ECOTOXICOLOGICAL AND GIS SPATIAL RISK ASSESSMENT OF ESTROGENIC ENDOCRINE DISRUPTING COMPOUNDS (E-EDCS) FROM MIDDLE GANGA

Thesis submitted to the

Saurashtra University, Rajkot, Gujarat

For the award of the degree of

Doctor of Philosophy in Wildlife Science

by

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DECLARATION

I, hereby, declare that the work conducted under this thesis titled **"Ecotoxicological and Spatial Risk Assessment of Estrogenic Endocrine Disrupting Compounds (E-EDCs) from Middle Ganga"** is a record of original and independent research work done by me and subsequently submitted for the award of the degree of **Doctor of Philosophy in Wildlife Science** to the **Saurashtra University, Rajkot (Gujarat)**. This research work has been carried out under the guidance and supervision of **Dr. Gautam Talukdar** and **Dr. Syed Ainul Hussain** of **Wildlife Institute of India, Dehradun**. The work has not formed the basis for the award of any other degree, diploma or any other qualification. I also declare that the thesis embodies my own work, analysis, observation, understanding and the particulars given in it are true to the best of my knowledge.

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CERTIFICATE

This is to certify that **Miss Ruchika Sah** thesis, **"Ecotoxicological and Spatial Risk Assessment of Estrogenic Endocrine Disrupting Compounds (E-EDCs) from Middle Ganga"** submitted for the degree of **Doctor of Philosophy in Wildlife Science at Saurashtra University, Rajkot, Gujarat**, embodies original research work carried under our guidance and supervision.

Miss Ruchika Sah has researched on this thesis for more than six terms under our supervision and guidance. The work presented in this thesis has not been submitted for any other degree. It meets all of the specifications stated forth in the ordinances of Saurashtra University in Rajkot, Gujarat, and the Wildlife Institute of India.

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I certify that the research work was appreciated by all who were present, and the comments made by the faculty and researchers have been appropriately included in the thesis.

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Ecotoxicological and GIS Spatial Risk Assessment of Estrogenic Endocrine Disrupting Compounds (e-EDCs) from Middle Ganga

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ABSTRACT

Chemical pollution threatens 60% of marine mammals, making it the second most significant threat to marine biodiversity. However, this alarming figure highlights the even greater risks faced by freshwater ecosystems, where biodiversity declines are happening at a faster rate than in marine environments. Freshwater ecosystems are especially vulnerable to the impacts of chemical pollution due to their limited water volume and flow, which reduces the dilution of contaminants. Their close proximity to pollution sources—such as agricultural runoff, industrial discharge, and urban waste—intensifies this exposure. Furthermore, restricted natural flushing and water exchange, along with nutrient loading and additional stressors like habitat degradation and invasive species, further amplify the impacts of pollution on freshwater biodiversity.

Among the array of chemical pollutants threatening freshwater biodiversity, estrogenic-Endocrine-Disrupting Chemicals (e-EDCs) have emerged as a critical ecological concern due to their persistence, bioaccumulation, mobility, and toxicity in aquatic environments.

Their ability to cause endocrine disruption, particularly through reproductive impairments, developmental abnormalities, and immune suppression, is concerning as it can result in population declines, altered species interactions, and lasting ecological imbalances.

The Ganga River, the second-largest river in India, spans approximately 2,525 kilometers and flows through the states of Uttarakhand, Uttar Pradesh, Bihar, Jharkhand, and West Bengal. It supports 36.1% of the nation's population (438.2 million) and holds profound religious, economic, and ecological significance. The river sustains a rich diversity of fauna, including several endangered riverine mammals such as the Gangetic dolphin (*Platanista gangetica*) and otter species, including the smooth-coated otter (*Lutrogale perspicillata*), Eurasian otter (*Lutra lutra*), and Asian small-clawed otter (*Aonyx cinereus*).

The Middle Ganga Reach (MGR), along the state of Uttar Pradesh, serves as an important habitat for endangered riverine mammals, yet faces intensified challenges to habitat suitability for these species due to various anthropogenic pressures, including EDC pollution from both point and non-point sources. Although research on e-EDCs in the river has progressed over the years, the spatiotemporal data remains incomplete and fragmented, particularly lacking in detailed characterization of point source contamination. This is a significant gap in our understanding of current risks and may result in inadequately informed and insufficient conservation efforts.

The objective of this thesis research was to investigate (a) the spatiotemporal distribution and load of e-E-EDCs in drains effluents and MGR (b) investigate and assess the estrogenic potential and ecological risk of selected e-EDCs to aquatic life, (c) identify high-risk zones spatially in river, and (d) assess the bioaccumulation potential of e-E-EDCs in the prey species of the Gangetic dolphin, quantify the associated ecological risks, and identify suitable biomonitors for evaluating e-EDCs exposure in the MGR.

Several questions were addressed through field surveys and laboratory studies.

The first objective addressed the spatiotemporal distribution and dynamics of e-EDCs in the MGR and the contribution of drain outfalls on these distributions. To investigate this, surface water samples were collected during the pre-monsoon (PreM; March-April) and post-monsoon (PostM; October) seasons in 2021 from 40 sites across the MGR, including 23 sites along the mainstem and 17 sites representing drain discharges (point-sources). Forty-two e-EDCs from four groups were quantified in the MGR, revealing a distribution profile of Plastic Additives (PAs) > Household and Personal Care Products (HPCPs) > Estrogens > Organochlorine Pesticides (OCPs). The PostM season showed a higher load of PAs, except for Bisphenol A (BPA), indicating increased surface runoff and atmospheric deposition. In contrast, other groups had higher concentrations in the PreM season, suggesting the pronounced impact of dilution during the PostM season. The spatiotemporal dynamics of certain e-EDCs were influenced by water quality parameters such as pH, dissolved oxygen, total dissolved solids, and conductivity. Notably, EDC concentrations at drain discharges were higher than those at river sites, particularly for PAs, HPCPs, and Estrogens. This pattern underscores the significant role of point sources, particularly drains, in introducing these contaminants into the river system. Riverward fluxes of e-EDCs from drains were substantial, ranging from 0.88 to 866.90 kg/year. Four key drains—three located in Varanasi (Assi, Khirkia, and Varuna) and one in Kanpur (Permiya) —were responsible for 81% of the total EDC mass flux entering the river. The high fluxes of Plastic additives in particular underscore the significant impact of urban and industrial wastewater on the MGR's contamination profile.

The second and third objectives aimed to assess whether e-EDC levels in the MGR pose a risk to aquatic biodiversity and to identify high-risk areas for targeted intervention. To evaluate this, the estrogenicity potential of the observed e-EDC concentrations was assessed, alongside a multi-tier ecological risk assessment. The results showed that 63% of the entire 600 km stretch exhibited high estrogenicity, indicating a significant presence of estrogenic compounds capable of disrupting endocrine functions in aquatic organisms. The multi-tier ecological risk assessment further classified the entire stretch of the MGR as a "high-risk" zone for at least two or more e-EDCs (**DEHP**: Di(2-ethylhexyl) phthalate; **DnBP**: Di-n-butyl phthalate; **BPA**: Bisphenol A and **TCS**: Triclosan) at all sampling sites. Additionally, the study highlights the identification of the top 10 high-risk contaminants among the 40 targeted e-EDCs: Di-n-butyl phthalate (DnBP), Estrone (E1), Di(2-ethylhexyl) phthalate (DEHP), Triclosan (TCS), Diethyl phthalate (DEP), Estriol (E3), Bisphenol A (BPA), Estradiol (E2), Triclocarban (TCC), and Gamma-

Hexachlorocyclohexane (γ-HCH). Prioritizing these high-risk e-EDCs focuses efforts on addressing the most pressing ecological threats, enabling efficient resource allocation, targeted mitigation strategies, informed policy development, and protecting vulnerable species like the Gangetic dolphin, while ensuring the long-term health and resilience of aquatic ecosystems.

The first component of fourth objective explored the bioaccumulation patterns and dynamics of e-EDCs, in prey fish of Gangetic dolphin. Additionally, it investigated whether dietary exposure to multi-class endocrine-disrupting chemicals poses health risks to Gangetic dolphins. To address this, the study was conducted in river around Varanasi district, a conservation priority stretch for the Gangetic dolphin, where 187 prey fish from 21 species were collected for analysis. Elevated e-EDCs bioaccumulation was observed across prey fish species, with DEHP and DnBP significantly contributing to the EDC burden.

The concentrations of persistent organochlorines in prey revealed a shift from dioxin-like Polychlorinated Biphenyls (PCBs) to non-dioxin-like PCBs. The prevalence of regulated 1,1,1-Trichloro-2,2-bis(4-chlorophenyl) ethane (p,p' DDT) and γ-HCH residues suggests regional non-compliance with regulatory standards. The concentration of some E-EDCs is dependent on the habitat, foraging behavior, trophic level and fish growth. The potential drivers of e-EDCs contamination in catchment includes agriculture, vehicular emissions, poor solid waste management, textile industry, and high tourist influx. Risk Quotients

(RQs) based on toxicity reference value were generally below 1, while the RQ derived from the reference dose highlighted a high risk to Gangetic dolphins from DEHP, DDT, DnBP, arsenic, PCBs, mercury, and cadmium, emphasizing the need for their prioritization within monitoring programs. The study also proposes a monitoring framework to provide guidance on monitoring and assessment of chemical contamination in Gangetic dolphin and habitats.

The second component of fourth objective aimed to identify the effective species for biomonitoring e-EDCs in site-specific habitats impacted by effluents within the MGR. To achieve this, 70 freshly caught specimens of two mollusc species, *Lamellidens marginalis* (a bivalve) and *Filopaludina bengalensis* (a gastropod), were collected in and around Varanasi city. Bioaccumulation in *F. bengalensis* and *L. marginalis* revealed higher contaminant levels downstream of drainage sites, with both species accumulating more plastic additives compared to other selected e-EDCs. Both species show potential as biomonitors for e-EDC contamination, with *L. marginalis*, in particular, being well-suited for site-specific monitoring in effluent-impacted areas of the Ganga River due to its sedentary nature and strong bioaccumulation capabilities.

From the sum of these studies, it can be concluded that e-EDC contamination, particularly from PAs, HPCPs, and estrogens is prevalent across all studied matrices. Given their high production, widespread usage, and known toxicities, it is recommended that these e-EDCs be included in regular, comprehensive monitoring initiatives as a proactive measure. **This**

thesis presents the first comprehensive spatiotemporal assessment of e-EDCs in the surface water and biota of the MGR. It characterizes the riverward fluxes of e-EDCs from drain discharges, marking a pioneering effort in this area. It is also the first work to quantify the dietary exposure risks of e-EDCs to riverine mammals and to explore the potential of macrobenthos as bioindicators of EDC contamination.

The overall findings of this work lay the groundwork for targeted mitigation measures, informed conservation actions, and policy development aimed at reducing EDC levels and safeguarding this ecologically fragile river stretch.

Additionally, this comprehensive research on e-EDCs in the MGR makes significant contributions to several United Nations Sustainable Development Goals (SDGs), primarily **SDG 6 (Clean Water and Sanitation)**, **SDG 14 (Life Below Water)**, and **SDG 15 (Life on Land)**. The thesis directly addresses **SDG 6 (particularly 6.1: Safe drinking water for all/6.3: Better Water Quality/6.6: Healthier Ecosystem)**, which aims to "improve water quality by reducing pollution, eliminating dumping, and minimizing release of hazardous chemicals and materials" by 2030. By providing the first comprehensive spatiotemporal assessment of e-EDCs in the Ganga River system, identifying major point sources, and quantifying riverward fluxes, this research provides crucial data for evidence-based policymaking and targeted interventions. The study's identification of four key drains responsible for 81% of total EDC mass flux enables focused remediation efforts, supporting efficient resource allocation for water quality improvement. Additionally, the research contributes to **SDG 14 and 15** by assessing ecological risks to aquatic biodiversity, particularly endangered species like the Gangetic dolphin. By establishing biomonitoring frameworks and identifying suitable indicator species, the thesis provides valuable tools for long-term ecosystem health assessment, supporting the targets of biodiversity conservation and sustainable ecosystem management. The research's holistic approach - examining water, prey fish, and molluscs - provides a comprehensive understanding of EDC impacts across the food web, enabling more effective conservation strategies for this ecologically significant river system.

***Keywords*: Estrogenic Endocrine disrupting compounds; Middle Ganga Reach; Gangetic dolphin; bio-monitors; surface water; ecological risk assessment, drain discharges, macrobenthos, biomonitor**

PREFACE STATEMENT

This thesis provides the first comprehensive baseline information on the occurrence, distribution, and spatiotemporal dynamics of estrogenic endocrine-disrupting compounds (e-EDCs) in the Middle Ganga Reach (MGR). It offers crucial insights into the contribution of multiple drain outfalls to the e-EDC load in the MGR. Additionally, for the first time, the dietary exposure risks of e-EDCs to Gangetic dolphins were quantified, and suitable biomonitor species were identified. The thesis is structured into seven chapters, outlined as follows:

Chapter 1

Chapter 1 offers a brief introduction to EDCs, provides an overview of the study area, and outlines the objectives and research questions guiding this thesis.

Chapter 2

This chapter of the thesis includes a comprehensive literature review of 22 years (2000-2020), which reviews the current state of literature on EDCs in riverine environments of India and their environmental fate and toxic impact on diverse aquatic taxa. The chapter places particular emphasis on the Ganga River, identifying critical research and data gaps that are subsequently addressed in detail in the experimental Chapters 4-6.

Chapter 3

This chapter provides an overview of the physical and chemical properties of forty-two target EDCs. These properties are crucial for understanding the environmental behavior, persistence, and toxicological impacts of the EDCs, guiding the analysis and interpretation of the experimental findings in Chapters 4 through 6.

Chapter 4

Chapter 4 addresses objectives 1 to 3 and research questions I, and II. It investigates the spatiotemporal occurrence and distribution of EDCs in the surface water of MGR, and assess the spatial influence of drains-outfalls on the EDC burden in MGR. The chapter also explores the influence of key environmental parameters on EDC distribution and attenuation. Additionally, it assesses the potential ecological risks and estrogenic activity of individual EDCs on aquatic organisms across various trophic levels, identifying potentially high-risk zones.

Chapter 5

Chapter 5 addresses objectives 4a-b and research question III, presenting findings on the bioaccumulation patterns of EDCs in various prey fish species of the Gangetic dolphin. It explores how abiotic and biotic factors influence these bioaccumulation profiles. The chapter also assesses the potential health risks posed by EDCs to Gangetic dolphins through dietary exposure and provides an issue-based framework for monitoring chemical pollution in their habitat.

Chapter 6

Chapter 6 is an extension of objective 4b (research question IV) and present the results of bioaccumulation profiles of EDCs in macroinvertebrates of MGR and their potential as biomonitor of EDC contamination in effluent-impacted habitats.

Chapter 7

Chapter 7 synthesizes the findings from Chapters 2 to 6 and offers suggestions for future research.

This PhD thesis is composed of several manuscripts (Annexure II). At the time of submission, one manuscript had already been published, while the second and third were in preparation for submission to peer-reviewed journals. The published manuscript is included in Chapter 5, with minor modifications for formatting consistency. I am the lead author of all the manuscripts, with Dr. Gautam Talukdar, Dr. S.A. Hussain, and Dr. Ruchi Badola serving as senior co-authors in different papers. While the work predominantly reflects my intellectual contributions, input from several co-authors has been acknowledged in the manuscript.

1. Sah, R., Talukdar, G., Megha Khanduri, Chaudhary, P., Ruchi Badola, & Syed Ainul Hussain. (2024). Do dietary exposures to multi-class endocrine disrupting

chemicals translate into health risks for Gangetic dolphins? An assessment and way forward. Heliyon, 10(15), e35130–e35130. *https://doi.org/10.1016/j.heliyon.2024.e35130*

2. Sah, R., Megha Khanduri, Chaudhary, P., Paul, K. T., Samridhi Gururani, Kirti Banwala, Paul, C., Jose, M. A., Bora, S., Ramachandran, A., Ruchi Badola, & Hussain, S. A. (2024). Dietary exposure of potentially toxic elements to freshwater mammals in the Ganga River basin, India. Environmental Pollution, 123928–123928. https://doi.org/10.1016/j.envpol.2024.123928

3. Sah, R., Baroth, A., & Hussain, S. A. (2020). First account of spatio-temporal analysis, historical trends, source apportionment and comprehensive ecological risk evaluation of banned organochlorine pesticides along the Ganga River. Environ. Pollut., 263, 114229. https://doi.org/10.1016/j.envpol.2020.114229

4. Sah, R., Talukdar, G., Ruchi Badola, & Syed Ainul Hussain. Spatiotemporal dynamics of estrogenic plastic additives in a heavily populated, rapidly urbanizing, and ecologically vital river landscape. Manuscript In preparation

5. Sah, R., Talukdar, G., Ruchi Badola, & Syed Ainul Hussain. Spatiotemporal dynamics of endocrine disrupting chemicals in Gangetic dolphin habitats and influence of socioeconomic and environmental factors. Manuscript In preparation

Conferences and Book Chapter

Sah, R., Talukdar, G., Hussain, S.A., Khanduri, M., Chaudhary, P., Badola, R. (2023). Beyond the surface: Assessing bioaccumulation potential of endocrine disruptors in a macrobenthos (Lamellidens marginalis) inhabiting an effluent-impacted urban river stretch (Ganga River). **3rd International Conference on River Health: Assessment to Restoration**. IIT(BHU) Varanasi, India. October 12-14, 2023.

Sah, R., Baroth, A., Hussain, S.A., Johnson, J.A., Ahada, C.P.S, Bhola, S. (2018). Pesticide Pollution and Surface Water Quality Across River Ganga: Spatial Distribution and Ecological Risk Assessment. **International Conference on Water: From Pollution to Purification**. School of Environmental Sciences and Advanced Centre of Environmental Studies and Sustainable Development (ACESSD), Mahatma Gandhi University, Kottayam, Kerala Varanasi, India. December 07-10, 2018.

- **Sah, R., Baroth, A., Hussain, S. A., Ahada, C., & Bhola, S. K.** (2019). *Assessment of physico-chemical parameters and organochlorine pesticides in* *water and sediment of the Ganga River: Preliminary status and trends*. In: Biodiversity Profile of the Ganga River. Publisher: Wildlife Institute of India.

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ABBREVIATIONS

e-EDC	Estrogenic Endocrine Disrupting Compounds
MGR	Middle Ganga Reach
PreM	Pre-Monsoon
PostM	Post-Monsoon
PAs	Plastic Additives
OCPs	Organochlorine Pesticides
HPCPs	Household and Personal Care Products
GD	Gangetic Dolphin
CPCB	Central Pollution Control Board
MLD	Million Litres Per day
TPD	Tonnes Per Day
CAS	Chemical Abstracts Service
PCBs	Polychlorinated Biphenyls
RQ	Risk Quotients
PNEC	Predicted No Effect Concentrations
PI	Prioritization Index
EEQs	Estradiol Equivalent Concentrations
WWTPs	Waste Water Treatment Plants
DO	Dissolved Oxygen
TDS	Total Dissolved Solids
ng/L	Nanogram Per Litre
mg/L	Miligram Per Litre
DF	Detection Frequency
PCB 44	2,2',3,5'-Tetrachlorobiphenyl
PCB 52	2,2',5,5'-Tetrachlorobiphenyl
PCB 77	3,3',4,4'-Tetrachlorobiphenyl
PCB 81	3,4,4',5-Tetrachlorobiphenyl

PCB 114	2,3,4,4',5-Pentachlorobiphenyl
PCB118	2,3',4,4',5-Pentachlorobiphenyl
PCB 138	2,2',3,4,4',5'-Hexachlorobiphenyl
PCB 153	2,2',4,4',5,5'-Hexachlorobiphenyl
PCB 156	2,3,3',4,4',5-Hexachlorobiphenyl
PCB 167	2,3',4,4',5,5'-Hexachlorobiphenyl
PCB 169	3,3',4,4',5,5'-Hexachlorobiphenyl
PCB 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl
PCB 189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
PCB 209	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl
DMP	Dimethyl phthalate
DEP	Diethyl phthalate
DnOP	Di-n-octyl phthalate
DEHP	Bis(2-ethylhexyl) phthalate
BBP	Butyl benzyl phthalate
DnBP	Di-n-butyl phthalate
BPA	Bisphenol A
α-HCH	alpha-1,2,3,4,5,6-hexachlorocyclohexane
β-HCH	beta-1,2,3,4,5,6-hexachlorocyclohexane
γ-HCH	gamma-1,2,3,4,5,6-hexachlorocyclohexane or Lindane
δ-HCH	delta 1,2,3,4,5,6-hexachlorocyclohexane
p,p'-DDE	1,1-Dichloro-2,2-bis (4-chlorophenyl) ethene dichlorodiphenyldichloroethylene
p,p'-DDD	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene
p,p'-DDT	1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl) ethyl]benzene
E1	Estrone
E2	Estradiol
E3	Estriol

EE2	Ethinyl estradiol
DES	Diethylstilbestrol
TCS	Triclosan
TCC	Triclocarban

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Chapter 1 Introduction and research rationale

1.1 Introduction

The International Programme on Chemical Safety, IPCS (2002) defines endocrine disruptors as “exogenous substances or mixtures that disrupts normal function(s) of the endocrine system and consequently cause adverse health effects in humans and wildlife” (IPCS, 2002).

These Endocrine Disruptors, or Endocrine Disrupting Chemicals (EDCs) can bind to endocrine receptors (ERs) in organisms, resulting in the activation, blocking and alteration of endocrine function (Bergman et al., 2012; Gore et al., 2015; Solecki et al., 2017). A specific subset of EDCs, known as ***estrogen-mimicking endocrine disruptor compounds (e-EDCs)***, are the chemicals (both natural and man-made) that mimic or induce responses akin to estrogen in organisms (Crisp et al., 1998; Campbell et al., 2006; WHO, 2012).

The category of EDCs exhibits high heterogeneity, encompassing natural and synthetic hormones, as well as various man-made chemicals that mimic estrogenic effects such as pesticides, pharmaceuticals, natural and synthetic hormones, surfactants, plasticizers and flame retardants (Crisp et al., 1998; Metcalfe et al., 2022). The release of EDCs into aquatic environments is driven by their extensive production, widespread use, and improper disposal, across sectors like households, agriculture, industry, and healthcare. EDCs are introduced into the aquatic environments through rainfall runoff, sewage discharge, landfill leachate, hospital waste, wastewater treatment plants, and livestock waste (Metcalfe et al., 2022).

Over the past few decades, the deleterious ecological impacts of these EDCs have been increasingly identified across a broad spectrum of aquatic taxa, including primary producers, zooplankton, insects, amphibians, fish, birds, turtles, and cetaceans (Table 2-2 & 2-3). Convincing evidence points their role in driving population and biodiversity decline by contribute to reproductive failure, developmental abnormalities, compromised immunity and endocrine dysfunction (Hutchings, 2000; Vos et al., 2000; Hickie et al., 2007; Hall et al., 2018; Desforges et al., 2018).

Considering that many of these EDCs possess the capability to elicit an estrogenic response at trace concentrations (Welshons et al., 2003; Vandenberg, 2014), it raises concerns that measurable amounts of these chemicals have been detected in diverse aquatic environments across the globe (Kortenkamp et al., 2012; Esteban et al., 2014; Yamazaki et al., 2015; Li et al., 2017; Chakraborty et al., 2021; Lalonde et al., 2018; Sah et al., 2020; Rex & Chakraborty, 2022; Liao et al., 2024). This is particularly concerning for densely populated, agriculturally active, rapidly urbanizing, extensively industrialized, rivers such as the Ganga. These rivers also support rich biodiversity, which often displays high degrees of endemism. Thus, high EDCs loads even in small stretches can lead to local extinctions (Alahuhta et al., 2019; Wliiliams-Subiza and Epele, 2021).

1.2 Objective

In the light of the above, the objectives of this doctoral work are:

1. To quantify the spatiotemporal distribution and load of e-EDCs in drains effluents and Ganga River

2. To investigate and assess the estrogenic potential and ecological risk of selected e-EDCs to aquatic life

3. Identification of high-risk zones

4. To comprehensively investigate the (a) occurrence, and driving factors of bioaccumulation patterns of EDCs, in fish species, at different trophic levels (b) potential health risks of EDCs to Gangetic dolphins through dietary routes (c) relevance of macro-invertebrates as biomonitor for EDCs exposure in effluent-impacted MGR habitats

1.3 Research Questions

This thesis will address the following research questions:

I. What is the spatiotemporal distribution of e-EDCs in MGR? What spatial influence does drain outfalls have on these distributions? How are e-EDCs distributions influenced by key environmental factors? (Objective I)

II. What are the estrogenic potency and ecological risks of e-EDCs to aquatic organisms at different trophic levels? What stretches represent vulnerable and high-risk zones? (Objective II and III)

III. What are the bioaccumulation patterns of e-EDCs at different trophic levels? What role do abiotic factors (physical-chemical properties of the aquatic environment) and biotic factors (physiological characteristics of aquatic organisms) play in influencing the bioaccumulation profiles of e-EDCs in diverse aquatic organisms? Does exposure to e-EDCs translate into health risks for Gangetic dolphins through the ingestion of contaminated prey? (Objective IV a and b)

IV. Which species might best fit the requirements for a biomonitor in effluent-impacted and e-EDCs -contaminated MGR habitats? (Objective IV b)

1.4 Significance of Study

The answers to these research questions will aid in designing robust monitoring strategies and implementing targeted mitigation measures to reduce the presence of e-EDCs in aquatic environments, which in turn can serve as a foundation for informed conservation initiatives. Furthermore, the study proposes a risk management and monitoring framework that can be used to augment the current biodiversity monitoring programs.

Chapter 2 Endocrine Disrupting Chemicals in Freshwater Environments: A review on literature

Abstract

This study presents a comprehensive review of estrogenic endocrine-disrupting chemicals (e-EDCs) contamination in Indian rivers, focusing on the Ganga River system. The objective was to synthesize and analyze literature data from 2000 to 2022 to assess EDC pollution, and identify research gaps.

The analysis reveals widespread contamination of both legacy and emerging EDCs across numerous rivers, highlighting significant challenges in freshwater ecosystem management. Key findings include: (1) Limited geographical coverage of EDC studies; (2) Insufficient monitoring of point-source discharges; (3) Absence of long-term monitoring programs; (4) Disproportionate focus on legacy contaminants over emerging EDCs; and (5) Significant data gaps in assessing EDC impacts on endangered species, particularly Gangetic River dolphins.

The literature review (2000-2022) indicates growing awareness of EDC issues but reveals persistent gaps in comprehensive, long-term studies. While research has intensified in some urban rivers, many rivers and emerging contaminants remain understudied. The review highlights an urgent need for standardized, wide-scale monitoring protocols across India's diverse river systems.

These research gaps limit our ability to develop targeted mitigation strategies and implement effective conservation measures. Addressing these challenges requires a multifaceted approach, including enhanced research efforts, improved monitoring systems, and targeted mitigation strategies. Such initiatives are crucial for safeguarding the ecological integrity of India's freshwater ecosystems and ensuring the survival of vulnerable species in the face of increasing anthropogenic pressures.

