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Groundwater poisoning by heavy metals has caused serious health hazards in the exposed population globally. Manganese (Mn) poisoning causing human health hazards is very meagerly reported worldwide. The present research elucidates for the first time the catastrophic effect of manganese causing cancer in the Gangetic plains of Bihar (India). The blood samples of $n=1146$ cancer patients were voluntarily obtained for the study, after their consent. Their household water samples were also collected for the study. All the samples were analysed for Mn contamination by Atomic Absorption Spectrophotometer. The study indicates high Mn contamination in the cancer patient blood samples with highest content as 6022 µg/L. Moreover, the cancer patient's household handpump water samples also contained elevated Mn contamination. The correlation coefficient study finds significant association between Mn contamination in blood of cancer patients and their handpump water. The carcinoma group of cancer patients mostly in Stage III & IV had significant Mn contamination in their blood (above WHO/BIS permissible limit). The geospatial study depicts Mn contamination in handpump water in the state of Bihar in correlation with cancer patient's blood samples. This novel finding is being reported in India for the first time, which correlates cancer with handpump drinking water. The long-term Mn exposure could be one of the causative agents for elevating cancer incidences. However, other confounding risk factors cannot be denied.

Keywords Manganese poisoning, Cancer patients, Geospatial study, Health risk assessment, Bihar

Worldwide, cancer incidents rank high among the leading causes of death and disability. It is estimated that 9.74 million deaths worldwide and 0.91 million deaths in India is due to different types of cancer. By 2040, the global cancer incidence is projected to reach 29.5 million cases, with a corresponding increase in death toll to 16.3 million. This is in addition to the 19.97 million new cases annually recorded globally (GLOBOCAN 2024)[1], and the 1.41 million new cases reported in India alone[2]. Cancer incidences in India are projected to escalate fivefold by 2025, with a 2.8-fold rise attributable to tobacco use and a 2.2-fold increase attributable to ageing and other risk factors, according to the Indian Council of Medical Research (ICMR). The United States and other developed nations are expected to have double that number of cancer cases[3]. Apart from general risk factors such as ageing, family history of cancer, tobacco, alcohol, obesity, viral infection, UV exposure; chemical exposure such as aflatoxins, asbestos, nitrosamines and environmental pollutants/ contaminants are amongst the potential risk factors accounting for 80–90% in developing different types of cancer[4]. The metallic pollutants such as Arsenic (As), Iron (Fe), Zinc (Zn), Cadmium (Cd) and Manganese (Mn) contamination in human biological samples in excess concentrations have been categorized as carcinogens[5]. From various studies it is depicted that Mn is a low mutagenic and carcinogenic metal[6]. But recently, few studies have found significant correlation between cancer incidences and Mn toxicity[7,8].

Essential trace elements play vital role in maintaining body homeostasis but can cause severe toxicity if consumed in excess. Mn is one of the essential trace elements and is the 5th most abundant metal on the earth that exists in the form of oxides, carbonates and silicates. This element is present in food, water, soil, and rock as a naturally occurring component. It acts as co-factor for numerous cellular enzymes involved in carbohydrate, nitrogen, oxygen radical neutralization, glycosaminoglycan, and cholesterol metabolism[9]. Mn has been reported to be toxic in higher doses for human beings[10,11] A major source of exposure to Mn is drinking water extracted

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from groundwater. The cause of water contamination is usually industrial pollution (anthropogenic), or sedimentary or igneous rocks (geogenic) deposits of Mn (e.g., augite and hornblende)[12,13].

Global incidences of high level of Mn in the groundwater have been reported in countries such as Nigeria, Bangladesh, China, Greece, Japan and other countries[14,15] In 1957, the first case of manganese poisoning in India was documented in a group of manganese miners in Chinchiwad, Maharashtra. These miners suffered from a wide range of symptoms, including aches and pains, weakness, clumsiness, anorexia, sleeplessness, emotional instability, and difficulty with gait[16]. Elevated levels of Mn in Murshidabad and 24 Pargana districts of West Bengal and Tumkur, Karnataka[17] have been detected in ground water but such findings have never been reported from the state of Bihar (India). Mn overexposure has etiology in diseases such as neuro degenerative diseases, cardiovascular toxicity, hepatotoxicity, reproductive and developmental toxicity[18]. However, a very few studies have been reported worldwide that would correlate high levels of Mn with cancer. Increased level of serum Mn in specific cancer types such as prostate, colorectal and breast cancer have been well reported[19–21].

The primary absorption site for dietary manganese involves divalent metal transporter 1 (DMT1). However, it may also be absorbed via the lungs after inhalation of Mn containing aerosols[22]. Smelters and welders often inhale Mn that is in the air[23]. Manganese upon inhalation crosses the blood-brain barrier through olfactory tract and via ZIP8 and ZIP14 transporters[24]. At the cellular level, an excess of Mn^{2+} accumulates in mitochondria, which causes toxicity. This is linked to the suppression of enzymes[25,26] in the mitochondria and the overproduction of H_2O_2 by mitochondrial superoxide dismutase, which causes oxidative stress in the cells[27,28] The Fenton reaction produces reactive oxygen species (ROS) and reactive nitrogen species (NOS), which have the potential to cause carcinogenicity and genotoxicity. The normal concentration of manganese in human blood is in the range of 4 to 15 µg/L[29–31].

Bihar (India) has seen a tremendous rise in cancer cases over the last few decades[32]. There may be more than one factors responsible for the development of cancer, but manganese as a trace element is certainly one of them because of the toxicity leading to carcinogenesis. Therefore, the purpose of this research is to ascertain the possible association between handpump drinking water, blood Mn content and different types of cancer in the state of Bihar.

Methods

Ethics approval

The Institutional Ethics Committee (IEC) Letter No. RMRI/EC/24/2020 on September 26, 2020, by the Rajendra Memorial Research Institute of Medical Sciences (MoU for Ethics Approval) in Patna, Bihar, India provided the ethics approval to carry out this work. All studied patients were informed about the research and their consent were obtained before the commencement of the study. The study protocols complied to all applicable ethical guidelines for human subject's research, including those established by the Indian Council of Medical Research, Government of India, and the Declaration of Helsinki.

Study area

The cancer patients were selected from the state of Bihar, located in the eastern part of India (between 24°-20'-10" N to 27°-31'-15" N latitude and 82°-19'-50" E to 88°-17'- 40" longitude) with total area of 94,163.00 sq. kms[33], which includes urban area 1,905.49 sq. kms and rural area 92,257.51 sq. kms and ranks 12th in terms of land area and 3rd in terms of population in the nation[34]. The population density is nearly 3 times of the national average (382 per square kilometer) (Fig. 1).

Selection of subjects

The study subjects were the patients registered in Mahavir Cancer Sansthan and Research Centre for the diagnosis and treatment for cancer. Altogether, $n = 1146$ cancer patients who voluntarily provided their consent were selected for the study. The inclusion criteria for the selection of the subjects were the confirmed cancer patients. The exclusion criteria for the study were the patients who were from the outside the state boundary and the patients did not had any other infectious disease. All the selected subject's written consent were obtained before the collection of their biological samples. In case of minor, their parent's written consent was obtained.

Collection of blood samples from the subjects

For the collection of blood samples, 5mL plain BD vacutainers were used, and 3mL peripheral venous blood was drawn from the left arm of the patients. The blood samples collected were stored at 4^0C until the experimental study was initiated.

Determination of manganese in blood

For the Mn determination, 0.5mL of cancer patients blood samples were added to 5mL of HNO_3 in a 25mL conical flask made up of glass, and the mixture was allowed to react overnight. The samples were heated on a hotplate for 90 to 120 min until the volume reached to 3mL. The pre-digested solution was then combined with 5mL of (6:1) mixture of HNO_3 and $HClO_4$ in the conical flask. The samples were again heated on the hotplate between 90 and 120 degrees Celsius until the solution volume reached to 2mL. Thereafter, 1mL of 1% HNO_3 was added, and then 10 mL of distilled water was added to make the final volume. Finally, the samples were subjected to manganese quantification analysis using the Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS) (Pinnacle 900T, Perkin Elmer, Singapore) operating at a wavelength of 279.48 nm (NIOSH 1994)[35].

Determination of manganese in water samples

The handpump water samples were collected from the household of the cancer patients. The samples were collected in 30 mL polypropylene collection bottles, and was immediately acidified with 2% HNO_3. Thereafter,

