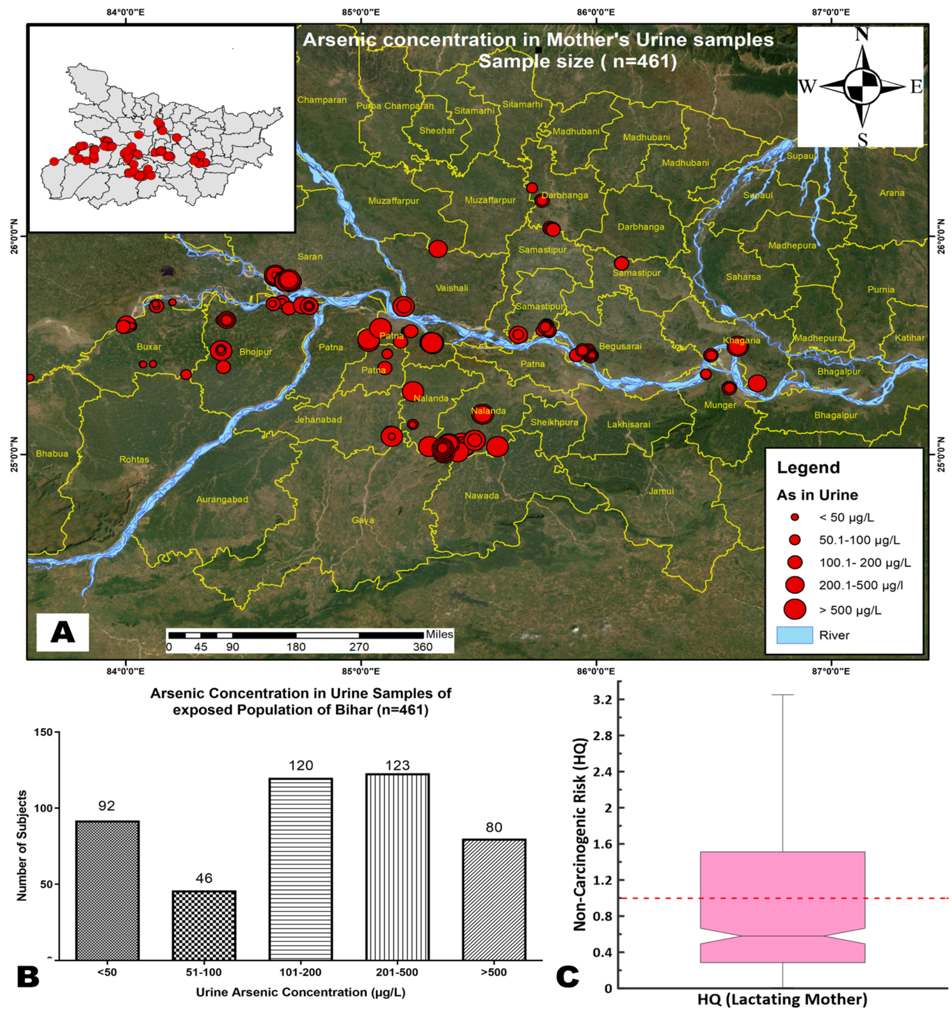

Fig. 5 **A** Showing geospatial maps of studied arsenic 11 hotspot districts with arsenic poisoning in the mother's urine samples of the subjects. **B** Showing the graph with the levels of arsenic concentration in the mother's urine samples of subjects of exposed districts. **C** Human health risk assessment (HQ) - non-carcinogenic risk in lactating mothers

Table 2 References of the health risk assessment studies

Parameters	Female	Children	References
IR	2.5	0.741	USEPA, 2008 & USEPA,2014
ED	67	0.25	Narsimha and Rajith,2018 & USEPA, 2008
EF	365	182	USEPA,2014
BW	55	7.4	USEPA, 2008 & ICMR,2009
AT	24,455	45.5	USEPA, 2008 & USEPA,2014
C(mg/L)			Present study

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

(<200 µg/Kg) [49]. The highest arsenic content in the rice was 821 µg/Kg (Fig. 7B).

Wheat arsenic concentration

The geospatial maps show significant arsenic content in the wheat samples of the subjects in arsenic hotspot 11 districts of Bihar (Fig. 8A). The arsenic content study in wheat was carried out in $n=279$ in the arsenic exposed population. The study showed that $n=105$ households had their wheat arsenic concentration below the permissible level, while $n=174$ households had arsenic content in their wheat samples more than the permissible limit (<100 µg/Kg) [49]. The highest arsenic content in the wheat sample was 775 µg/Kg (Fig. 8B).