2.1 Introduction

Endocrine disruption is among the most significant effects of contaminants on aquatic ecosystems, with far-reaching implications on human and wildlife. Endocrine-disrupting substances (EDCs) are chemical compounds in the environment (air, soil, or water supply), food sources, personal care products, and manufactured products that can imitate or counteract the actions of endogenous hormones like estrogen and androgen or interfere with the synthesis and metabolism of endogenous hormones and hormone receptors in humans and wildlife (Kavlock et al., 1996).

EDCs in aquatic environments have emerged as a critical ecological concern due to their persistent, bioaccumulative, mobile, and toxic properties (WHO,2012; Metcalfe et al., 2022). EDCs commonly detected in aquatic environment mainly include pesticides (such as organochlorine pesticides), polychlorinated biphenyls (PCBs), bisphenol A (BPA), polybrominated diphenyl ethers (PBDEs), phthalates, natural and synthetic hormones, and household and personal care products (HPCPs) etc. (Pignotti et al., 2017; Pal et al.,2010; Annamalai, 2015).

Despite regulations on some legacy EDCs, these contaminants persist at high levels, even in remote ecosystems, underscoring their resilience and ongoing risks to ecological health (Campbell et al., 2006). At the same time, many emerging EDCs remain unregulated, particularly in developing countries, due to significant data gaps, resource limitations, and regulatory lag, which hinder effective monitoring and enforcement. These challenges are further exacerbated by policymakers' limited awareness of the dangers posed by these chemicals, leading to inadequate control measures and oversight. This lack of regulation allows the chemicals to infiltrate river systems, increasingly threatening riverine biodiversity.

The increasing evidence of the risks posed by EDCs to various species has spurred global efforts to monitor their presence in aquatic environments. However, despite the well-documented health impacts on aquatic biodiversity, particularly apex predators, significant monitoring gaps persist, especially in freshwater systems within developing nations. These gaps hinder a comprehensive understanding of EDCs' effects, making it challenging to develop effective mitigation strategies. Furthermore, to effectively manage EDCs in aquatic environments, it is imperative to understand their sources, fate and transport behaviour (Pal et al., 2010).

In the light of the above, this chapter provides an in-depth analysis of the sources, fate, status, and occurrence of various EDCs in freshwater ecosystems. It also critically identifies research and data gaps in the study of EDCs within India's freshwater systems, highlighting key areas for future investigation, with an emphasis on the Ganga River.

2.2 EDCs in the Freshwater Environment

2.2.1 Sources, Fate, and Transport

The primary driver of EDC pollution in freshwater environments is intricately linked to their extensive production and widespread usage across various sectors, including agriculture, healthcare, veterinary, industry, household, and personal care (Table 2-1).

Agriculture is a major sector contributing to EDCs in the Indian freshwater environment. The total arable land in the India stood at 1,80,888 thousand Ha in 2018-19, with 197,320 thousand Ha of total cropped area and a cropping intensity of 141.60% (PIB, 2022). The Net Sown Area contributed to nearly 45% of land use in the country during this period (DES, 2023). India is also one of the world's major pesticide manufacturers (Abhilash and Singh, 2009) and the total pesticide production has increased from 216703 MMT in 2018 to 255090 MMT in 2020 (GoI, 2023). Apart from being produced indigenously, a number of pesticides such as Atrazine, DDT and Diclofol are also imported from China, Europe and Israel (PPQS, 2023), all of which are used in agriculture to prevent crop loss. However, improper pesticide application, overuse, or poor soil management can lead to greater pesticide residue being washed away. Pesticides are introduced into rivers primarily through agricultural runoff, which occurs when rainfall or irrigation water washes over fields treated with pesticides and carries these chemicals into nearby water bodies. This process is exacerbated during heavy rains, which can lead to increased runoff (Eichelberger & Lichtenberg, 1971; Vryzas, 2018; Siddiqui, 2019).

Another major source of EDCs in freshwater environments is discharge of industrial wastewater. There were an estimated 11073 chemical, 10106 metal, 4501 pharmaceuticals, 11732 rubber and plastic products, 13539 textiles, 3949 leather operating factories in India in the year 2019-2020 (MoSPI, 2020). These industries are a major producer of EDCs such as plasticizers, PCBs, phthalates and alkyl phenols, as well as pharmaceuticals including synthetic steroid hormones. Alarmingly, a number of industrial clusters are located in biodiversity-rich river basins. Similarly, municipal wastewater is also a major source of EDCs to freshwater environment. The discharge of treated, partially treated and/or untreated municipal wastewater into rivers introduces a diverse array of chemicals, including pharmaceuticals, personal care products, natural/synthetic steroids and other down-the-drain EDCs.

Effluent from wastewater treatment plants (WWTPs) is a major source of EDCs in aquatic environments, often exceeding other sources in impact (Kasprzyk-Hordern et al., 2009; Inam et al., 2019; Luo et al., 2019). Treated effluents contribute to EDCs in the environment because current wastewater treatment plants (WWTPs) are not fully equipped to remove these complex chemicals. EDCs, which include pharmaceuticals, personal care products, and industrial chemicals, often resist conventional treatment processes and can pass through WWTPs largely unchanged (Inam et al., 2019; Luo et al., 2019). Furthermore, bioassays have determined the potential of WWTPs and STP effluent to adversely affect aquatic organisms through endocrine disruption (Kusk et al., 2011).

These effluents lead to EDC accumulation not only at discharge points but also downstream, causing widespread contamination that disrupts aquatic ecosystems, including altering wildlife reproductive and developmental processes. For instance, feral carps downstream of EDC-discharging STPs show elevated plasma vitellogenin concentrations (Petrovic et al., 2002). Consequently, these point sources significantly impact riverine wildlife through the mobility of effluents and bioaccumulation of contaminants.

Besides WWTPs/STPs, EDCs may enter aquatic environments through multiple pathways including sewer leaks (Phillips & Chalmers, 2009), improper waste disposal management in hospitals (Pironti et al., 2021; Khan et al., 2021), animal waste runoff (Liu et al., 2018), direct discharges of untreated industrial or household wastewater (Tong et al., 2022), and atmospheric deposition (Annamalai et al., 2015). Furthermore, intense stormwater runoff, aging sewers, sewer overflow/leakage exacerbates the diffusion of EDCs in urban rivers (Hwang & Foster, 2006; Zgheib et al., 2012).

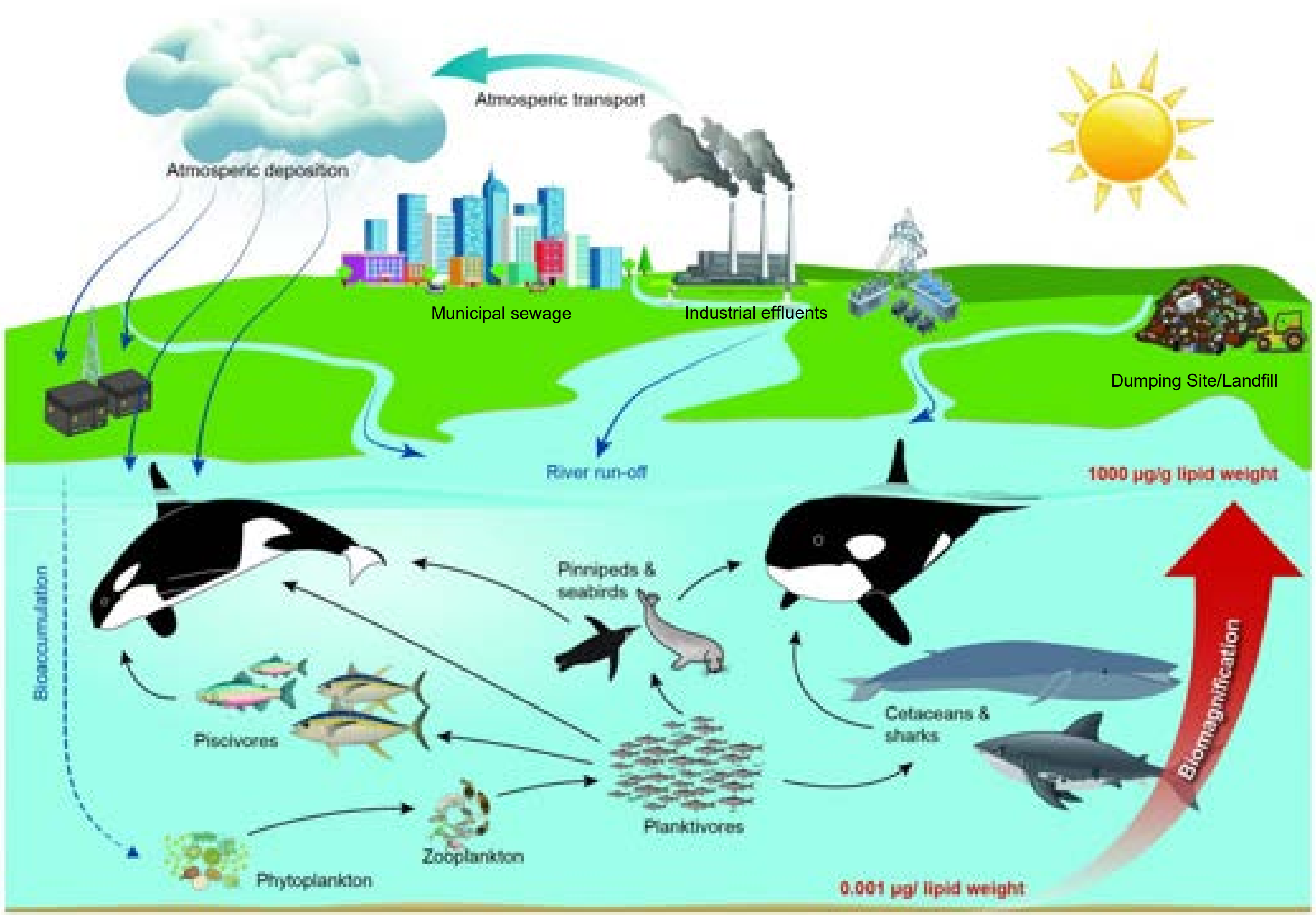


Figure 2-1: Source, Fate, and Routes of EDCs in the aquatic environment

Figure credit: Aarhus University

The spatial and temporal distribution of EDCs in aquatic environments is a function of numerous variables including population, land-use, regulatory effectiveness, transport through other media such as aquifers, aquatic biogeochemistry, air-surface exchange processes and weather-related phenomena (Lammel and Stammler, 2012; Macías-Zamora et al., 2014; Ryberg and Gilliom, 2015). The EDCs are transported to aquatic environments, through diverse pathways, including rainfall, sewage discharge, landfill leachates, hospital waste, indiscriminate wastewater release, manure, and aquaculture wastewater (Campbell et al., 2006; Metcalfe et al., 2022). Upon release into water bodies, EDCs undergo diverse fate mechanisms that are often related to their persistence and partitioning. These mechanisms include atmospheric deposition, dispersion, sorption onto sediments, remobilization, and dissolution (Figure 2-1). The mobility of EDCs is further shaped by factors such as water flow, sedimentation, water chemistry, and sediment characteristics. The bio-available fractions are subsequently taken up by biota, potentially resulting in bioaccumulation, biomagnification, and trophic transfer. However, the trophic transfer and biomagnification of EDCs may vary with chemical, environmental, biological, and ecological factors (Russell et al., 1999; Mackay et al., 2018). Additionally, the fate of EDCs is influenced by transformation processes such as photodegradation and microbial degradation, which contribute to the gradual breakdown of these chemicals over time. Understanding the intricate interplay of these fate and transport mechanisms is essential for assessing the potential risks posed by EDCs to aquatic ecosystems and their inhabitants (Wang et al., 2020; He et al., 2022).

2.3 Impact on freshwater environment

2.3.1 Mode of EDC action

EDCs affect a wide range of organisms in the environment. However, their modes of action remain more or less similar across taxa as described in Figure 2-2 (La Merrill et al., 2019). Each of these characteristics highlights a key characteristics (KCs) in which EDCs may interfere with normal endocrine functioning.

1. Receptor Interaction: e-EDCs may bind and activate hormone receptors
2. Receptor Blockage: e-EDCs may hinder normal receptor function by blocking them.
3. Receptor Expression Alteration: e-EDCs can influence the levels at which hormone receptors are produced.
4. Signal Transmission Disruption: They disrupt the signaling processes in hormone-sensitive cells, affecting protein synthesis, RNA expression, protein modifications, and ion transport.
5. Epigenetic Modification: e-EDCs may induce epigenetic changes in cells that produce or respond to hormones.
6. Hormone Synthesis Disruption: e-EDCs may interfere with the normal production of hormones.
7. Hormone Transport Alteration: EDCs can impact the movement of hormones across cell membranes.
8. Circulation and Distribution Impact: They can alter hormone circulation and concentration within the body.

9. Hormone Metabolism Interference: e-EDCs may disrupt the breakdown or clearance of hormones from the body.

Cell Fate Alteration: These chemicals can affect the survival or development of hormone-producing or hormone-responsive cells.

2.3.1 Toxicological Profiles: Impact on wildlife

Figure 2-2 and Table 2-3 broadly describes the effects of EDCs at different ecological levels in aquatic ecosystems. Through their effects on individuals and populations of species, they have the potential to affect both ecosystem structure and function. Owing to their influence on the endocrinology of organisms, EDCs often have far-reaching repercussions for the potential to affect both ecosystem structure and function. Studies have established their effects on the survival, growth and behaviour in multiple taxa including primary producers, primary consumers and higher consumers including aquatic mammals large mammals in densely populated rivers such as the Yangtze and Ganga face severe threats from various anthropogenic activities, including flow modification, habitat loss, degradation, and chemical pollution (Cazzolla Gatti, 2016). These combined threats exacerbate pressures on these ecosystems, putting already vulnerable species at even greater risk. Despite ongoing efforts, there remains a significant gap in species-specific, exposure-specific, and effect-based data concerning aquatic mammals in India, particularly those classified as threatened. This gap highlights a critical area for future research.

Table 2-1: Sources, pathways, and impact of key EDCs

EDC class	Potential Subgroup	Origin/Application	Pathways to aquatic environments
Persistant Organic Pollutants (POPs)	Organochlorines such as organo chlorine pesticides (OCPs), Polychlorinated Biphenyls (PCBs)	Agrochemicals Insecticides Termiticides Rodenticides Pesticides Electrical equipment, paints, and other industrial applications	Agricultural runoff Industrial effluents Drain discharges Untreated sewage
Plastic additives	Plasticizers such Phthalates (PAEs) Bisphenol A PTEs such as Lead, cadmium,	PVC plastics Packaging materials HPCPs Children's toys Medical devices Fragrances Dyes Medical devices Fishing nets	Agricultural runoff Industrial effluents Drain discharges Leaching from extensive dumping of plastics waste Landfill Leachates Untreated sewage
Household Personal Care Products (HPCPs)	Anti-microbials such as Triclosan (TCS), Triclocarban (TCC)	Soaps, shampoos Toothpastes Mouthwashes Perfumes Insect repellents Sunscreens	Industrial/Municipal Wastewater effluents Aquaculture Agricultural runoff Untreated sewage
Estrogens	Natural hormones such as Estrone (E1), Estradiol (E2), Estriol (E3) and synthetic estrogens such as Ethinyl estradiol (EE2) and Diethyl stilbesterol (DES)	Biologically produced and excreted natural hormones Synthetic steroids Contraceptives	Industrial/Municipal Wastewater Hospital waste Agricultural runoff Livestock/Aquacultur e waste Untreated sewage

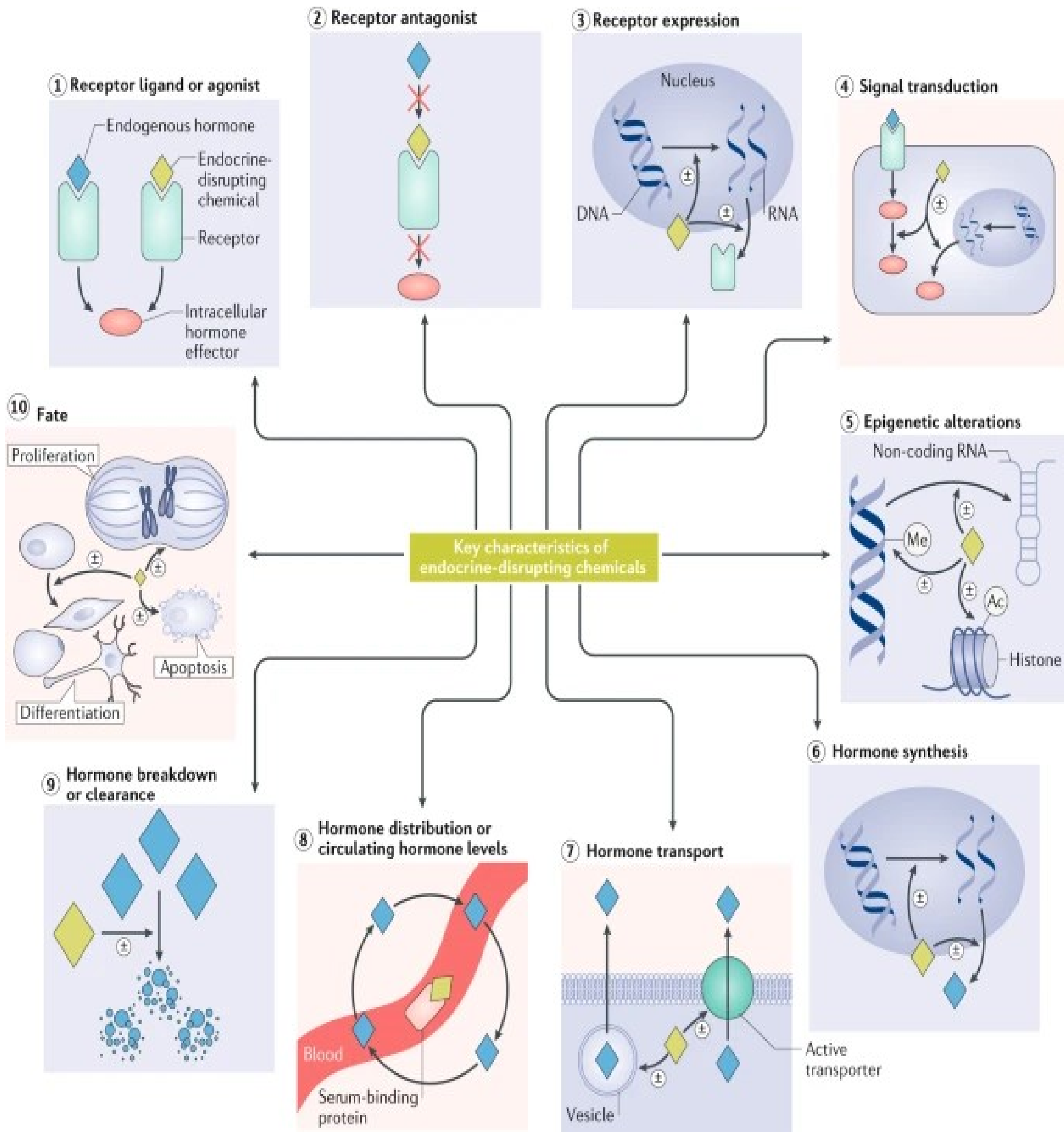


Figure 2-2: Mode of action Endocrine Disrupting Chemicals

(Adapted from La Merrill et al., 2019; Depicted EDC actions include amplification and attenuation of effects. Ac, acetyl group; Me, methyl group).

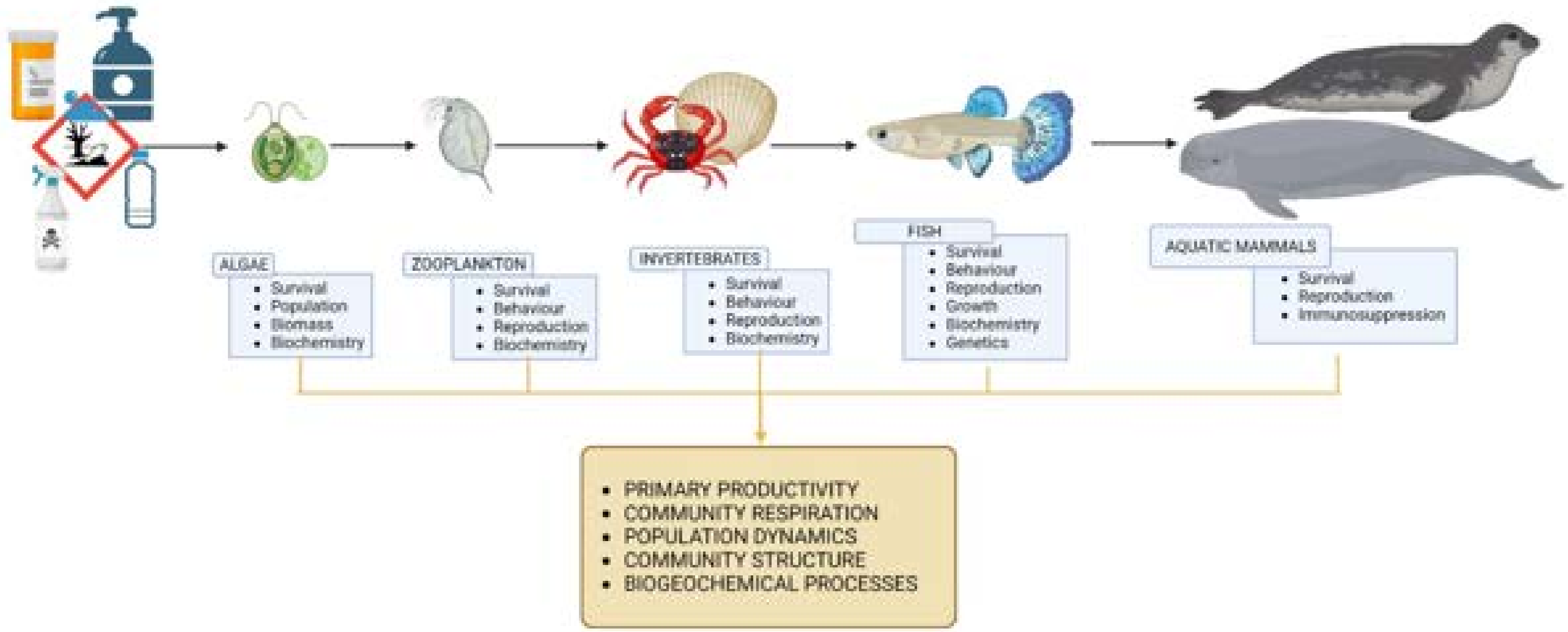


Figure 2-3: Reported effects of EDCs at various ecological levels and potential ecosystem effects.

(Created with BioRender.com)

Table 2-2: A detailed account of EDCs effects at various trophic levels				
Group	**Organism**	**Contaminant**	**Effect**	**Reference**
Primary producers	Algae	Alkyl Phenols and Steroid Hormones	Photosystem energy fluxes	Perron and Juneau, 2011
		Triclosan	Algal growth	White et al., 2005
		Phenolic Compounds	Inhibit growth and chlorophyll content	Zhang et al., 2015; Wang et al., 2018
Primary Consumers	Zooplankton	Estrogens	Inhibits moulting	Plahuta et al., 2017
		Phenolic Compounds	Effects on population assemblages	Severin et al., 2003
	Benthic invertebrates	Pesticides	Effects on development, age structure and population size	Di Marzio et al., 2013
		Estradiol	Decrease nematode diversity	Essid et al., 2021
		Cadmium	Affects reproduction	Kluytmans et al., 1988
		Tributylin, Dibutylin, Triphenyltin	Induction of Imposex, Sterilization	Bryan et al., 1986; Gibbs et al., 1988; Grilo and Rosa, 2017
		BPA, and Phthalate (DEHP)	ERR expression (crucial for normal development and reproduction)	Park and Kwak, 2010
		Estrogenic Sewage Effluent	Stimulation of embryo production and Delayed hatching	Langston, 2020
Higher Consumers	Fish	Chlorinated Hydrocarbons	Decreased hatching success	Hansen et al., 1985
		Bisphenols	Inhibit growth	Wu et al., 2022
		Estrogen (E2)	Slower anti-predator responses and effects on survival and population	Rearick et al., 2018
		Sewage Treatment Plant Discharge	Intersex in male fish	Tetreault et al., 2012

Group	Organism	Contaminant	Effect	Reference
	Amphibians	Pesticides	Immunosuppressive effects and developmental anomalies	Hayes et al., 2006
		Endosulfan	Reduced mating success and suppressed pheromonal communication	Park et al., 2001
		benzo(a) pyrene, triclosan	Trans-generational effects, reduced size at metamorphosis, reduced recruitment	Regnault et al., 2018
Top Carnivores	Reptiles	Pesticides	Abnormality of gonads, population decline in alligators	Guillette Jr., 1994, Boggs et al., 2010
			Affects Adrenal glands	Di Lorenzo et al., 2020
		PCBs	Reversal of temperature-dependent sex determination in turtles	Crews, 1995
	Birds	Methoxychlor	Demasculinizing effects on male sexual behaviour	Ottinger et al., 2002
		DDTs, Methoxychlor, OCP	Behavioural changes, feminization of males, ovotestis	Ulfstrand et al., 1971; Fry, 1995
		DDT	Reduced egg laying	Halldin, 2005
		DDT	Population decline	Lundholm, 1997

2.4 Exposures of EDCs in the freshwater environment

2.4.1 EDC Research in Freshwater Environments: Status and Challenges

Monitoring systems offer detailed insights into contaminant occurrence, distribution, sources, transport, and fate, accurately capturing their dynamics in the environment.