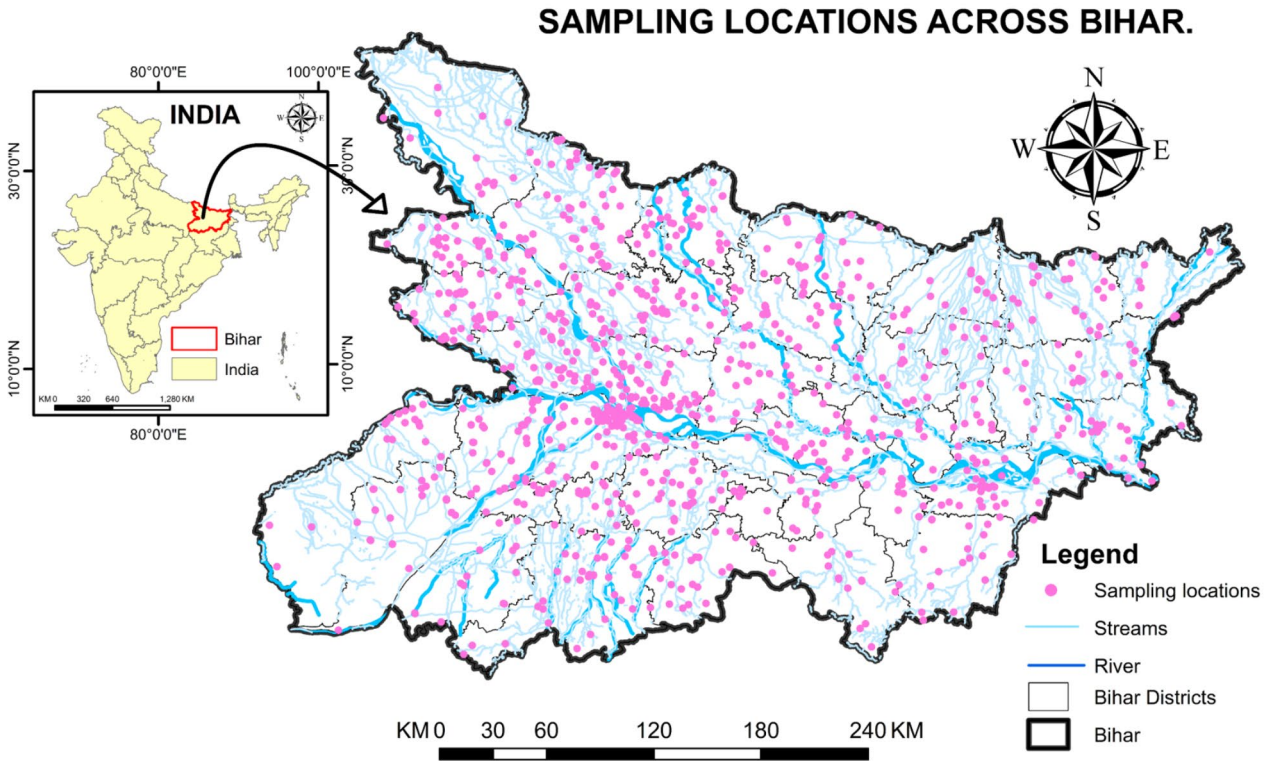

Fig. 1. Location sites of the studied cancer patients (Household Water sample sites).

Parameters	Male	Female	Children	References
IR	2	2	1	USEPA, 2008 & USEPA,2014[34,35]
ED	64	67	12	Narsimha and Rajith,2018, WHO 2013[36,37]
EF	365	365	365	USEPA,2014; Ahada and Suthar 2017[38,39]
BW	65	55	15	ICMR,2009; Narsimha and Rajith,2018[36,40]
AT	23,360	24,455	4380	USEPA, 2008 &USEPA,2014[34,35]
RfD	0.14	0.14	0.14	USEPA,2002; USEPA(IRIS),2011[31,41]
C(mg/L)				Present study

Table 1. Human Health risk assessment (non-carcinogenic risk).

the water samples were filtered using 0.45 µm syringe filter and were evaluated for Mn concentration using Graphite Furnace based Atomic Absorption Spectrophotometer (GF-AAS) of Perkin Elmer model number Pinnacle 900 T (USA) (NIOSH 1994)[35].

Quality control

A dilution of the manganese standard (1000 mg/L) and the standard stock solution was prepared (PerkinElmer Singapore's (CAS no. Pb7439921; Lot No. 25170PBY1; PE No N9300175)). Throughout the study period, the calibration correlation coefficient remained at 0.999. Mn had a detection limit of 0.07 µg/L in blood and 0.03 µg/L in water. For quality assurance, 10% of the samples were retested for the final confirmation.

Geospatial study

The Arc-GIS software (10.3.1) was utilized for geo-spatial analysis. The samples GPS coordinates were superimposed on the shape file of the Bihar district, with Google Maps serving as the basis map. The map showed both bubble and dot representations of the manganese levels in the blood.

Human health risk assessment

In this study, the method of the Environmental Protection Agency (EPA) was used for the calculation of human health risk assessment. Health risk assessment is a way to assess the health of the exposed population due to drinking of high Mn-contaminated handpump water. As per the recent studies, it is assumed that groundwater

is the route of exposure for manganese toxicity of the exposed population of males, females, and children. The average daily dose provides all the information that shows the magnitude of exposure, exposure duration, ingestion rate, frequency, etc. The equation used for the calculation of risk assessment is given below Table 1.

$$ADD = (C \times IR \times EF \times ED)/(BW \times AT)$$

where, ADD = Average Daily Dose. C = Concentration of Mn in groundwater. IR = Ingestion rate. EF = Exposure frequency. ED = Exposure duration. BW = Body weight. AT = Average time.

Hazard quotient

The hazard quotient is the ratio of potential risk of ingestion, inhalation, and dermal exposure of any chemicals to the level at which no adverse effects are expected. Hazard quotient is calculated by dividing the average daily dose with reference dose. The reference dose for manganese is 0.14 mg/kg/day. The carcinogenic risk is not associated yet with manganese contamination but the non-carcinogenic risk has been reported[44–46].

$$HQ = ADD/RfD$$

where, HQ = Hazard Quotient. ADD = Average daily dose. RfD = reference dose i.e. 0.14 mg/kg/day. If the hazard quotient is greater than 1 then it shows, that it has the possible potential of a non-carcinogenic health effect and if the HQ value is less than 1 then it shows no possibility of a non-cancer health effect.

Statistical analysis

The statistical analysis was performed based on the SPSS 16.0 software. The correlation coefficient was measured and graphed. The statistical analysis yielded the data by applying two tailed ANOVA at 95% confidence interval (Table 2).

Results

Age wise

A total of $n = 1146$ cancer patients were analyzed for the blood Mn concentration in the different districts of Bihar, India. The average age of the participants was 45 years (ranging from 2 to 92 years of age) in the study. The $n = 767$ participants out of 1146 were females (67%) and 379 males (33%) respectively. Blood Mn level was found to be < 15 µg/L in $n = 238$ (20.77%) patients while $n = 908$ (79.23%) patients had Mn in blood > 15 µg/L. The patients between the age group 41 to 60 had very high level of blood Mn (ranging from 110 to 550 µg/L) (Figs. 2 and 3).

District wise study

Maximum numbers of patients were recorded from Patna district (116) followed by Vaishali (60), East Champaran (57), Muzaffarpur (52), Siwan (48) and Saran (55) the rest of $n = 758$ patients were from other districts of the state. (Fig. 4).

Cancer type wise

Out of these 1146 confirmed cancer cases, the breast cancer cases were $n = 381$ (33.25%), hepatobiliary and gastrointestinal cancer $n = 309$ (26.96%), cervical cancer $n = 64$ (5.58%) and other cancer types such as oral, nasal, renal, adnexal, penile cancer etc., $n = 398$ (34.78%) respectively. Further, these cancer types were categorized broadly as carcinoma $n = 972$ (84.8%), leukemia $n = 113$ (9.86%), lymphoma $n = 35$ (3%) and sarcoma $n = 26$ (2.27%) respectively (Fig. 5).

S.No	Parameters	For Mn in blood	For Mn in water
1	No. of Observation	1146	1146
2	Min. (µg/L)	0.07	5
3	Max. (µg/L)	6022.00	630.20
4	Avg. (µg/L)	201.60	65.30
5	SD	386.27	74.38
6	Var	149206.86	5532.78
7	SE	11.41	2.20
8	Q1 (µg/L)	17.95	28.9595
9	Median (µg/L)	46.93	38.54
10	Q3 (µg/L)	203.05	59.75
11	*RSD (%)*	191.61	113.92

Table 2. Statistical data analysis. (Min minimum, Max maximum, Avg. Average, SD standard deviation, Var Variance, SE Standard Error, Q1 first quartile, Q3 third quartile, RSD relative standard deviation).

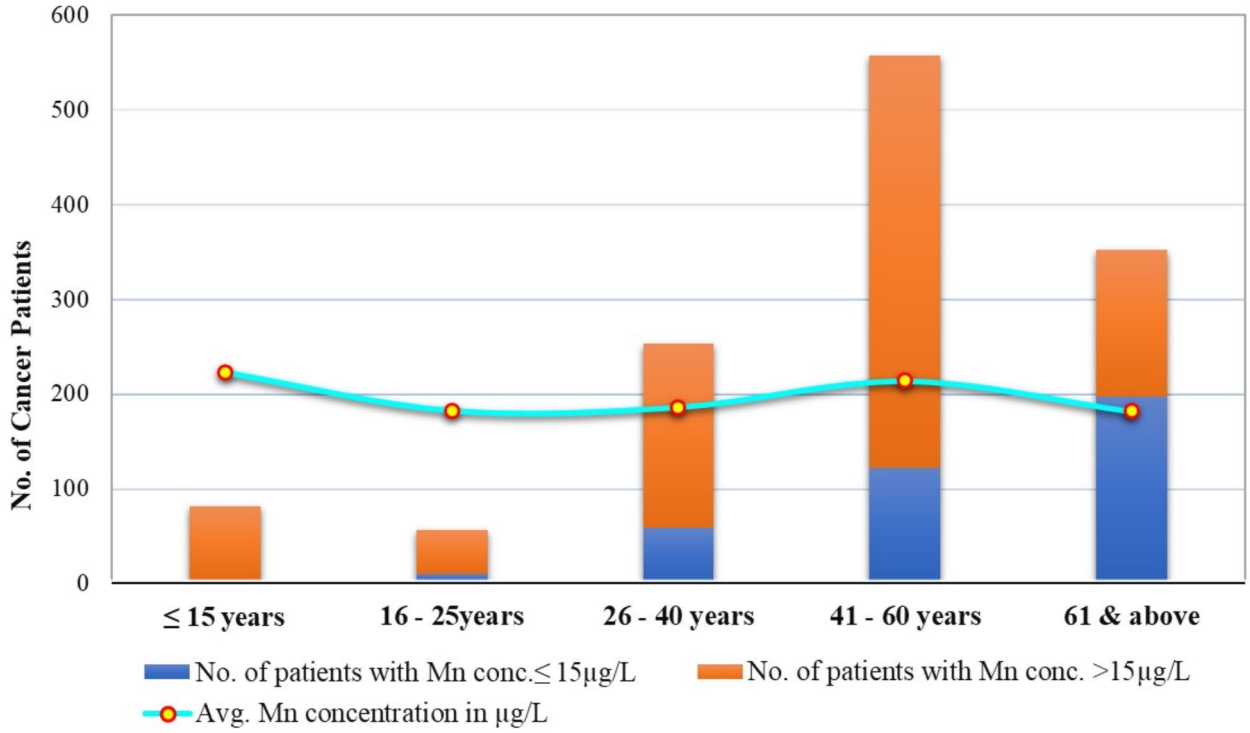

Fig. 2. Age wise distribution of number of cancer patients in Bihar with corresponding average Mn concentration in µg/L. The average Mn content in blood was 199 ± 10.52 µg/L.