Potato arsenic concentration

The geospatial maps show significant arsenic contamination in the potato samples of the subjects in arsenic hotspot 11 districts of Bihar (Fig. 9A). The arsenic content study in potato samples was carried out in $n=168$ in the arsenic exposed population. The study showed that $n=163$ households had their potato arsenic concentration below the permissible level, while $n=5$ households had arsenic content in their potato more than the

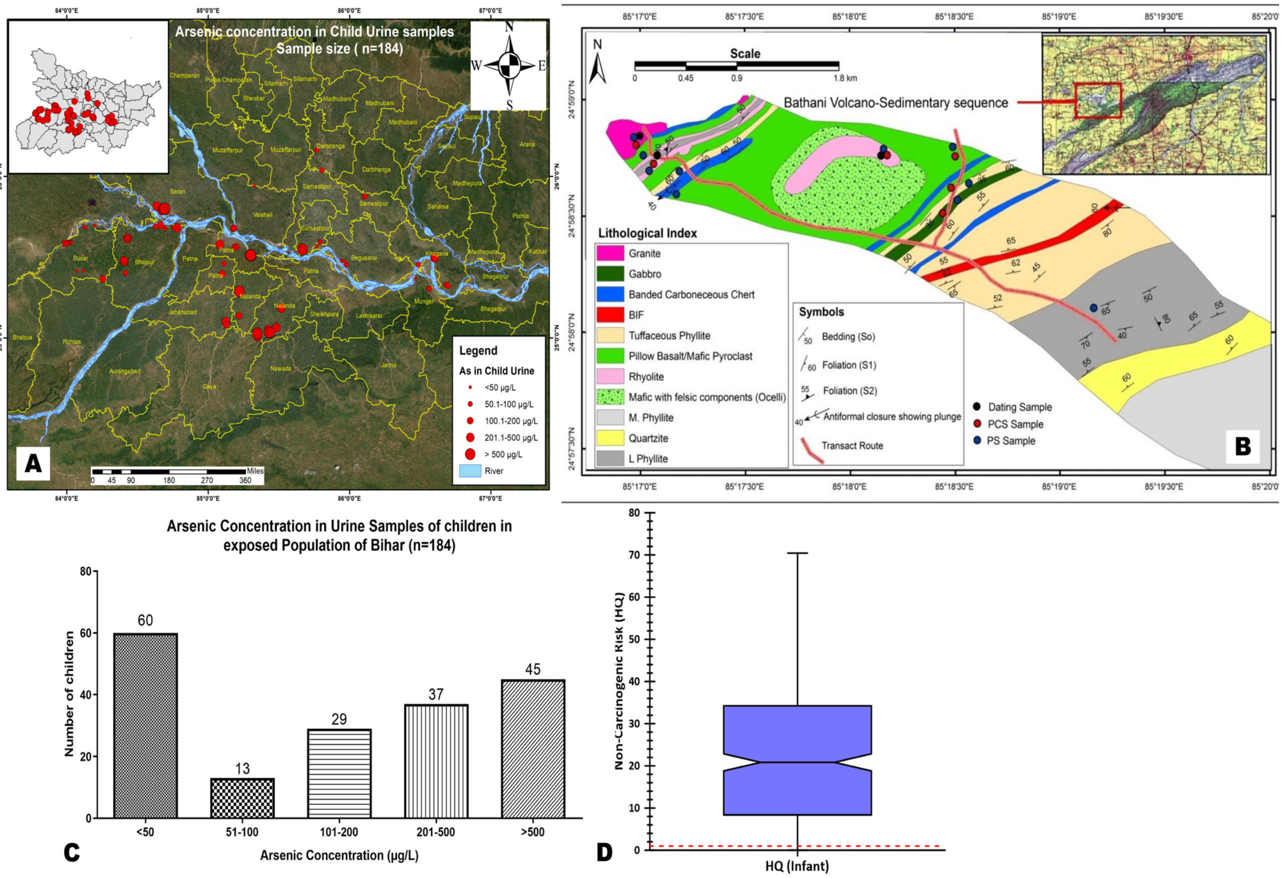

Fig. 6 **A** Showing geospatial maps of studied arsenic 11 hotspot districts with arsenic poisoning in the child's(infants) urine samples of the subjects. **B** Illustration related to the cause of arsenic poisoning. **C** Showing the graph with the levels of arsenic concentration in the mother's urine samples of subjects of exposed districts. **D** Human health risk assessment (HQ)- non-carcinogenic risk in Children (infants)

permissible limit (<500 µg/Kg) [49]. The highest arsenic content in the potato was 1450 µg/Kg (Fig. 9B).

Human health risk assessment

The groundwater arsenic concentration and breast milk arsenic concentration is used for the estimation of health risk assessment of mother's and children population respectively. The results reveal that 37.23% samples of ground water and 97.08% samples of breast milk exceeded the threshold limit of hazard quotient. It means 37.23% mother's population and 97.08% of children have potential risk of non-carcinogenic health effect. If the carcinogenic risk is taken in account, then it was found that, 85.96% of ground water samples and 98.41% of breast milk samples have crossed the limit of ILCR (Incremental lifetime cancer risk) which indicates that 85.96% of mother's and 98.41% of children is on the verge of causing cancer in the near future (Fig. 10; Table 2).