Table 2-3: Toxicological impact reported in aquatic mammals exposed to EDCs			
Species	**EDC**	**Effect**	**Reference**
Indo-Pacific finless porpoise *(Neophocaena phocaenoides)*	BPA and alternatives	Alteration in Tetraiodothyronine, testosterone, and cortisol levels	Guo et al., 2023
Indo-Pacific humpback dolphin (*Sousa chinensis*)	Phthalates and metabolites	Thyroid function and lipid disruption	Dziobak et al., 2022; Xie et al., 2023
Indo-Pacific humpback dolphin (*Sousa chinensis*)	PCBs and derivatives	raise tetraiodothyronine, testosterone, and cortisol level	Guo et al., 2022
Northern fur seal *(Callorhinus ursinus)*	PCBs, OCPs	Gene expression	Soulen et al., 2022
Common Seal (*Phoca vitulina)*	PCBs	Population collapse	Reijnders, 1986
Common dolphins (*Delphinus delphis*) and Harbour porpoises (*Phocoena phocoena*)	Persistent organic pollutants (PCBs, DDE, PBDE)	Impact on reproduction and mortality	Pierce et al., 2008
Sea lions (*Zalophus californias*)	DDTs	Premature pupping	Delong et al., 1973
Saimaa ringed seal (*Phoca hispida saimensis*)	Hg	Population decline	Hyvärinen et al., 1997
St. Lawrence Beluga Whales (*Delphinapterus leucas*)	Hg and Pb	Chronic lesions and reproductive impairment	Béland et al., 1993
Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*)	OCPs	Endocrine disruption in individuals of unprotected waters	Liu et al., 2022
La Plata dolphin (*Pontoporia blainvillei)*	PCBs, DDTs	Transplacental transfer	Barbosa et al., 2018
Mekong Irrawaddy dolphin (*Orcaella brevirostris*)	DDT, Hg	Population decline	Dove, 2022
Galapagos Sea Lions (*Zalophus wollebaeki*)	PCBs, PBDEs	Accumulation	Alava et al., 2009
Harbor seal (*Phoca vitulina*)	Metals (Ag, Cd, Ni, Pb, and Zn)	Hypersensitivities caused by chronic exposure	Kakuschke et al., 2011

Species	EDC	Effect	Reference
Coastal cetaceans (*S. chinensis, N. phocaenoides, S. coeruleoalba, P. microcephalus, K. sima, B. acutorostrata, G. macrorhynchus, L. hosei , B. physalus*)	Triclosan and triclocarban	Thyroid function disruption	Hinther et al., 2011; Guo et al., 2023
Cetaceans (Indo-Pacific humpback dolphin, Bottle nose dolphin	DDT	Cytotoxicity and reproductive dysfunctions	Galligan et al., 2019; Yu et al., 2019; Guo et al., 2023
Coastal cetaceans (*S. chinensis, N. phocaenoides, S. coeruleoalba, P. microcephalus, K. sima, B. acutorostrata, G. macrorhynchus, L. hosei , B. physalus*)	BPA and alternatives	Affect endocrine and immune functions, stress responses and lipid metabolism	Lunardi et al., 2016; Guo et al., 2023
St. Lawrence Estuary belugas and minke whales	PCBs, OCs and hexabromobenzene (HBB)	Expression of thyroid- and steroid-related genes	Simond et al., 2019

The environmental concentrations of these contaminants are critical because their bioavailability allows them to enter the food web and affect ecosystems.

As emerging contaminants, EDCs have only recently garnered the attention of the scientific community. In the global context of EDC research, an intricate network of international and interdisciplinary collaborations, advanced infrastructure and shared initiatives defines the approach towards understanding the exposures and impacts of EDCs. Leading research institutions and environmental agencies worldwide engage in robust studies encompassing diverse ecosystems and species. The emphasis lies on standardized methodologies, global data harmonization, and collaborative efforts to establish a unified understanding of the universal implications of EDC exposure.

A review of existing literature from 2000 - 2023 (Figure 2-4) reveals an increasing number of published research on endocrine disruptors in aquatic environments, specifically rivers, with a maximum of 1061 publications in the year 2022. This timeframe (2000-2023) has witnessed a growing interdisciplinary approach, incorporating fields such as environmental science, toxicology, ecology, and public health, to comprehensively understand the dynamics of EDCs in river environments. Researchers have explored various aspects, including sources and levels of contamination, effects on aquatic organisms and ecosystems, and the development of mitigation strategies.

As shown in Figure 2-4, developing countries have yet to prioritize research on EDCs in their rivers, with China being a notable exception. China hosts the majority of studies on EDCs, even surpassing the United States in research contributions. This higher research output in China can be attributed to the country's proactive recognition of the potential health and environmental impacts posed by EDCs. China’s substantial research funding and robust academic infrastructure provide the necessary resources for comprehensive studies and publications. Additionally, the country's historical evaluation criteria for funding and career promotion have incentivized researchers to actively contribute, reinforcing China’s leadership in addressing the complex challenges posed by EDCs on both national and global scales. Figure 2-5 compares the number of publications from

India and China between 2000 and 2023. As shown, research on Indian rivers has been sporadic and limited, hindering a comprehensive understanding of EDC contamination, its sources, and potential impacts. This is particularly concerning given the persistent upward trends in population growth, rapid urban development, and industrialization within the country, all of which are closely associated with the large-scale production and widespread discharge of EDCs into rivers.

The figure 2-6 illustrates the global research focus on EDCs from 2000 to 2023 through a network visualization. At the core of this network is the theme of "endocrine disruption," reflecting its central role in the scientific exploration of EDCs. The visualization reveals distinct clusters of research, including the investigation of pharmaceuticals and pesticides in water systems (green cluster), risk assessment of specific EDCs like Bisphenol A in surface water (red cluster), and the biological impacts of EDCs on molecular and reproductive health (yellow cluster). The size of the dots (nodes) in the network represents the frequency or

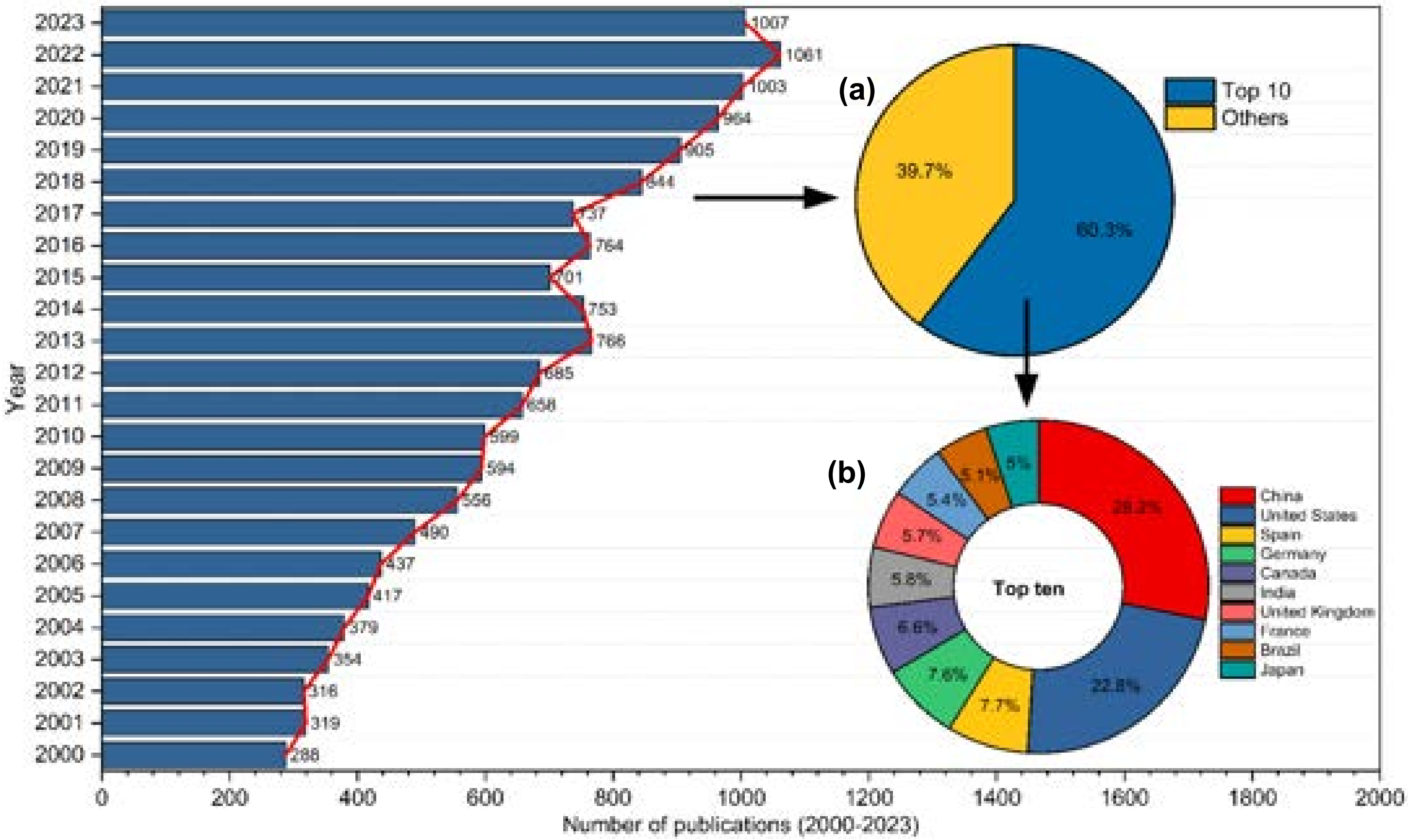


Figure 2-4: (a) Number of documents on EDCs research published between 2000 and 2023 (b) Top global contributors on EDCs research in river ecosystems from 2000 - 2023

(Data retrieved from Scopus)

prominence of each research topic, with larger nodes indicating areas that have been more heavily studied and central to research discussions. In contrast, smaller nodes represent topics that, while still relevant, have received less attention or are more specialized. These smaller nodes could indicate emerging areas of study or potential gaps in the current research landscape. The presence of these smaller nodes suggests that while some areas of EDC research are well-explored, others might benefit from further investigation to address these gaps and develop a more comprehensive understanding of EDC impacts.

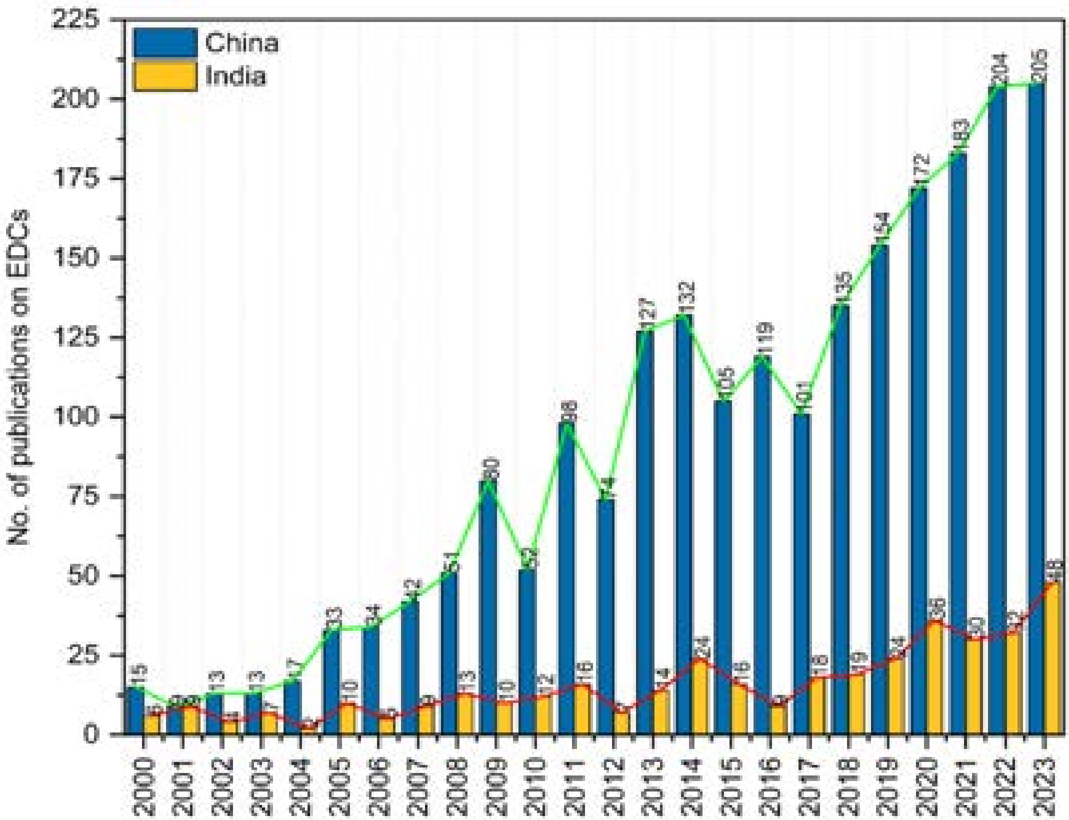


Figure 2-5: Comparison of literature published in India and China regarding EDCs in riverine systems ***(Data retrieved from Scopus)***

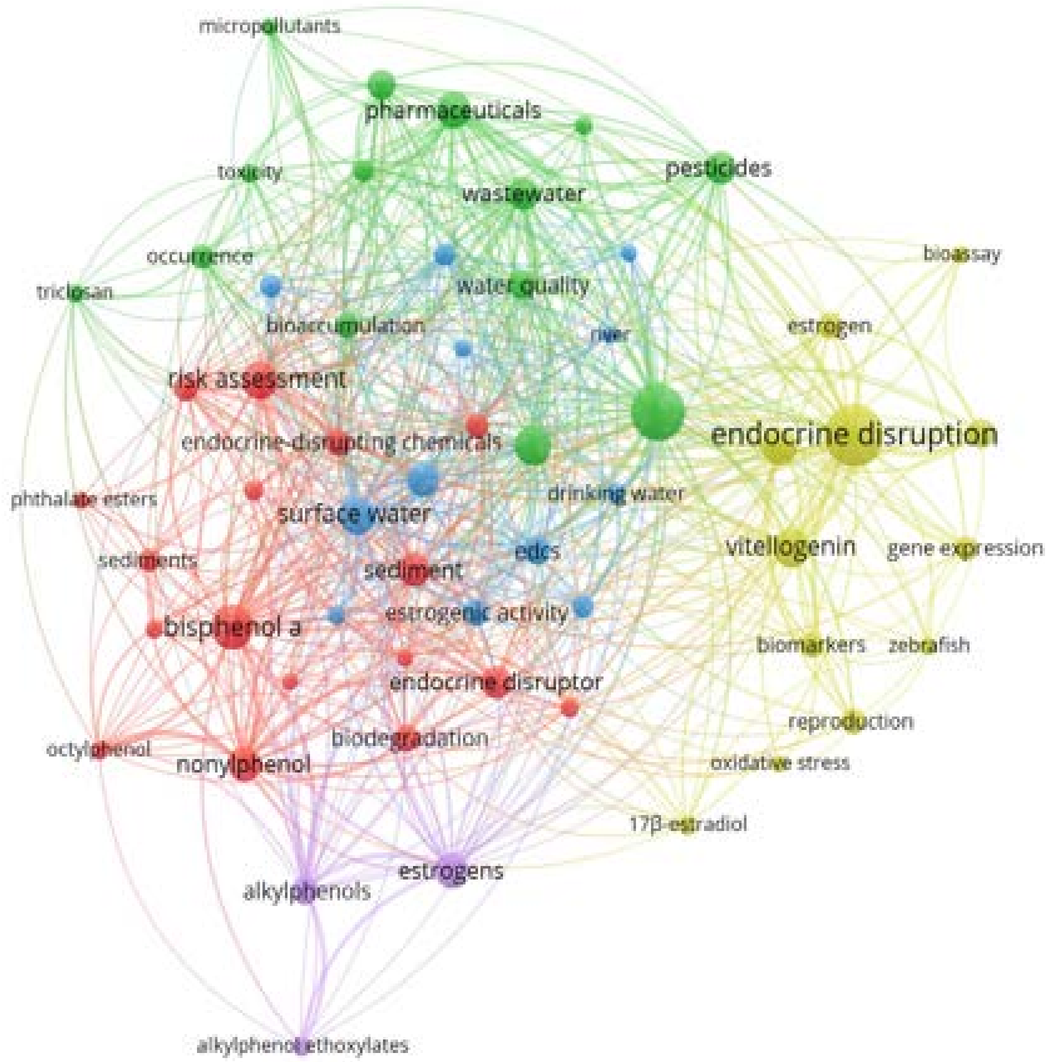


Figure 2-6: Research focus on EDCs between 2000 and 2023

(Data retrieved from Scopus)

Overall, the figure encapsulates the multifaceted and interconnected nature of global EDC research over the past two decades, highlighting both well-explored areas and those that may require additional focus to address research gaps and emerging challenges. The subsequent section presents EDC research on the abiotic and biotic components of various Indian rivers, which is essential for identifying knowledge gaps and determining the scope of future studies to address the challenges posed by EDCs.

2.4.2 EDC Research on the Abiotic and Biotic Components of Indian Rivers: Identifying Knowledge Gaps with a focus on the Ganga River

Within the Indian context, EDC research is evolving amidst the country's distinct environmental dynamics and regulatory landscape. The Figure 2-7 provides a time-progressive overview of research on EDCs in India's freshwater environments from 2010 to 2020. It shows a clear shift in focus over time, with earlier research (represented by blue nodes) concentrated on legacy EDCs like DDT and PAHs, while more recent studies (represented by yellow nodes) have increasingly focused on phthalates, pesticides, and personal care products. The prominence of larger nodes underscores well-established research areas, while the smaller nodes highlight emerging topics or gaps in the current research landscape. Addressing these gaps could offer deeper insights into the presence and effects of less-studied EDCs in freshwater ecosystems.

Nevertheless, as indicated by Figure 2-7, research interest in EDCs has broadened significantly between 2000 and 2023, with substantial progress made in studying EDCs in India's freshwater environments over the past two decades. From initial identification and baseline studies to advanced analytical techniques and integrated monitoring programs, the field has evolved to address complex ecological and human health challenges posed by EDCs.

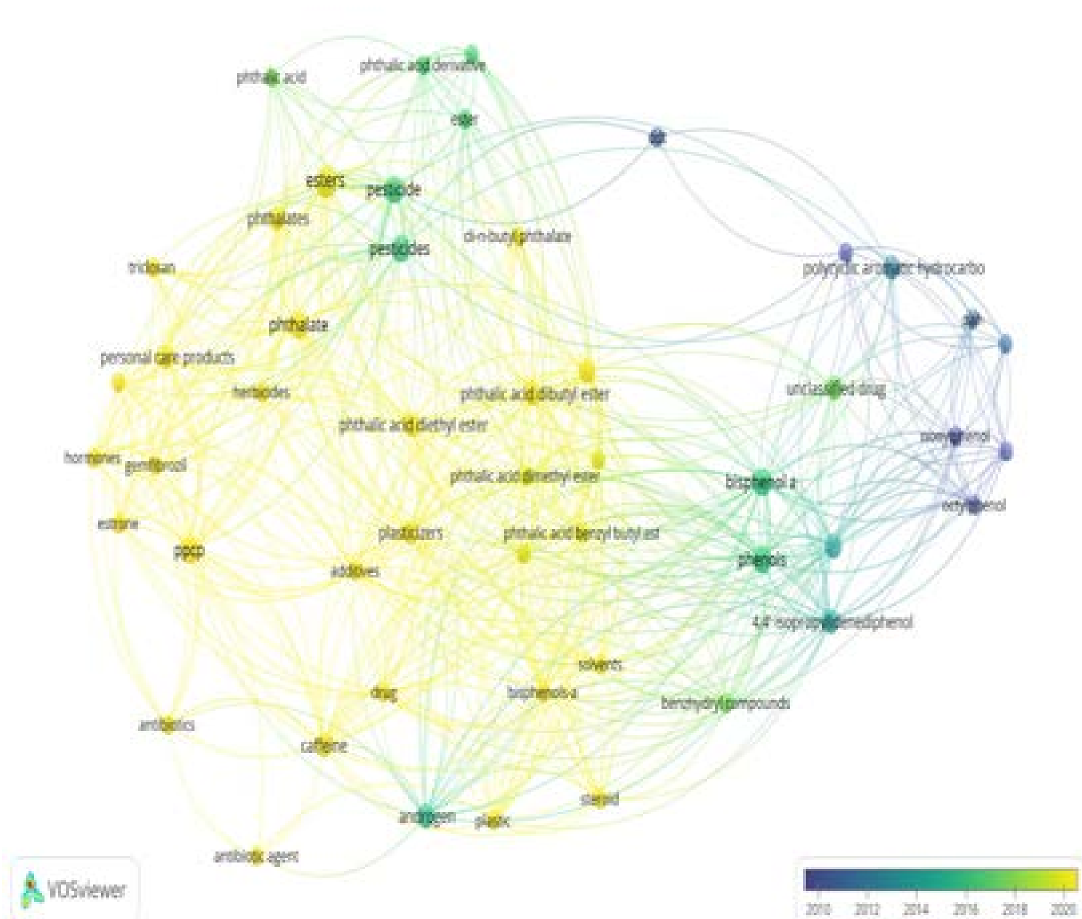


Figure 2-7: Time-Progressive Insights into EDC Group Research in freshwater environments of India

(Data retrieved from Scopus)

The presence and impact of EDCs in freshwater ecosystem have become a significant environmental concern, especially in heavily populated, rapidly urbanizing, and developing nations such as India. Figure 2-8 to 2-11 highlights the concentrations of EDCs in the surface water across various Indian rivers over a 22-year period.

Figure 2-8 represents spatiotemporal distribution of BPA concentrations across various rivers in India from 2000 to 2021. The analysis reveals diverse patterns of pollution. The Ganga River consistently shows high levels of BPA, with concentrations reaching 803 ng/L in 2016 (Mukhopadhyay & Chakraborty, 2021) and 710 ng/L in 2019 (Chakraborty et al., 2020). The Yamuna River had the highest recorded BPA concentration among these rivers, with a staggering 6312.325 ng/L in 2018 (Lalwani et al., 2020). The Hindon River also exhibited significant contamination, with a BPA concentration of 249.5 ng/L in 2018 (Lalwani et al., 2020). Other rivers like the Kathajodi and Mahanadi showed moderate levels of BPA, with concentrations of 140 ng/L and 130 ng/L respectively, in 2018 (Lalwani et al., 2020). The Sarda River, however, displayed much lower BPA levels, at 19.9 ng/L in 2018 (Lalwani et al., 2020).

Rivers such as the Cooum and Adyar had BPA concentrations of 692.333 ng/L and 407.5 ng/L, respectively, in 2018 (Lalwani et al., 2020), reflecting the impact of urban pollution. Similarly, the Palar River recorded a concentration of 44.9 ng/L in 2018 (Lalwani et al., 2020). The Chithrapuzha River had a BPA concentration of 72.4 ng/L in 2018 (Lalwani et al., 2020). The Mutha and Mula Mutha rivers recorded BPA levels of 188 ng/L and 193 ng/L, respectively, in 2018 (Lalwani et al., 2020), while the Mula River itself had a concentration of 172 ng/L in the same year (Lalwani et al., 2020). The Mula, Pawana River reported a BPA level of 237 ng/L in 2018 (Lalwani et al., 2020).

Further, rivers like the Kanhan (249 ng/L), Satluj (389 ng/L), and Beas (265 ng/L) also showed varying levels of BPA contamination (Lalwani et al., 2020). These findings indicate significant regional differences in BPA pollution, largely influenced by local industrial and urban activities. The potential sources of BPA contamination in these rivers likely include industrial discharges from plastic manufacturing and processing plants, urban runoff containing plastic debris and consumer products, and wastewater effluents from treatment plants that inadequately remove BPA. Additionally, agricultural runoff, particularly in regions with extensive use of plasticizers in farming, can contribute to BPA levels in rivers. These sources collectively highlight the pervasive nature of BPA pollution across different environmental matrices. Nevertheless, despite the extensive dataset, there is a clear need for continuous monitoring and more comprehensive studies across these rivers. There is a notable lack of consistent long-term data for these rivers, which limits our understanding of temporal trends and the effectiveness of pollution control measures.

Most studies focus on a single year or short time spans, leaving gaps in understanding the ongoing impact of BPA on these river systems. Addressing this research gap requires a concerted effort to conduct longitudinal studies across all major rivers, with a focus on both heavily polluted and less impacted rivers to develop a complete picture of BPA contamination in India.

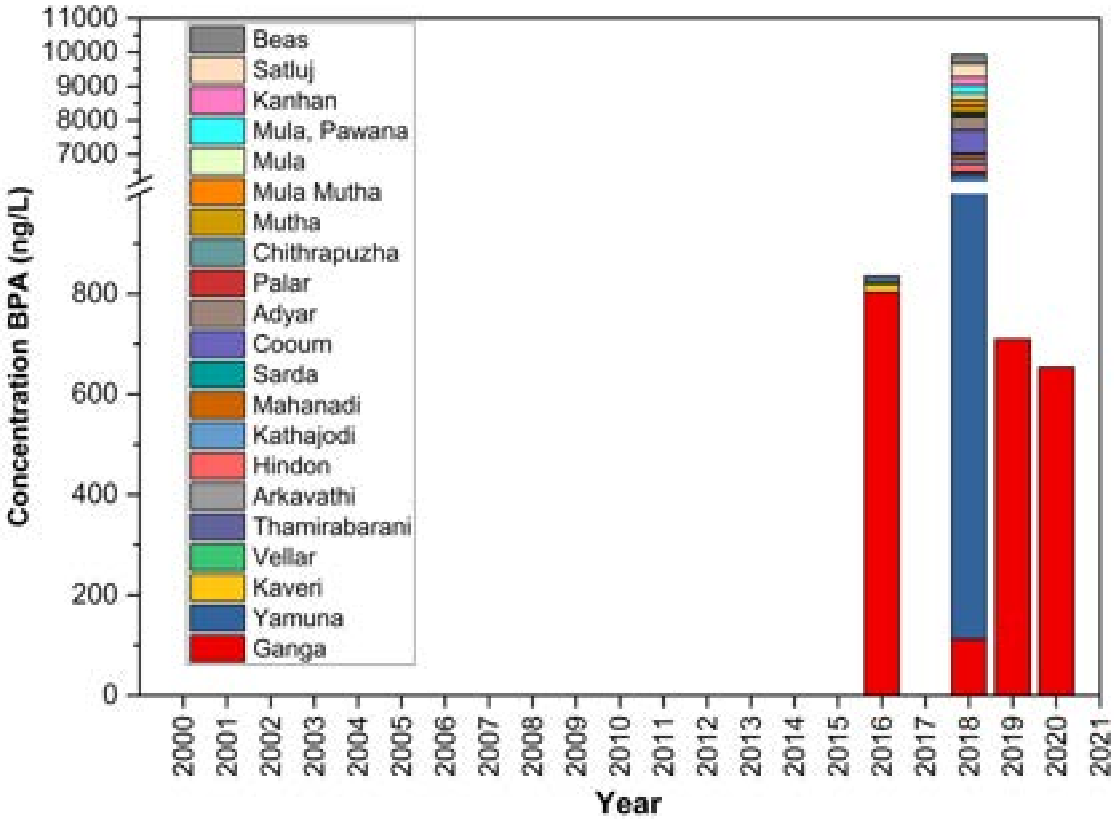


Figure 2-8: BPA in surface water of India from 2000 to 2022

Figure 2-9 represents spatiotemporal distribution of household and personal care products (HPCPs) concentrations across various rivers in India from 2000 to 2021. The spatiotemporal distribution of HPCPs concentrations across Indian rivers, including the Ganga, reveals significant variability, both geographically and over time. Specifically, data for the Ganga River in 2020 shows an HPCP concentration of 30.6 ng/L (Singh & Suthar, 2021). This value is notably lower compared to other rivers such as the Gomti, where a concentration of 3410 ng/L was recorded in 2016 (Nag et al., 2018). Similarly, the Tamiraparani River reported a concentration of 944 ng/L in 2009 (Ramaswamy et al., 2011), while the Kaveri River exhibited concentrations of 65 ng/L in 2009 and 123.6 ng/L in 2016 (Ramaswamy et al., 2011; Vimalkumar et al., 2018). The Vellar River displayed the lowest concentration among the studied rivers at 25.3 ng/L in 2016 (Vimalkumar et al., 2018). The Torsa River in 2017 exhibited a higher concentration of 166.2 ng/L (Sarkar et al., 2020), reflecting substantial contamination from local sources such as agricultural runoff and domestic waste.