Cancer stage wise

Out of total participants $n = 1146$, $n = 32$ (2.8%) cancer patients were in stage I, $n = 173$ (15.1%) in stage II, $n = 414$ (36.1%) in stage III and $n = 526$ (45.9%) in stage IV respectively (Fig. 6A). There has been significant rise in the levels of Mn contamination in the blood samples in relation to the cancer stage. The stage III and IV cancer patients had higher Mn contamination in their blood. (Fig. 6B).

Correlation coefficient

A statistically significant correlation ($r = 0.062$ and $p < 0.05$) was observed between the manganese concentration in the handpump water samples and the blood samples of the cancer patients (Fig. 7).

Manganese concentration in cancer patient's blood samples

The Mn concentration in blood samples of the cancer patients showed normal levels < 15 µg/L in $n = 235$ (20.5%) patients, while $n = 331$ (28.8%) had levels between 16 and 50 µg/L, $n = 159$ (13.8%) between 51 and 100 µg/L, $n = 249$ (21.7%) between 101 and 500 µg/L, $n = 98$ (8.5%) between 501 and 1000 µg/L, $n = 48$ (4.1%) and between 1001 and 5000 µg/L respectively. Strikingly, the highest level of Mn observed was 6022 µg/L in one patient having liver cancer while $n = 56$ (4.8%) patients had Mn level below 1 µg/L. The average Mn content in blood was 199 ± 10.52 µg/L (Fig. 8).

Manganese concentration in cancer patient's household handpump water samples

The Mn concentration in handpump water samples of the cancer patient's household showed [Bureau of Indian Standards (BIS)] normal levels < 100 µg/L in $n = 972$ (84.8%), while $n = 97$ (8.4%) had levels between 100 and 200 µg/L, $n = 40$ (3.4%) between 200 and 300 µg/L, $n = 30$ (2.6%) between 300 and 400 µg/L and $n = 7$ (0.6%), above 400 µg/L respectively. The average Mn content in handpump water was 65.30 ± 2.198 µg/L (Fig. 9).

Geospatial analysis

The geospatial analysis of manganese concentration in blood samples of cancer patients showed significant distribution in the middle Gangetic plain region of Bihar. However, high concentration was also found in south western and north eastern part of the state (Figs. 10 and 11). The geomapping also showed significant correlation between handpump household water Mn concentration with blood Mn concentration of cancer patients (Fig. 12A). The Mn concentration in handpump water samples exceeding the BIS limits (100 µg/L) in comparison to blood Mn concentration (WHO limit 15 µg/L) of cancer patients showed significant correlation. However, there were $n = 15$ blood Mn concentrations samples had inverse relation between handpump water and blood (Fig. 12).

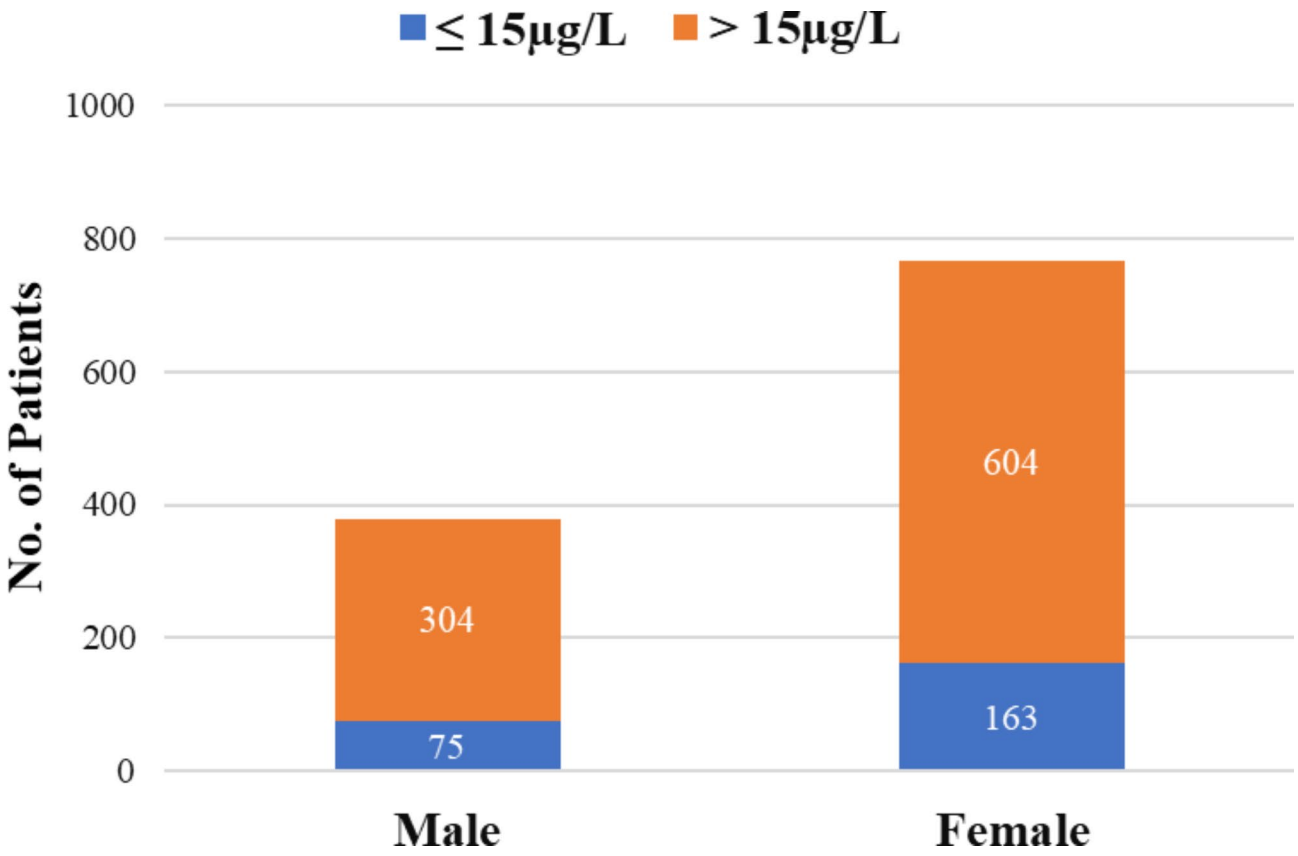

Fig. 3. Comparison of Mn concentration in the blood of male and female with cancer incidences from different sites of Bihar ($n = 1146$).

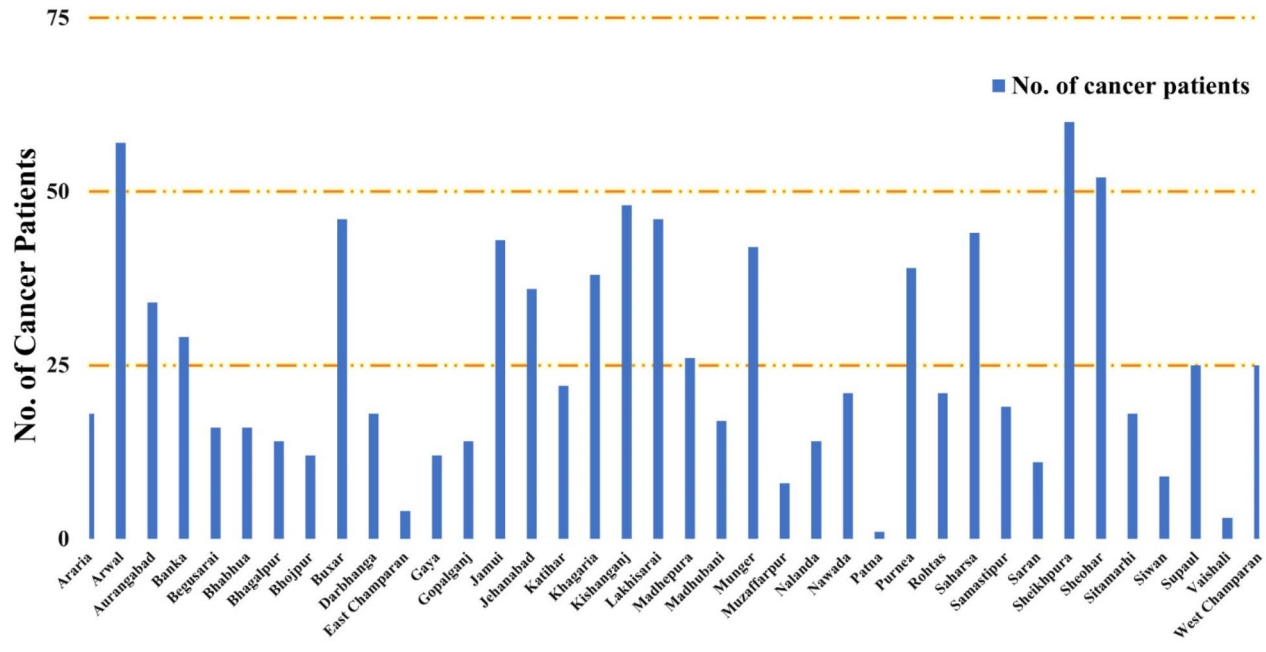

Fig. 4. District wise distribution of cancer patients from different districts of Bihar ($n = 1146$).

