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Dietary exposure of potentially toxic elements to freshwater mammals in the Ganga river basin, India☆

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ABSTRACT

The threatened Gangetic dolphin (*Platanista gangetica)* and smooth-coated otter (*Lutrogale perspicillata)* occuring in the Ganga River Basin (GRB), are experiencing a decline in their population and distribution range owing to multiple anthropogenic pressures, including pollution by Potentially Toxic Elements (PTEs). Apex predators primarily encounter contaminants through dietary exposure. Yet, notable gaps persist in our understanding of the risks associated with the ingestion of PTE-contaminated prey for Gangetic dolphins and smooth-coated otters. In this study, we examined the occurrence and spatial variation of PTEs in the prey (fish) of both these riverine mammals across three major rivers of the Basin, while also evaluating the associated risk of ingesting contaminated prey. Our assessment revealed no statistical variation in bioaccumulation profiles of PTEs across the three rivers, attributable to comparable land use patterns and PTE consumption within the catchment. Zn and Cu were the most dominant PTEs in the prey species. The major potential sources of pollution identified in the catchment include agricultural settlements, vehicular emissions, and the presence of metal-based additives in plastics. Zn, As and Hg accumulation vary with the trophic level whereas some PTEs show concentration (Hg) and dilution (As, Cr, Pb and Zn) with fish growth. The Risk Quotient (RQ), based on the dietary intake of contaminated prey calculated using Toxicity Reference Value was consistently below 1 indicating no significant risk to these riverine mammals. Conversely, with the exception of Co and Ni, the Reference Dose-based RQs for all other PTEs indicated a substantial risk for Gangetic dolphins and smooth-coated otters through dietary exposure. This study serves as a pivotal first step in assessing the risk of PTEs for two threatened riverine mammals in a densely populated river basin, highlighting the importance of their prioritization in regular monitoring to reinforce the ongoing conservation efforts.

1. Introduction

Globally, freshwater biodiversity is threatened by multiple stressors including climate change, habitat degradation, invasive species and pollution (Reid et al., 2019). Riverine mammals are among the groups most affected by pollution, with serious repercussions for the populations of freshwater cetaceans and otters (Huang et al., 2012; Cazzolla Gatti, 2016; Kean et al., 2021).

The Gangetic dolphin (*Platanista gangetica*) and smooth-coated otter (*Lutrogale perspicillata*) are apex predators inhabiting several stretches of major rivers within the Ganga River Basin (GRB) ((Hussain, 2002; WII-GACMC, 2018; Das et al., 2022). *P. gangetica* (henceforth GD) and *L. perspicillata* (henceforth SCO), characterized by their status as apex predators, restricted home ranges, slow population growth rates, and low population densities are highly vulnerable to various human-induced impacts (Xie et al., 2021; Gkotsis et al., 2022).

The occurence of GD is restricted to the riverine habitats of northern India owing to its physiological and ecological requirements (Das et al., 2022; Das et al., 2024). The species, classified as "Endangered" on the IUCN Red List (IUCN, 2023), has experienced a population decline of over 50% since 1957 (Behera et al., 2013; Das et al., 2022; IUCN, 2023; Das et al., 2024) and a 24% reduction in its range within the GRB

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(Paudel and Koprowski, 2020). SCO is a large-sized otter widely distributed in India, Pakistan, Bangladesh Nepal, Indo-China, Malaysia, Sumatra and Java (Hussain et al., 2018; Hussain and Choudhury, 1997; Nawab and Hussain, 2012a; IUCN, 2023). Despite its wide distribution, the vulnerable species is experiencing a decline (Hussain et al., 2018), this is particularly concerning, with projections (IUCN, 2023) indicating a potential population reduction of over 30% in the next three decades.

Rivers in the Ganga Basin, such as the Ganga, Chambal, Yamuna, Ghaghra, Gandak, and Kosi, serving as important habitats for GD (Das et al., 2022, 2024) and SCO (Hussain and Choudhury, 1997) face challenges in habitat suitability due to the overlap with significant human settlements. As a result, these riverine mammals are exposed to a multitude of anthropogenic threats, including hunting, accidental mortality by gillnet entanglement, habitat degradation, dams and other infrastructure, boat traffic, prey availability, climate change, and pollution by diverse contaminants (Behera et al., 2013; Sah et al., 2020; IUCN, 2023). Potentially Toxic Elements (PTEs) elevate this danger by accumulating in their habitats, ultimately reaching high levels in these apex predators (Kannan et al., 1993; Nair, 2009; Banyal and Kumar, 2014; Paul, 2017; Sarah et al., 2019; Siddiqui et al., 2019; National Mission for Clean Ganga, 2023). PTEs include metals and metalloids, of varying environmental significance, such as Cadmium (Cd), Lead (Pb), Mercury (Hg), Arsenic (As), Copper (Cu), Chromium (Cr), Nickel (Ni), Zinc (Zn), that have been recognized for their persistence, widespread sources, and potential risk to humans and aquatic biota (Nieder et al., 2018; Pourret and Hursthouse, 2018). PTEs have been documented to be associated with neurotoxicity, immunotoxicity, cytotoxicity and genotoxicity in aquatic mammals (Wise et al., 2008; Meaza et al., 2020). Several studies have also reported them to cause reproductive anomalies (Hyvärinen and Sipilä, 1984; Béland et al., 1992; Thomas et al., 2021) and even local extinctions (Gutleb, 2000).

Nonetheless, despite global evidence on the effects of PTEs on aquatic mammals, the risk posed to GD and SCO by PTEs in India is yet to be assessed and quantified.

Given their threatened status, it is imperative to comprehensively monitor and assess PTE-related health risks in these endangered flagship species to bolster ongoing conservation efforts and preserve aquatic ecosystem integrity. However, their threatened status often makes conventional biomonitoring efforts challenging and unethical. The reliance of GD and SCO on fish as their primary prey Nawab and Hussain, 2012b; IUCN, 2024) provides a valuable and indirect method for biomonitoring PTEs and assessing the risks posed by them to these species. Over the past few decades, the screening-level ecological risk assessment approach, based on dietary tissue residue guidelines, has been successfully used to explore the potential health risk posed by contaminant exposure to aquatic mammals (Xie et al., 2020; Ye et al., 2021). This approach assesses the dietary exposure risk of contaminants to the target species by quantifying the concentration of contaminants in the prey base. Furthermore, studies report variations in PTE bioaccumulation in fish depending on species, habitat, feeding habits and geographical factors, and such variations seldom follow any uniform patterns (Lin et al., 2020; Córdoba-Tovar et al., 2022; Yan et al., 2022). This necessitates the assessment of contamination in different species occupying different niches and trophic levels, at various sites, even within the same river.