Geological perspective

From the geological perspective, as depicted on the map of Bihar (Fig. 1A, amp and B, 2 A&B, 3 A&B, 4 A&B, 5 A, 6 A, 7 A and 8 A), the alarming hotspots whether in groundwater, mother's milk or urine or the food samples are defining the regions from where the samples have been collected show a definitive proximity to the Gangetic flow regime on either of its bank. It is clearly the oscillation zone of river Ganga which is defined by the conspicuous crests and troughs perpendicular to its flow and is what defines the pockets of moderate to high arsenic contamination. Arguably the river morphology has seen a remarkable change temporally and has thus influenced areas which at one point in time during the last 50 years of reference were not as much affected. Thus, Ganga River continues to act as an extensive receptacle of sediments which based on river morphology, change in channel architecture, and effect of biological activity has clearly accentuated the anomalous concentration level within aquifers and the sediments accumulating along this zone. With the belief that the arsenic laden sediments are primarily derived from the extra peninsula region, the levels of contamination are significantly higher within the oscillation zone of Ganga River than the districts in north Bihar which are presumably closer to the source

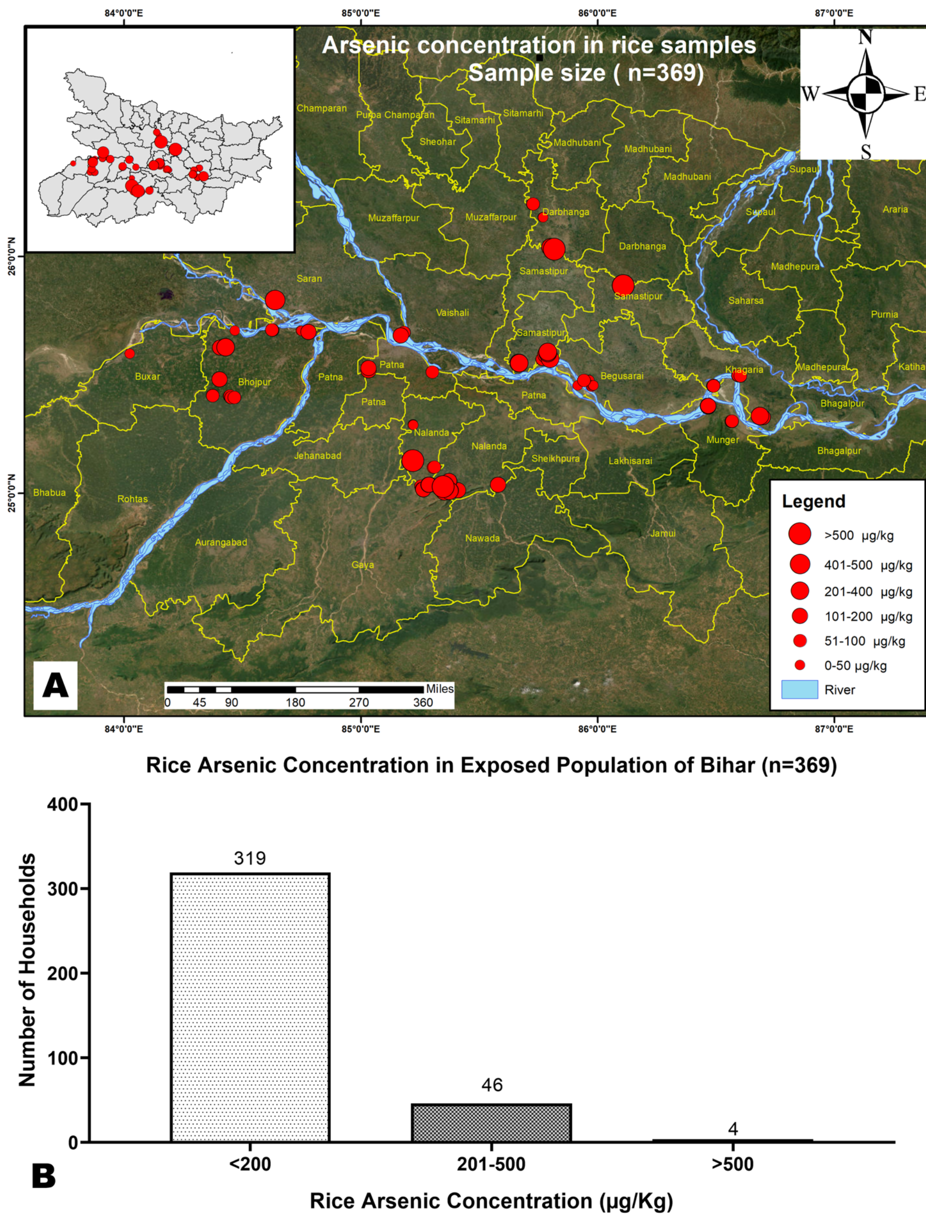

Fig. 7 **A** Showing geospatial map of studied arsenic 11 hotspot districts with arsenic poisoning in the rice samples of the households. **B** Showing the graph with the levels of arsenic concentration in the rice samples of households of exposed districts

of contamination. Whether in Darbhanga or Champaran district of Bihar (based on studies carried out by Geological Survey of India), the level of contamination is not as high as that found within the flow regime of River Ganga where most of these rivers draining the Himalayas make a confluence with the larger order stream [50, 51]. Thus, the arsenic exposed area of Bihar vouches the alarming concentration within breastmilk, blood, urine or groundwater samples.