The Arkavati River showed one of the highest concentrations at 942.5 ng/L in 2019 (Gopal et al., 2021), indicating significant pollution from urban and industrial activities. In contrast, the Yamuna River, despite being heavily industrialized, had a lower concentration of 25.5 ng/L in 2019 (Biswas & Vellanki, 2021). The Cauvery River recorded the lowest concentration among the studied rivers, with 10.39 ng/L in 2019 (Renganathan et al., 2021).

These findings underscore the heterogeneous nature of HPCP pollution across different river systems in India, influenced by various regional factors including population density, industrial activities, and wastewater management practices. Additionally, there are significant spatiotemporal gaps in the dataset that limit a comprehensive understanding of HPCP pollution in Indian rivers, particularly the Ganga River. For the Ganga, data is available only for the year 2020, leaving a substantial temporal gap that prevents the assessment of long-term trends in HPCP concentrations.

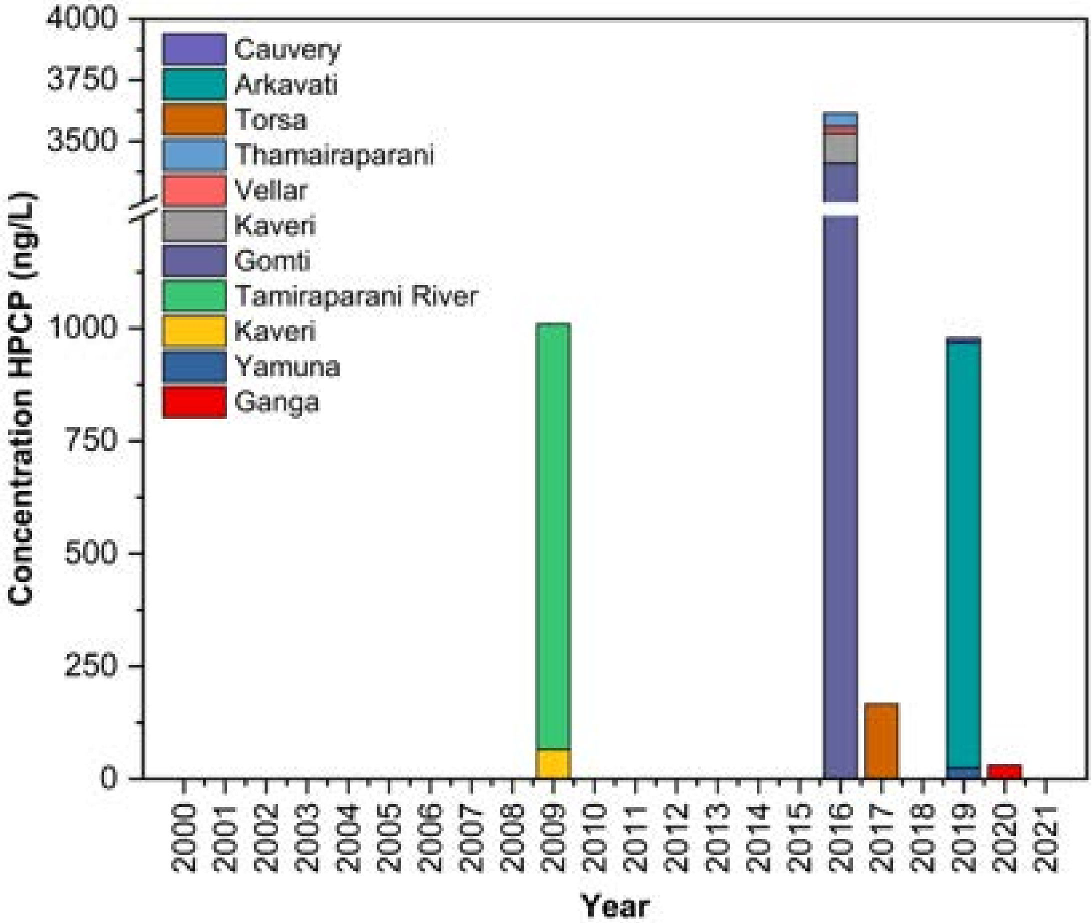


Figure 2-9: HPCPs in surface water of India from 2000 to 2022

The dataset for the Ganga includes data only for 2020 from upstream locations (Haridwar and Rishikesh) in Uttarakhand, leaving substantial temporal and spatial gaps in understanding the pollution dynamics across the entire river system. The middle and lower sections of the Ganga, which are likely to be more polluted due to high population density and increased anthropogenic activities, are not represented in the dataset. This lack of comprehensive data hinders the ability to assess long-term trends in HPCP concentrations and their potential impacts on the river's biodiversity, including the endangered Gangetic dolphin. This lack of continuous data makes it challenging to evaluate whether HPCP levels are increasing, decreasing, or remaining stable over time, thereby complicating efforts to predict future pollution scenarios and their potential impact on the river's biodiversity. The spatial gaps are also concerning, as data is only available for a limited number of rivers, with large sections of the Ganga basin and other important river systems lacking adequate monitoring.

Figure 2-10 represents spatiotemporal distribution of phthalates (PAEs) concentrations across various rivers in India from 2000 to 2021. The spatiotemporal distribution of PAEs across various Indian rivers, including the Ganga, Kaveri, Vellar, Thamiraparani, and Adyar, reveals notable variations pollution levels over time. In the Kaveri River, PAE concentrations were recorded at 182.35 ng/L in 2014, which significantly decreased to 7.04 ng/L by 2019, suggesting a possible improvement in pollution control measures or changes in regional industrial activities (Selvaraj et al., 2014; Elaiyaraja et al., 2022). Similarly, the Vellar River showed a PAE concentration of 8.26 ng/L in 2019, while the Thamiraparani River had a considerably lower concentration of 0.56 ng/L in the same year (Elaiyaraja et al., 2022). Conversely, the Adyar River exhibited an extremely high PAE concentration of 78,000 ng/L in 2021, indicating severe contamination likely driven by intense industrial and urban activities (Chandra & Chakraborty, 2023). The Ganga River recorded PAE concentrations of 176.1 ng/L in its lower reaches in 2016 (Mukhopadhyay & Chakraborty, 2021), with a significant rise to 1600 ng/L by 2019, as documented in a comprehensive study of the entire river system (Chakraborty et al., 2021). This increasing trend in the Ganga indicates growing pollution pressure across the entire river, posing serious risks to the river's ecological health.

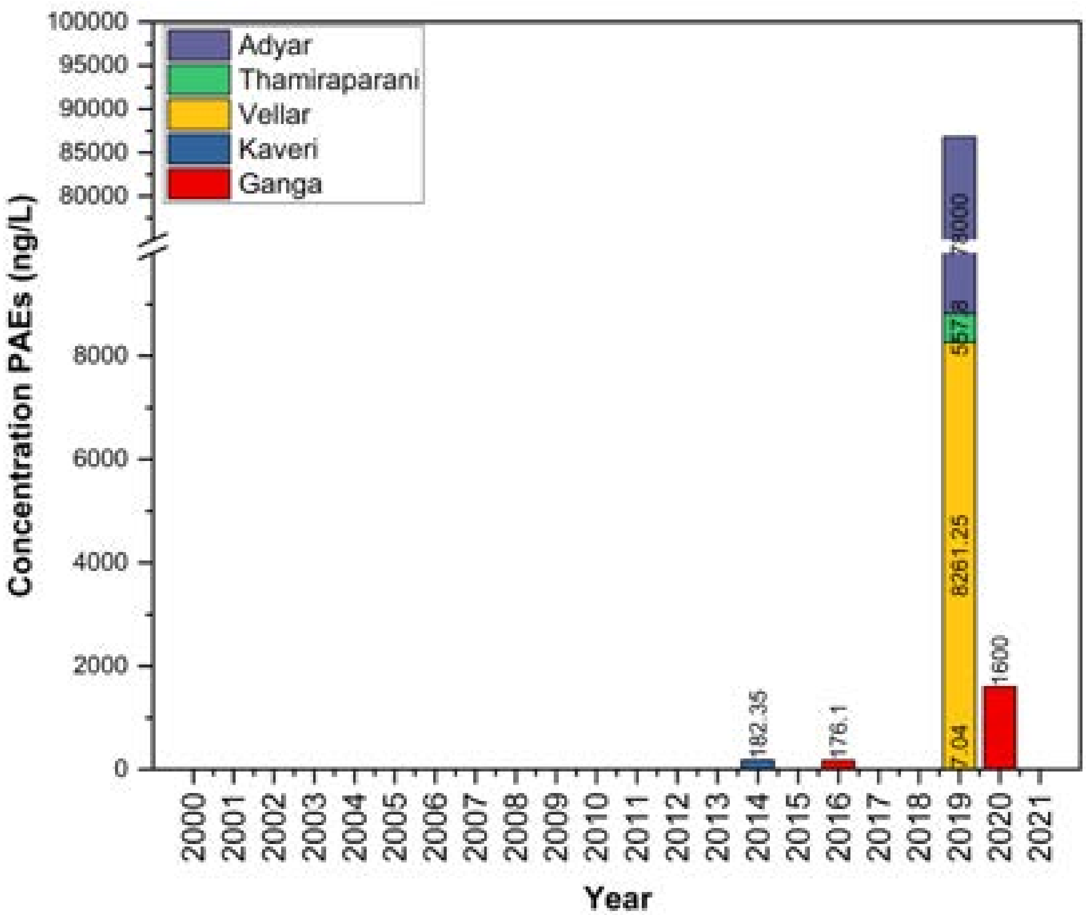


Figure 2-10: Phtalates (PAEs) in surface water of India from 2000 to 2022

Despite these findings, the dataset reveals critical spatiotemporal gaps, particularly in the Ganga River. The data points for the Ganga are limited to two years (2016 and 2019), with the 2019 data representing a significant jump in PAE levels across the entire river system. Additionally, the lack of data from the early 2000s through to 2015 prevents a comprehensive analysis of long-term trends in PAE contamination. The limited spatial coverage also fragments the assessment, making it difficult to correlate PAE concentrations with potential sources of pollution or to understand the broader implications for the river's biodiversity. In addition, the spatial gaps across Indian rivers in the dataset are significant, with data primarily concentrated on a few rivers like the Ganga, Kaveri, and Adyar, while many other critical river systems remain unmonitored. This uneven coverage limits the ability to fully understand the extent of PAE pollution across the diverse and ecologically important river basins in India.

Figure 2-11 represents spatiotemporal distribution of estrogen concentrations (natural and synthetic estrogen), concentrations across various rivers in India from 2000 to 2021. The spatiotemporal distribution of estrogen concentrations across various Indian rivers, including the Ganga, Vrishabhavathi, Periyar, Ahar, and Yamuna, reveals significant variations over time and between rivers. The Ganga River showed a notably high estrogen concentration of 1001.2 ng/L in 2017 (Satyanarayana et al., 2022), which then dramatically decreased to 6.3 ng/L by 2019 (Singh & Suthar, 2021). It's important to note that the sampling locations for the Ganga River in the studies were markedly different in terms of land use, population density, livestock, water flow, and other anthropogenic pressures. The study conducted by Singh & Suthar, 2021, focused on a segment of the river in Uttarakhand (Rishikesh and Haridwar), a region characterized by lower population density, low livestock, high flow, and reduced industrial activity compared to Uttar Pradesh (Prayagraj and Varanasi).

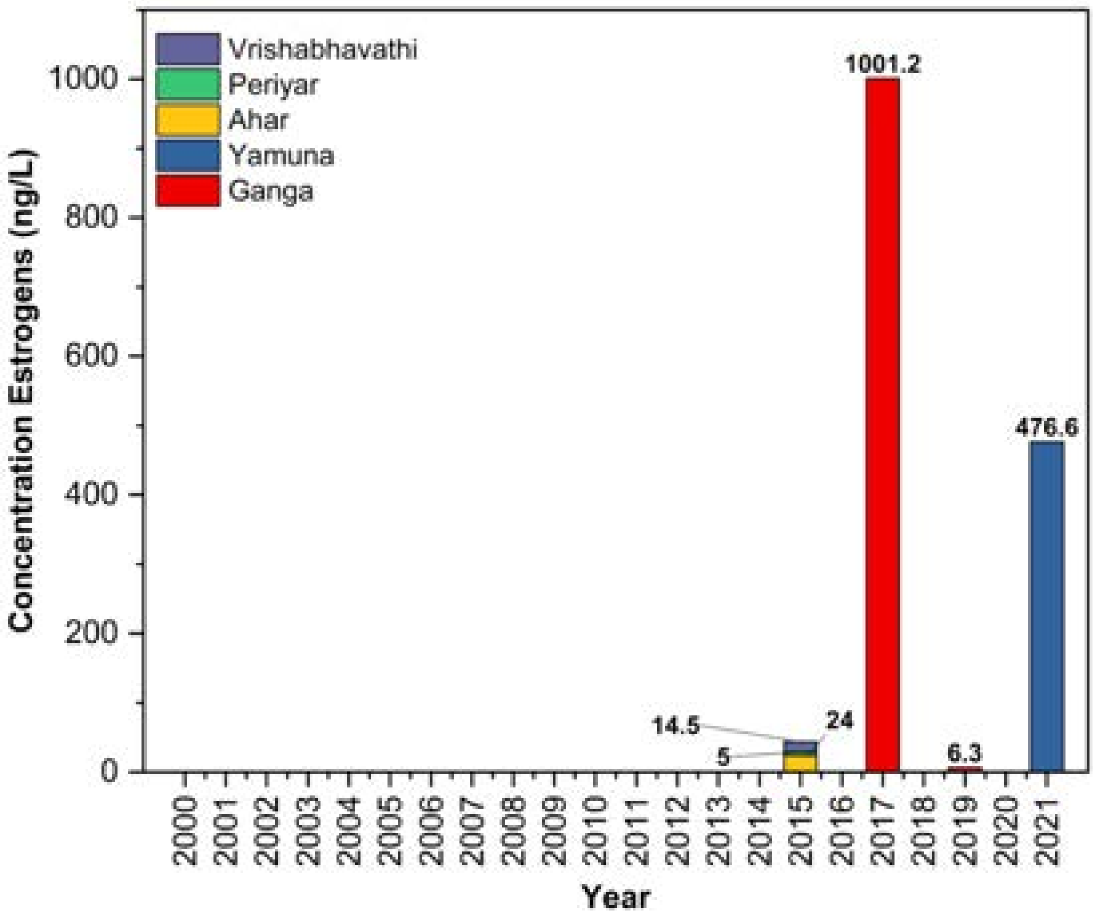


Figure 2-11: Estrogens in surface water of India from 2000 to 2022

This distinction highlights the influence of regional factors on pollutant levels, with Uttarakhand's comparatively pristine environment likely contributing to the lower estrogen concentrations observed in their findings. The Vrishabhavathi River, recorded an estrogen concentration of 14.5 ng/L in 2015, while the Periyar River reported a much lower concentration of 5.0 ng/L in the same year (Tue et al., 2022; Khalid et al., 2018). The Ahar River had a concentration of 24.0 ng/L in 2014 (Williams et al., 2019). The study also highlights a significant estrogen concentration of 476.6 ng/L for the Yamuna River in 2021, indicating ongoing contamination issues in this river system.

The spatiotemporal analysis of Estrogens levels across India highlights substantial spatial differences in estrogen pollution across rivers, with the Ganga showing particularly high levels in 2017. Despite the insights provided by the available data, there are significant spatiotemporal gaps in the monitoring of estrogen concentrations, particularly in the Ganga River. The data for the Ganga is limited to just two years, 2017 and 2019, with sampling locations that differ significantly in land use, population density, and anthropogenic pressures. This variation in sampling locations complicates the assessment of long-term pollution trends and the effectiveness of pollution control measures across the entire river system. The absence of consistent data across other years, especially before 2017 and after 2019, further limits the ability to understand the overall trajectory of estrogen pollution in the Ganga and its potential impacts on biodiversity.

These gaps are particularly concerning for the Ganga River's ecological health, which supports a rich diversity of species, as estrogen contamination is known to cause endocrine disruption in aquatic organisms, leading to reproductive issues and population declines (Campbell et al., 2006; Rearick et al., 2018). Without comprehensive and continuous monitoring across different sections of the Ganga, it is challenging to develop targeted conservation strategies or to mitigate the adverse impacts of rising estrogen levels on the river's ecosystem.

Figure 2-12 represents spatiotemporal distribution of pesticides concentrations (with endocrine disruption properties), concentrations across various rivers in India from 2000 to 2021. In 2003, a study by Sarkar et al. documented the pesticide concentrations in several rivers, revealing that the Ram Ganga River had the highest concentration at 90.0 ng/L. This high level of contamination is indicative of significant agricultural runoff and possibly untreated wastewater in the region. Additional rivers studied by Sarkar et al., 2003, include the Kosi River, which recorded a concentration of 50.0 ng/L, highlighting moderate contamination levels, and the Gomti River, which had a concentration of 65.0 ng/L, indicating ongoing pesticide contamination likely exacerbated by agricultural activities and urban runoff. Other rivers studied by Sarkar et al., 2003, include Gori River (55 ng/L), Saryu River (48 ng/L), Ladhiya (45 ng/L), Lohawati (40 ng/L) Sharda (30 ng/L) and Dhuail (35 ng/L) rivers all of which reflect varied levels of pesticide contamination likely influenced by local agricultural practices.

The Ganga River, has been subjected to several studies to monitor pesticide concentrations across different stretches and years, revealing significant spatial and temporal variability. In 2006, a study conducted by Singh et al., 2006 reported a pesticide concentration of 90 ng/L in the middle stretch of the Ganga. However, the study was conducted at one sampling location in Unnao district of Uttar Pradesh. By 2008, Ghose et al. 2009 documented a dramatic pesticide level in the Lower Ganga, in West Bengal, with concentrations reaching 1,584.43 ng/L. This surge is indicative of severe pollution, likely driven by agricultural runoff and industrial discharges in the region. In 2016, Chakraborty et al. 2016, reported a pesticide concentration of 62 ng/L in the lower stretch of Ganga, reflecting a temporary decrease in contamination levels. However, this trend did not last, as Mondal et al. 2018, recorded a staggering increase to 3,010 ng/L in 2018, highlighting the recurring and severe contamination issues in this region.

The pesticide concentration in the Ganga's middle-lower stretch, in Bihar, was recorded at 366.8456 ng/L (Leena et al., 2011) in 2011. In 2012, a more comprehensive study by Mutiyar & Mittal, 2013, covered the entire stretch of the Ganga, reporting a notably lower pesticide concentration of 28.37 ng/L. Meanwhile, in 2017, our study (Sah et al.,2020) conducted across the entire stretch of the Ganga and found a concentration of 1,267 ng/L, further confirming the widespread and persistent nature of pesticide pollution along the river.

The temporal dataset for Ganga River reveals substantial temporal gaps in the monitoring of pesticide contamination in the Ganga River. The available data is sporadic, with significant intervals between studies, such as between 2003, 2006, 2008, 2011, 2012, 2017, and 2018. These gaps hinder the ability to assess long-term trends and make it challenging to correlate contamination levels with specific agricultural or industrial activities, or regulatory changes. The sporadic nature of the data complicates efforts to understand the full impact of pesticide pollution on the river's ecosystem.

In addition to these temporal gaps, the spatial analysis of the studies shows that monitoring

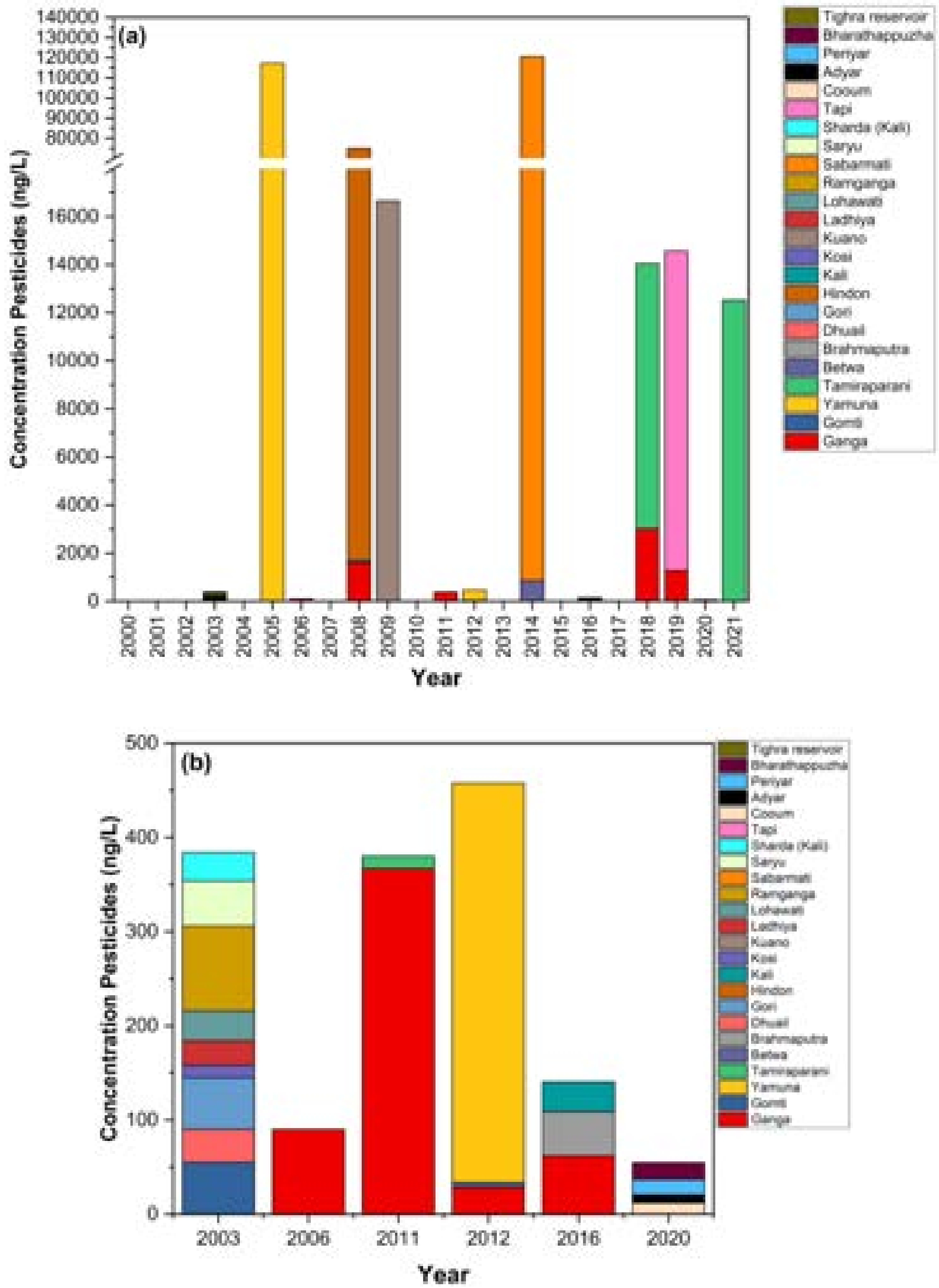


Figure 2-12: (a) Pesticides in surface water of India from 2000 to 2022 (b) Detailed view of pesticide concentrations in the year 2003, 2006, 2011, 2012, 2016, and 2020

has been conducted in a fragmented manner, focusing on specific sections of the Ganga rather than providing comprehensive coverage across the entire river. Most studies have been isolated to certain regions, leaving large stretches of the river unmonitored. This piecemeal approach limits our understanding of the overall contamination patterns and makes it difficult to develop effective, river-wide management strategies. However, the study conducted by Mutiyar & Mittal, 2013 and Sah et al. in 2020 represents a more comprehensive effort to monitor pesticide levels across the entire Ganga, providing valuable insights that highlight the need for more uniform and consistent monitoring practices.

The Yamuna River exhibited extremely high pesticide concentrations in both 2005 and 2012. In 2005, the concentration was recorded at 117,296 ng/L (Aleem & Malik, 2005), indicating severe contamination, likely due to intense agricultural runoff and industrial discharge. In 2012, pesticide levels were recorded at 221 ng/L (Kumar et al., 2012) and 627.5 ng/L (Saha et al., 2012), showing that while pollution remained significant, it was somewhat reduced compared to the 2005 peak. The Hindon River reported a pesticide concentration of 73,384.62 ng/L in 2008 (Ali et al., 2008), highlighting severe contamination. The Gomti River showed fluctuating levels of pesticide contamination. In 2008, the concentration was 113.02 ng/L (Malik et al., 2008), while in 2012, it drastically reduced to 5.23 ng/L (Mutiyar & Mittal, 2013), suggesting potential improvements in pollution control or variations in pesticide use. The Kuano River reported a high pesticide concentration of 16,650 ng/L in 2009 (Singh & Mishra, 2009), indicative of substantial contamination, likely from agricultural runoff. The Sabarmati River showed an alarming pesticide concentration of 119,776.3 ng/L in 2014 (Hashmi & Menon, 2015), reflecting severe pollution, likely due to industrial effluents and agricultural runoff. In the same year, the Betwa River had a concentration of 859 ng/L (Bhat & Padmaja, 2014), indicating significant pesticide contamination, though lower compared to the Sabarmati. The Thamirabarani River exhibited rising pesticide levels over time. In 2011, the concentration was 13.12 ng/L (Kumarasamy et al., 2011b), which increased to 11,030 ng/L in 2018 (Arisekar et al., 2019b) and further to 12,530 ng/L by 2021 (Arisekar et al., 2021b). This trend suggests escalating pollution in the region. The Tapi River recorded a concentration of 13,291.67 ng/L in 2019 (Hashmi et al., 2019), highlighting significant pesticide contamination, likely from agricultural activities in the region.

The Brahmaputra River recorded a relatively lower concentration of 47 ng/L in 2016 (Chakraborty et al., 2016), suggesting moderate contamination levels compared to other rivers.

The spatiotemporal assessment of the available dataset for pesticide contamination reveals considerable temporal gaps in the monitoring of pesticide concentrations across rivers of India. Many rivers have been studied sporadically, with large intervals between studies. For instance, the Thamirabarani River was studied in 2011, 2018, and 2021, leaving gaps that make it difficult to track the progression of contamination over time. Similarly, the Yamuna River was studied in 2005 and 2012, but there is no data available before or after these years to provide a continuous assessment of pollution trends.

Spatially, the dataset covers a limited number of rivers, with some, like the Brahmaputra, only being monitored once or twice and many other significant rivers not represented in the dataset. This lack of comprehensive spatial coverage prevents a full understanding of pesticide pollution across India's diverse river systems.

Environmental monitoring provides essential data on the presence and distribution of pollutants in aquatic ecosystems, but biological monitoring is vital to fully comprehend their impact on living organisms and the overall health of these environments. This chapter not only assesses the spatiotemporal distribution of key EDCs in surface water but also extends this analysis to biological samples collected from rivers across India between 2000 and 2022. This integrated approach is particularly important as it helps to identify data gaps, guiding future studies towards more targeted and effective monitoring strategies.