Considering the identified research gaps, the overarching objective of this work is to screen PTEs that pose risk to GD and SCO. This study aims to specifically address the following key research questions: (i) What is the current contamination status of PTEs in the prey base of GD and SCO in GRB, India? (ii) What factors influence the observed patterns of bioaccumulation of PTEs in the prey? (iii) What risks do these apex predators face through consumption of PTEs contaminated prey?

The study's findings will help screen PTEs that pose risks to the GD and SCO in the three rivers, reinforcing ongoing conservation efforts.

2. Materials and methods

2.1. Study sites

Three rivers, namely Kosi, Gandak, and Ghaghra, of the GRB were selected for the present study, and multiple sites were selected for sampling in each river stretch based on land-use patterns, anthropogenic pressures and GD and SCO distribution. The study area is part of the Middle Gangetic plains, characterised by high rainfall, and extensive agriculture settlements. Most of the study sites occur in the state of Bihar, which is characterised by a predominantly rural population accounting for 88.71% of the state's total population and inhabiting 92,257.51 km^2 of the state's area as opposed to the remaining 11.29% of its urban population inhabiting only 1095.49 km^2 of the state's area (Census of India, 2011). A total of twelve sites were selected from three rivers (Fig. 1). Further details of the study area are provided in Supplementary Information (Text S1 and Table S1).

2.2. Sample collection

Species of the genus *Platanista* feed predominantly on fish, with prey selectivity determined by prey size rather than species, owing to their narrow oesophagi (Takahashi and Yamasaki, 1972). The prey size distribution is generally dominated by prey items <20–30 cm in size (Sinha et al., 1993; Choudhary et al., 2006; Kelkar et al., 2018). Otters are also known to primarily consume small and medium-sized fish of average length of 5–15 cm, while larger fish are generally opportunistically consumed as they are harder to capture than smaller fish (Erlinge, 1968; Rowe-Rowe, 1977; Anoop and Hussain, 2005; Nawab and Hussain, 2012b). Thus, the sampling efforts focused on collecting samples within the preferred size ranges of these species. A total of 276 freshly caught fishes comprising of 20 species were obtained from fishermen in the rivers in the post-monsoon season of 2021 (December 2021–January 2022). Fish samples were collected from four sites along the Ghaghra (N = 80, 7 species), Gandak (N = 92, 9 species) and Kosi (N = 104, 10 species) Rivers, respectively, for the present study. A total of 9 benthopelagic, 7 pelagic, and 4 demersal fish species were collected from all sampling sites.

The total length and weight of each individual were recorded on-site. The species were identified on-site, packed in a sealed bag, and kept in an ice-box for transportation to the laboratory, where they were stored in a deep freezer at −50 °C until further processing. Details of the fish species collected from each site are given in Table 1 and Table S2. Data on trophic levels and habitat preference of each species were obtained from Fish Base (Froese and Pauly, 2023).

2.3. Sample preparation and analysis

Freeze-dried and ground whole fish samples were microwave-digested in a nitric acid-hydrogen peroxide solution. Briefly, approximately 0.4 g sample was digested using 4.0 mL of nitric acid (Merck, 69%) and 0.5 mL of hydrogen peroxide (Merck, 50%) in an Anton Paar Multiwave Go Microwave Digester at 120 °C for 15 min, ramped to 200 °C in 15 min and digested at 200 °C for 30 min. After cooling, the digested sample was diluted to 25 mL with ultrapure water.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent ICP-MS-7850) was utilized to identify and quantify the PTEs Cr, Co, Ni, Cu, Zn, As, Cd, Hg, and Pb in the prey species. A seven-point calibration was performed using multi-element calibration standard-2A and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use/United States Pharmacopeia (ICH/USP) Oral Target Elements Standard A (Agilent Technologies, USA). Scandium (Sc), Yttrium (Y), Indium (In), Terbium (Tb) and Bismuth (Bi) were used as internal standards for Cr, Co, Ni, Cu, Zn, As, Cd and Pb. Gold (Au) was used as internal standard for Hg.