A few high values have been observed within the non-arsenic affected districts as well and which need to be examined from the geological perspective. Figure 5B indicates that out of 15 water samples collected from Nalanda, Gaya and Jehanabad district (primarily non arsenic affected areas), none of the results show a concentration beyond 10 ppb which supports the observation of aquifers being free of arsenic contamination. But, as per Fig. 2D (blood sample) Fig. 3D (breast milk) and Fig. 4D (urine sample), there are only a few instances where the human body parameters show increased levels. Understanding the area of interest geologically, the sampled area to the north of NE-SW trending Rajgir hills is occupied by rocks constituting the volcano sedimentary sequence. The Rajgir hills represents the Rajgir-Munger

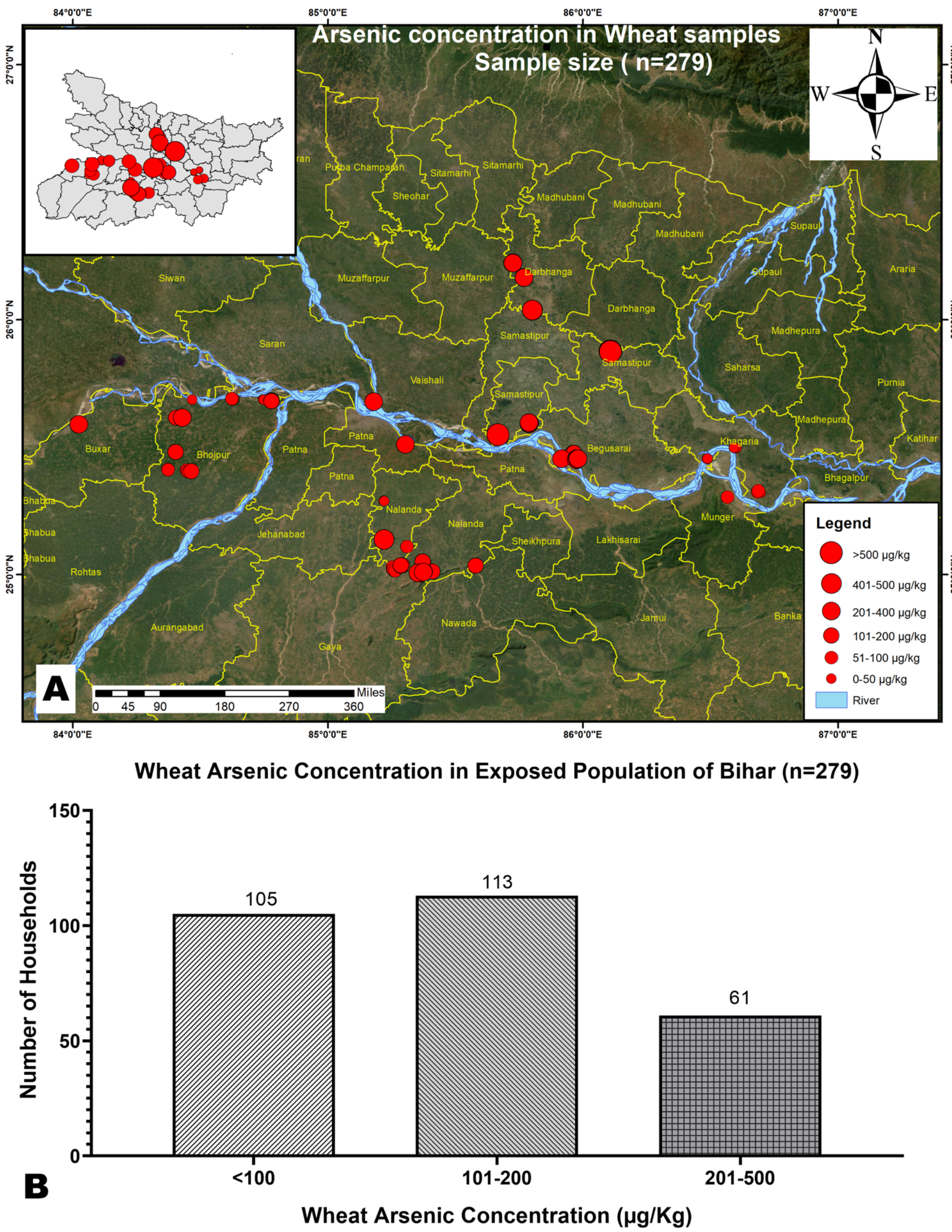

Fig. 8 **A** Showing geospatial map of studied arsenic 10 hotspot districts with arsenic poisoning in the wheat samples of the households. **B** Showing the graph with the levels of arsenic concentration in the wheat samples of households of exposed districts

metasediments formed under lacustrine conditions comprising quartzites and phyllites which overlain the basement rocks i.e. the granites and its variants. Principally, they aren't a proven source for arsenic based on their mineralogical constitution. But, the volcano sedimentary package reveals emplacement of diverse mafic and felsic rocks and they together justify for multistage and multi-source magmatism in the area. The presence of pillow lava marks the eruption of these rocks in subaqueous environment [52, 53] (Fig. 9. Supplement 3).