Despite the acknowledged importance of biological monitoring, its systematic and comprehensive implementation in India remains lacking. Figures 2-13 represent the concentrations of various EDCs detected in the biotic components of Indian rivers between 2000 and 2022, illustrating the disparity in biomonitoring studies compared to data on surface water. Most of our knowledge about EDC concentrations in the biotic compartments of Indian lotic ecosystems comes from studies on pesticides. Given the extensive agricultural land and the high demand for chemical pesticides, the scientific community's focus on assessing the risks posed by pesticides to aquatic ecosystems is well-founded. However, India's growing industrial sector, rapid population growth, unplanned urbanization, and inadequate wastewater management infrastructure necessitate in-depth studies on the occurrence, distribution, and risks of a diverse range of EDCs in the country's riverine ecosystems.

The data reveals significant insights into the concentration of pesticides in biota across various Indian rivers over a span of two decades, highlighting the varying levels of contamination and their potential impact on riverine ecosystems. In 2000, a study by Sarkar et al. documented relatively low pesticide concentrations in the Ganga River (10 ng/g), the Kosi River (23 ng/g), the Dhuali River (61 ng/g), the Ramganga River (56 ng/g), the Gori River (17 ng/g), and the Saryu River (49 ng/g). These values reflect a certain degree of pesticide presence, although the concentrations were comparatively moderate at the turn of the century.

However, by 2005, the Gomti River exhibited a dramatic increase in pesticide concentration, with levels reaching 2210.17 ng/g (Malik et al., 2007). This sharp rise indicates significant contamination, likely driven by increased agricultural runoff and possibly industrial discharges. In 2007, the Ganga River showed an alarming concentration of 6930 ng/g, while the Gomti River further increased to 7665 ng/g (Singh & Singh, 2007). These extremely high levels suggest a worsening pollution scenario, particularly in these agriculturally intensive regions.

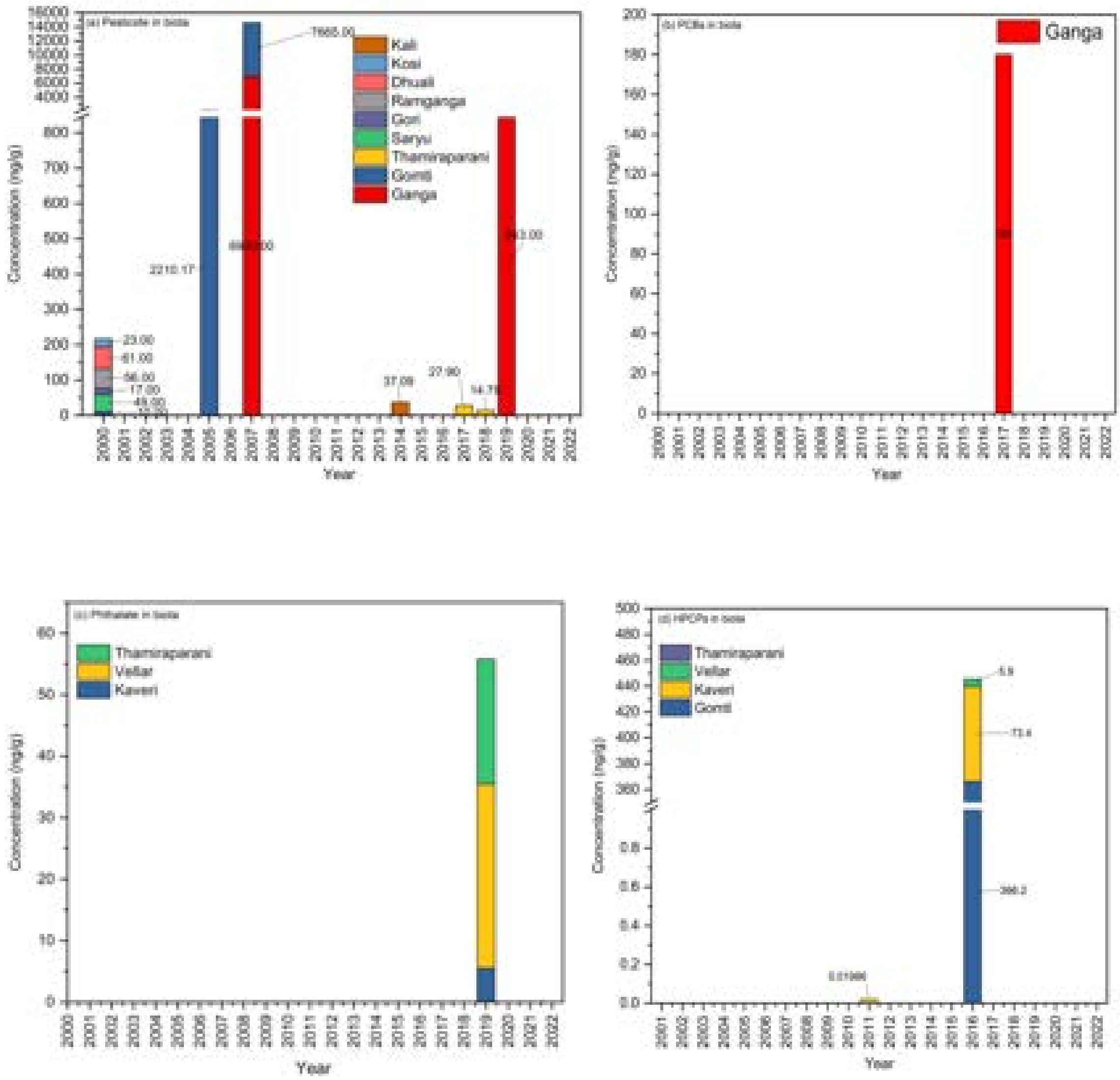


Figure 2-13: EDCs in biota of Indian rivers from 2000 to 2022 (a) Pesticides (b) PCBs (c) Phthalates (d) HPCPs

By 2014, the Kali River recorded a pesticide concentration of 37.08611 ng/g (Maurya et al., 2016), which, while lower than the levels seen in the Ganga and Gomti, still indicates persistent contamination. The Thamiraparani River was monitored in 2017 and 2018, showing concentrations of 27.90025 ng/g and 14.79 ng/g, respectively (Arisekar et al., 2021b; Arisekar et al., 2019b). These values suggest a potential improvement in water quality or changes in pesticide usage practices in the region. However, by 2019, the Ganga River was once again reported with a high concentration of 843 ng/g (Kaur et al., 2021), indicating ongoing and significant pollution issues.

The data highlights several spatiotemporal gaps in the monitoring of pesticide concentrations across Indian rivers, particularly concerning the Ganga River. The inconsistent monitoring across different rivers and years makes it difficult to draw comprehensive conclusions about the overall state of India's river systems. To address these gaps, there is a pressing need for more systematic, long-term monitoring programs that cover all major rivers, with a focus on consistent data collection over time. This approach would provide a more accurate picture of the spatiotemporal trends in pesticide contamination, helping to inform better management and conservation efforts aimed at protecting India's aquatic biodiversity.

While there is data available for the Ganga in 2000, 2007, and 2019, the large gaps between these years make it challenging to assess consistent trends in pesticide contamination over time. These gaps leave questions about the effectiveness of pollution control measures and the potential seasonal or yearly variations in pesticide levels, which are crucial for understanding the long-term impact on the river's ecosystem. In addition, the fluctuating levels of pesticide contamination, especially the extremely high concentrations recorded in 2007 and 2019, pose significant risks to the biodiversity of the

Ganga River. Pesticides can accumulate in the tissues of aquatic organisms, leading to bioaccumulation and biomagnification through the food chain, ultimately affecting not just fish populations but also apex predators, including the endangered Gangetic dolphin. The lack of continuous data hampers the ability to develop targeted and effective conservation strategies, leaving critical gaps in the understanding of the river's ecological health.

The Ganga River was specifically monitored for PCB contamination, and in 2017, the concentration was found to be 180 ng/g (Figure 2-10 d). This level of PCB contamination is particularly alarming due to the persistent nature of PCBs, which are known to remain in the environment for extended periods and pose significant risks to both wildlife and human health through bioaccumulation and biomagnification. Notably, the Ganga is the only river that was monitored for EDC contamination over the extended period from 2000 to 2022. In contrast, no other river in the dataset was monitored consistently across this entire timeframe. This lack of continuous monitoring in other rivers leaves significant gaps in our understanding of how EDCs have impacted different river ecosystems over time, limiting the ability to track long-term trends and to develop comprehensive strategies to address the pollution in India's diverse aquatic systems.

Phthalate contamination (Figure 2-10 c) in biota was recorded primarily in the year 2018 (Elaiyaraja et al., 2022). The Thamiraparani River had the highest concentration at 59.3 ng/g, indicating significant pollution. The Vellar River followed with a concentration of

47.6 ng/g, and the Kaveri River had the lowest phthalate concentration among these rivers at 44.9 ng/g. Although the differences in these values are not vast, they highlight the widespread presence of phthalates across these water bodies, raising concerns about their long-term environmental impact.

The concentration of HPCPs in the biota varied significantly across different rivers and timeframes (Figure 2-10 d). For example, in 2011, the Kaveri River recorded a low concentration of 19.86 ng/g (Shanmugam et al., 2014), suggesting minimal contamination at that time. However, by 2016, the situation had drastically changed, with the Kaveri River showing a much higher concentration of 73.4 ng/g (Vimalkumar et al., 2018). This was followed by the Gomti River, which reported an extremely high concentration of 366.2 ng/g in the same year, indicating severe contamination, possibly due to extensive urban and agricultural activities (Nag et al., 2018). The Vellar river recorded a concentration of 5.9 ng/g, in 2016 (Vimalkumar et al., 2018).

The occurrence of these chemicals in the Ganga River is a matter of much concern, given the rich biodiversity the river supports. This biodiversity includes some of the most threatened species on the planet, including the Gangetic shark *Glyphis gangeticus* (CR), Gharial *Gavialis gangeticus* (CR), Red-crowned roofed turtle *Batagur dhongoka* (CR), and the Gangetic dolphin *Platanista gangetica* (EN) (IUCN, 2024). The contamination of their habitats by EDCs could potentially exacerbate the threats faced by species, particularly when considering how even trace environmental concentrations can translate to individual- and population-level impacts, as well as trans-generational effects (Crisp et al., 1998; Campbell et al., 2006; WHO,2012).

Freshwater cetaceans represent a gravely threatened group, with the Chinese River Dolphin recently being driven to functional extinction by multiple stressors (Turvey et al., 2007). The Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*) is also facing a severe population decline owing to multiple threats, including pollution (Xiong et al., 2019; Mogensen et al., 2022). It is reasonable to assume that the Gangetic dolphin would be under the same level of threat from pollution as its other relatives.

However, despite ongoing conservation initiatives in India and substantial global evidence on the population-level impacts of EDCs on aquatic mammals, the research landscape concerning EDCs in dolphin habitats has not seen significant advancement (Figure 2-14). The analysis EDCs in aquatic species, as depicted in Figure 2-14, reveals that several legacy contaminants, such as PCBs, DDTs, and HCHs, and other organochlorine pesticides have been consistently detected in both the Gangetic dolphin (GD) and its prey species. However, the data is predominantly outdated, with most studies dating back to 1988-1996, as shown in Figure 2-10. This temporal limitation is compounded by the fact that the studies focus almost exclusively on legacy pollutants, with emerging EDCs, such as those found in plastic additives, HPCPs, pharmaceuticals and other newer industrial chemicals, largely ignored.

The concentration levels of these legacy contaminants in the Gangetic dolphin and its prey are alarming, with PCBs reaching concentrations as high as 56732 ng/g in dolphins. Similarly, DDTs show substantial bioaccumulation, with concentrations of 27031 ng/g and in dolphins, and much higher levels than in prey species. These findings indicate a significant risk of bioaccumulation and biomagnification within the food web, which could have severe implications for the health and survival of this threatened flagship species.

The absence of current and comprehensive data on EDCs leaves the Gangetic dolphin and other aquatic species potentially vulnerable to unidentified and unmonitored threats. This highlights a critical need for up-to-date and extensive research into the presence and effects of both legacy and emerging EDCs on these species.

2.5 Conclusion

An analysis of literature data on rivers in India, as covered in this chapter, reveals that many rivers are highly contaminated with both legacy and emerging EDCs. Despite significant advancements, several challenges persist in the research on EDCs in India's freshwater environments. These challenges are particularly concerning for densely populated, agriculturally active, rapidly urbanizing, extensively industrialized, yet biodiverse rivers such as the Ganga. Some key research and data gaps identified in this chapters are:

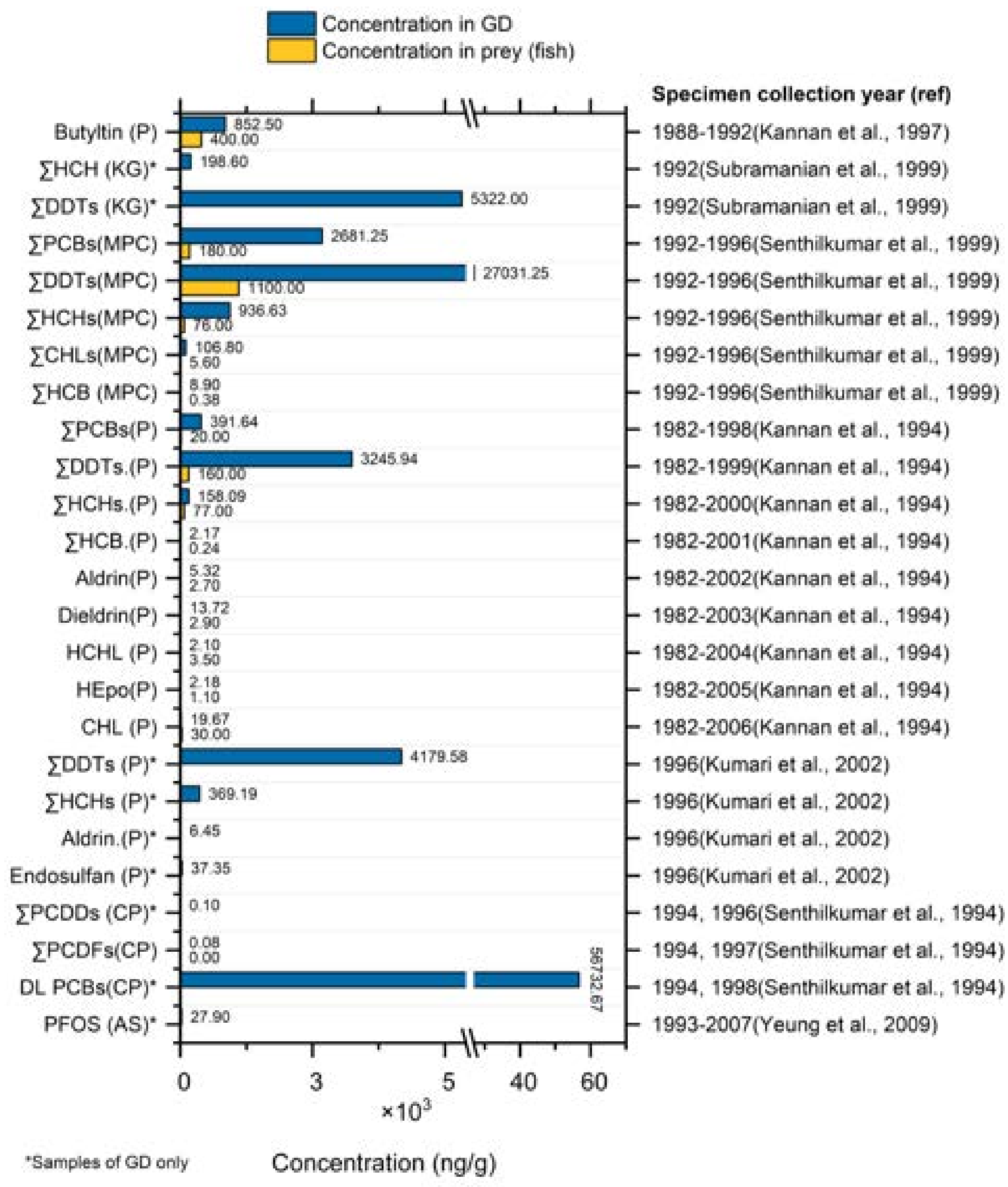


Figure 2-14: Contaminants concentrations, including EDCs, detected in Gangetic Dolphin

(KG: Kahalgaon; MPC: Mariandhar, Patna, Chapra; P: Patna; CP: Chappra, Patna; AS: Archived Samples, Patna University)

a) **Limited Geographical Coverage**: Many regions and rivers remain under-researched, resulting in significant gaps in geographical data. This lack of comprehensive coverage impedes the ability to assess the full extent of EDC contamination and its impacts across the country.

b) **Incomplete Understanding of contribution of point-sources:** The understanding of pollutant contributions to the Ganga River is severely constrained by the lack of monitoring and quantification of critical point-source discharges, such as those from wastewater treatment plants (WWTPs), sewage treatment plants (STPs), and drains. Despite their significant role in contributing to the river's pollution, these discharges are largely unmonitored, resulting in an incomplete understanding of their impact. This lack of precise data on the volume and contaminant load from these sources hinders the ability to accurately assess their contribution to the river's overall pollution burden. Consequently, efforts to develop targeted and effective management strategies are undermined, as policy interventions may be based on insufficient information. Addressing this gap is essential for a comprehensive understanding of pollution dynamics in the Ganga River and for the formulation of more effective conservation and pollution mitigation strategies.

c) **Lack of Long-Term Studies:** There are no long-term monitoring programs tracking EDC concentrations and their effects over extended periods. Existing information is fragmented and lacks comprehensive spatiotemporal studies, making it difficult to observe chronic impacts, trends, and potential future risks. The scarcity of historical data hinders the ability to understand trends and long-term impacts of EDCs. This gap in historical context limits the effectiveness of current and future environmental management and conservation efforts. For threatened species (such as Gangetic dolphin), this implies that potential past exposures and their long-term effects on current populations are not well understood, making it difficult to implement proactive conservation measures.

d) **Focus on Legacy Contaminants:** Research has predominantly concentrated on legacy contaminants such as pesticides and PCBs, with less attention given to emerging classes of EDCs, such as hormones, BPA, and personal care products. This imbalance implies that the potential threats posed by emerging contaminants to species, particularly threatened. are not fully understood or addressed. This imbalance necessitates expanding research efforts to include these emerging contaminants to fully comprehend their impacts on freshwater ecosystems.

e) **Research and Data Gaps in Monitoring EDCs Impact on Gangetic River Dolphins:** The most recent studies on EDCs in Gangetic River dolphins are from 1988-1996, indicating a significant temporal gap in current data. Despite a substantial body of global evidence outlining the impacts EDCs on aquatic mammals, their risk-based assessments are alarmingly scarce for this endangered cetacean.

Addressing these gaps is crucial for developing a comprehensive understanding of EDC pollution and its ecological implications in India's freshwater environments. Enhanced research efforts, improved monitoring systems, and targeted mitigation strategies are essential to safeguard these ecosystems and ensure the ecological integrity and survival of vulnerable species.

Chapter 3 Selected EDCs for further research

3.1 Physicochemical properties

This chapter focuses on the key properties of forty-two EDCs targeted in this study, including nineteen polychlorinated biphenyls (PCBs), seven plastic additives, nine pesticides, five hormones, and two household and personal care products. These EDCs were selected mainly based on their consumption, detection frequency in literature and toxic impacts on aquatic mammals. Understanding the key physical and chemical properties of various EDCs targeted in this study is essential for assessing their environmental behavior, potential for bioaccumulation, and impact on aquatic ecosystems. The provided data serve as a foundation for further analysis and interpretation of the risks associated with these contaminants.

The EDCs are highly heterogeneous and can be broadly classified in two categories:

A. Natural: These chemicals are found in human and animal such as natural estrogens or hormones like estrone (E1), estradiol (E2), estriol (E3) etc.
B. Synthetic: Those that are man-made or synthesized such as polychlorinated biphenyls (PCBs), plastics additives (e.g. bisphenol A, phthalates), pesticides (e.g. dichlorodiphenyltrichloroethane (DDT), pharmaceutical agents (e.g. Ethinyl

estradiol (EE2) and diethylstilbestrol (DES), house hold and personal care products (anti-microbials – Triclosan and triclocarban).

Polychlorinated Biphenyls (PCBs)

PCBs are synthetic organic chemicals consisting of two linked benzene rings with varying numbers of chlorine atoms. Their chemical stability, low flammability, and insulating properties have led to their widespread use in electrical equipment, paints, and other industrial applications. However, these same properties make PCBs persistent environmental pollutants with significant bioaccumulation and biomagnification potential. PCBs have been banned in India since the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2001, but legacy pollution remains an issue due to historical usage. PCBs are strictly regulated under various environmental protection laws in India, including the Hazardous Waste (Management, Handling, and Transboundary Movement) Rules.

Plastic Additives

Plastic additives such as phthalates are used to enhance the flexibility, durability, and longevity of plastic products. These chemicals are commonly found in products like PVC plastics, medical devices, toys, and packaging materials.

BPA is an organic synthetic compound used primarily in the production of polycarbonate plastics and epoxy resins. It is found in a variety of consumer goods, including water bottles, food containers, dental sealants, and the linings of metal cans. The use of BPA in certain products, particularly those used by infants and children, is restricted under BIS regulations due to its potential health risks. However, they can leach into the environment, posing risks to wildlife and humans due to their endocrine-disrupting properties. India is a significant producer and consumer of plastic additives due to its large plastic manufacturing industry.

Pesticides

Pesticides are chemicals used to control pests in agriculture but can accumulate in the environment, posing risks to non-target species including aquatic life.

Estrogens/Hormones

Hormones are biologically active compounds that regulate various physiological processes. Synthetic and natural hormones can disrupt endocrine systems in wildlife and humans when they enter the environment. India produces various hormones for medical and agricultural purposes, such as growth promoters and contraceptives.

Household and Personal Care Products

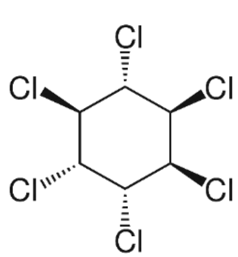
Household and personal care products can introduce various chemicals into the environment through regular use and disposal. These include antimicrobial agents, preservatives, and surfactants found in products like soaps, shampoos, detergents, and disinfectants.

The CAS No., chemical structure, physico-chemical properties, and IUPAC name of target EDCs are provided in Table 3-1 and Figure 3-1

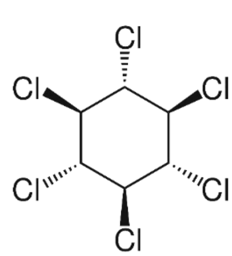
Table 3-1: Physical and Chemical Characteristics of target EDCs				
EDC Group	**Common Name**	**Abbreviation**	**Molecular Weight**	**K_{ow}**
Polychlorinated Biphenyls (PCBs-19)	2,4'-Dichlorobiphenyl	PCB 8	223.09	5.07
	2,4,4'-Trichlorobiphenyl	PCB 28	257.5	5.62
	2,2',3,5'-Tetrachlorobiphenyl	PCB 44	292	5.82
	2,2',5,5'-Tetrachlorobiphenyl	PCB 52	292	5.84
	3,3',4,4'-Tetrachlorobiphenyl	PCB 77	292	6.36
	3,4,4',5-Tetrachlorobiphenyl	PCB 81	292	6.36
	2,2',4,5,5'-Pentachlorobiphenyl	PCB 101	326.4	6.43
	2,3,3',4,4'-Pentachlorobiphenyl	PCB 105	326.4	6.65
	2,3,4,4',5-Pentachlorobiphenyl	PCB 114	326.4	6.65
	2,3',4,4',5-Pentachlorobiphenyl	PCB118	326.4	6.74
	2,2',3,4,4',5'-Hexachlorobiphenyl	PCB 138	326.4	6.87
	2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153	360.88	7.02
	2,3,3',4,4',5-Hexachlorobiphenyl	PCB 156	360.88	7.18
	2,3',4,4',5,5'-Hexachlorobiphenyl	PCB 167	360.88	7.27
	3,3',4,4',5,5'-Hexachlorobiphenyl	PCB 169	360.88	7.42
	2,2',3,4,4',5,5'-Heptachlorobiphenyl	PCB 180	395.32	7.44
	2,3,3',4,4',5,5'-Heptachlorobiphenyl	PCB 189	395.32	7.71
	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	PCB 209	498.6	8.18
Plastics Additives	Dimethyl phthalate	DMP	194.18	1.6
	Diethyl phthalate	DEP	222.24	2.47
	Di-n-octyl phthalate	DnOP	390.6	8.1
	Bis(2-ethylhexyl) phthalate	DEHP	390.6	7.6
	Butyl benzyl phthalate	BBP	312.4	4.73
	Di-n-butyl phthalate	DnBP	278.34	4.5
	Bisphenol A	BPA	228.29	3.32

EDC Group	Common Name	Abbreviation	Molecular Weight	K_{ow}
Pesticides	alpha-1,2,3,4,5,6-hexachlorocyclohexane	α-HCH	290.8	3.8
	beta-1,2,3,4,5,6-hexachlorocyclohexane	β-HCH	290.8	3.78
	gamma-1,2,3,4,5,6-hexachlorocyclohexane or Lindane	γ-HCH	290.8	3.72
	delta 1,2,3,4,5,6-hexachlorocyclohexane	δ-HCH	290.8	4.14
	1,1-Dichloro-2,2-bis (4-chlorophenyl) ethene dichlorodiphenyldichloroethylene	p,p'-DDE	318	6.6
	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene	p,p'-DDD	320	6.02
	1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl) ethyl]benzene	p,p'-DDT	354.5	6.51
Hormones	Estrone	E1	270.4	3.67
	Estradiol	E2	272.4	5.07
	Estriol	E3	288.4	4.76
	Ethinyl estradiol	EE2	296.4	4.342
	Diethylstilbestrol	DES	268.3	1.6
Household and personal care products	Triclosan	TCS	289.5	2.47
	Triclocarban	TCC	315.6	8.1