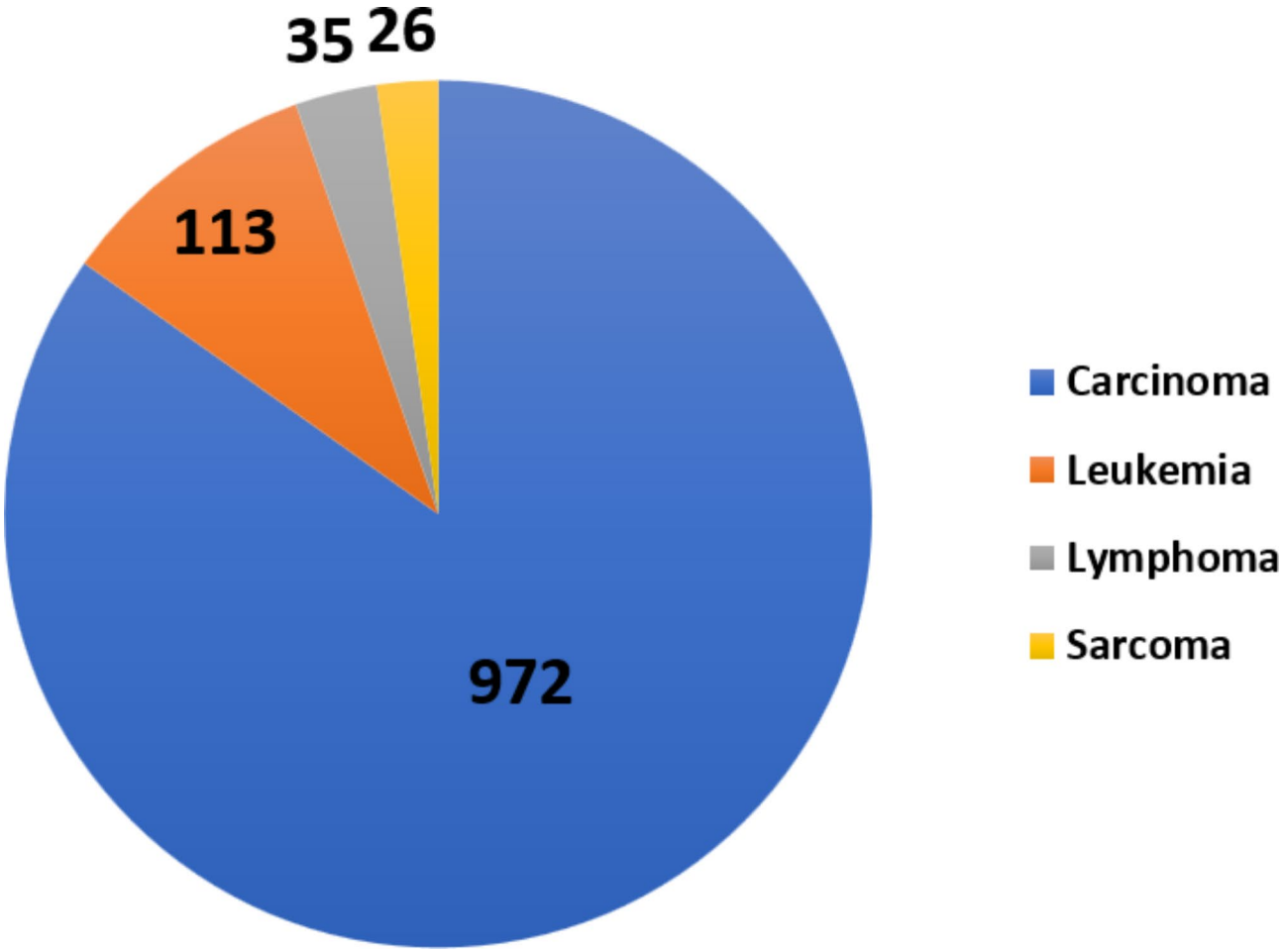

Fig. 5. Cancer type distribution ($n = 1146$).

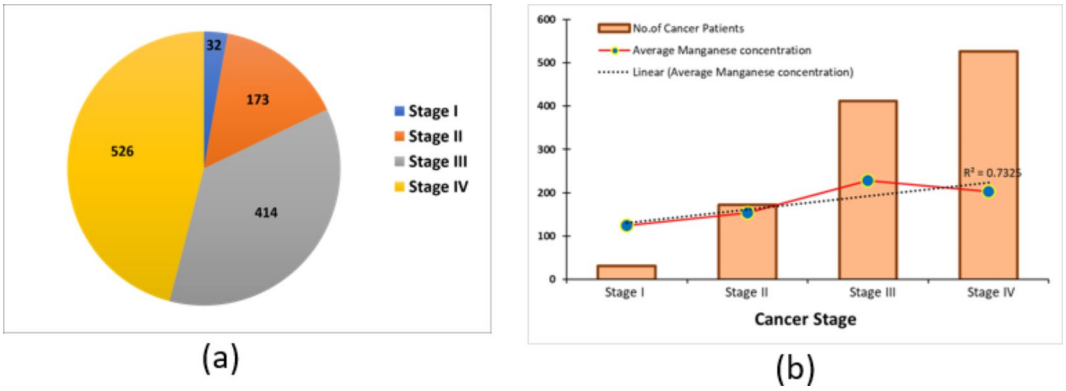

Fig. 6. (**A**) Stagewise distribution of cancer patients ($n = 1146$). (**B**) Stagewise distribution of cancer in relation to average blood Mn concentration in patients ($n = 1146$).

Health assessment

Being the representative non-carcinogenic risk, the HQ data manifested that 4.54% of females, 5.52% of males, and 6.58% of children are at higher risk of non-carcinogenic consequences (at CI 95%). The highest risk of non-carcinogenic was observed in children due to their low body weight as compared to adult males and females (Fig. 13A). The present study shows significant carcinogenic association between the Mn exposure in the cancer patients with different cancer types for the first time, besides non-carcinogenic risks (Fig. 13B).

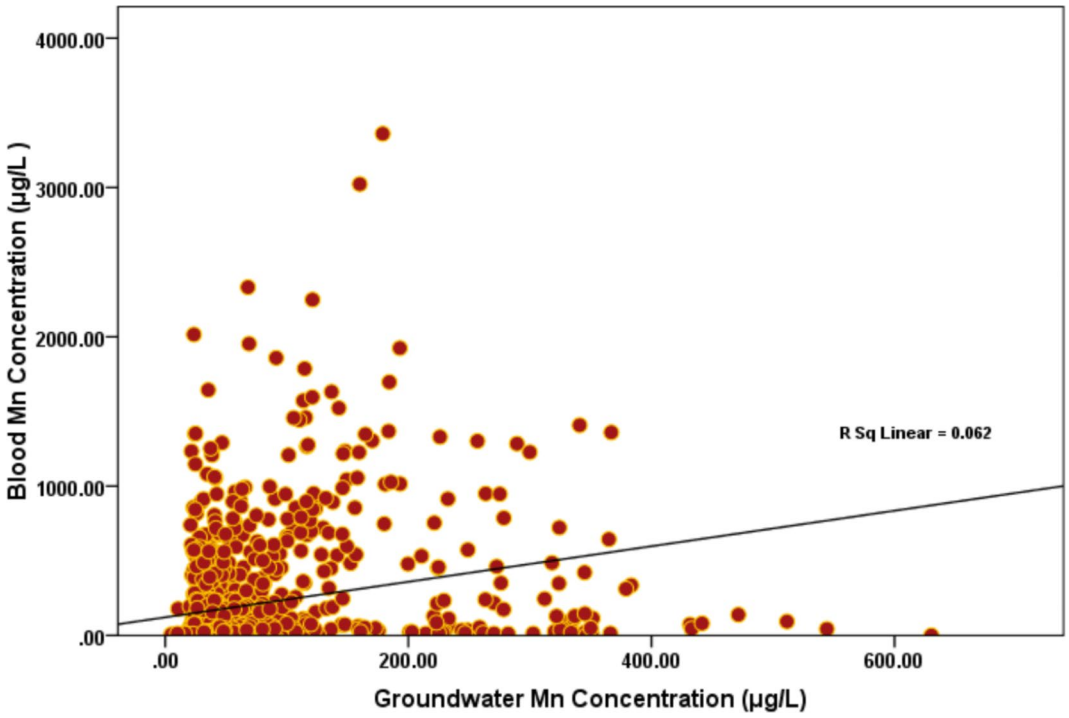

Fig. 7. Correlation coefficient study between Cancer patient's blood and their handpump water Mn concentration ($n = 1146$) ($r^2 = 0.062$).