The occurrence of a lithological setup as diverse as these advocates for an upwelling mechanism which are derivative of volcanic rocks as they appear on the surface. The area also coincides with the trace of the Munger Saharsa Ridge Fault aligned with the northern faulted face of Rajgir hills along which several hotsprings are located and they derive gases from some hot unknown magmatic source. These volcanic rocks may have derived some arsenic rich plumes defining multistage and multi-source magmatism in the area which ideally are confined to this zone only [54, 55].

But this hypothesis cum observation has to be affirmed by extensive sampling in the area of various available media viz. rocks, water (from aquifers at various depths)

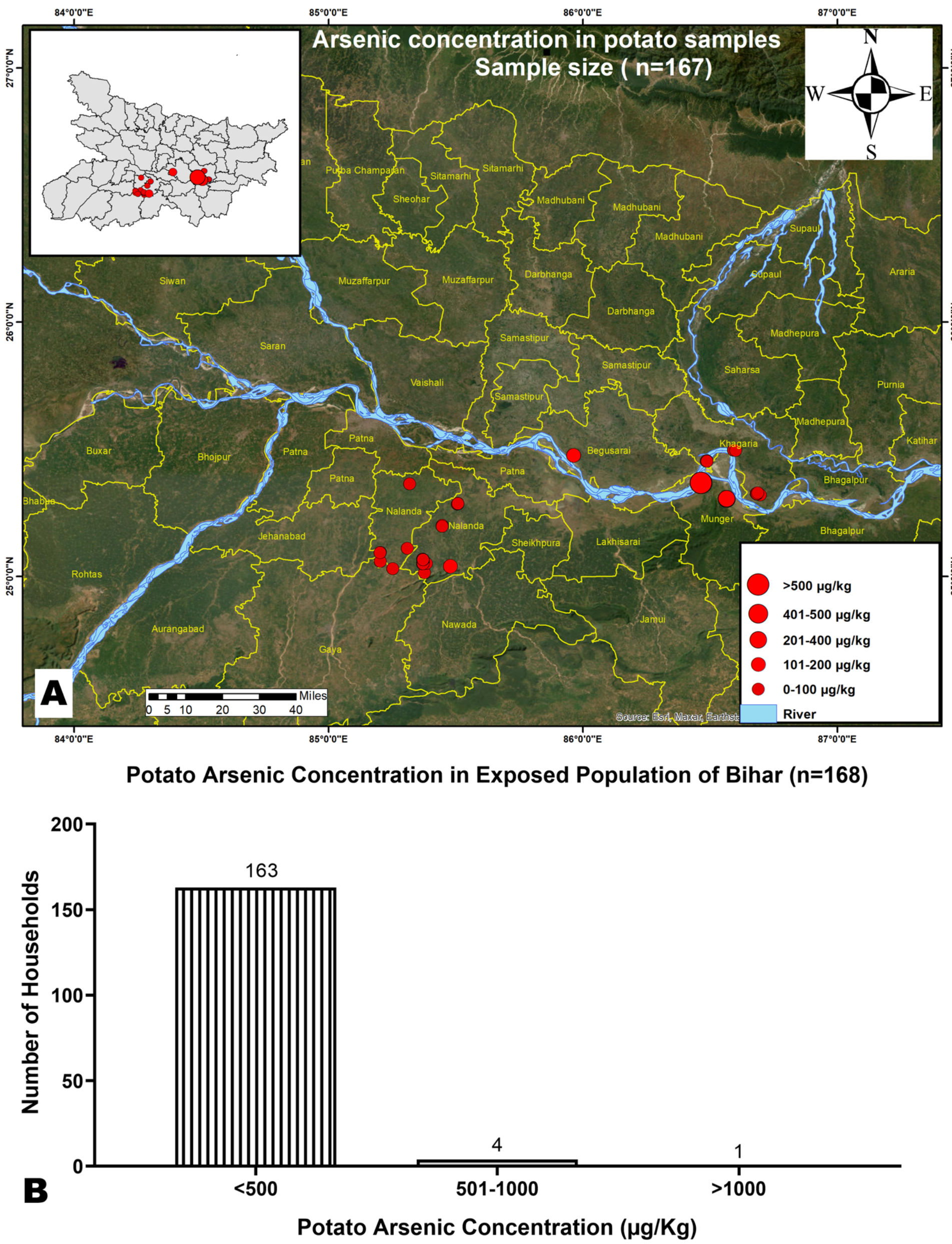

Fig. 9 **A** Showing geospatial map of studied arsenic 10 hotspot districts with arsenic poisoning in the potato samples of the households. **B** Showing the graph with the levels of arsenic concentration in the potato samples of households of exposed districts

soil and stream sediments to affirm this observation as the water samples do not have anomalous concentration. The few cases which have yielded high levels in mother's milk, blood or urine could also have migrated from arsenic affected areas recently or post contamination. With earth's crust containing (on average) 1.5ppm arsenic, it is unlikely to be the direct source for increased concentration if not supported by prevalence of arsenic bearing minerals [56].