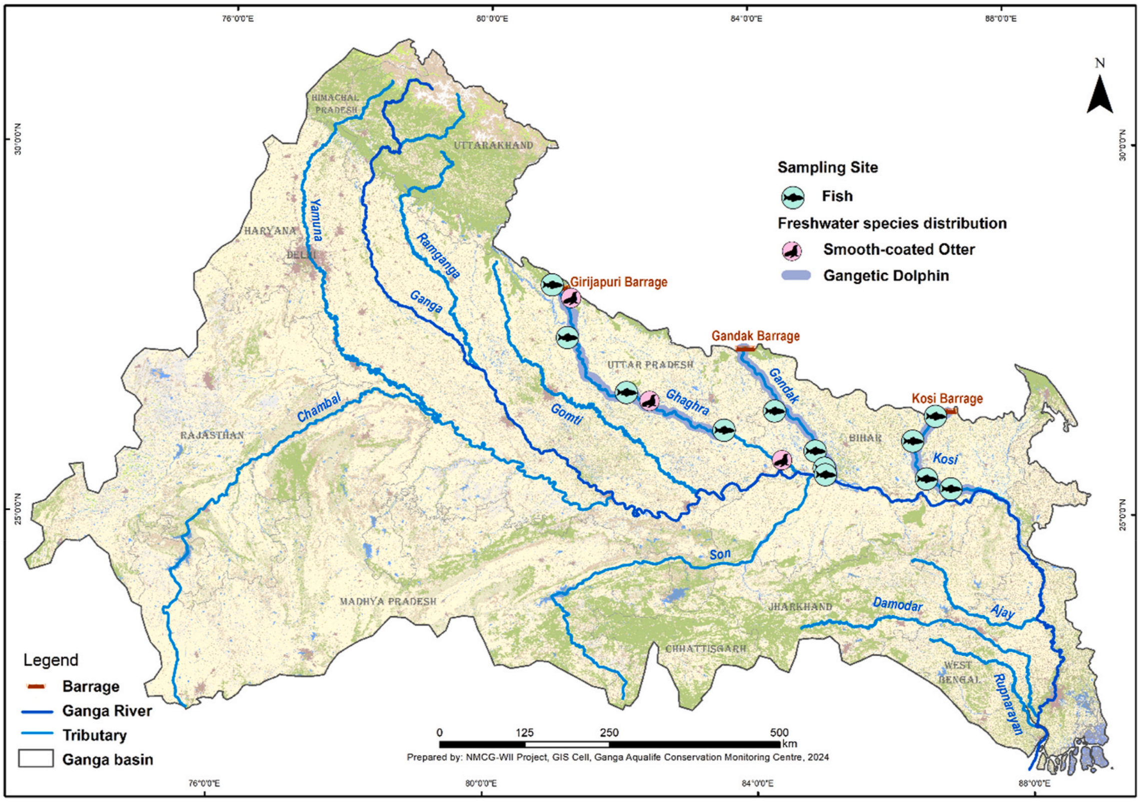

Fig. 1. Study Area representing the sampling sites and GD and SCO habitat in Kosi, Gandak, and Ghaghra River of Ganga River Basin, India.

Table 1
Details of fish species collected from Kosi, Gandak, and Ghaghra.

Species	Trophic level	Feeding habit	Habitat preference	Number of individuals
Ailia coilia[a]	3.6 ± 0.6	carnivorous	Pelagic	11
Aspidoparia jaya	3.3 ± 0.4	carnivorous	Pelagic	9
Aspidoparia morar	3.2 ± 0.4	carnivorous	Pelagic	63
Channa striata	3.6 ± 0.47	carnivorous	Benthopelagic	8
Cirrhinus reba	2.5 ± 0.2	omnivorous	Benthopelagic	12
Clupisma garua	3.7 ± 0.59	carnivorous	Demersal	9
Eutropiichthys vacha	3.9 ± 0.63	carnivorous	Demersal	12
Mystus cavasius[a]	3.4 ± 0.4	carnivorous	Benthopelagic	30
Mystus tengara[a]	3.2 ± 0.40	omnivorous	Benthopelagic	10
Mystus vittatus[a]	3.1 ± 0.1	omnivorous	Benthopelagic	10
Notopterus notopterus	3.5 ± 0.0	carnivorous	Demersal	7
Puntius chola[a]	2.5 ± 0.1	omnivorous	Benthopelagic	20
Puntius conchonius[a]	2.9 ± 0.33	omnivorous	Benthopelagic	16
Puntius sophore[a]	2.6 ± 0.1	omnivorous	Benthopelagic	10
Raiamas bola	3.4 ± 0.4	omnivorous	Demersal	5
Rasbora rasbora[a]	3.2 ± 0.4	omnivorous	Benthopelagic	10
Rhinomugil corsula	2.4 ± 0.2	omnivorous	Pelagic	13
Salmophasia bacaila	3.2 ± 0.40	omnivorous	Pelagic	11
Setipinna taty	3.6 ± 0.6	carnivorous	Pelagic	5
Xenentodon cancila	3.9 ± 0.62	carnivorous	Pelagic	6

[a] *Species commonly occurring in GD stomach contents* (Kelkar et al., 2018).

2.4. Quality control and quality assurance

The analytical data quality was guaranteed through the implementation of laboratory quality assurance and quality control methods, including the use of in-house standard operating procedures, calibration with standards, analysis of reagent blanks, recovery of SRM and analysis of replicates. The accuracy and precision of the analytical procedure was tested by recovery measurements using SRM (ERM-CE278k-mussel tissue) from European Reference Materials. The percentage recoveries of the PTEs in the SRM ranged from 90.9% to 106%.

The precision of the analytical procedures, expressed as the relative standard deviation (RSD), ranged from 5 to 10%. Two blanks and one SRM sample for each batch (10 samples) were analysed. Linear calibration curves were obtained with the R^2 value of 0.999–1.000 and calibration verification standard deviation was $<\pm 5\%$. The detected concentrations for most elements were above their respective detection limits. For concentrations below the detection limit, half of the detection limit was used in calculations.

2.5. Screening level ecological risk assessment (SLERA)

The SLERA, concerning potential health hazards from consuming prey contaminated with PTEs, utilizes Risk Quotients (RQ) based on two dietary tissue guidelines intended for human and mammalian exposure (Hung et al., 2004, 2007; Yu et al., 2020): the reference dose (RfD; mg kg^{-1} ww day^{-1}) and toxicity reference value (TRV, mg kg−1 ww day^{-1}).

The RfD is generally used in regard to human health, and the TRV in reference to animal health. The calculated values of Maximum Allowable Concentration based on Reference Dose (MAC_{RfD}) and Toxicity Reference Value (MAC_{TRV}) for GD and SCO are provided in Table S3. The TRV of PTEs to GD and SCO was calculated based on the no observable adverse effect dose for mammalian test species, relative to body weight scaling procedure (bodyweight of the GD or SCO/bodyweight of the test species) as described by Sample et al. (1996) and Hung et al. (2004, 2007). The RQ was determined from the ratio of the observed concentration and the Maximum Allowable Concentration (MAC) of a specific PTE in fish for human or mammalian consumption. An RQ greater than 1.0 implies a health risk to GD and SCO from consuming fish contaminated with a particular PTE, potentially inducing adverse biological effects in these threatened riverine mammals.

The scarcity of comprehensive biological data on GD and SCO may constrain the precision of SLERA, owing to uncertainties in biological and exposure parameters including body weight, consumption habits, fraction ingested, exposure duration, and frequency. Despite these limitations, the SLERA methodology utilized in this study aims to approximate a worst-case scenario, adopting a conservative approach to screen PTEs that pose risk to GD and SCO for comprehensive assessment in order to enhance their protection**.** Notwithstanding its constraints, the SLERA methodology employed in this study seeks to simulate a worst-case scenario, employing a conservative approach to screen PTEs that pose risk to GD and SCO. This initial screening serves as a basis for a more thorough assessment aimed at bolstering the protection of these species.