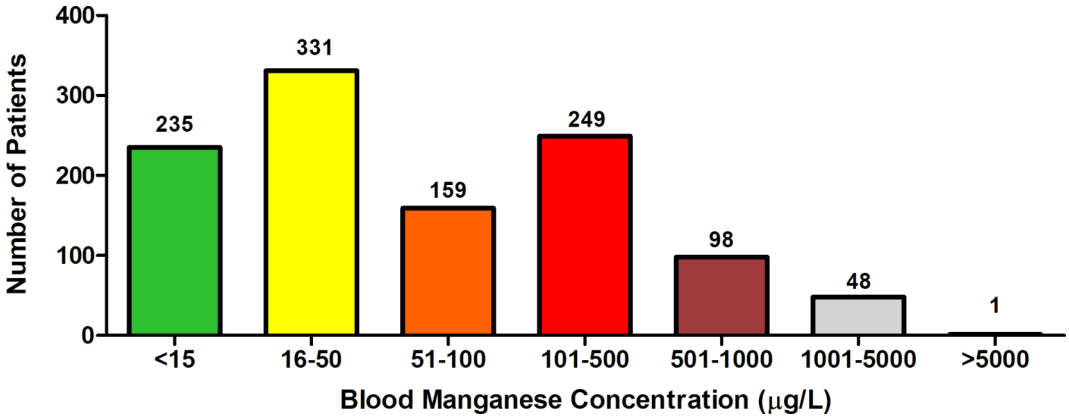

Fig. 8. Mn concentration in blood samples of cancer patients ($n = 1146$).

Discussion

In the present investigation, the study was conducted on $n = 1146$ cancer patients to understand the Mn exposure levels in them. The findings showed that two third of the studied patients were female cancer patients while one third were the male cancer patients. The gender wise Mn contamination in blood showed that 7% of the male cancer patients had Mn concentration < 15 µg/L while 93% had Mn concentration > 15 µg/L which depicts that Mn poisoning in male patient is higher in the percentage level. As far as the cancer patients from the various districts of Bihar is concerned, the maximum number of cancer patients with Mn poisoning were majorly from Patna district (116 cases) followed by Vaishali district (60 cases), East Champaran district (57 cases), Saran district (55 cases), Muzaffarpur district (52 cases), Vaishali district (48 cases) and the rest $n = 758$ from other districts of the state. However, the low Mn concentration in the blood samples of cancer patients were observed in Kaimur, Sheikhpura and Arwal districts of the state. Moreover, there was significant correlation observed between Mn contaminations with the higher cancer stages. The stage III and IV had the maximum Mn contaminations with higher cancer stages. One of the reasons for this is that rural patient's come to this cancer institute in very late stage due to ignorance and lack of early screening facilities.

The geospatial study of Mn concentrations in blood samples of $n = 1146$ cancer patients revealed substantial dispersion in Bihar's middle Gangetic plain area. However, significant levels were observed in the state's south

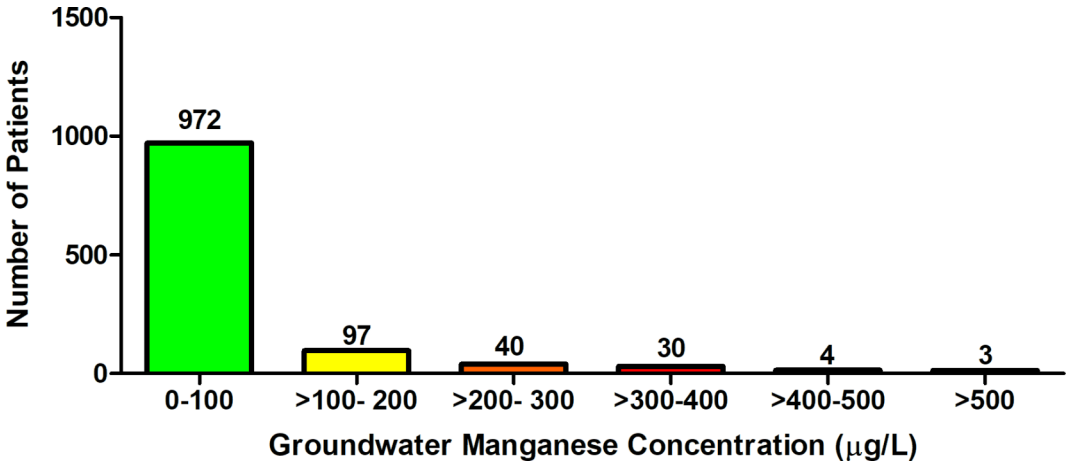

Fig. 9. Mn concentration in household handpump water samples of cancer patients ($n = 1146$).

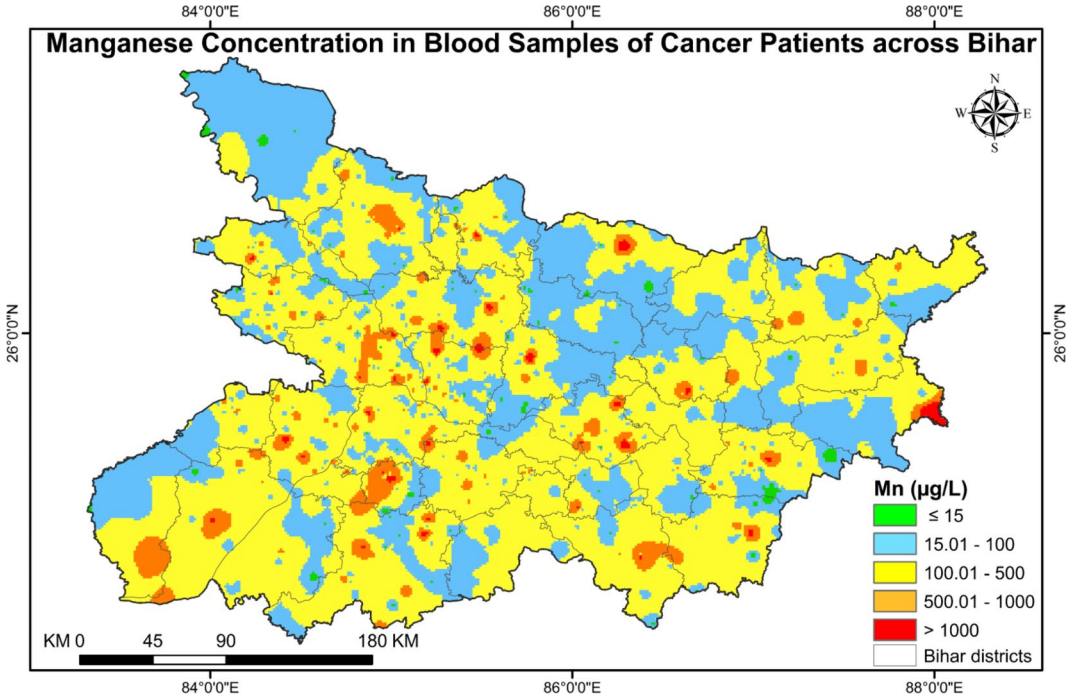

Fig. 10. Mn concentration in blood samples of cancer patients across Bihar ($n = 1146$).

western and north eastern region. This denotes that the water source in this region has higher concentration of Mn which these cancer patients were drinking for a long-time period. Geologically, Bihar is essentially an alluvium dominated topography with 92.7% alluvium and rest comprising rock exposure. To the north of Ganga, an in-situ soil profile is difficult to achieve as the sediments being transported from the extra peninsular region through Himalayan bound rivers comprise the thick sequence of alluvium in almost all districts of North Bihar. Likewise, the rivers draining from the rocky uplands of South Bihar (flowing from south to north) carry sediments. The permissible limit for Mn in drinking water is BIS standards for water as 100 µg/L.[47] WHO standard for Mn in soil is 500 mg/Kg and in vegetable is 0.42–6.64 mg/Kg. Blood Mn levels typically vary from 4 to 15 µg/L, whereas urine levels typically range from 1 to 8 µg/L. Along with iron, it is one of the most common metals found in Earth's crust and a transition metal. Although it does not occur in nature in its pure (elemental) form, it is a component of more than 100 minerals[29]. The compounds containing manganese in its most ecologically and physiologically significant forms ($Mn^{2+,}$ Mn^{4}+, or Mn^{7+}) are among its eleven oxidation states. Its complex formation capabilities are extensive, allowing it to bind with oxygen, sulfur, chlorine, carbonates, and silicates, among many other elements[2948] Several major health problems have resulted from either little or excessive Mn exposure. Overexposure to it may induce neurological damage, while its deficiency is linked to skin lesions and bone modeling and remodeling illnesses[4950] The majority of the population's manganese exposure comes from