Discussion

Environmental pollutants are posing severe health threats to the humans. Infants are more vulnerable to these pollutants. Arsenic in the recent times in the Gangetic plains of Bihar has shown severe health hazards in the exposed population. Most of the studies carried out are on the adults, but the present study reveals for the first time the impact of arsenic exposure on the infants in the Gangetic plains of Bihar. In the present study, the maximum age group in arsenic exposed subjects were observed in 85% of the subjects with the age group between 17 and 30 years, while 15% subjects were in the age group between

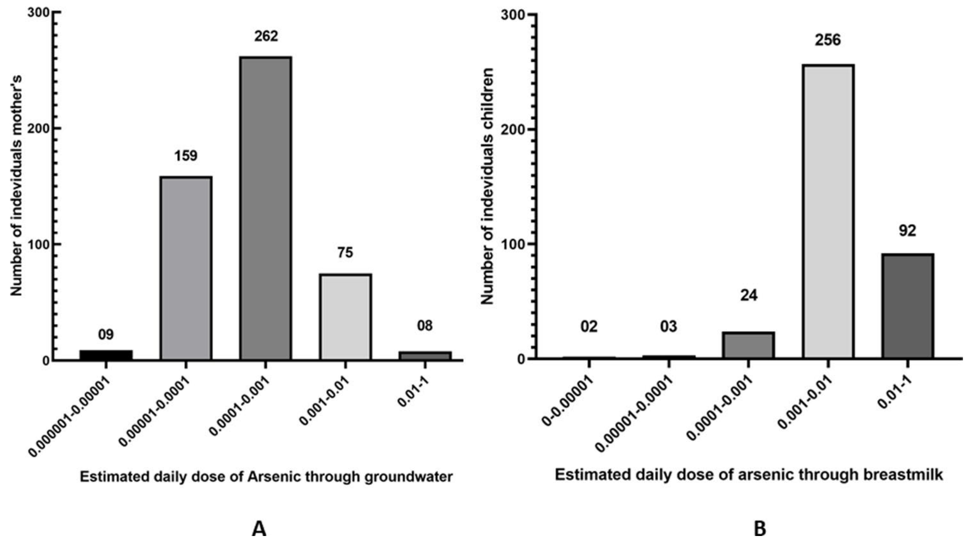

Fig. 10 Graph of estimated daily dose of arsenic through consumption of arsenic contaminated groundwater and breastmilk for mother's and children population respectively. **A**(Mother's), **B**(Children)

31 and 60 years. The arsenic concentration, in studied $n=513$ water samples, 88% water samples had arsenic concentration below the WHO levels of 10 µg/L. Only 12% water samples had arsenic concentration in water samples between 11 and 550 µg/L with maximum concentration as 551 µg/L. The blood arsenic concentration in the studied arsenic exposed subjects in $n=205$ samples, 41% had the blood arsenic content in the normal range that is below 10 µg/L, while 59% blood samples had arsenic content above the permissible limit with highest content as 732 µg/L. The reason for the excessive arsenic content in their blood is due to the intake of staple foods such as rice, wheat and potato which were contaminated with arsenic. The breastmilk arsenic content in arsenic exposed population in the studied $n=378$ subjects, 44% subjects had arsenic content below permissible limit that is <1 µg/L, while 56% subjects had arsenic content above the permissible limit with highest arsenic content as 458 µg/L. In the studied mother's urine arsenic concentration in arsenic exposed population in $n=461$ subjects, 20% had arsenic content below permissible limit of 50 µg/L. However, 80% subjects had arsenic content in their urine above the permissible limit with maximum arsenic concentration as 1038 µg/L. In the child's urine arsenic concentration studied carried out in arsenic exposed $n=184$ child subjects, 33% child subjects had arsenic content in their urine below permissible limit (50 µg/L), while 67% child subjects had arsenic content above permissible limit with maximum arsenic concentration as 1031 µg/L.

In the studied food samples in the rice, the arsenic concentration in the studied 369 rice samples, 86% had arsenic content below FAO permissible limit of 200 µg/Kg [57]. However, 14% rice samples had arsenic content above the permissible limit with maximum arsenic concentration as 821 µg/Kg. In the studied food samples in the wheat, the arsenic concentration in the studied 279 wheat samples, 37% had arsenic content below FAO permissible limit of 100 µg/Kg [58]. However, 63% wheat samples had arsenic content above the permissible limit with maximum arsenic concentration as 775 µg/Kg. In the studied food samples in the potato, the arsenic concentration in the studied 168 potato samples, 97% had arsenic content below FAO permissible limit of 500 µg/Kg [49] However, 3% potato samples had arsenic content above the permissible limit with maximum arsenic concentration as 1450 µg/Kg.