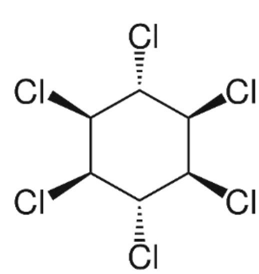
3.2 Chemical Structure



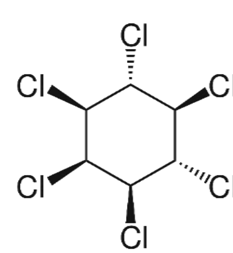
α-HCH



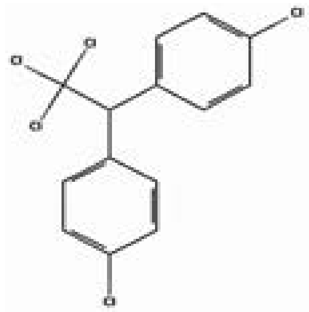
β-HCH

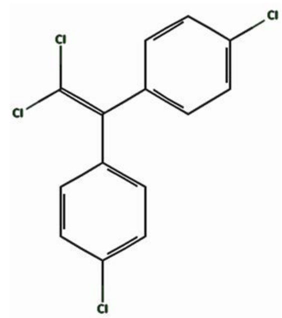


γ-HCH



δ-HCH


p,p’ DDT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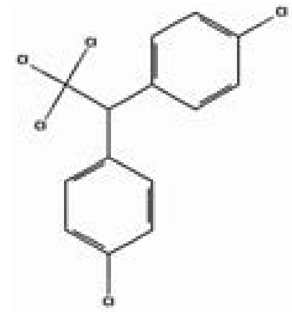
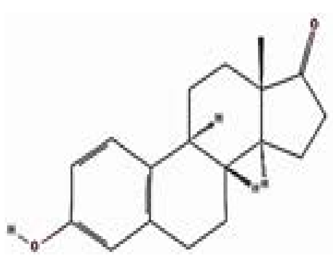
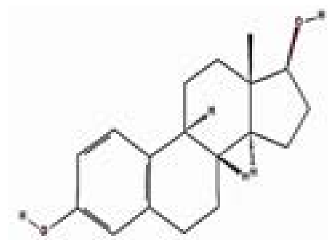
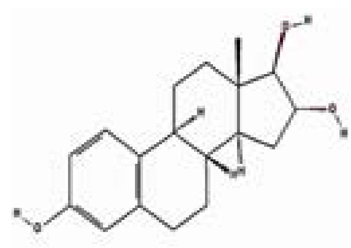
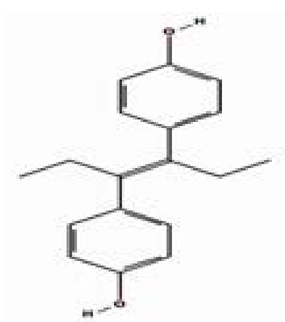
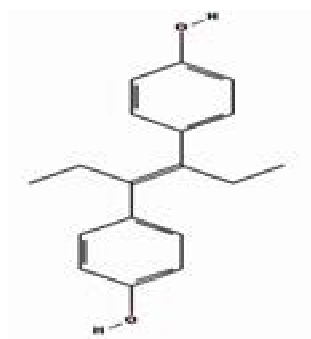

p,p’ DDD

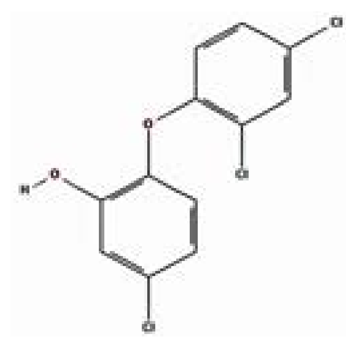

Estrone

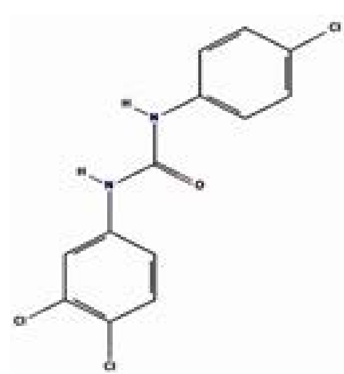

Estradiol

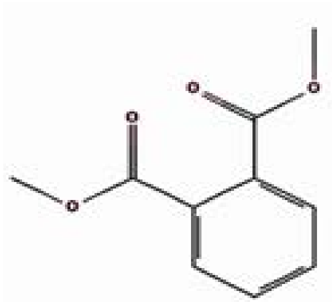

Estriol


Diethylstilbestrol

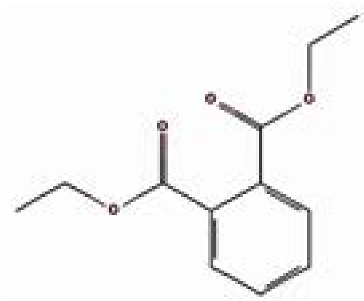

Ethynylestradiol


Triclosan

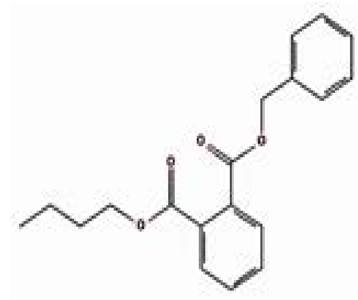

Triclocarban



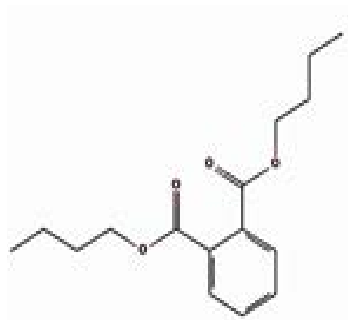
DMP



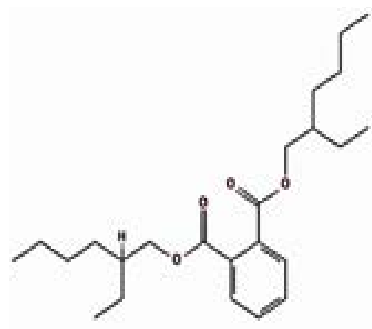
DEP



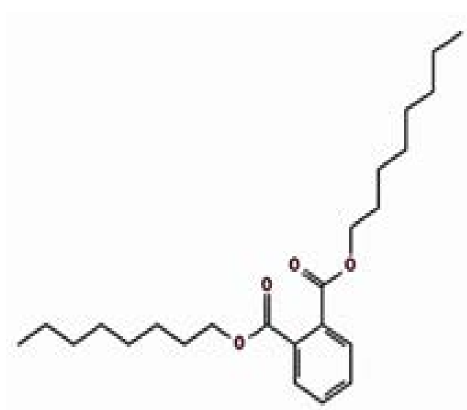
BBP



DnBP



DEHP



DnOP

Chapter 4 Mapping the risk down the drain: Insights into the occurrence, seasonal dynamics, estrogenic load, and ecological risks of multiclass EDCs in MGR

Abstract

This study presents the first comprehensive assessment of e-EDCs in the MGR, providing baseline data on their occurrence, spatiotemporal distribution, and ecological risks. The study analyzed various e-EDCs, including plastic additives, personal care products, estrogens, and pesticides, across different seasons and locations.

Results revealed significant contamination, with plastic additives (phthalates and BPA) emerging as predominant e-EDCs class. Seasonal dynamics showed higher concentrations of BPA, Estrogens and HPCPs ($p<0.05$) during pre-monsoon, while phthalates increased post-monsoon ($p<0.05$). Drain discharges were identified as major contributors, with three drains accounting for 81% of the total EDC load. Water quality parameters, including conductivity, total dissolved solids, and pH, influenced EDC distributions of some e-EDCs.

A multi-tier ecological risk assessment identified ten priority e-EDCs for routine monitoring: DnBP, E1, DEHP, TCS, DEP, E3, BPA, E2, TCC, and γ-HCH. Risk stratification revealed 63% of the MGR as high risk and 25% as moderate risk, with the entire study area potentially at risk from multiple e-EDCs.

This study highlights the urgent need for improved wastewater management, enhanced monitoring programs, and targeted remediation strategies. The findings provide crucial insights for informed conservation efforts, particularly for the endangered Gangetic Dolphin. Future research should focus on food-web bioaccumulation and trophodynamics of priority e-EDCs to further understand their ecological impact in this conservation-priority stretch of the Ganga River.

4.1 Introduction

Chemical pollution, climate change, invasive species, and habitat degradation threaten about 27% of all freshwater species catalogued on the IUCN Red List, facing the risk of extinction (Tickner et al., 2020; Williams-Subiza and Epele, 2021). Among the array of anthropogenic stressors that threatens freshwater biodiversity, the occurrence of endocrine-disrupting chemicals/compounds (EDCs) in aquatic environments has emerged as a critical ecological concern due to their persistent, bioaccumulative, mobile, and toxic properties (Wang & Zhou, 2013; Mueller et al., 2022). Notably, not all EDCs are inherently persistent and some are categorized "pseudo-persistent" due to their persistent sources, and incomplete removal in conventional wastewater treatment plants (Ebele et al., 2017; OECD et al., 2019).

EDCs comprise a highly diverse class of compounds, including both naturally occurring hormones and synthetic substances and can be found in a wide array of sources, encompassing various chemical classes. Examples of EDCs include natural hormones like estrogens and androgens, as well as man-made substances such as pesticides (e.g., DDT, atrazine), pharmaceuticals (e.g., birth control pills, hormone replacement therapies), plasticizers (e.g., bisphenol A, phthalates), potentially toxic elements (e.g., lead, mercury), industrial chemicals (e.g., polychlorinated biphenyls, dioxins), surfactants (e.g., nonylphenol), and ingredients found in household and personal care products (e.g., parabens, triclosan) (Endocrine Society, 2022; Diamanti-Kandarakis et al., 2009).

The extensive production, widespread and increasing usage, and inadequate disposal of EDCs across various sectors including agriculture, healthcare, industry, and households is responsible for their continuous release to freshwater environments through multiple pathways such as rainfall, sewage discharge, landfill leaching, hospital waste, wastewater treatment plants (WWTPs), and livestock waste.

Over recent decades, there has been growing recognition of the detrimental effects of EDCs on a wide range of aquatic taxa, ranging from primary producers, zooplankton, insects, amphibians, fish, birds, turtles, to apex predators, resulting in skewed population dynamics in regional populations of aquatic animals (Table 2-2 and Table 2-3).

Despite their documented adverse effects, including individual- and population- level impacts, EDCs are frequently omitted from routine monitoring programs (Vos et al., 2000; Desforges et al., 2018).

This oversight is especially alarming for rivers with a huge population, already under pressure from anthropogenic factors like habitat loss, over-exploitation, and climate change. The Middle Ganga Reach (MGR), along Uttar Pradesh, with one of the highest populations in India, intense urbanization, and extensive agriculture, exemplifies this, as it supports multiple endangered species of flora and fauna while facing heightened habitat suitability challenges due to various anthropogenic stressors in its catchment. The MGR, the longest stretch of the Ganga River (~1000 km), is an important habitat for endangered Gangetic dolphins and other important flora and fauna yet faces intensified habitat challenges due to anthropogenic pressures (WII-GACMC, 2019; Aquatic Biodiversity Conservation in Ganga River Basin, 2024).

The MGR flows through the state of Uttar Pradesh, the most populous state in India, with a population of approximately 200 million. The state is also one of India's top contributors to mismanaged plastic waste, generating approximately 375,950 tonnes per annum (UPPCB, 2019). The MGR ecosystem faces additional pressure from 56 open drains, which discharge approximately 1,705 million liters per day (MLD) of domestic and industrial effluents into the river, highlighting their potential role as major conduits for EDCs from the catchment area (CPCB, 2018).

Significant levels of legacy EDCs, including organochlorine pesticides, polychlorinated biphenyls (PCBs), and potentially toxic elements (PTEs), have been detected across various environmental matrices in the river (Figure 2-8 to Figure 2-13). ***However, research has predominantly focused on these legacy EDCs, while emerging EDCs remain under-investigated***.

Seasonal surveys are imperative for understanding capture the temporal fluctuations in EDC concentrations, which can be influenced by factors such as agricultural cycles, consumption patterns, industrial discharges, and varying flow conditions. This knowledge is vital for developing targeted conservation strategies and mitigation efforts that address peak EDC threats to the ecosystem. ***However, the existing studies are fragmented and lack a comprehensive assessment of the seasonal dynamics of these pollutants.***

Furthermore, despite substantial knowledge of their contribution to the organic load in the MGR and the growing ecological concerns related to EDCs, ***the evident knowledge gaps regarding contributions of EDC contamination from drain outlets to the receiving river are worrisome***. Understanding the impact of these point sources on the distribution characteristics of EDCs in the MGR is crucial for controlling their release and implementing targeted mitigation strategies to preserve ecological integrity.

Given the above, the study aims to specifically address the following key research hypothesis: (i) The occurrence and spatiotemporal distribution of e-EDCs in MGR is influenced by the drain outfalls (ii) The spatiotemporal dynamics of e-EDC are influenced by key environmental factors (iii) The occurrence of e-EDC in MGR pose risks to aquatic organisms at different trophic levels.

The overarching objective of this chapter is to understand the occurrence and spatiotemporal dynamics of EDCs in the Middle Ganga Reach (MGR) and identify vulnerable and high-risk zones.

4.2 Materials and methods

4.2.1 Study area, sampling sites and sampling methodology

The investigated study area is a ~600 Km stretch of MGR from Kanpur to Ghazipur in Uttar-Pradesh (UP) and lies between north latitudes 26° 33' 40.608" N-25° 34' 56.64" N, and east longitudes, 80° 18' 3.096" E - 83° 36' 6.48" E (Figure 4-1). The characterization of MGR is provided in Table 4-1. The stretch holds immense significance due to its religious, economic, and socio-cultural importance and is an important habitat for diverse flora and fauna including the endangered Gangetic Dolphin (IUCN, 2024). The area experiences a humid subtropical climate with hot summers (April-June, >40°C), cool winters (November-January, <10°C), and a monsoon season (July-October). During its 600 km course, the MGR is joined by four tributaries: the Yamuna, Pandu, Tamas, and

Gomti. The river receives considerable amounts of wastewater daily from industries and municipal sewage systems from cities such as Kanpur, Unnao, Prayagraj, Mirzapur, and Varanasi, leading to significant water quality deterioration (UPPCB, 2019). The MGR catchment area includes 16 cities/towns and 82 villages, generating an estimated 873.9 million litres per day (MLD) of sewage (UPPCB, 2019). However, there is a ~53% gap between sewage generation and treatment.

Table 4-1: Characterisation of MGR	
River length (Km)	600
Geographical Area (Sq. Kms)	240928
Demography (Billions) Census 2011[a]	0.2
Total cropped Area (000 thousand Hectares, 2013-2014)[b]	25896
Cropping Intensity[#] (%) 2013-2014	145
Total pesticide consumption[c] (%)2016-2017	71.03
Annual precipitation*	585.2

**Average of total annual rainfall in East Uttar Pradesh and West Uttar Pradesh*
aCensus of India Office
bDES, 2024
cStates/UTs Zonal Conferences on Inputs (Plant Protection)
#Cropping Intensity = Gross Cropped Area x 100/ Net Sown Area

Approximately 591 grossly polluting industries surround MGR, such as tannery, sugar, pulp and paper, chemicals, petrochemical, distillery, textile, electroplating, metallurgical, etc., and discharge treated, untreated, and/or partially treated wastewater into the river. Based on the Biological Oxygen Demand (BOD) load, 3.5-8.8 mg/L, the stretch has been designated as critically polluted (UPPCB, 2019). Majority of these industrial settlements are in urban city centres such as Kanpur, Prayagraj, and Varanasi. A brief description of these major pollution hotspots in the MGR is provided below.

Kanpur:

Kanpur City district spans an area of 3155 km^2, with a total population of 4581000 (Urban: 66%; and Rural: 34%) (District Kanpur Nagar, Government of Uttar Pradesh | Industrial Capital of Uttar Pradesh | India, n.d.). Kanpur is a highly industrialized area, with the growth rate of industries approximated at 12% annually (District Industrial Profile of Allahabad, 2011). The Kanpur-Unnao Industrial belt contributes approximately 26 MLD of industrial effluent into the Ganga via drains, in addition to the 560 MLD of untreated municipal sewage discharged into the river (UPPCB, 2019). The district's industrial sector is predominantly composed of leather and textile industries. Large-scale industries here include fertilizer and cement, and machines and tools. Tanneries constitute upto 58% of the Grossly Polluting Industries (GPIs) polluting the Ganga (Dwivedi et al., 2018), employing compounds like aliphatic amines, non-ionic surfactants, oils, and pigments, which eventually contaminate the environment when discharged into rivers with the effluent, capable of exerting mutagenic and genotoxic effects on organisms (Alam et al., 2010). The industry also utilises chromium salts, much of which are released into the rivers with industrial wastewater, polluting the riverine environment with potentially toxic elements (PTEs) including Zn, Mn, Cu and Pb as well (Bhuiyan et al., 2011; Kumar et al., 2023). In addition to PTEs, the Ganga River is also reported to be contaminated due to the extensive use of pesticides in the Kanpur area, which has a net sown area of 190,000 hectares. (Dwivedi et al., 2018; Economics and Statistics Division State Planning Institute Planning Department, Uttar Pradesh, 2022).

Prayagraj:

Prayagraj division, comprising of districts Pratapgarh, Prayagraj, Kaushambi and Fatehpur, covers an area of 15131 km^2, with a total population of 15,694,623 projected for 2023 (Urban: 16%, Rural: 84%) (District Prayagraj, 2024). The division consists of 16 towns and 7652 villages. The catchment generates and discharge more than 215 MLD of municipal sewage into the Ganga at Prayagraj (UPPCB,2019). Besides its resident population, Prayagraj also experiences a high tourist footfall owing to its cultural significance. Prayagraj saw the arrival of 14492831 tourists in 2021 (District Wise Domestic and Foreign Tourist Visits in Uttar Pradesh in Year 2021, 2021). Mass-bathing events have been observed to precede a deterioration in water quality by various researchers (Sinha et al., 1991; Vidyarthi et al., 2020). The river is joined by the Yamuna at Sangam, beyond which the water quality is observed to be deteriorated owing to the domestic and industrial pollutant load of the Yamuna (Sharma et al., 2014). In addition to the industrial wastewater brough by the Yamuna, Prayagraj's own industries may potentially contribute to contaminants in the environment. The major industries include Agro-based, textiles, paper, leather, and chemical industries (District Industrial Profile of Allahabad, 2011b; UPPCB, 2019). Prayagraj District was recorded to have a net sown area of 336000 Ha in 2020, and the Ganga has been recorded to be contaminated by OCPs in this region (Raghuvanshi et al., 2014).

Varanasi:

Within the Varanasi district, there are 38 towns and 1327 villages, with a population of 3,676,841(Urban: 43.44% and Rural: 56.56%) projected to reach approximately 4,300,000 in 2023 (2011 Census). Varanasi City, situated within the study area, is one of the world's oldest inhabited cities and a major tourist destination. However, the city's historical layout poses challenges for sewage infrastructure upgrades due to spatial constraints and historical preservation concerns. Varanasi generates around 330 MLD of sewage, with sewage treatment plants having a capacity of 421.8 MLD, yet only approximately 210 MLD is utilized, resulting in a treatment gap of 120 MLD (UPPCB, 2017). The textile industry is the dominant industrial sector in the local economy, ranking just behind tourism in significance.Diverse chemical classes such as phthalates, alkyl-phenol ethoxylates, azo dyes, flame retardants, chloro-phenols, chlorinated aromatics, organotins, heavy metals, perfluorinated chemicals, etc play crucial roles in various stages of textile production (CPCB, 2019). Varanasi city has three primary sewage discharge points: the Nagwa Drain (previously known as the Assi River), the Khirkia Drain, and the Varuna River (Figure 4-1).

Sampling campaigns were conducted in the Pre-Monsoon (March-April) and Post-monsoon (October - November) of the year 2021. Surface water samples were collected from a total of 40 sampling sites across MGR, including **23** sites located in the mainstream, and **17** sites representing drain discharge (Table 4-2). At each site, 4–5 grab samples were collected, at an average depth of 0-15 cm below surface water, bulked together to form a composite sample. At drain-outfall sites, water was sampled at the drain mouth, or mixing of drain discharge and river water. Additional detailed characteristics of the sampling sites are provided in Table 4-2 to Table 4-4. The samples were collected in pre-cleaned amber glass bottles, labelled, and pH-adjusted to 2.5–3. They were stored in an ice box (4°C) for shipping and processed within 24-48 hours. The physical and chemical properties of the surface water were assessed online using a ProDSS Multiparameter Digital Water Quality Meter (YSI, USA).

4.2.2 Chemicals and reagents

The thesis included forty-two e-EDCs that belong to five different groups. The target e-EDCs and their physicochemical properties, are presented in Table 2-2. Details of the internal standards and materials used in this study are provided in Annexure I. The quantification of the target PAs was performed using Ultra-High Performance Liquid Chromatography–Tandem Mass Spectrometry (UHPLC–MS/MS) and Gas Chromatography–Tandem Mass Spectrometry (GC-MS-MS). Detailed information about the instrumental parameters can be found in Annexure I.

4.2.3 Sample treatment and instrument analysis

The refrigerated samples were first filtered through a 0.7 mm mesh Whatman glass microfiber filters (GE Healthcare Life Sciences, UK).

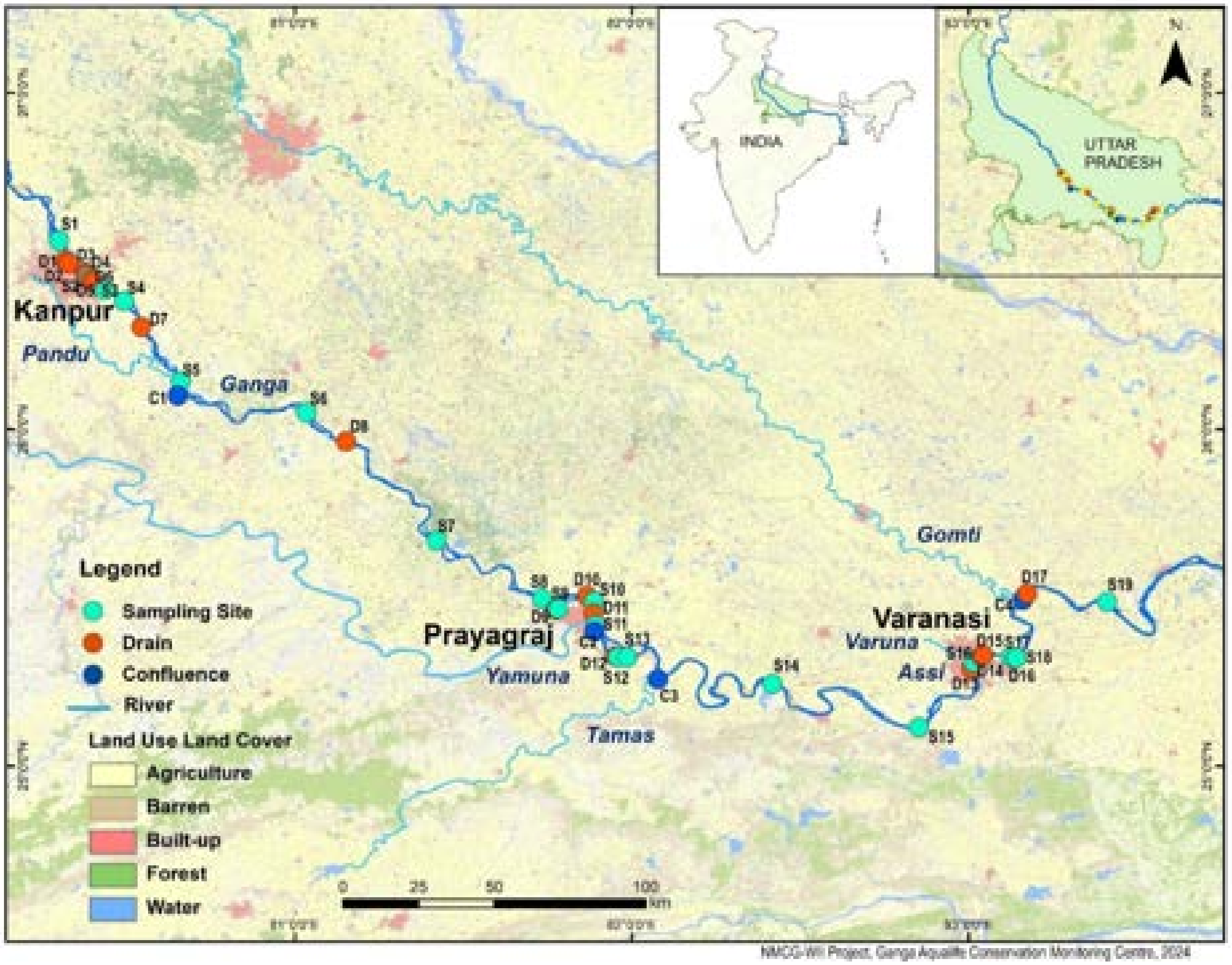


Figure 4-1: Sampling locations in the MGR, including river sites (S), drain discharge points (D), and tributary confluences (C)

Table 4-2: Site Description with coordinates

Site No.	Site ID*	Site Name	District	Latitude	Longitude
1	S1	Fattepur	Kanpur	26.56128	80.30086
2	D1	Permiya		26.50112	80.32036
3	D2	Ranighat		26.49391	80.3291
4	D3	Gola Ghat		26.46676	80.37722
5	D4	Satti Chaura		26.46	80.38056
6	S2	1 Km D/S Satti Chaura		26.45112	80.38805
7	D5	Dabka Nalla		26.44482	80.39433
8	D6	Shetla Bazaar		26.43709	80.40373
9	S3	3 Km D/S Shetla Bazaar		26.4137	80.43648
10	D7	City Jail Drain	Unnao	26.30443	80.54536
11	S4	Chandrika Ghat		26.14197	80.66195
12	C1	Pandu River Conf		26.11688	80.66392
13	S5	Bhitoura	Fatehpur	26.05049	81.03466
14	S6	Dalmau	Raebareily	25.96462	81.15246
15	S7	Manikpur	Pratapgarh	25.66972	81.42156
16	S8	Shrimgverpur	Prayagraj	25.49732	81.73499
17	D8	Kodar		25.46566	81.77625
18	S9	Kodar 500m D/S		25.46769	81.78181
19	D9	Rasoolbad Samsan Ghat		25.50534	81.86861
20	S10	ALBD Phaphmau Bridge		25.48803	81.88915
21	D10	Solari		25.45366	81.88692
22	S11	2km D/S Solari		25.41837	81.89241
23	C2	500m D/S Sangam		25.41621	81.90139
24	D11	Mannaiya		25.32361	81.95908
25	S12	500m D/S Mannaiya		25.32005	81.9805
26	S13	2.5km D/S Mannaiya		25.27515	82.08833
27	C3	Tamas Conf	Mirzapur	25.26456	82.21982
28	S14	Mirzapur		25.17344	82.89341
29	S15	Adalpura		25.21084	83.00665
30	D12	Nagwa	Varanasi	25.29033	83.00852
31	S16	Assi Ghat		25.30888	83.01508
32	D13	Khirkia		25.32556	83.03914
33	D14	Varuna		25.32923	83.04424
34	S17	Kunda Kala		25.32331	83.12623
35	D15	Mughalsarai		25.32126	83.14078
36	S18	500m D/S Mughalsarai		25.32211	83.14206
37	C4	Gomti Conf		25.50896	83.17147
38	S19	Saidpur	Ghazipur	25.51312	83.17429
39	S20	Chochakpur		25.48761	83.41164

****S, D, and C denote sites in the river, drains, and confluences of tributaries, respectively.***

Table 4-3: Variation in flow and organic load of priority drains monitored by CPCB, in Uttar Pradesh

State	Priority Towns	Mixed Priority Drains	Domestic Priority Drains	Total Priority Drains	Pre-Monsoon, 2018		Post-Monsoon, 2018	
					Flow (MLD)	BOD Load (TPD)	Flow (MLD)	BOD Load (TPD)
Uttar Pradesh	13	16	40	56	1704.54	121.52	1642.09	84.94

**(UPCB.2019)*

Table 4-4: Seasonal Characteristics of drains in Uttar Pradesh *(CPCB, 2018)*

District	**Priority drains**	**Pre-Monsoon, 2018**		**Post-Monsoon, 2018**		**Pollution Sources (Status)**	**Sampling Point**	
		Flow (MLD)	Organic Load (TPD)	Flow (MLD)	Organic Load (TPD)		**Latitude**	**Longitude**
Kanpur	Permiya Nala	189.81	1.61	166.51	2.05	Domestic (Flow)	26.301960	80.191209
Kanpur	Ranighat Drain	1.33	0.29	1.08	0.25	Domestic (Flow)	26.294670	80.192970
Kanpur	Golaghat Nala	3.56	0.2	1.13	0.18	Domestic (Flow)	26.275620	80.223900
Kanpur	Satti Chaura	0.84	0.04	1.29	0.08	Domestic (Flow)	26.273280	80.225090
Kanpur	Shetla Bazaar	22.74	14.14	11.73	1.81	Mixed (Flow)	26.260430	80.243020
Unnao	City Jail Drain	7	1.18	27.5	3.36	Mixed (Flow)	26.225800	80.304500
Raebareilly	Loni Drain	31	1.63	12.4	0.07	Mixed (Flow)	26.431000	80.599000
Prayagraj	Rasulabad Drain	68.07	0.76	52.05	1.57	Domestic (Flow)	25.495200	81.848500
Prayagraj	Kodar Drain			24.62	1.14	Domestic (Tapped)	25.46566	81.77625
Prayagraj	Solari Drain	22.64	0.22	93.06	4.84	Domestic (Flow)	25.272700	81.879300
Prayagraj	Mavaiya Drain	71.09	1.06	71.09	3.2	Domestic (Flow)	25.232400	81.545000
Prayagraj	Mannaiya	0.89	0.04			Mixed (Flow)	25.193100	81.575000
Varanasi	Nagwa Drain	198	22.57	138.93	11.93	Domestic (Flow)	25.165800	83.003500
Varanasi	Varuna Drain	571.3	31.59			Mixed (Flow)	25.194500	83.023900
Varanasi	Khirkiya Nala	25.57	3.96	180.72	12.27	Mixed (Flow)	25.193400	83.021800
Varanasi	Mughal Sarai	19.56	0.57	11.58	0.25	Domestic (Flow)	25.32126	83.14078

****MLD=Million Litres per Day; TPD=Tonnes per Day; CPCB=Central Pollution Control Board.***

Following filtration, the target analytes were extracted using a Waters Solid Phase Extraction manifold with Oasis HLB 6cc/500mg cartridges (Waters Corporation, USA). The cartridges were first conditioned with 10 mL of HPLC-grade methanol (Merck, USA), followed by 10 mL of Dichloromethane:n-Hexane solution (1:1 v/v; Merck, USA) and finally by 10 mL ultrapure water. These were then vacuum dried for 1 hour. Samples were loaded into the cartridges to separate the target analytes, which were then eluted using 10 mL Dichloromenthane:n-Hexane (1:1 v/v) solution followed by 12 mL Dichloromethane:Methanol (v/v) solution and finally by 10 mL Methanol (Merck, USA).