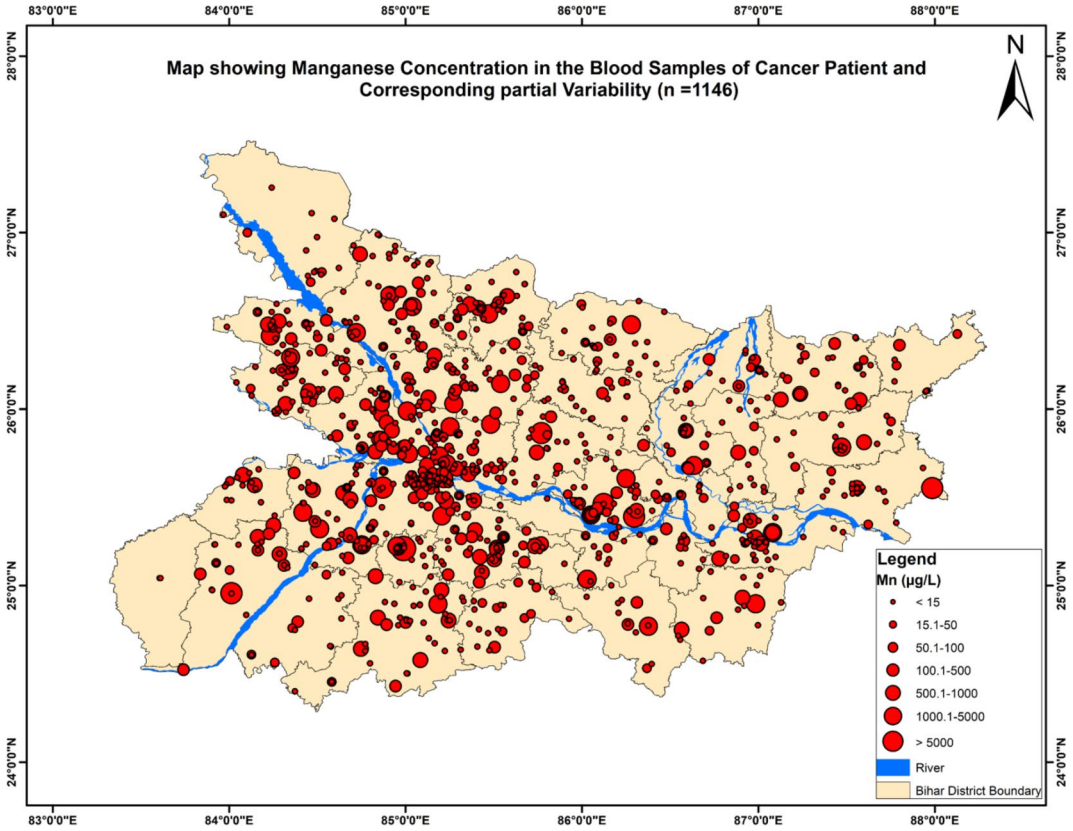

Fig. 11. Geomapping showing blood Mn concentration in cancer patients from different sites of Bihar ($n = 1146$).

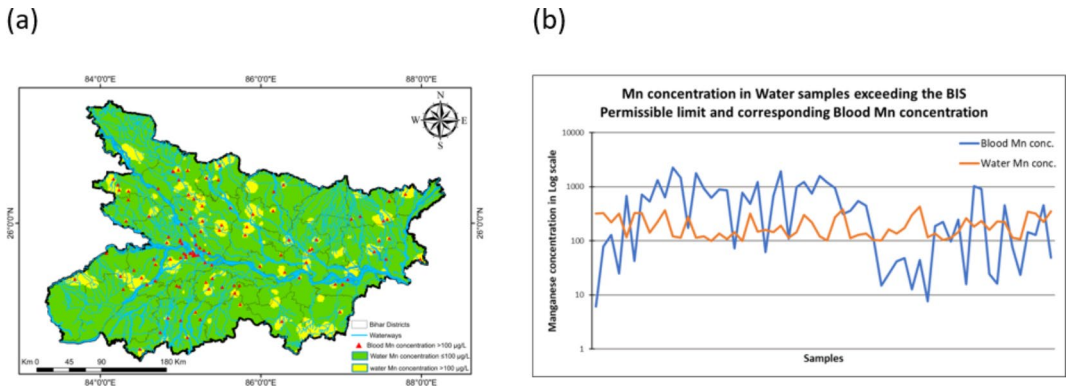

Fig. 12. (**A**) Geomapping showing handpump household water Mn concentration in comparison with blood Mn concentration of cancer patients from different sites of Bihar ($n = 1146$). (**B**) Line graph indicating the relationships among the Mn concentrations in water samples, blood samples and permissible limit prescribed by BIS.

food such as rice and whole grains. In addition to shellfish, other healthy sources include tea, beans, seeds, chocolate, and leafy green vegetables are the major source of Mn exposure[29,51–55] Geologically, anoxic conditions do lead to enrichment of heavy metals like Mn and in general the higher spot values appear to be confined to fluvial bodies including Ganga particularly in districts of Patna, Bhagalpur etc. along with the belt of Kosi flood affected areas[56–58] The activity of manganese has been reported[59] to be high under waterlogging conditions. Pockets with water logging and anoxic conditions thus needs to be studied in detail for enrichment of transported Mn geogenically from distant sources (like Himalayas) and possible increased Mn concentration[60–62].

Mn is absorbed in the human body through gastrointestinal tract and is distributed into various vital tissues such as liver, pancreas, bone, kidney and brain. The chronic Mn exposure causes decrease in the levels of choline in the hypothalamus and thalamus[63]. The alpha synuclein usually attributes to the Mn homeostasis in neurons

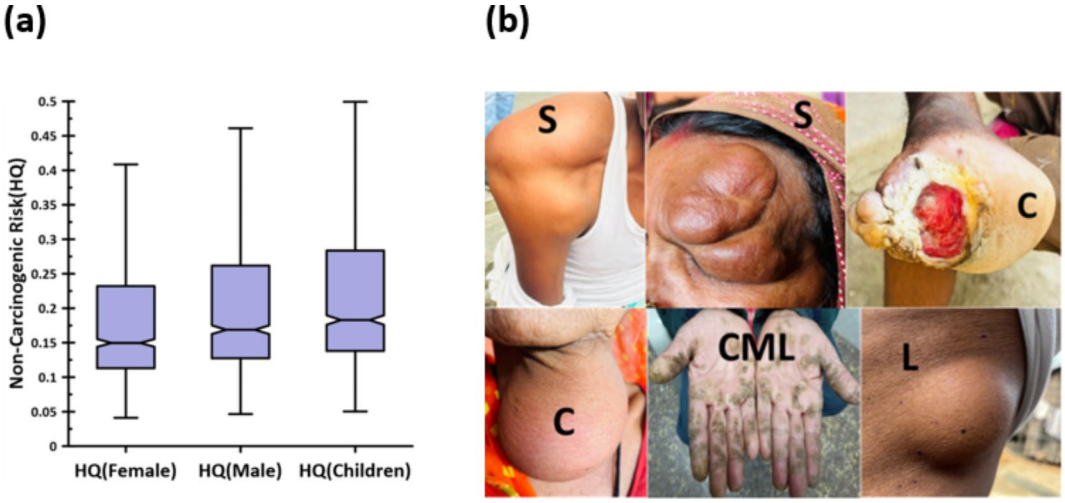

Fig. 13. (**A**) Non-carcinogenic risk (HQ) in female vs. Male Vs Children.(**B**) Showing cancer type in correlation with Mn exposure in the exposed cancer patients. (S-sarcoma, C-carcinoma, CML- Leukemia & L-lymphoma).

causing neurotoxicity[64]. However, various studies have validated that due to long term exposure of Mn could lead to mutation in the SLC30A10 gene resulting in diseases such as parkinsonism with hypermagnesemia along with dystonia, polycythemia and chronic liver disorders[65–67].

In the present study, the Mn concentration in drinking water was with highest concentration as 630 µg/L with $n = 174$ water sources having Mn levels higher than the BIS permissible limit of 100 µg/L. The correlation coefficient study shows significant positive correlation between blood Mn concentration versus Mn contaminated handpump water. The study indicates that water is the major source of cancer caused in the studied cancer patients. The Mn concentration in blood samples of the cancer patients showed that out of $n = 1146$, only 20% of the patients had normal Mn levels (< 15 µg/L), while rest 80% patients had high Mn concentration with highest levels as 6022 µg/L. The health assessment study shows that there is non-carcinogenic risk in correlation with water Mn levels and blood Mn levels in the exposed population. But, the present study reports Mn contamination in the cancer patients for the first time. Zhang et al.[68], in their study have reported the relationship between Mn concentrations in rural drinking water with cancer incidences and mortality in Huai'an city in China. Bacquart et al.,[69] reported in their study the Mn contamination in groundwater of West Bengal of India, which denotes the natural presence of Mn in the Gangetic plains of India.

Moreover, the increased Mn concentration in the cancer patients causes release of reactive oxygen species (ROS), which leads to disruption in various signaling pathways. It also interferes the functions of the cell organelles such as mitochondria, Golgi complex including heat-shock proteins (HSP70 and HSP40)[70]. Under the cytotoxic condition, Mn concentrations causes collapsed functions of Golgi complex and mitochondria leading to cellular trafficking[71]. Mn superoxide is found in mitochondria is suppressed due to higher Mn concentrations in blood leading to the cause of carcinogenesis[1072–75] Spangler & Reid[76] have reported the Mn exposure through airborne and water exposure in the population of North Carolina in United States. The study reports that for each log increase in the groundwater Mn concentration there was a corresponding county level increase of 12.10 deaths/100,000 population in all site cancer rates while for airborne was 8.10 deaths/100,000 population in all site cancer. Yan et al.[77] have correlated the Mn and other heavy metals for the cause of liver and gastric cancer in the exposed population of China. Wojcik et al.[78] in a study in United States, found Mn and other heavy metals in the toenails in correlation with breast cancer.

Similar studies have been carried out on other heavy metals such as arsenic in which authors have documented the association between these heavy metals and cancer incidences in the Gangetic plains of Bihar along with the assessment of the disease burden[79–87].

Conclusion

The study indicates that there has been significant manganese contamination found in the blood samples of the cancer patients. This novel finding is the first reporting in India, which correlates Mn contamination in handpump drinking water with exposed humans. The long-term Mn exposure could be one of the causes of the cancer incidences in the exposed area. Hence, further studies are required in this field with emphasis on areas with high Mn contamination in food chain and prevalence of cancer incidences. There is need of medical interventions from the government agencies to combat the present Mn poisoning issues. This will enable the cancer incidence rate to be controlled in the exposed population in the specific locations from this finding. Furthermore, remedial process of Mn poisoning needs to be implemented in the exposed regions.

Data availability

The data can be shared on reasonable request from the corresponding author.