The biomagnification of the arsenic contamination in the studied humans through the intake of their food samples is the first finding ever reported in these 11 districts of Bihar. Moreover, the major chunk of the population is at very high risk as they are consuming arsenic contaminated food, which through the breast milk is reaching to their infants. From the results, it reveals that in the arsenic exposed population inhabiting in the districts along the river Ganges – Buxar, Bhojpur, Saran, Patna, Vaishali, Samastipur, Beugusarai, Khagaria, Nalanda, Darbhanga and Munger are at the verge of high risk of disease burden. The infants are at very high risk for the disease burden in the future as their mothers are lactating high arsenic content in their breast milk. This can lead to mental disorders, low intelligence, low memory etc. in these infants. Rebelo & Caldas, 2016 have studied the toxic effects of arsenic, lead, mercury and cadmium in breast milk and risks caused to the breastfed infants in Brazil [24]. Furthermore, the study also depicted that there is no safe dose of exposure established for arsenic or lead. Mohammadi et al., 2022 studied the contamination of breast milk with arsenic, lead, mercury and cadmium in Iran and reported that these toxic heavy metals had high content in the milk colostrum [59]. Freire et al., 2022 reported high heavy metal such as arsenic, mercury, lead and cadmium content in pooled donor breast milk in Spain [60]. Sharafi et al., 2023 reported high arsenic content in 73% of studied lactating mother's breastmilk in Iran [61]. The calculated HQ was also very high in the studied infants due to arsenic toxicity caused through breastmilk. Salmani et al., 2018 studied the arsenic exposure in breast fed infants in the first month after their birth in Iran in the study 53% of lactating mother's breastmilk was contaminated with arsenic [33]. Bassil et al., 2018 reported 63% of lactating mother's breastmilk contaminated with arsenic in Lebanon [62]. The arsenic exposure was found to be through the cereal and fish intake by the exposed population. Similar other studies were also reported from the studied countries China, Iran, Turkey, Germany and India [63–68].

This study carried out in Iran, evaluated the concentrations of lead (Pb), arsenic (As), and chromium (Cr) in the breast milk of 100 urban mothers in the city Hamadan, and assessed the associated health risks for infants. Breast milk samples were collected at 2, 6, 8, and 12 months postpartum, with heavy metal concentrations measured. The median concentrations of Pb, As, and Cr were 41.90, 0.50, and 3.95 μg/L, respectively. Notably, 94% of samples exceeded the WHO lead contamination limit. The hazard quotient (HQ) for Pb and arsenic exceeded acceptable levels in 61% and 10% of samples, respectively, indicating a potential health risk for infants [69]. Another cross-sectional study carried out in Iran, studied breast milk from 100 healthy lactating mothers in Hamadan city, focused on aluminum and various minerals and trace elements. Samples were collected at 1, 2, 6, 7, and 12 months postpartum from ten government health centers. The study reported levels of sodium, zinc, calcium, iron, copper, magnesium, and aluminum. The mean concentrations were 0.75 μg/mL for iron, 1.38 μg/mL for zinc, and 0.191 μg/mL for aluminium, with 95% of participants showing harmful levels of aluminum. Additionally, zinc deficiency was observed in 50% of samples, highlighting potential health risks [70]. Another study from Iran, carried out the exposure study of toxic metals. This systematic review assessed the risks of arsenic in breast milk for newborns and infants. In the study, the arsenic levels ranged from 0.04±0.70 to 27.75±28.30 μg/L, with a pooled average concentration of 0.11 μg/L, suggesting that infant breast milk consumption poses a minimal cancer risk [71].

According to the findings of this research, it is abundantly evident that the presence of these heavy metals is evidence that mothers are exposed to arsenic poisoning. This not only has a detrimental effect on the health of the infant, but it also has a detrimental effect on the health of the mother as well. It was discovered that prolonged exposure to arsenic in newborns or children up to the age of 5, may result in decreased intellectual quotient (IQ) scores. This was discovered via observation. There have been reports that various arsenic concentrations have been discovered [72]. In 55% of the breastmilk samples, the amount of arsenic was more than the WHO permitted limit of <1 μg/L, in the mothers inhabiting the arsenic exposed region. Similar study was conducted in West Bengal (India) where mothers were lactating arsenic contaminated breast milk and were feeding to their infants unknowingly [73]. Due to the fact that arsenic may be taken up by plants via the soil's surface, it is also possible to assert that food crops, in addition to drinking water, should be regarded as an essential means by which individuals take in arsenic [74]. Previous research has shown that irrigating food crop plants with water containing arsenic may make the soil more likely to retain arsenic via the adsorption of arsenic on soil exchange complexes [75]. In the present study, the high arsenic content in the rice and wheat denotes that through the food chain the arsenic biomagnification has taken place in the mothers which in turn are lactating high arsenic contaminated breastmilk which are fed by their infants accidentally. Infants who are vulnerable to the effects of arsenic exposure via drinking water are an additional factor that might be regarded a significant source. There is evidence that the transmission of arsenic to the mammary glands is reduced, which ultimately protects the neonates from being exposed to arsenic [76, 77]. It has also been claimed that the foetus and babies are protected from arsenic exposure owing to arsenic methylation when the mother is pregnant and while the mother is nursing the baby [50, 78]. But, in contrast to these studies, no studies have reported the real time arsenic poisoning in the children in the Gangetic plains of Bihar (India). The present study clearly reports that in 55% of the exposed lactating mothers had arsenic content in their breastmilk higher than the WHO permissible limit. The same day child's urine arsenic content was also observed in 65% of the arsenic exposed children. The study throws light that the mothers who lactated the arsenic in their breast milk and same day only 50% of the arsenic was released through the child's urine. This denotes that the rest 50% of the arsenic is accumulated in the child's body and could be highly toxic to the vital organs of the body such as brain, liver, kidney, heart, lungs etc. Moreover, the child's day to day activity will also be in catastrophic condition, which needs to be catered through the medical interventions.