4.2.4 Estrogenic potency of target e-EDCs

To assess the estrogenic effect of the target EDCs in the MGR, the estradiol equivalent concentrations (EEQs) of the target e-EDCs in the MGR surface water were calculated as follows (Liu et al., 2017):

$$\mathbf{EEQ_T = \Sigma EEF \times C} \qquad \textit{Equation (1)}$$

EEQ_T = Sum of EEQ of the individual estrogen

EEF = Estradiol equivalency factor of estrogen

C = Concentration of target e-EDCs

The EEF values of the target EDCs were taken from literature and are as follows:

4.2.5 Multi-tier Ecological Risk Assessment

4.2.5.1 Tier-1: Screening-Level Ecological Risk Assessment

The ecological risks posed by 42 Endocrine Disrupting Chemicals (EDCs) in the surface waters of MGR were evaluated using a deterministic quotient approach. Chronic and sub-lethal risk quotients (RQs) were calculated by dividing the median concentration of each chemical in water by the predicted no-effect concentration (PNEC) according to Equation (2). The initial risk assessment categorized the EDCs into different ranks: low risk if RQ < 0.01, moderate risk if 0.01 ≤ RQ < 0.1, and high risk if RQ ≥ 1 (Bu et al., 2013; Liu et al., 2017).

$$\mathbf{RQ = Cm/PNEC} \qquad \textit{Equation (2)}$$

Where,

Cm refers to the highest quantified concentration of a single e-EDC at each site, derived from the seasonal datasets.

PNEC is derived by the most sensitive toxicity data with appropriate Assessment Factors (AFs) of 10, 20, 100, or 1000 depending on test endpoints of No Observed Effect Concentration (NOEC), Effective Concentration for 10% of the population (EC10), Lowest Observed Effect Concentration (LOEC), Effective Concentration for 50% of the population (EC50) (Bu et al., 2013; European Medicines Agency, 2018; Ecological Risk Assessment | US EPA, 2024).

4.2.5.2 Tier-2: A semi-probabilistic approach

A semi-probabilistic risk assessment was employed to evaluate the ecological risks posed by environmental pollutants. This approach integrates deterministic and probabilistic elements. Initially, measured concentrations of target chemicals at various sampling sites were compared to the Predicted No Effect Concentration (PNEC), which serves as the threshold for determining potential harm to aquatic organisms. The deterministic component involved directly comparing these concentrations to the PNEC. The probabilistic aspect accounted for variability and uncertainty in exposure levels and species sensitivity, allowing for an estimation of the frequency with which chemical concentrations exceeded the PNEC at different sites. This method, as shown in Equation 3, provided a comprehensive assessment of the potential risks to aquatic life by incorporating both fixed values and variable probabilities (US EPA, 1992; US EPA, 1998).

$$\boldsymbol{F = \left(\frac{n}{N}\right) * 100} \qquad \textit{Equation (3)}$$

where,

F = Frequency of PNEC exceedance

n = Number of sites with concentrations >PNEC

N = Total number of sampling sites for indidual e-EDCs.

The resulting value represents the proportion of sites where potential health impacts are anticipated (Liu et al., 2017).

4.2.5.3 Tier-3: prioritization indexes: An optimized risk assessment

An optimized risk assessment approach was applied to prioritize pollutants based on their ecological impact. In this process, prioritization indexes (PI) were developed to streamline the evaluation by incorporating multiple factors, such as the frequency of pollutant concentrations exceeding the PNEC, the relative toxicity of each chemical, and their environmental prevalence across the study sites (Equation 4). This optimized method enabled a more targeted risk evaluation, focusing on chemicals most likely to cause harm. By ranking pollutants according to their potential risk, the approach facilitated the allocation of resources towards managing the highest priority contaminants, ensuring a more efficient and effective mitigation strategy (NORMAN Association, 2013, Liu et al., 2017).

$$\mathbf{PI} = \mathbf{RQ} \ \mathbf{x} \ \mathbf{F} \qquad \textit{Equation (4)}$$

Where,

PI = Prioritization index

RQ = Risk quotient calculated in Tier I,

F = Frequency of PNEC exceedance

4.2.6 Statistical Analysis

Chemical concentrations were expressed as the range; average ± standard deviation, and reported as ng/L. Before statistical analysis, data < LOQ were converted to ½ LOQ.

Shapiro–Wilk tests and Levene's tests were utilized to test assumptions for normal distribution and homogeneity of variance respectively. As the assumptions were violated non-parametric equivalents were used as the assumptions were violated. Mann-Whitney U test was conducted to test variations of statistical significance for EDCs concentrations among sites, and seasons. Spearman relationship analysis was used to identify the impact of environmental parameters on EDCs distribution in MGR. Inverse distance weighted (IDW) analysis was used to predict and visualize/identify the vulnerable or high-risk zone in the MGR region using ArcGIS 10.6 software.

4.2.7 Quality control and quality assurance

Strict quality control and quality assurance measures were taken during sample collection and laboratory analysis. Field blanks and laboratory procedural blanks were designed to improve the precision of the experimental. Recoveries of the e-EDCs ranged from 89% to 102.3%, with relative standard deviations (RSDs) of < 15%. The detection limits for each target analyte were determined by adding three times the standard deviation of 10 replicate measurements at the lowest matrix-spiked calibration standard concentration to the blank value of the sample. Additionally, concentrations of all quantifiable e-EDCs were blank-corrected by subtracting the mean values of background contamination present in the blanks to account for any potential interference and ensure more precise measurements.

4.3 Results and Discussion

4.3.1 Concentrations profile of e-EDCs in MGR

The concentrations of target e-EDCs, with detection frequencies (DFs) ≥10%, in MGR are presented in Figure 4-2 and Table 4-5. In Figure 4-5, the concentrations of target e-EDCs are presented on a logarithmic scale to accommodate the wide range of values observed across different e-EDCs, enabling clear visualization of both high and low concentration data points. The median, average±standard deviation and range of the total concentration of 42 e-EDCs (Σ_{42}EDCs) were **10048.14** ng/L and, **10638.5±5935.15** ng/L respectively, with a range of **2254.68–27141.16** ng/L. Overall, 21 out of the 42 EDCs, were detected in the water samples, with DFs ranging from 10 (p,p' DDD) –100%. Plastics additives such as phthalates (658.86-8285.77 ng/L) and BPA (139.82-9395.64 ng/L) were the most abundant and prevalent (DF: 88-100%) group, demonstrating their widespread occurrence in the MGR waters.

Extensive production and widespread use of plastic additives contribute to their dominance and prevalence in the environment, with dispersion occurring at every stage from synthesis to degradation (Hahladakis & Lacovidou, 2018). The low levels of BPA compared to certain phthalates, such as DEHP, DEP, and DnBP, can be explained by how it is chemically incorporated within the structure of the plastic. This strong chemical bonding reduces the likelihood of BPA leaching or evaporating (volatilizing) into the environment. As a result, BPA remains more stable within the product, making it less likely to be released compared to phthalates, which are not chemically bonded and can more easily escape into the surrounding environment (Tijana Vasiljevic & Harner, 2021). Moreover, the high concentration of plastic additives (Phthalates + BPA) suggests a strong link to inadequate solid and plastic waste management in the densely populated catchment area (Chakraborty et al., 2021).

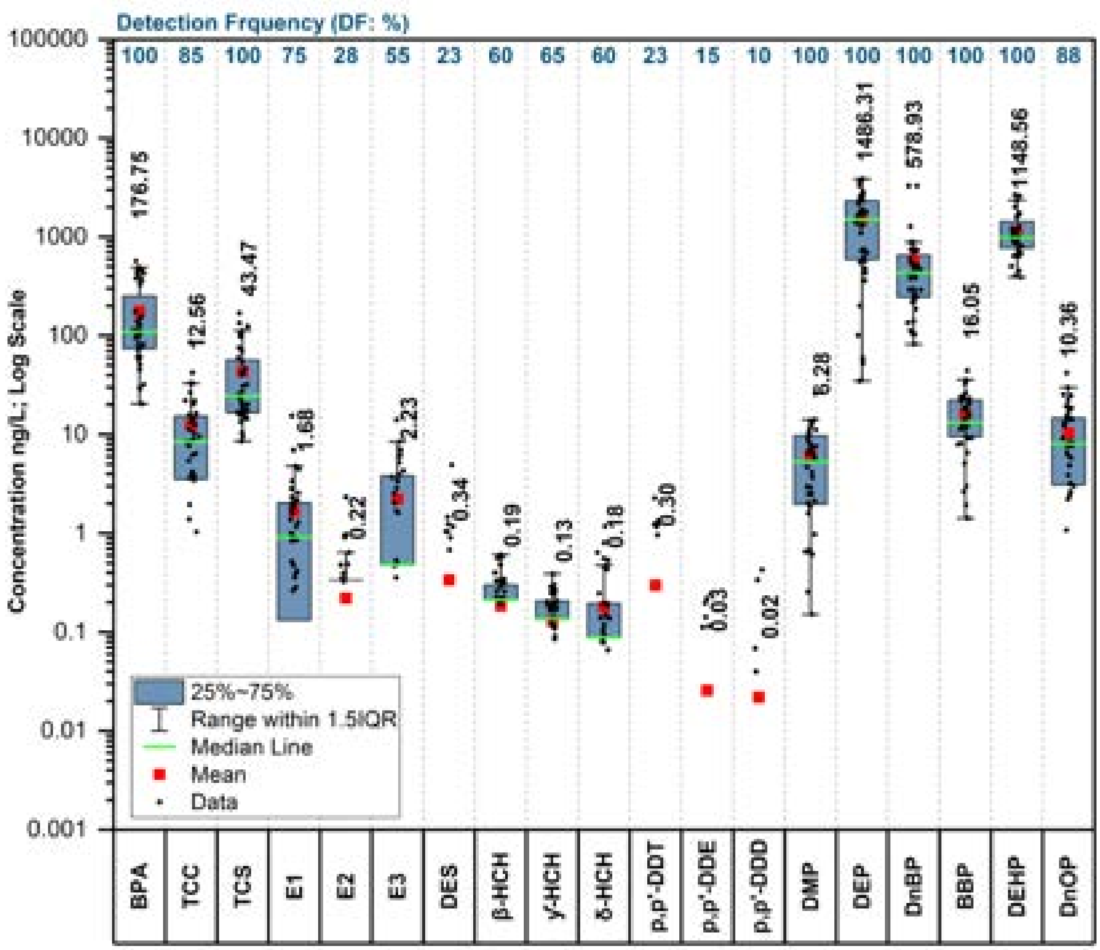


Figure 4-2: Average concentration (ng/L) and detection frequency (DF:%) of target e-EDCs in MGR

Detection frequency (DF %) is highlighted in blue colour

In MGR, the mean concentration of the individual e-EDCs followed the order: DEP (1486.31±1027.93 ng/L) > DEHP (1148.56±568.64) > DnBP (578.93±680.67) > BPA (176.75±157.59) > TCS (43.47±24.63 ng/L). The contribution of DEP, DEHP, DnBP, BPA, and TCS accounted for 43 %, 33%, 17%, 5%, and 1.2% of the total e-EDCs concentrations, respectively.

The high levels of DEP, DEHP, and DBP observed in the MGR, both in this study and previous research (Chakraborty et al., 2021), can be attributed to their widespread use as additives in various industrial products and household items (Rishikesh Mankidy et al., 2013; Wen & Gao, 2017; Gao et al., 2019; Luo et al., 2021), underscoring their role as major phthalate contaminants in aquatic environments. Additionally, although low molecular weight phthalates such as DEP and DnBP are primarily used as plasticizers in plastic products, they also have secondary applications in cosmetics and personal care products. For example, DEP is used in perfumes, as an ethanol denaturant, and in adhesives, printing inks, and cellulose esters, while DBP is commonly found in nail polish, varnishes, paints, and adhesive formulations (Gómez-Hens and Aguilar-Caballos, 2003; Latini, 2005). Also, for phthalates, a relatively low removal rate was achieved compared to other target e-EDCs (Guo et al., 2022). The fact that these phthalates were the highest contributors to total e-EDC contamination is concerning, as they have all been listed as priority environmental pollutants by the U.S. Environmental Protection Agency (European Commission, 2007). Similarly, BPA is extensively used in medical equipment, food and beverage packaging containers, aerospace materials, construction materials, toys, electronic devices, water pipe linings, thermal and carbonless paper coatings, casting, etc. (Tijana Vasiljevic & Harner, 2021).

Triclosan (TCS) and triclocarban (TCC) had concentrations lower than plastic additives but higher than other e-EDCs groups, making household and personal care products (HPCPs) the second most prevalent and significant group of e-EDCs in the MGR. The observed variation in concentrations and detection frequencies between TCS (43.47 ng/L; DF: 100%) and TCC (12.56 ng/L; DF: 85%) can be attributed to differences in their consumption patterns, chemical properties, and degradation rates in environmental matrices (refs). Specifically, triclosan is widely used in liquid soaps, whereas triclocarban is primarily found in solid soap bars. As a commonly used antimicrobial in consumer products and medical disinfectants, TCS is one of the most frequently detected organic compounds in surface water (Li et al., 2021a; Singh and Suthar, 2021). Their relatively higher residual concentrations of are largely relevant to their relatively higher consumption in daily life. It is important to highlight that our sampling in 2021 coincided with the COVID-19 pandemic (particularly Pre-Monsoon sampling), a period marked by increased use of hygiene products. This surge in usage likely contributed to elevated down-the-drain discharges of triclosan into aquatic ecosystems.

Compared to the other two groups discussed above, estrogens represented a minor proportion. The low levels of Estrogens could be attributed to their low solubility in water and high susceptibility to biodegradation and photolytic degradation (Petrie et al., 2015;

Zuo et al., 2013). Additionally, due to their significant log Kow, Estrogens are expected to be absorbed onto the solid phases (Pal et al., 2010). Among the detected estrogens, average concentrations ranged from 0.01 ng/L to 16.95 ng/L, with the highest concentration recorded for Estriol (E3) at 2.23 ng/L and closely followed by Estrone (E1) at 1.68 ng/L. Ethinylestradiol (EE2) concentrations were below detectable limits (<DL). The DF for Estrone (E1), Estradiol (E2), Estrone (E3), and Diethylstilbestrol (DES) were 75%, 28%, 55%, and 23%, respectively. The elevated concentration of E3 can be attributed to its high excretion rates from the human body, E3 is a primary estrogen metabolite, particularly during pregnancy, and is excreted in significant amounts through urine (Adeel et al., 2017). Additionally, E1. E2, and E3 are interconnected through metabolic pathways in the environment. Under aerobic microbial conditions, these estrogens can be interconverted; for instance, E2 can be oxidized to E1, and E1 can be reduced back to E2 or further metabolized to E3 (Casey et al., 2003; Goeppert et al., 2015). This dynamic transformation under aerobic conditions ensures that all three estrogens are present in varying concentrations depending on the environmental context. Besides human excreta, livestock farming and the application of animal manure to agricultural land is also identifies to be significant contributors to estrogen levels in the MGR (Kjær et al., 2007). The lowest EDC concentrations (<DL-0.30 ng/L) were found among organochlorine pesticides (OCPs), with p,p' DDT showing the highest levels within this group.

Except for α-HCH, all six OCPs were detected in both seasons. The HCHs (β, γ, and δ isomers) had a higher detection frequency, ranging from 60% to 65%, within the pesticide group, indicating their pervasive presence in the MGR. The pesticides levels noted in the present study are significantly lower than our previous study (Sah et al., 2020), suggests that regulatory measures and enforcement efforts for banned and regulated pesticides are proving effective. However, the continued presence of even low levels of contamination indicates ongoing challenges, such as illegal use, improper disposal, and legacy pollution.

4.3.1 Spatiotemporal variations of e- EDCs in drains and MGR

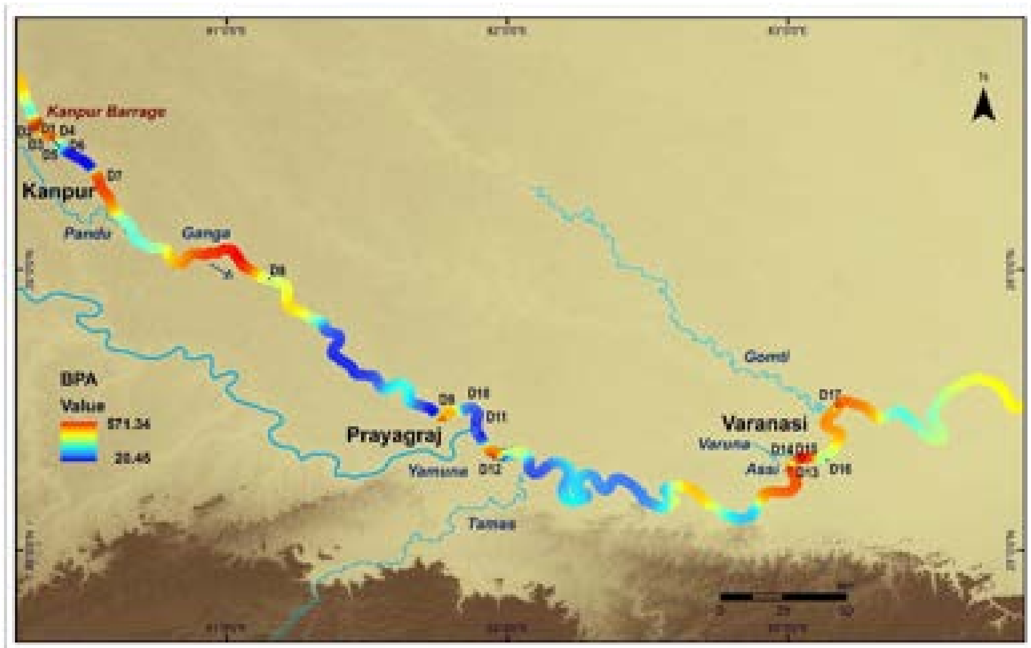
Seasonal variations of EDCs are depicted in Figure 4-6 and Table 4-5 (group-wise). The total EDCs (ΣEDCs) concentration was significantly higher ($p<0.001$) during the Pre-Monsoon (PreM) season compared to the Post-Monsoon (PostM) season. Figure 4-7, and Figure 4-10 to Figure 4-14 illustrates the seasonal variations in the proportion of different EDC groups. Notably, Plastic additives show a significant increase ($p<0.001$) during the PostM season, while ΣHPCPs ($p<0.001$), ΣEstrogens ($p=0.11$), and ΣPesticides ($p=0.30$) exhibit a declining trend. The elevated levels of plasticizers observed in the PostM season can be explained by several contributing factors. Primarily, the increased use of plasticizers during this period, particularly in agricultural practices and industrial activities, significantly contributes to their heightened presence in the environment.

Table 4-5: Group-wise EDCs concentration in MGR

Site ID	Site Name	EDCs group (ng/L)			
		ΣPlastic Additives	**ΣHPCPs**	**ΣEstrogens**	**ΣPesticidess**
S1	Fattepur	2388.82	15.85	0.88	4.56
D1	Permiya Drain	5900.39	139.30	16.95	4.21
D2	Ranighat Drain	2629.04	36.54	1.92	8.38
D3	Gola Ghat Drain	6060.29	39.67	2.22	3.27
D4	Satti Chaura Drain	2654.72	32.16	0.65	3.54
S2	1 Km D/S Drain	2548.24	14.67	0.01	3.75
D5	Dabka Nala Drain	5627.59	41.54	14.67	8.47
D6	Shetla Bazaar Drain	2761.33	54.31	8.90	5.10
S3	1km D/S Drain	2517.02	19.56	1.76	1.44
S4	5 Km D/S Drain	1018.61	13.45	0.01	1.87
D7	City Jail Drain	15028.24	69.75	1.33	2.08
S5	Chandrika Ghat	3848.25	26.80	5.96	2.27
C1	Pandu River Conf	4747.58	79.03	0.01	0.36
S6	Bhitoura	7018.69	22.94	2.38	21.48
D8	Dalmau	4498.62	66.43	4.56	2.21
S7	Manikpur	2272.02	64.06	5.35	4.97
S8	Shrimgverpur	4208.42	30.94	0.01	2.33
D9	Kodar STP	17597.12	255.05	14.31	8.17
S9	500m D/S	2474.88	86.97	2.52	2.93
D10	Rasoolbad Drain	5378.25	24.11	2.43	3.40
S10	Phaphamau	2381.58	25.89	1.98	4.85
D11	Solari Drain	998.83	124.77	6.80	13.52
S11	2km D/S Drain	4792.82	39.20	2.09	3.83
C2	Sangam	8799.63	31.55	7.76	0.88
D12	Mannaiya Drain	5029.42	43.89	6.91	2.77
S12	500m D/S Drain	2870.73	20.85	0.28	4.18
S13	2.5 Km D/S Drain	5974.19	25.60	5.11	1.98
C3	Tamas Conf	5732.11	28.40	1.40	5.25
S14	Katni Ghat	3900.87	36.72	5.64	2.74
S15	Adalpura	5812.20	29.58	0.40	14.10
D13	Assi Drain	8840.64	111.42	6.82	0.06
S16	Assi Ghat	4377.32	26.46	4.23	3.24
D14	Khirkia Drain	6498.27	158.29	3.39	0.60
D15	Varuna Drain	15975.54	177.03	12.92	4.64

Site ID	Site Name	EDCs group (ng/L)			
		ΣPlastic Additives	**ΣHPCPs**	**ΣEstrogens**	**ΣPesticidess**
S17	Kunda Kala	3666.81	22.96	5.80	0.14
D16	Mughalsarai Drain	4329.43	121.48	12.66	3.32
S18	500 m D/S Drain	3304.45	24.60	1.47	1.55
C4	Confluence Gomti	1523.40	18.18	0.01	3.18
D17	Saidpur	4561.37	31.06	5.61	3.44
S19	Chochakpur	2760.30	10.54	0.97	4.81
	Range	998.83-17597.12	10.54-255.05	0.01-16.95	0.06-21.48
	Median	4353.36	31.86	2.48	3.36
	Average±Standard Deviation	5082.70±3667.66	56.04±52.46	4.48±4.46	4.36±4.07

Moreover, the PostM season is marked by substantial rainfall, which enhances surface runoff and atmospheric depositions. These processes effectively transport plasticizers from various sources, including urban areas, agricultural lands, and industrial zones, into aquatic systems. Previous studies also suggest that estrogen and HPCP concentrations are generally lower in the wet season than in the dry season, mainly due to the dilution effect caused by increased precipitation during the wet season (Lei et al., 2020; Lu et al., 2021). However, when examining contaminant-specific seasonal dynamics (Figure 4-8), distinct seasonal patterns emerged. The concentrations of TCS, TCC, DMP, and DnBP were notably higher in the PreM season, with mean levels, except for DMP, being 1.8 to 2.5 times greater than those observed in the PostM season. In contrast, the concentrations of E3, δ-HCH, DEP, BBP, DEHP, and DnOP were significantly elevated during the PostM season, with mean concentrations ranging from 1.9 to 187 times higher than in the PreM season.