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References:

1. Bray, F. et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA A Cancer Journal for Clinicians* **74**(3), 229–263. https://doi.org/10.3322/caac.21834 (2024).
2. International Agency for Research on Cancer. *World Health Organization* (2018).
3. Kumar, A. et al. Arsenic causing gallbladder cancer disease in Bihar. *Scientific Reports* **13**(1), 4259. https://doi.org/10.1038/s41598-023-30898-0 (2023).
4. Heidelberger, C. Chemical carcinogenesis. *Cancer* **40**(1 Suppl), 430–433. https://doi.org/10.1002/1097-0142(197707)40:1+%3c430::aid-cncr2820400703%3e3.0.co;2-b (1977).
5. Coradduzza, D. et al. Heavy metals in biological samples of cancer patients: A systematic literature review. *Biometals* **37**(4), 803–817. https://doi.org/10.1007/s10534-024-00583-4 (2024).
6. Assem, F. L., Holmes, P. & Levy, L. S. The mutagenicity and carcinogenicity of inorganic manganese compounds: A synthesis of the evidence. *Journal of Toxicology and Environmental Health: Part B Critical Reviews* **14**(8), 537–570. https://doi.org/10.1080/10937404.2011.615111 (2011).
7. Spangler, J. G. Air manganese levels and chronic liver disease mortality in North Carolina counties: An ecological study. *International Journal of Environmental Research and Public Health* **9**(9), 3258–3263. https://doi.org/10.3390/ijerph9093258 (2012).
8. Skalny, A. V. Bioelementology as an interdisciplinary integrative approach in life sciences: Terminology, classification, perspectives. *Journal of Trace Elements in Medicine and Biology* **25**(Suppl 1), S3–S10. https://doi.org/10.1016/j.jtemb.2010.10.005 (2011).
9. Erikson, K. M., Syversen, T., Aschner, J. L. & Aschner, M. Interactions between excessive manganese exposures and dietary iron-deficiency in neurodegeneration. *Environmental Toxicology and Pharmacology* **19**(3), 415–421. https://doi.org/10.1016/j.etap.2004.12.053 (2005).
10. ATSDR. *Toxicological Profile for Manganese (Draft for Public Comment)* (US Department of Health and Human Services, Public Health Service, 2008).
11. Podgorski, J., Araya, D. & Berg, M. Geogenic manganese and iron in groundwater of Southeast Asia and Bangladesh—Machine learning spatial prediction modeling and comparison with arsenic. *The Science of the Total Environment* **833**, 155131. https://doi.org/10.1016/j.scitotenv.2022.155131 (2022).
12 Islam, M. S. & Mostafa, M. G. Occurrence, source, and mobilization of iron, manganese, and arsenic pollution in shallow aquifer. *Geofluids* https://doi.org/10.1155/2023/6628095 (2023).
13. Kondakis, X. G., Makris, N., Leotsinidis, M., Prinou, M. & Papapetropoulos, T. Possible health effects of high manganese concentration in drinking water. *Archives of Environmental Health.* **44**(3), 175–178 (1989).
14. Frisbie, S. H., Ortega, R., Maynard, D. M. & Sarkar, B. The concentrations of arsenic and other toxic elements in Bangladesh's drinking water. *Environmental Health Perspectives* **110**(11), 1147–1153 (2002).
15. Niyogi, T. P. Chronic manganese poisoning. *IndianJ. IndustMed.* **3**, 3–13 (1958).
16. Ramakrishnaiah, C. R., Sadashivaiah, C. & Ranganna, G. Assessment of water quality index for the groundwater in Tumkur Taluk, Karnataka State, India. *E-Journal of Chemistry* **6**(2), 523–530 (2009).
17. O'Neal, S. L. & Zheng, W. Manganese toxicity upon overexposure: A decade in review. *Current Environmental Health Reports* **2**(3), 315–328. https://doi.org/10.1007/s40572-015-0056-x (2015).
18. Asaad, J. I., Al-Mamoori, A. M., Shukri, H. & Ghlem, H. A. A. Microelements levels of Iron, Manganese, Zinc, Copper, Lead, Cadmium, and Nickel in the serum samples of Iraq prostate cancer patients. *International Journal of PharmTech Research* **9**(9), 341–346 (2016).
19. Choi, R. et al. Serum trace elements and their associations with breast cancer subgroups in Korean breast cancer patients. *Nutrients* **11**(1), 37 (2018).
20. Milde, D., Novák, O., Stuzka, V., Vyslouzil, K. & Machácek, J. Serum levels of selenium, manganese, copper, and iron in colorectal cancer patients. *Biological Trace Element Research* **79**, 107–114 (2001).
21. Nadaska, G., Lesny, J. & Michalik, I. *Environmental Aspect of Manganese Chemistry* 1–16 (2012). http://heja.szif.hu/ENV/ENV_100702-A/env100702a.pdf.
22. Korczynski, R. E. Occupational health concerns in the welding industry. *Applied Occupational and Environmental Hygiene* **15**(12), 936–945 (2000).
23. Zoni, S., Bonetti, G. & Lucchini, R. Olfactory functions at the intersection between environmental exposure to manganese and Parkinsonism. *Journal of Trace Elements in Medicine and Biology* **26**(2–3), 179–182 (2012).
24. Gunter, T. E., Gavin, C. E. & Gunter, K. K. The case for manganese interaction with mitochondria. *Neurotoxicology* **30**(4), 727 (2009).
25. Zheng, W., Ren, S. & Graziano, J. H. Manganese inhibits mitochondrial aconitase: A mechanism of manganese neurotoxicity. *Brain Research* **799**(2), 334–342 (1998).
26. Chen, J. Y., Tsao, G. C., Zhao, Q. & Zheng, W. Differential cytotoxicity of Mn(II) and Mn(III): Special reference to mitochondrial [Fe-S] containing enzymes. *Toxicology and Applied Pharmacology* **175**(2), 160–168 (2001).
27. Fernandes, J. et al. From the cover: manganese stimulates mitochondrial H2O2 production in SH-SY5Y human neuroblastoma cells over physiologic as well as toxicologic range. *Toxicological Sciences* **155**(1), 213–223 (2017).
28. Khan, K. et al. Manganese exposure from drinking water and children's academic achievement. *Neurotoxicology* **33**(1), 91–97 (2012).
29. ATSDR. *Toxicological profile for manganese (Draft for Public Comment)* (U.S. Department of Health and Human Services, Public Service, 2012).
30. Zhang, L. L. et al. Baseline blood levels of manganese, lead, cadmium, copper, and zinc in residents of Beijing suburb. *Environmental Research* **140**, 10–17 (2015).
31. USEPA. Integrated Risk Information System (IRIS) (2002). http://cfpub.epa.gov/ncea/iris/index.cf-m?fuseaction=iris.showSubstanceList. Accessed 21 June 2024.
32. Kumar, A. et al. Arsenic exposure in Indo Gangetic plains of Bihar causing increased cancer risk. *Scientific Reports* **11**(1), 2376. https://doi.org/10.1038/s41598-021-81579-9 (2021).
33. Roy, L. B., Bhushan, M. & Kumar, R. Climate change in Bihar, India: A case study. *Journal of Water Resource and Hydraulic Engineering* **5**(3), 140–146 (2016).
34. Ranjan, R. K. Millennium Development Goals and Poverty in Bihar–A Regional Approach. *IRJMSH* **7**(1), 390–398 (2016).
35. 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).
36. USEPA. *U.S. Environmental Protection Agency Washington, DC 20460, EPA/600/R-07/045F* (2008).
37. USEPA. *Human Health Evaluation Manual, Supplemental Guidance: Update of Standard Default Exposure Factors* (2014). https://www.epa.gov/sites/default/files/2015-11/documents/oswer_directive_9200.1-120_exposurefactors_corrected2.pdf
38. Narsimha, A. & Rajitha, S. 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 (2018).