2.6. Statistical analyses

Data were analysed by descriptive statistics and expressed as Range, and Mean ± Standard Deviation. The concentration of PTEs in fish is expressed as ng/g wet weight (ww). The assumptions for homogeneity of variance and normal distribution were tested with Levene's tests and Shapiro–Wilk tests respectively. As the assumptions were violated, the variations of the PTEs between rivers and niche were analysed using non-parametric Kruskal-Wallis H-test. Spearman's rank coefficient was used to evaluate the role of ecological and biological factors in bioaccumulation of PTEs in prey fishes. Additionally, linear regression was applied to study the relationship between tissue PTE concentrations and the trophic levels of prey species. Models were validated through residual vs fit plots, normal Q-Q Plots and residual vs leverage plots. Only the models fulfilling these criteria were assessed. Statistical tests results were considered significant at p-value <0.05 and < 0.01.

3. Results and discussion

3.1. PTE concentrations in prey base of GD and SCO

The mean concentrations of PTEs detected in the prey species in the three rivers are presented in Fig. 2 and Table S4. While the observed bioaccumulation pattern of ΣPTEs in three rivers was noted as Ghaghra (4449.10–49622.50 ng/g ww) > Gandak (10967.00–53101.86 ng/g ww) > Kosi (15681.70–42657.68 ng/g ww), a Kruskal-Wallis test indicated no significant ($p > 0.05$) differences in PTE bioaccumulation among prey fish across the three rivers (Fig. 2). The similarity in land use, particularly the prevalence of agricultural settlements, across the catchments of the three rivers may account for the absence of variations in the accumulation of PTEs in prey species across these rivers (Fig. S1). In Gandak and Kosi, the PTE concentrations in prey species followed the trend Zn > Cu > Cr > Hg > Pb > Ni > As > Co > Cd. A slight variation in accumulation for As and Pb was observed in the Ghaghra River, with the trend Zn > Cu > Cr > Hg > As > Ni > Pb > Co > Cd. (See Table S4).

Similar dominance and trends of some of these PTEs have also been noted in the tissue of the GD sampled along the Ganga River, with the element concentrations following the order Fe > Zn > Cu > Mn > Ni > Cd > Pb in dolphin tissues (Kannan et al., 1993).

Zn, Co and Cu are essential elements and fish rely on their intake from water and diet to facilitate essential processes, including growth, development, protein metabolism, immune-biochemical plasticity, and resistance against various stresses (Zhang and Wang, 2005; Wood et al., 2012; Guo et al., 2016; Kumar et al., 2017; Don Xavier et al., 2018; Kumar et al., 2020).

In the present study, the concentrations of Cu and Zn in the prey species, across all three rivers, were found to be higher than the other toxic elements. The elevated zinc levels can also be attributed to its natural abundance primarily influenced by chemical weathering and increased erosion in the flood-prone catchment area (Arya and Singh, 2021).

The river catchments of Gandak, Kosi, and Ghaghra are characterised by a predominantly agricultural land use (Fig. S1) interspersed with some degree of built-up area (Singh et al., 2017a,b; Parida et al., 2022; Anand et al., 2018). In the state of Bihar, where the three rivers flow and confluence with the Ganga River, there exists a total cultivated area of 5.71 million hectares (ICAR, 2023), characterized by a cropping intensity of 144% (Government of Bihar, 2024). Despite considerable

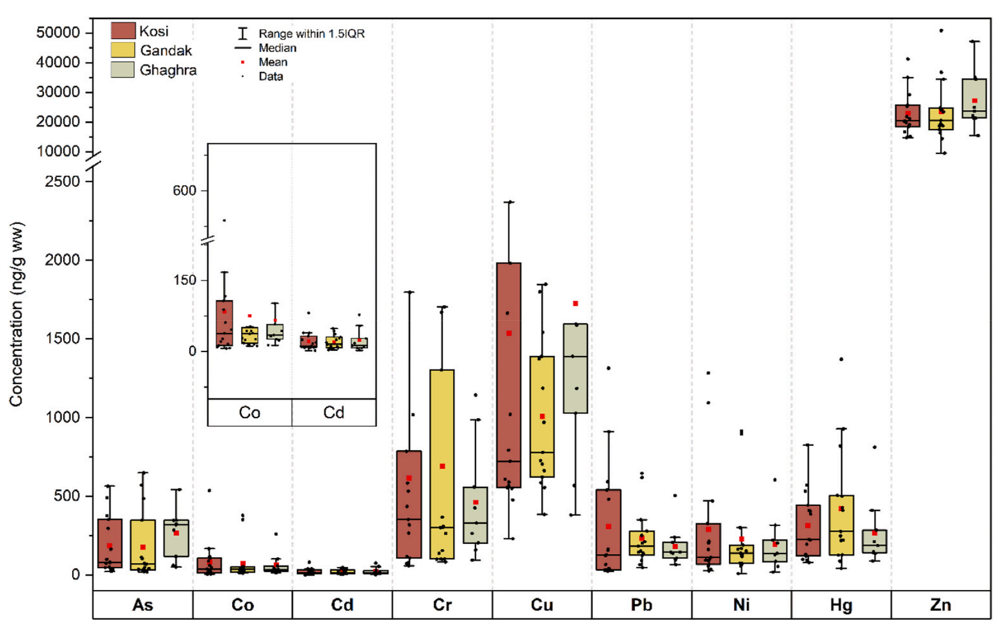

Fig. 2. River-wise concentrations of PTEs (ng/g ww) in fish tissues collected from Gandak, Kosi and Ghaghra Rivers, India.

growth in the built-up area, the catchment lacks heavily industrialised zones, distinguishing it from other rivers, like the Ganga, where evidence of PTE pollution is prevalent (Singh et al., 2020; Parween et al., 2021).

Therefore, the primary origin of anthropogenic contributions of PTEs in the examined rivers appears to be non-point source pollution, specifically emanating from agricultural run-off. Agrochemicals, including fertilisers and pesticides, often contain PTEs such as Cd, Pb, Cu, Zn and As (Alloway, 2013; Alengebawy et al., 2021). Many regions within the investigated catchment, are contending with zinc-deficient soils, spurring an increased need for zinc-based fertilizers (ICAR, 2023). Application of PTE-contaminated irrigation water, sewage sludge, livestock and poultry manures are also recognized as anthropogenic sources of PTEs in agricultural soil. The high annual monsoon floods in these rivers create conditions conducive to the influx of PTEs via surface runoff from the agriculturally dominated catchment (Ghosh et al., 2014; Sämann et al., 2019).