The geological perspectives also report that arsenic poisoning in the subjects in the Gangetic plains of Bihar is due to the oscillation movement of river Ganga along its course. The studied districts were Buxar, Bhojpur, Patna, Saran, Samastipur, Begusarai, Khagaria, Munger, Nalanda and Darbhanga. The selected district Nalanda was thought to be arsenic free region of the state, but this study reveals for the first time the arsenic poisoning in the exposed population. Moreover, the strong hypothesis related to the tectonic movements in the Rajgir hills area, clearly demonstrates the presence of arsenic in the sediments as well as in the water [50, 51]. In the present study the high arsenic content in the breastmilk of the exposed population of the districts of Bihar was found to be in the following increasing order Khagaria > Saran > Begusarai > Samastipur > Buxar > Bhojpur > Darbhanga > Munger > Vaishali > Patna > Nalanda.

The hazard quotient study carried also correlates that the infants are more vulnerable to carcinogenic risk followed by their lactating mothers. This risk study also correlates that arsenic poisoning is directly correlated to the child's arsenic poisoning [43, 51, 79–81]. This could further worsen the basic activities, growth, mental growth of the child with the growing age. Hence, there is urgent intervention required to combat the problem in the exposed population especially the lactating mothers and their infants.

Conclusions

The present study concludes that arsenic poisoning is prevalent in the Gangetic plains of Bihar in the studied districts - Buxar, Bhojpur, Patna, Saran, Samastipur, Begusarai, Khagaria, Munger, Darbhanga and Nalanda. The study reports for the first time in these studied districts of Bihar with arsenic poisoning in the biological samples of the lactating mother as well as in their infants. The study demonstrates a direct correlation between contaminated breast milk and infant arsenic toxicity. This could result in substantial health risks for both mothers and infants, as it could harm the development of their children. In addition, neonates who consumed arsenic through their mother's breast milk eliminated only 50% of the arsenic content, while the remaining arsenic content accumulated in their bodies, posing serious health risks. The carcinogenic risk was also very high in the exposed infants followed by their mothers. Therefore, it is essential to implement medical intervention urgently in order to address the current problems. The mothers can be awared for using arsenic free water and food which can prevent her from exposure of arsenic & child through breastmilk. The formulation of health policies by the state government is necessary to mitigate the risk of exacerbated health conditions in newborns who have been exposed to arsenic poisoning.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12940-024-01115-w.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Acknowledgements

The authors are thankful to institute Mahavir Cancer Sansthan and Research Centre, Patna for providing necessary laboratorial and infrastructural facilities. Moreover, the authors are thankful to Indian Council of Medical Research, Government of India (ICMR F.No. 5/10/FR/79/2020-RBMCH, Dated. August 09, 2021) for the financial assistance of the entire research work and partially from DST-WTI project, Government of India (DST/TMD-EWO/WTI/2K19/EWFH/2019/201).

Author contributions

The entire experimental work was conceptualized by A.K and A.K.G. The field work sampling was carried out by R.A, K.K, N.K.C, S.A, A.K. In the manuscript writing A.K, R.A, K.K, D.K., A.B., S.S, S.K., A.S., M.S., and A.K.G., contributed the majority of writing activities, but support was also provided by M.A., T.P., K.S.V. Literature search was done by A.S., R.A, K.K., M.K., N.K.C., P.K.N., & S.A. Figures were developed by A.K., R.A., K.K., S.K. and A.B. The study design was carried out by A.K., and A.K.G. The experimentation was carried out by M.K., R.A, K.K., S.A, P.K.N., and data analysis by A.K., A.K.G., M.A., D.K., A.S., M.S., S.S. and A.B. The final manuscript writing was done by A.K., R.A, D.K., K.K, A.B., S.K., M.S., and A.K.G. All authors read and approved the final manuscript.

Funding

The fund for this research work was provided by the grant from Indian Council of Medical Research (ICMR F.No. 5/10/FR/79/2020-RBMCH, Dated. 09/08/2021), Government of India.

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethical approval

The entire research work was approved from 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, which granted the ethical clearance with IEC Letter No. RMRI/EC/24/2020 dated September 26, 2020. Before the investigation began, all patients were briefed about the study's aims, and signed informed consent was obtained. The study was carried out in accordance with Indian Council of Medical Research, Government of India ethical guidelines for research involving human subjects and the ethical standards of the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 11 July 2024 / Accepted: 9 September 2024
Published online: 20 September 2024

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