39. *WHO. World Health Statistics* (2013).
40. USEPA (US Environmental Protection Agency). *A Risk Assessment–Multiway Exposure Spreadsheet Calculation Tool* (United States Environmental Protection Agency, 1999).
41. Ahada, C. P. & Suthar, S. Assessment of human health risk associated with high groundwater fluoride intake in southern districts of Punjab, India. *Exposure and Health* **11**(4), 267–275. https://doi.org/10.1007/s12403-017-0268-4 (2019).
42. ICMR (Indian Council of Medical Research). *Nutrient 566 Requirements and Recommended 567 Dietary Allowances for Indians* (Indians National Institute of Nutrition, 2009).
43. U.S. Environmental Protection Agency. *Integrated Risk Information System (IRIS) on Manganese* (National Centre for Environmental Assessment, Office of Research and Development, 2011). Retrieved from https://iris.epa.gov.
44. Markiv, B., Expósito, A., Ruiz-Azcona, L., Santibáñez, M. & Fernández-Olmo, I. Environmental exposure to manganese and health risk assessment from personal sampling near an industrial source of airborne manganese. *Environmental Research* **224**, 115478. https://doi.org/10.1016/j.envres.2023.115478 (2023).
45. Ghosh, G. C. et al. Human health risk assessment of elevated and variable iron and manganese intake with arsenic-safe groundwater in Jashore, Bangladesh. *Scientific Reports* **10**(1), 5206. https://doi.org/10.1038/s41598-020-62187-5 (2020).
46. Sharma, G. K. et al. Evaluating the geochemistry of groundwater contamination with iron and manganese and probabilistic human health risk assessment in endemic areas of the world's largest River Island, India. *Environmental toxicology and pharmacology* **87**, 103690. https://doi.org/10.1016/j.etap.2021.103690 (2021).
47. BIS. Methods of Sampling and Test (Physical and Chemical) for Water and Wastewater: Part 59 Manganese (First Revision), IS 3025 (Part 59) (2006).
48. Stokes, P. M. & NRCC (National Research Council of Canada). *Manganese in the Canadian Environment* (NRCC, 1988). [Cited in Health Canada, 2019.]
49. Crossgrove, J. & Zheng, W. Manganese toxicity upon overexposure. *NMR in Biomedicine* **17**(8), 544–553. https://doi.org/10.1002/nbm.931 (2004).
50. Racette, B. A. et al. Pathophysiology of manganese-associated neurotoxicity. *Neurotoxicology* **33**(4), 881–886. https://doi.org/10.1016/j.neuro.2011.12.010 (2012).
51. Keen, C. L., Ensunsa, J. L. & Clegg, M. S. Manganese metabolism in animals and humans including the toxicity of manganese. *Metal Ions in Biological Systems* **37**, 89–121 (2000).
52. Watts, D. L. The nutritional relationships of manganese. J. Orthomolec. Med. 219–222 (1990).
53 Barbeau, A. Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C. Cotzias). *Neurotoxicology* **5**(1), 13–35 (1984).
54. Cowan, D. M. et al. Manganese exposure among smelting workers: relationship between blood manganese-iron ratio and early onset neurobehavioral alterations. *Neurotoxicology* **30**(6), 1214–1222. https://doi.org/10.1016/j.neuro.2009.02.005 (2009).
55. Freeland-Graves, J. H., Mousa, T. Y. & Kim, S. International variability in diet and requirements of manganese. *J. Trace Elem. Med. Biol.* **38**, 24–32 (2016).
56. IOM. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc* 285–324 (Institute of Medicine, Food and Nutrition Board. Washington, DC, National Academy Press, 2001).
57. USEPA. *Analysis of National Occurrence of the 1998 Contaminant Candidate List (CCL) Regulatory Determination Priority Contaminants in Public Water Systems* (Office of Water. EPA 815-D-01-002, 2002). https://www.epa.gov/sites/production/files/2014-09/documents/support_cc1_nation-occur_analysis.pdf.
58. Kohl, P. M. & Medlar, S. J. *Occurrence of manganese in drinking water and manganese control* (American Water Research Foundation, American Water Works Association and IWA Publishing, 2006).
59. Ljung, K. & Vahter, M. Time to re-evaluate the guideline value for manganese in drinking water?. *Environmental Health Perspectives* **115**, 1533–1538 (2007).
60. Ganvir, P. S. & Guhey, R. An implication of enhanced rock weathering on the groundwater quality: A case study from Wardha Valley Coalfields, Central India. Weathering and Erosion Processes in the Natural Environment. https://doi.org/10.1002/9781394157365.ch9 (2023).
61. Singh, V. B., Madhav, S., Pant, N. C. & Shekhar, R. (Eds.). *Weathering and Erosion Processes in the Natural Environment* (Wiley, 2023).
62. Civardi, J. & Tompeck, M. Iron and Manganese Removal Handbook 2nd Edn. (American Water Works Association, 2015).
63. Bagga, P. & Patel, A. B. Regional cerebral metabolism in mouse under chronic manganese exposure: Implications for manganism. *Neurochemistry International* **60**(2), 177–185. https://doi.org/10.1016/j.neuint.2011.10.016 (2012).
64. Dučić, T. et al. Alpha-synuclein regulates neuronal levels of manganese and calcium. *ACS Chemical Neuroscience* **6**(10), 1769–1779. https://doi.org/10.1021/acschemneuro.5b00093 (2015).
65. Hutchens, S. et al. Hepatic and intestinal manganese excretion are both required to regulate brain manganese during elevated manganese exposure. *American Journal of Physiology Gastrointestinal and Liver Physiology* **325**(3), G251–G264. https://doi.org/10.1152/ajpgi.00047.2023 (2023).
66. Nishito, Y. et al. Direct comparison of manganese detoxification/efflux proteins and molecular characterization of ZnT10 protein as a manganese transporter. *The Journal of Biological Chemistry* **291**(28), 14773–14787. https://doi.org/10.1074/jbc.M116.728014 (2016).
67. Zogzas, C. E., Aschner, M. & Mukhopadhyay, S. Structural elements in the transmembrane and cytoplasmic domains of the metal transporter SLC30A10 are required for its manganese efflux activity. *The Journal of Biological Chemistry* **291**(31), 15940–15957. https://doi.org/10.1074/jbc.M116.726935 (2016).
68. Zhang, Q. et al. Study on the relationship between manganese concentrations in rural drinking water and incidence and mortality caused by cancer in Huai'an city. *BioMed Research International* **2014**, 645056. https://doi.org/10.1155/2014/645056 (2014).
69. Bacquart, T. et al. A survey of arsenic, manganese, boron, thorium, and other toxic metals in the groundwater of a West Bengal, India neighbourhood. *Metallomics* **4**(7), 653–659. https://doi.org/10.1039/c2mt20020a (2012).
70. Fernandes, J. et al. Transcriptome analysis reveals distinct responses to physiologic versus toxic manganese exposure in human neuroblastoma cells. *Frontiers in Genetics* **10**, 676. https://doi.org/10.3389/fgene.2019.00676 (2019).
71. Carmona, A. et al. Manganese accumulates within golgi apparatus in dopaminergic cells as revealed by synchrotron X-ray fluorescence nanoimaging. *ACS Chemical Neuroscience* **1**(3), 194–203. https://doi.org/10.1021/cn900021z (2010).
72. Gavin, C. E., Gunter, K. K. & Gunter, T. E. Mn2+ sequestration by mitochondria and inhibition of oxidative phosphorylation. *Toxicology and Applied Pharmacology* **115**(1), 1–5. https://doi.org/10.1016/0041-008x(92)90360-5 (1992).
73. Chuang, T. C. et al. Human manganese superoxide dismutase suppresses HER2/neu-mediated breast cancer malignancy. *FEBS Letters* **581**(23), 4443–4449. https://doi.org/10.1016/j.febslet.2007.08.021 (2007).
74. Zejnilovic, J., Akev, N., Yilmaz, H. & Isbir, T. Association between manganese superoxide dismutase polymorphism and risk of lung cancer. *Cancer Genetics and Cytogenetics* **189**(1), 1–4. https://doi.org/10.1016/j.cancergencyto.2008.06.017 (2009).
75. Ebert, E. C. Mechanisms of colon cancer binding to substratum and cells. *Digestive Diseases and Sciences* **41**(8), 1551–1556. https://doi.org/10.1007/BF02087899 (1996).
76. Spangler, J. G. & Reid, J. C. Environmental manganese and cancer mortality rates by county in North Carolina: An ecological study. *Biological Trace Element Research* **133**, 128–135. https://doi.org/10.1007/s12011-009-8415-9 (2010).

77. Yan, J. et al. The association between trace metals in both cancerous and non-cancerous tissues with the risk of liver and gastric cancer progression in northwest China. *Journal of Pharmaceutical and Biomedical Analysis* **242**, 116011. https://doi.org/10.1016/j.jpba.2024.116011 (2024).
78. Wojcik, K. M. et al. Seasonal patterns in trace elements assessed in toenails. *Environmental Advances* **15**, 100496. https://doi.org/10.1016/j.envadv.2024.100496 (2024).
79. Kumar, A. & Ghosh, A. K. Arsenic and Cancer. In *Environmental Exposures and Human Health Challenges* 106–132 (IGI Global, 2019).
80. Kumar, A. et al. Arsenic contamination in groundwater causing impaired memory and intelligence in school children of Simri village of Buxar district of Bihar. *J. Ment. Health Hum. Behav.* **24**, 132–138. https://doi.org/10.4103/jmhhb.jmhhb_31_18 (2019).
81. Kumar, A. et al. Arsenic causing gallbladder cancer disease in Bihar. *Scientific Reports* **13**(1), 4259. https://doi.org/10.1038/s41598-023-30898-0 (2023).
82. Kumar, A. et al. *Severe disease burden and the mitigation strategy in the arsenic-exposed population of Kaliprasad Village in Bhagalpur District of Bihar* (Biological Trace Element Research, 2023). https://doi.org/10.1007/s12011-023-03822-w.Advanceonlinepublication.10.1007/s12011-023-03822-w.
83. Kumar, A. et al. 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 (2022).
84. Kumar, A. et al. Assessment of arsenic exposure in the population of Sabalpur village of Saran District of Bihar with mitigation approach. *Environmental Science and Pollution Research International* https://doi.org/10.1007/s11356-021-13521-5.Advanceonlinepublication.10.1007/s11356-021-13521-5 (2021).
85. Kumar, A. et al. *Assessment of arsenic exposure and its mitigation intervention in severely exposed population of Buxar district of Bihar* (Toxicol. Environ. Health Sci., 2021). https://doi.org/10.1007/s13530-021-00086-6.
86. Kumar, A. & Ghosh, A.K. Assessment of arsenic contamination in groundwater and affected population of Bihar. N. Kumar (ed.), *Arsenic Toxicity: Challenges and Solutions*. https://doi.org/10.1007/978-981-33-6068-6_7 (2021).
87. Sakamoto, M., Kumar, A., Choudhary, D. K., Bishwapriya, A. & Ghosh, A. 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 (2024).

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Author contributions

A.K & A.K.G: Conceptualization, data curation, supervision, and writing – editing original draft of the manuscript.R.K, G.K, A.B, S.K & K.K.: Data curation, writing, preparation of geospatial maps, graphs etc.N.K.C., S.A., M.M., M.K., M.M.: Collection of the samples, analyzation of samples, literature study.R.K., A.S., V.R., D.K. & M.S. : Data curation, validation, and supervision. All authors edited and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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