Vehicular emissions and associated traffic-related activities may also contribute to the concentration of PTEs in nearby water bodies through multiple pathways. Airborne deposition from exhaust emissions, wear and tear of vehicle components, runoff from roads, oil and fluid leaks, brake lining dust, and traffic-induced soil disturbance all play a role. These pollutants, including Pb, Cd, Zn, Cu, and Fe, can be transported into water bodies through stormwater runoff during rainfall events (Adamiec et al., 2016; Duan and Tan, 2013; Lin et al., 2020; Men et al., 2018; Singh et al., 2018). PTEs such as Zn, Cu, and Pb are emitted into the atmosphere from various sources. Their long-range atmospheric transport, leads to their deposition in soils and eventual introduction into aquatic ecosystems through runoff processes (Nicholson et al., 2003).

It is noteworthy, that the concentration of Cd, Ni, Cu, Zn, and Pb in dolphin prey in the present study is comparable to and often even lower than that observed by Kannan et al. (1993). As previously mentioned, this can be attributed to limited presence of built-up areas and industrial settlements along these rivers, which restricts the input of PTEs from point sources. Additionally, the use of PTE-added pesticides in the basin has either remained consistent or reduced, owing to efforts for their regulation and public awareness (Agnihotri, 2000; Directorate of Plant Protection Quarantine and Storage, 2023). Similarly, regulatory actions such as the restriction on the addition of lead mixtures to petroleum, generation and disposal of hazardous wastes, and awareness campaigns (Bihar State Pollution Control Board, 2024; UNEP, 2021) likely contributed to maintaining stable PTE concentrations over the specified period. Nevertheless, a decrease in their input may not necessarily be translated to low risk due to the persistent, accumulative, and toxic nature of PTEs.

Less explored but significant, the anthropogenic inputs of PTEs are also associated with metal-based additives in plastics. These elements, found in small quantities within additives like UV and heat stabilizers (Cd, Pb), inorganic pigments (Cd, Cu, Pb) and organic pigments (Co), serve to enhance both the functional and aesthetic aspects of plastics (Hahladakis and Iacovidou, 2018; Turner & Filella, 2021). The inclusion of PTEs in these additives, combined with improper plastic usage, recycling, and disposal, raises concerns about their unintended release into freshwater environments.

The Gangetic plains, including areas in Bihar, are known for arsenic-enriched groundwater, making the river-groundwater interface a significant source of arsenic contamination in rivers (Rahman et al., 2021; Wallis et al., 2020).

3.2. Correlations among PTE concentrations and ecological, and biological variables

Previous studies have recorded an effect of diet, habitat use and size of fish on the accumulation of trace elements in their tissues (Signa et al., 2017; Wang et al., 2019). Hence, an investigation into the contamination patterns of PTEs in fish was conducted, exploring their correlation with ecological and biological variables to elucidate the contamination dynamics of PTEs within species and riverine food web. The following sections discuss the results of our assessments of variations of PTE contamination with these factors.

3.2.1. Relationship with ecological factors (niche and trophic level)

PTE accumulation in fishes is known to vary phylogenetically (Jeffree et al., 2010), and by feeding habits and habitat preferences (Lin et al., 2021; Pragnya et al., 2021; Jiang et al., 2022). An Independent Samples Kruskal-Wallis test indicated significant variation in tissue PTE concentrations of As, Co, Cd, Cr, Cu and Ni among species occupying different positions in the water column. Significant variations were observed for As (<0.01) and Co (<0.05) concentrations between pelagic and benthopelagic species, and between demersal and benthopelagic species. Significant variations were observed only between the benthopelagic and demersal groups for Cd (<0.01) and Cu (<0.05), while the concentrations varied significantly between pelagic and benthopelagic groups for Cr (<0.01) and Ni (<0.05) (Fig. 3).

Similar results were observed by Yi et al. (2017), where highest concentrations of heavy metals appeared in the fish living in the pelagic middle-lower layers. Higher concentrations of elements in benthopelagic species have been attributed to their uptake from prey in the pelagic zone (Rejomon et al., 2010; Abdolahpur Monikh et al., 2012).

Demersal fishes accumulated the highest concentrations ($p > 0.05$) of Hg in the present study, indicating the affinity of this element with the benthic sediments and its transfer from benthic sediment and prey to fish (Wong et al., 1997; Zhou and Wong, 2000; Hosseini et al., 2013). The recovery of sediments from Hg contamination is known to be slow (Dianne Kopec et al., 2018), and this could result in the persistence of this contaminant in sediments for long periods, thus being available for uptake by the river's biological communities for a longer time.

As and Zn are known to biodilute (Revenga et al., 2012; Montañez et al., 2018), and Hg is known to biomagnify across aquatic food webs (Guo et al., 2016). Our data supports the same trends (Fig. 4), with Zn ($p < 0.05$) and As ($p < 0.01$) showing significant negative correlations, while Hg showed a significant ($p < 0.01$) direct relationship with the trophic level of the prey species. Further exploration of these relationships through linear regression models revealed significant influence of trophic levels on the tissue concentrations of Zn ($p < 0.05$, $R^2 = 0.16$), As ($p < 0.01$, $R^2 = 0.17$) and Hg ($p < 0.01$, $R^2 = 0.18$).

3.2.2. Relationship with biological factors

A Spearman's correlation analysis (Fig. 4) indicates a significant direct relationship between Hg accumulation and average length ($p < 0.01$) and weight ($p < 0.01$). In contrast, Cr ($p < 0.05$), Co ($p < 0.01$), Ni ($p < 0.05$), Zn ($p < 0.01$), As ($p < 0.01$) and Pb ($p < 0.05$) show an inverse relationship with the average length of the species. The metals Co ($p < 0.05$) and Zn ($p < 0.01$) also show a significant, inverse relationship with the average weight of the fish species. Previous studies have also reported growth dilution of several PTEs including Cr, Ni, Zn, Cu and Pb in fish (Canli and Atli, 2002; Merciai et al., 2014; Jiang et al., 2022). On the other hand, growth constant and efflux rate constant have been identified as key drivers of Hg accumulation in fish, resulting in its positive relationship with fish size (Dang and Wang, 2012).

The above findings indicate that various factors play a role in influencing the bioavailability, bioaccumulation, and dynamics of PTEs in the riverine ecosystem. Bioaccumulation and biodilution are themselves governed by a number of factors including site-specific food web structures and environmental factors. These relationships need to be explored further for efficient modelling, prediction and risk assessment for the conservation of threatened habitats and species. Moreover, our results indicating the relationship between the average length and weight of species and the PTE concentrations need to be investigated further as both length and weight could act as proxies for other drivers such as species, diet, sexual maturity, health, and habitat quality

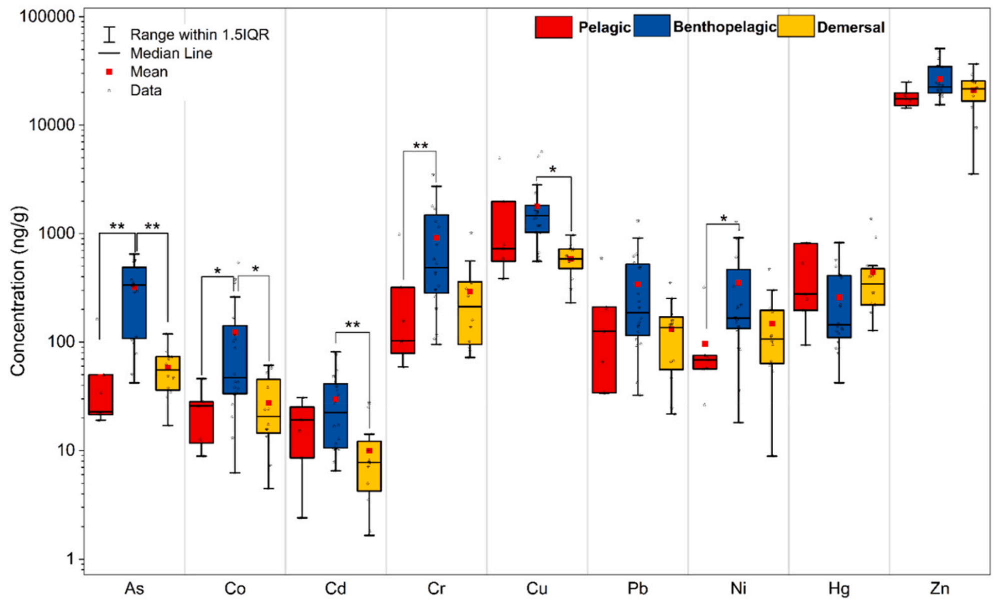

Fig. 3. Variations in tissue PTE concentrations (ng/g ww) in tissues of fish species collected from Gandak, Kosi and Ghaghra Rivers, India, with habitats.

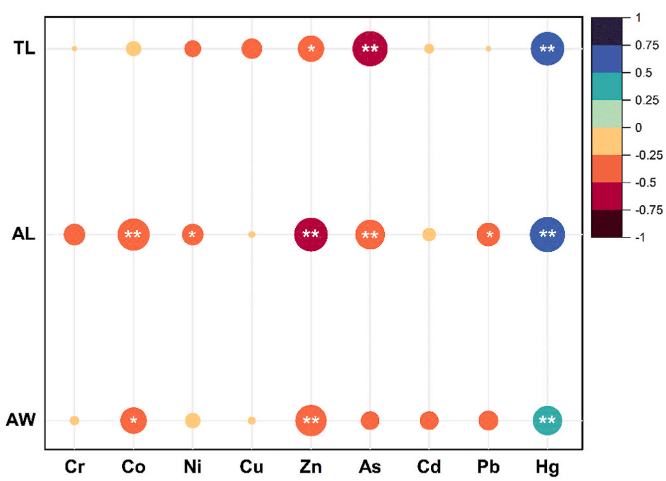

Fig. 4. Correlation of tissue PTE concentrations with ecological factor (TL: Trophic level) and biological traits (AW: Average weight; AL: Average Length) Correlation significant at * = $p < 0.05$; ** = $p < 0.01$.

(Richter et al., 2000; Moutopoulos and Stergiou, 2002; Romanuk et al., 2011; Mozsár et al., 2015; Zhang et al., 2020; Hasan et al., 2021).

3.3. Risk assessment

Figs. 5 and 6 present a summary of the Risk Quotients (RQs), based on MAC_{TRV} and MAC_{RfD}, derived from the assessment of PTEs in prey-fish species and their potential impact on GD and SCO. The RfD, a frequently used metric in human health risk assessment, plays a pivotal role in assessing potential adverse health impacts linked to exposure to environmental contaminants. It is more stringent, and conservative than the TRV, thus providing a thorough and cautious evaluation of potential adverse health effects associated with exposure to environmental contaminants by incorporating increased safety factors for enhanced protection.

3.3.1. Gangetic dolphin (GD)

In general, the average risks associated with PTEs to GD, as assessed by MAC_{TRV}, consistently remained low with all RQs falling within the range of <0.001 to 0.701(Fig. 6). However, the 95th percentile data exceeded this threshold for Arsenic (RQ_{TRV} = 1.226–1.564), in all the three rivers, indicating the widespread contamination and high potency of this metalloid (Fig. 5 and Fig. S2-S4).

According to the MAC_{RfD}, all PTEs, except Co and Ni, posed a significant risk of exposure to GD through dietary exposure (Fig. 5). The average RQ in Kosi, Gandak, and Ghaghra rivers extends from 1.068 to 30.536, 1.004 to 29.837, and 1.196 to 44.048, respectively. The average RQ values, calculated using the RfD, indicate that both Co and Ni pose a low risk to GD, as they consistently remain below 1. Nevertheless, the 95th percentile RQ for Ni ranged from 1.215 to 2.855 across the three rivers. The RQ_{RfD} in all the rivers reveals a consistent pattern: As > Hg > Cr > Zn > Cu > Cd > Ni > Co.

In line with our findings, coastal species like the Indo-Pacific humpback dolphin (*Sousa chinensis*) have also been documented to face risks from consuming prey contaminated with Hg, As, Zn, and Cu (Lin, et al., 2020; Xie et al., 2020).

PTEs toxicities have been linked to various immunotoxic and neurotoxic consequences in marine mammals (Bowles, 1999; Desforges et al., 2015; López-Berenguer et al., 2020). Chromium is recognized for its cytotoxic and genotoxic effects on cetaceans (Wise et al., 2008; Meaza et al., 2020). Hg is reported to have neurotoxic, nephrotoxic, hepatotoxic and immunotoxic effects in cetaceans (Kershaw and Hall, 2019). While the immunotoxic and neurotoxic impacts of PTEs are extensively studied, these pollutants can also contribute to reproductive impairments in aquatic mammals (Hyvärinen and Sipilä, 1984; Béland et al., 1992). As has been observed to have lower toxicity in marine mammals as arsenobetaine, the most abundant organoarsenic compound that accumulates, is known to have low carcinogencity and toxicity (Neff, 1997). However, other authors report its potential for carcinogenecity and endocrine disruption (Golub et al., 1998).

The effects of Cu toxicity are little understood in aquatic mammals. In humans, acute Cu toxicity causes gastrointestinal reactions, and chronic toxicity is often associated with liver function (Fraga, 2005). Similar responses may be expected for other mammals including freshwater dolphins and otters. The research on terrestrial mammals, including hamsters, mice, rats, and rabbits has provided evidence that arsenic can induce developmental toxicity, leading to outcomes such as malformation, mortality, and growth retardation.

Given the observed high toxicity in animal studies and its documented effects on human health, legitimate concerns arise regarding

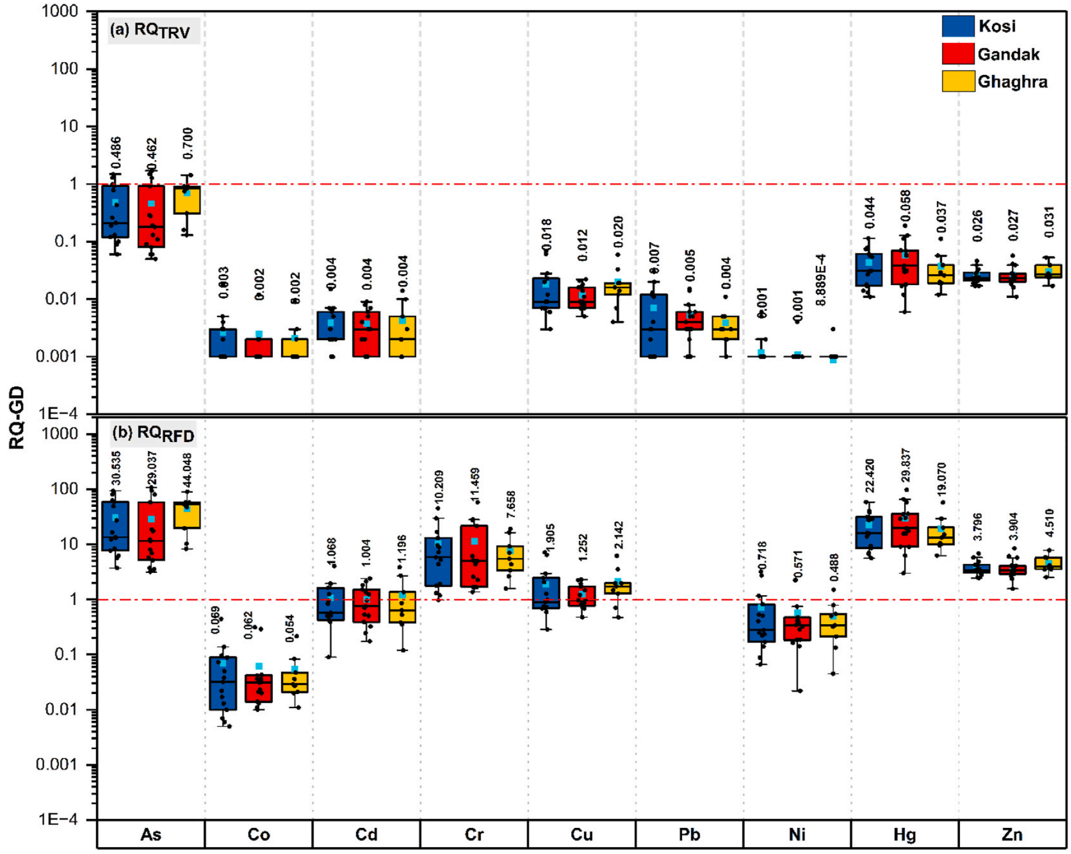

Fig. 5. Mean Risk Quotient (RQ; Log scale) for each PTE in the three rivers calculated for Gangetic dolphin from (a) Toxicity Reference Values (TRV), and (b) Reference Dose (RfD).

potential risks to aquatic mammals. The high RfD-based RQ values revealed in the present findings bring much needed attention to the risks PTEs pose to threatened GD in the GRB, emphasizing the need to prioritize these contaminants in regular monitoring programs aimed at GD conservation efforts.

3.3.2. *Smooth-coated otter (SCO)*

Apart from other mjor rivers in the GRB, the SCO was recorded from the Ghaghra River. Details of the average RQ calculated, based on TRVs, for the PTEs in Ghaghra are given in Fig. 6 and Fig. S5. All PTEs had $RQ_{TRV} < 1$, except for As, which posed a high risk ($RQ_{TRV} = 1.679$). Notably, RQ values at the 50th and 95th percentiles also exceeded the benchmark Fig. S5.

The RfD based RQ values indicated high risk from all elements except for Co (RQ 0.211–0.978). Based on MAC_{RfD}, As (105.47) posed highest risks to SCO followed by Hg (45.66), Cr (18.34), Zn (10.80), Cu (5.13), Cd (2.86), and Ni (1.17).

Otters may acquire high concentrations of persistent contaminants from the fish they prey on, with bioconcentration in the levels of 90–95% (Ruiz-Olmo et al., 2000a,b), and prey and direct contamination are known to have resulted in local extinction of the otter *Lutra lutra* in various regions of Europe (Gutleb, 2000).

In addition to the accumulation of these contaminants in tissues of otters, there is evidence indicating the maternal transfer of these elements from mothers to offspring (NOM-027-SSA1-1993, 1993; Chen et al., 2009; White et al., 2009; Basu et al., 2005; Croteau et al., 2005; Yates et al., 2005; Scheuhammer et al., 2007; Brown et al., 2021).

Elevated concentrations of Co, Zn, and Cd have been noted in diseased and emaciated sea otters compared to healthy individuals, suggesting potential immunotoxic effects of these PTEs in otters (Kannan et al., 2006). Mercury is commonly regarded as the most detrimental metal to otters (Kruuk et al., 1997; Gutleb et al., 1998; Lemarchand et al., 2010), and the high RQ_{RfD} for Hg observed in the present study is concerning. Despite this, it is important to highlight that the otters have been known to eliminate significant amounts of Hg and other PTEs through their hair during moulting, potentially making it a significant and efficient method of eliminating toxic elements (Mason and MacDonald, 1986; Hyvarinen et al., 2003; Strom, 2007).

Therefore, exploring the potential of otter fur for future biomonitoring of PTEs could offer a valuable non-invasive approach.

However, high elimination rates for PTEs may not be sufficient to safeguard these animals against PTE toxicity. For instance, Hg poisoning has been reported in a specimen of Eurasian otter, *Lutra lutra,* wherein the liver Hg concentrations were below the lowest observed adverse effect level 3.4 μg/g (Kim et al., 2023).

Cd has been observed to affect the health of the baculum, a reproductive bone in the North American river otter, potentially affecting reproductive success in the species (Thomas et al., 2021). Similar to GD, the present state of knowledge regarding the effects of PTEs on otters is quite limited, and effects must be predicted from responses established for other mammals including other mustelids. For instance, the elements Cr, Cu, Cd, Pb, As and Hg have a potential association with the decline of the mink, a semi-aquatic carnivorous mustelid, in Georgia, North Carolina and South Carolina (Osowski et al., 1995). Pb has also been observed to affect baculum health in the American mink (Fraschetti, 2021).

Considering the consequences of PTE exposure observed for the survival, reproduction and populations of otters and other aquatic mustelids, the risks indicated by the present study reflect a potential threat to SCO populations in the GRB as well.

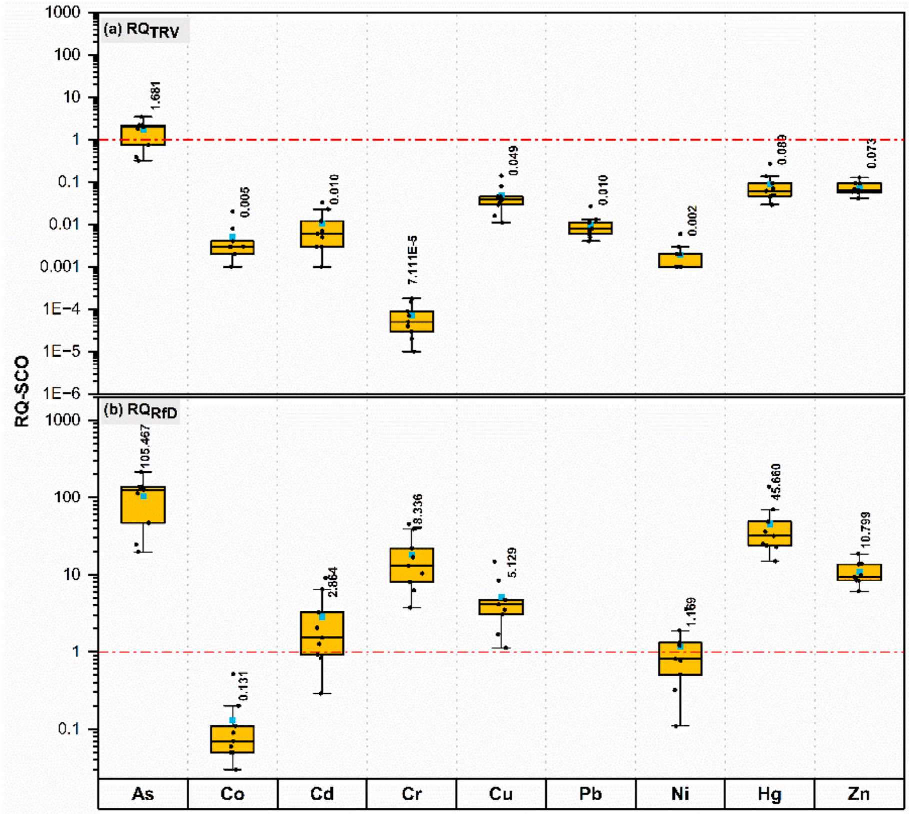

Fig. 6. Mean RQ (Log scale) for each PTE in the three rivers calculated for Smooth-coated otter from (a) Toxicity Reference Values (TRV), and (b) Reference Dose (RfD).

4. Conclusions

In the present study, SLERA was conducted and 9 PTEs were screened that may pose potential risks to GD and SCO through dietary pathways.

The bioaccumulation of PTEs revealed no significant variation across the three rivers attributed to the comparable land-use pattern and anthropogenic influence within the catchment. Non-point sources, including runoff from agricultural settlements, vehicular emissions, and improper plastic disposal serve as the primary drivers of PTE pollution in all three rivers, which is further exacerbated by flooding in the catchment areas.

Some PTEs show significant variation in bioaccumulation across habitats, with higher concentrations generally noted in benthopelagic fish species. Zn, As and Hg accumulation are dependent on the trophic level in the present study. Several elements also show concentration and dilution with fish growth. Of these relationships, the property of biomagnification, displayed by Hg, is of particular concern as it may have more serious repercussions for higher trophic levels, such as our threatened apex predators.

The risk assessment through the dietary intake of contaminated prey, using both TRV and RfD, indicate varying degrees of risk to the GD and SCO. In the present study, the risk posed by PTEs based on TRV was generally low (RQ $<$ 1), whereas risk based on RfD revealed that all PTEs, except Co, posed a high risk through dietary exposure in these riverine mammals.

This study serves as a screening-level tool, offering valuable insights for the screening and prioritization of PTEs within routine monitoring programs designed to support conservation efforts for GD and SCO. While this study primarily focused on evaluating the health risks posed to GD and SCO by PTEs, it is imperative to acknowledge individual and cumulative risks from other emerging and toxic contaminants. Further investigations and holistic monitoring programs across large spatial and temporal scales in habitats of GD and SCO are necessary to enhance the efficacy of current monitoring strategies aimed at conserving these endangered riverine mammals.

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CRediT authorship contribution statement

Ruchika Sah: Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Megha Khanduri:** Writing – original draft, Formal analysis, Data curation. **Pooja Chaudhary:** Formal analysis, Data curation. **K. Thomas Paul:** Formal analysis. **Samridhi Gururani:** Formal analysis, Data curation. **Kirti Banwala:** Data curation. **Chitra Paul:** Data curation. **Mebin Aby Jose:** Data curation. **Sarita Bora:** Data curation. **Aishwarya Ramachandran:** Visualization. **Ruchi Badola:** Supervision, Resources, Project administration, Funding acquisition. **Syed Ainul Hussain:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.123928.

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