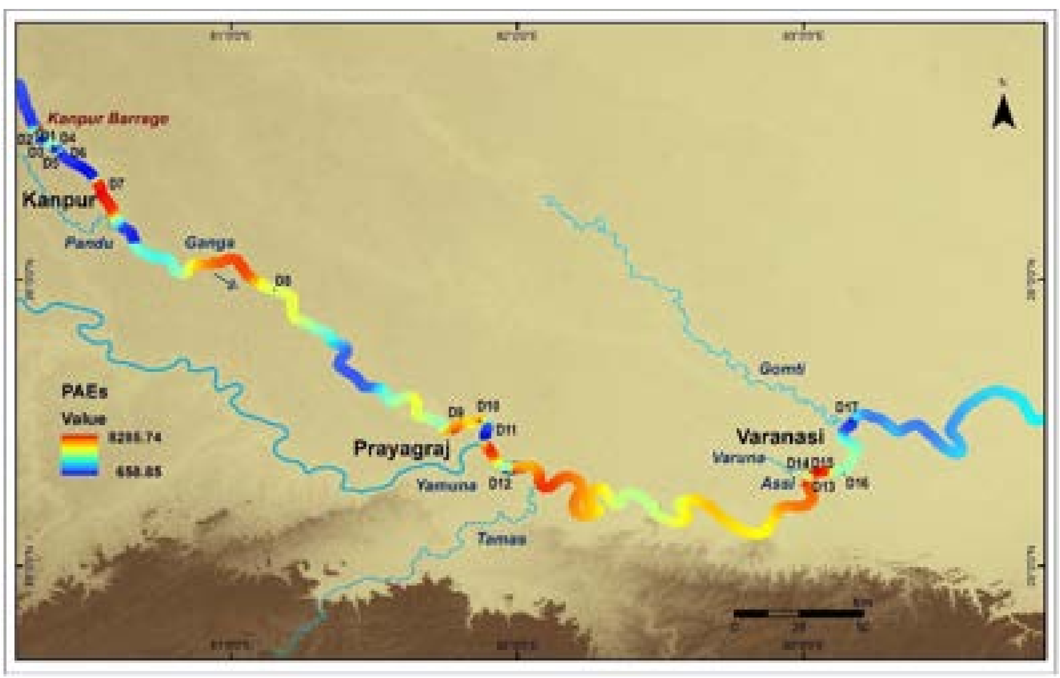


Figure 4-3: Spatial distribution of average concentrations (ng/L) of (a) BPA and (b) Total Phthalate Esters (ΣPAEs) along the MGR in 2021

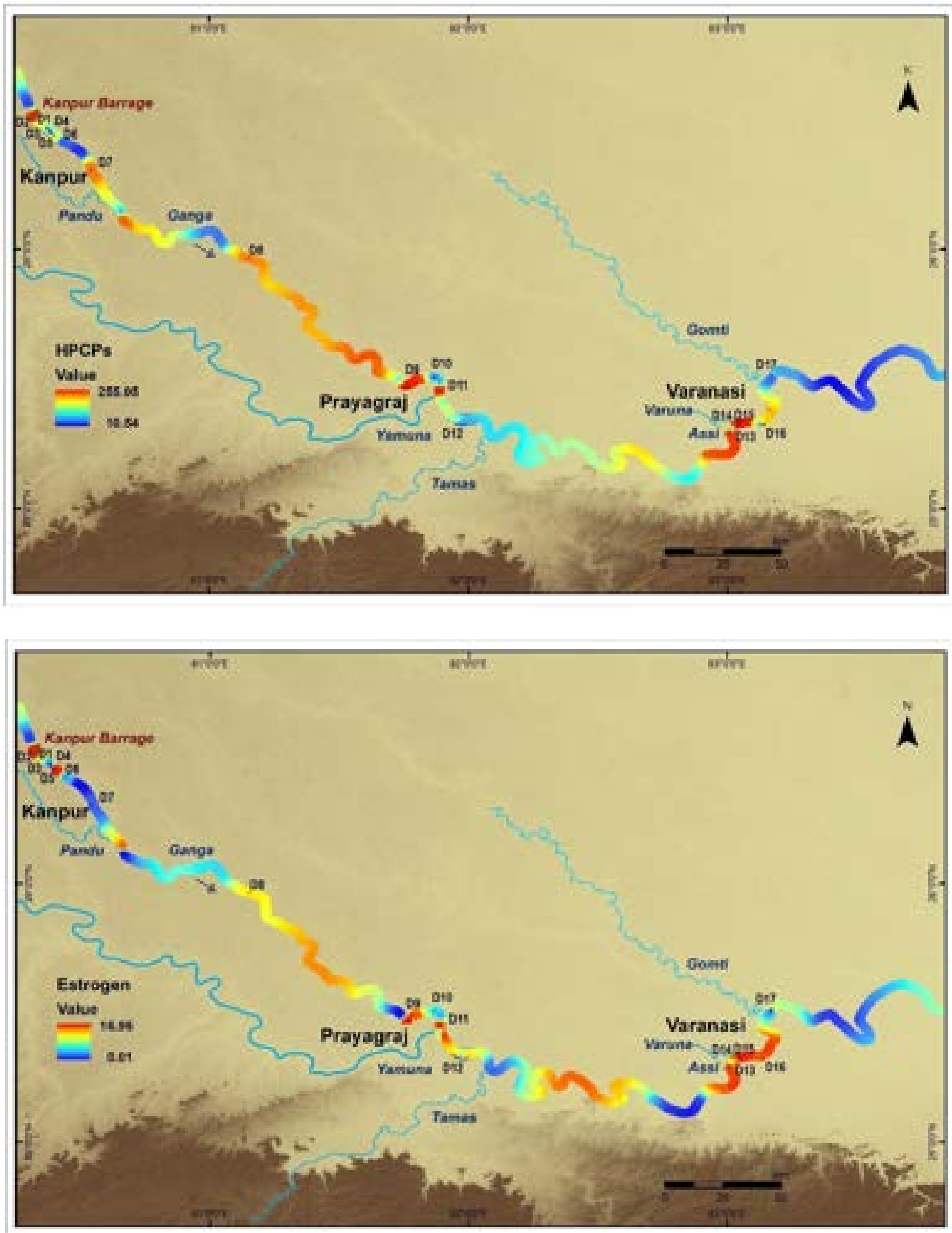


Figure 4-4: Spatial distribution of average concentrations (ng/L) of (a) ΣHPCPs and (b) ΣEstrogens along the MGR in 2021

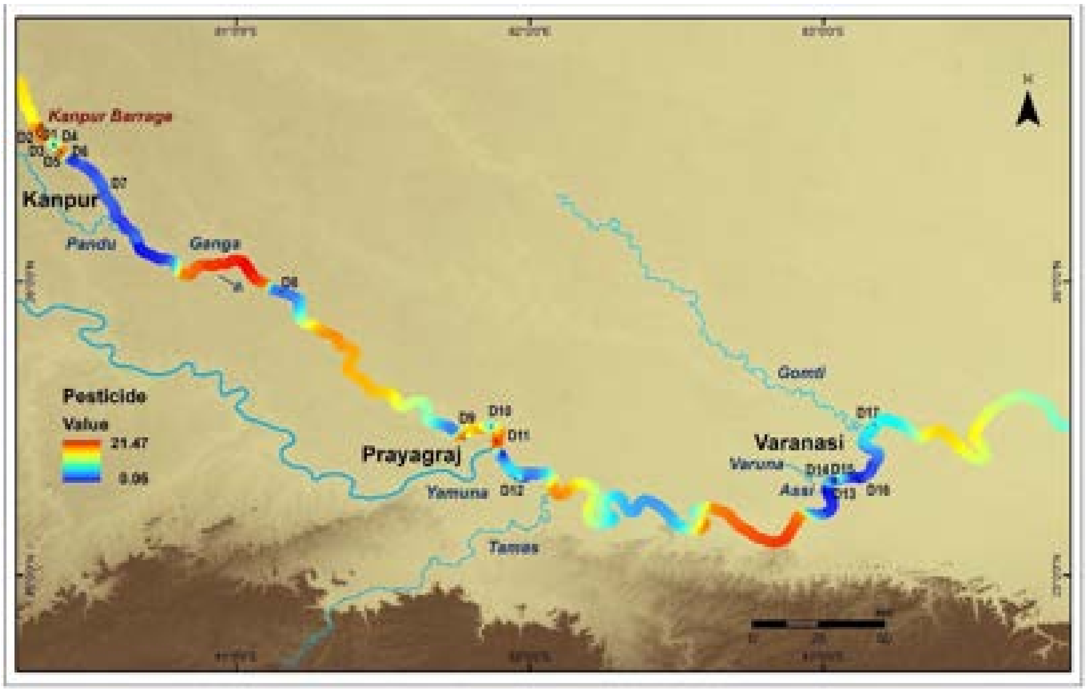


Figure 4-5: Spatial distribution of average concentrations (ng/L) of Pesticides (ng/L) along the MGR in 2021

The box plot (Figure 4-9) illustrates the spatial variability of various EDCs between rivers and drains in the MGR. The concentrations are presented on a log scale (ng/L) to account for the wide range of observed values. Significant differences between river and drain concentrations are highlighted with p-values (Figure 4-9 & Table 4-5). The drains contribution towards the high EDC load ($p<0.05$) is presented in Table 4-6. Except for pesticides, generally, higher EDCs concentrations were observed in drains outfalls than rivers. However significant spatial variation is observed for BPA, TCs, E1, and E2 (Figure 4-9). WWTPs and STPs are identified as significant contributors to these EDCs pollution

(Belhaj et al., 2015; Pessoa et al., 2014), as the drains studied typically carry treated, partially treated, or sometimes untreated domestic wastewater, therefore resulting in the high concentrations observed in drain discharges.

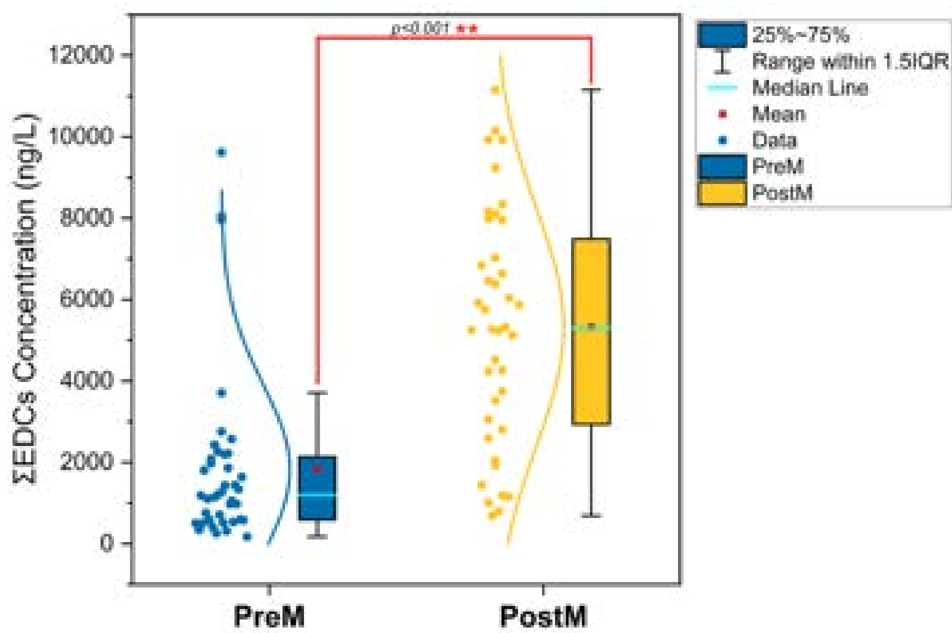


Figure 4-6: Temporal characteristics of Σe-EDCs (ng/L) in PreM (March-April 2021) and PostM (October 2021) seasons

The lack of spatial variability in phthalate concentrations between drains and rivers suggests that both point and non-point sources contribute equally to phthalate levels. This is due to the widespread use of phthalates in consumer products and industrial processes, leading to consistent input from direct discharges and diffuse runoff. Additionally, their persistence and ability to disperse through air and water result in uniform distribution across river systems.

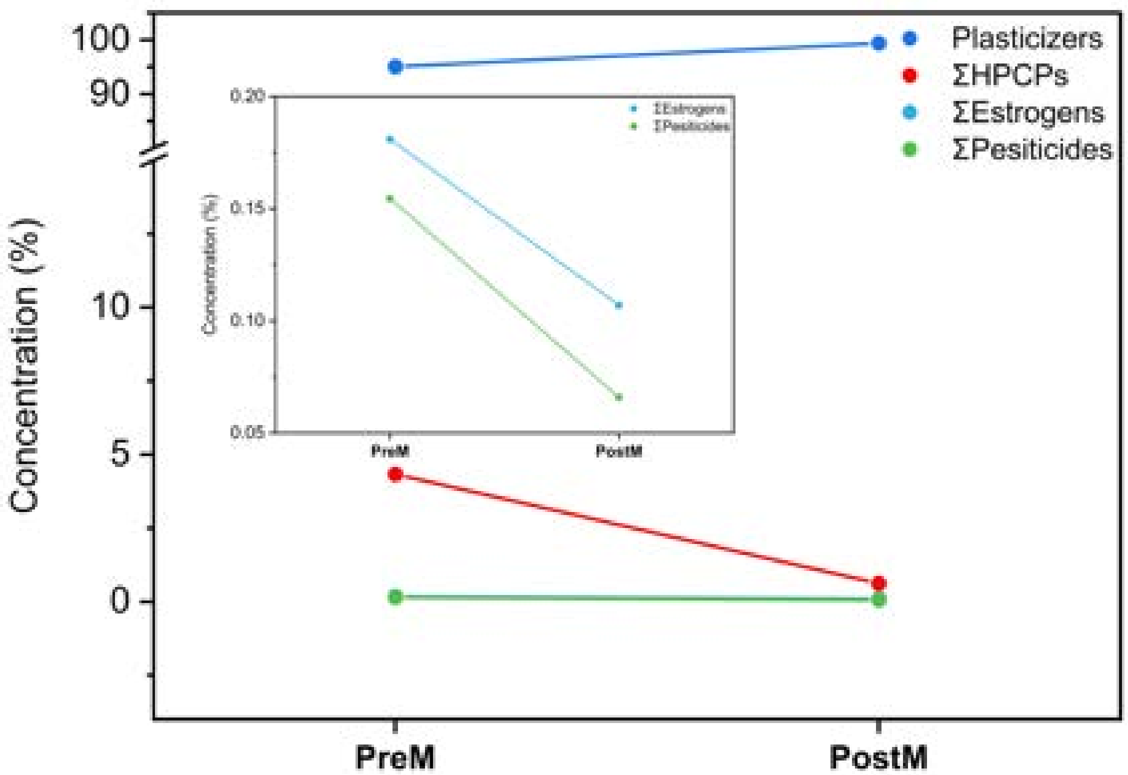


Figure 4-7: Proportion (%) of group-wise e-EDCs distributions along PreM (March-April 2021) and PostM (October 2021) seasons

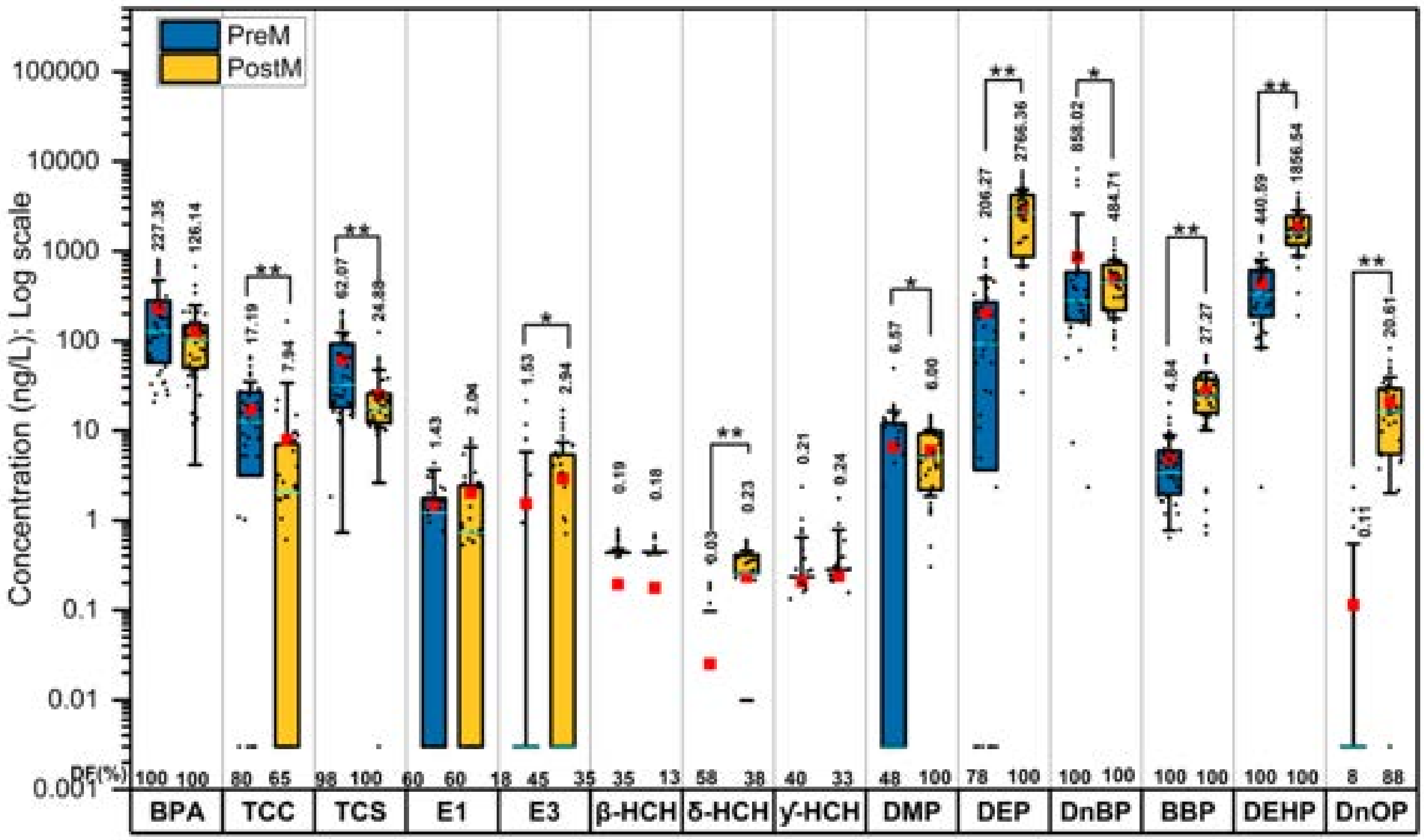


Figure 4-8: Temporal characteristics of target e-EDCs in PreM (March-April 2021) and PostM (October 2021) seasons

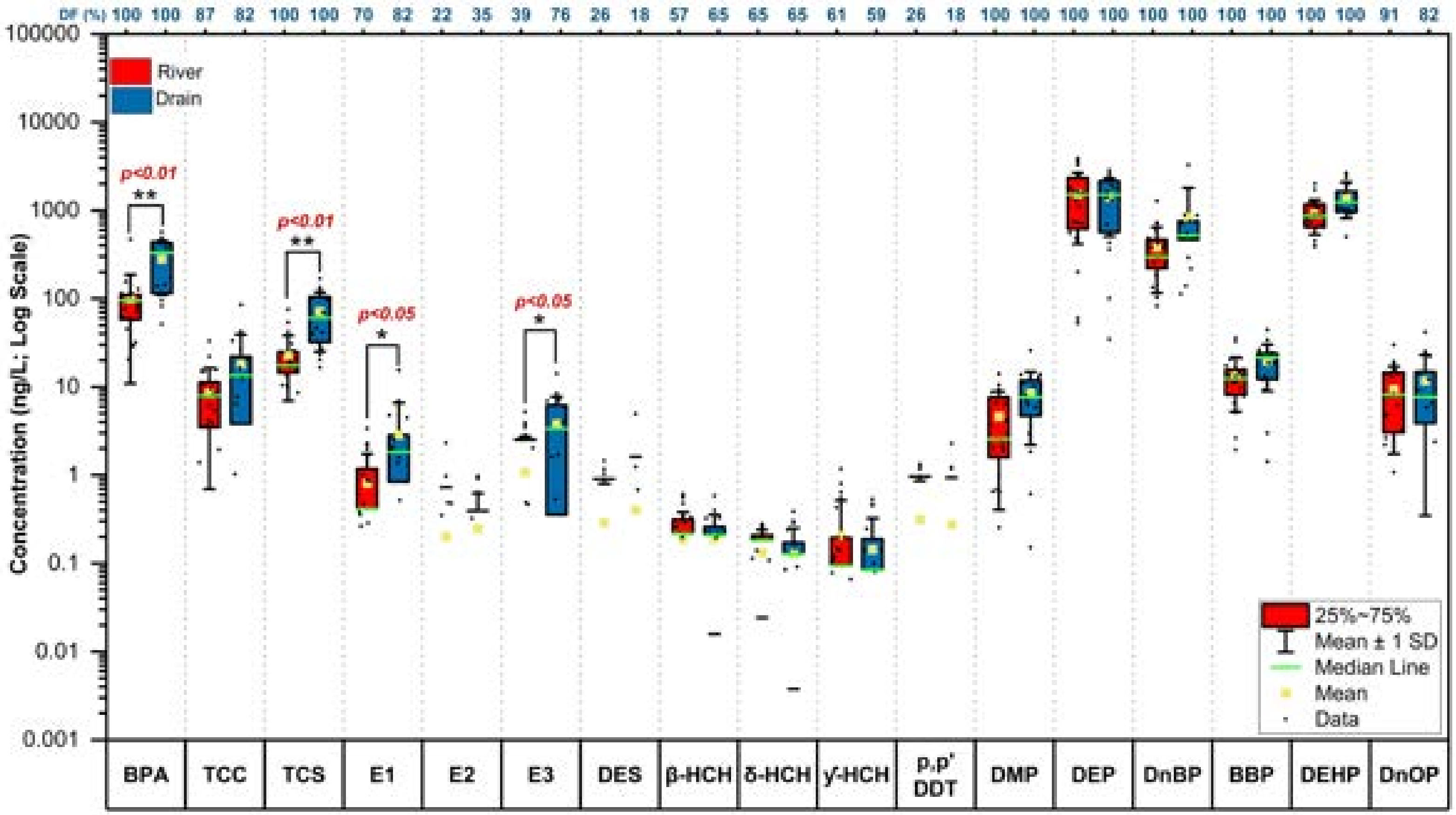


Figure 4-9: Spatial characteristics (Log scale) of target e-EDCs in drain outlets and receiving river

Statistical significance is indicated by p-values of $p < 0.01$(**) and $p < 0.05$ (*)

Table 4-6: Spatial variation of EDC concentrations (with p<0.05) in drains across MGR

District	Code	Site Name	BPA	TCS	E1	E3
Kanpur	**D1**	Permiya Drain	446.37	112.68	1.585	14.12
	D2	Ranighat Drain	115.31	20.16	1.375	0.535
	D3	Gola Ghat Drain	428.945	27.115	0.52	1.695
	D4	Satti Chaura Drain	113.06	32.1575	0	0
	D5	Dabka Nala Drain	144.355	41.54	6.95	7.04
	D6	Shetla Bazaar Drain	95.045	46.625	1.82	6.17
Unnao	**D7**	City Jail Drain	379.68	69.746	0	0.355
Raebareilly	D8	Dalmau	139.295	60.01	2.55	1.61
Prayagraj	**D9**	Kodar Stp	330.265	169.3	15.465	0
	D10	Rasoolbad Drain	82.02	23.075	2.1	0
	D11	Solari Drain	51.51	104.365	2.835	3.955
	D12	Mannaiya Drain	492.42	40.085	1.965	0
Varanasi	**D13**	Assi Drain	411.08	97.99	0.84	5.975
	D14	Khirkia Drain	365.95	124.885	0	3.38
	D15	Varuna Drain	571.345	134.58	4.465	8.445
	D16	Mughalsarai Drain	176.145	99.45	4.77	6.955
Ghazipur	**D17**	Saidpur	441.25	16.58	1.3	4.305

The highlighted cells represent the top five most e-EDC-contaminated drain sites for the EDCs showing significant spatial variation.

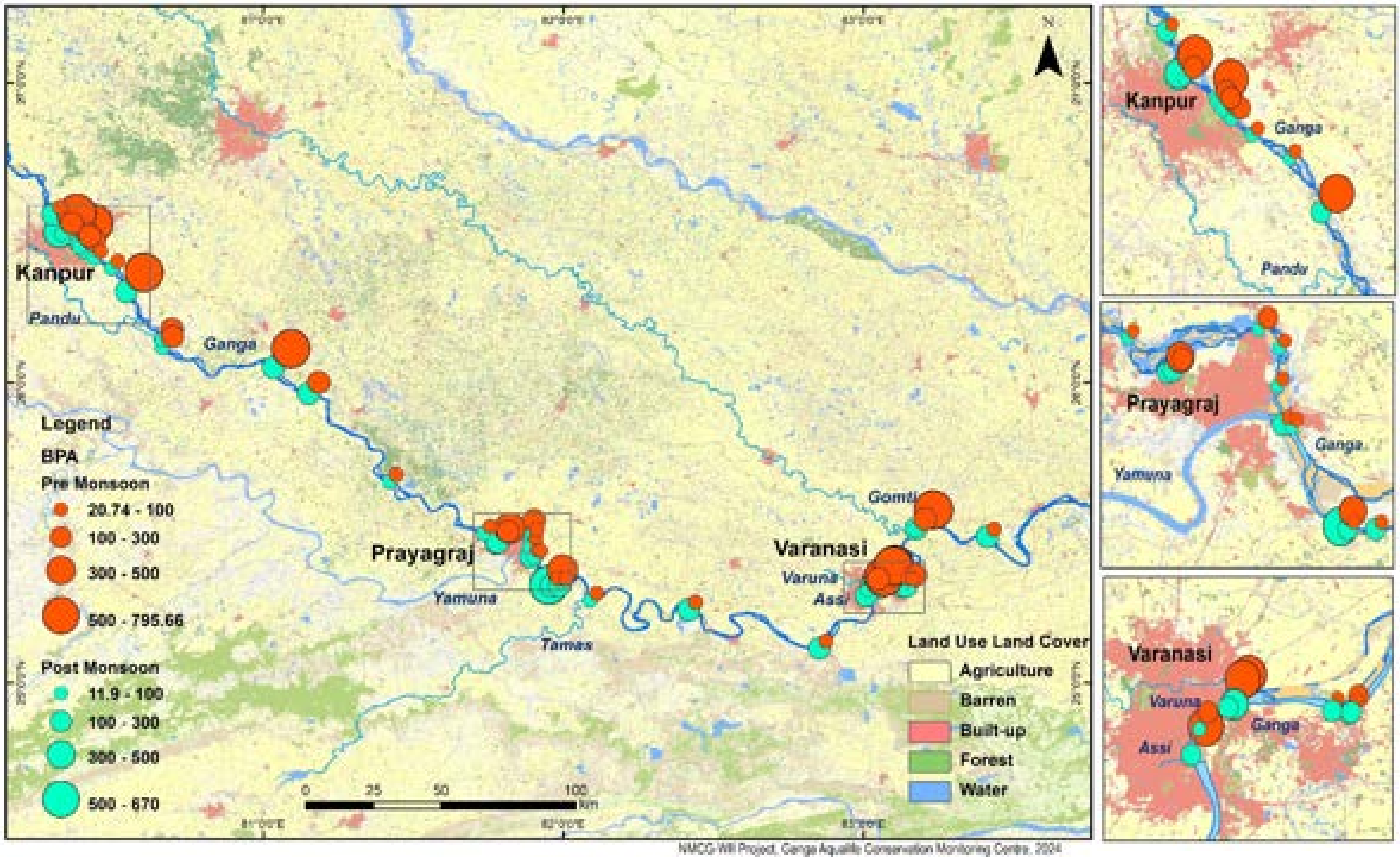


Figure 4-10: Spatiotemporal characteristics of BPA along the MGR

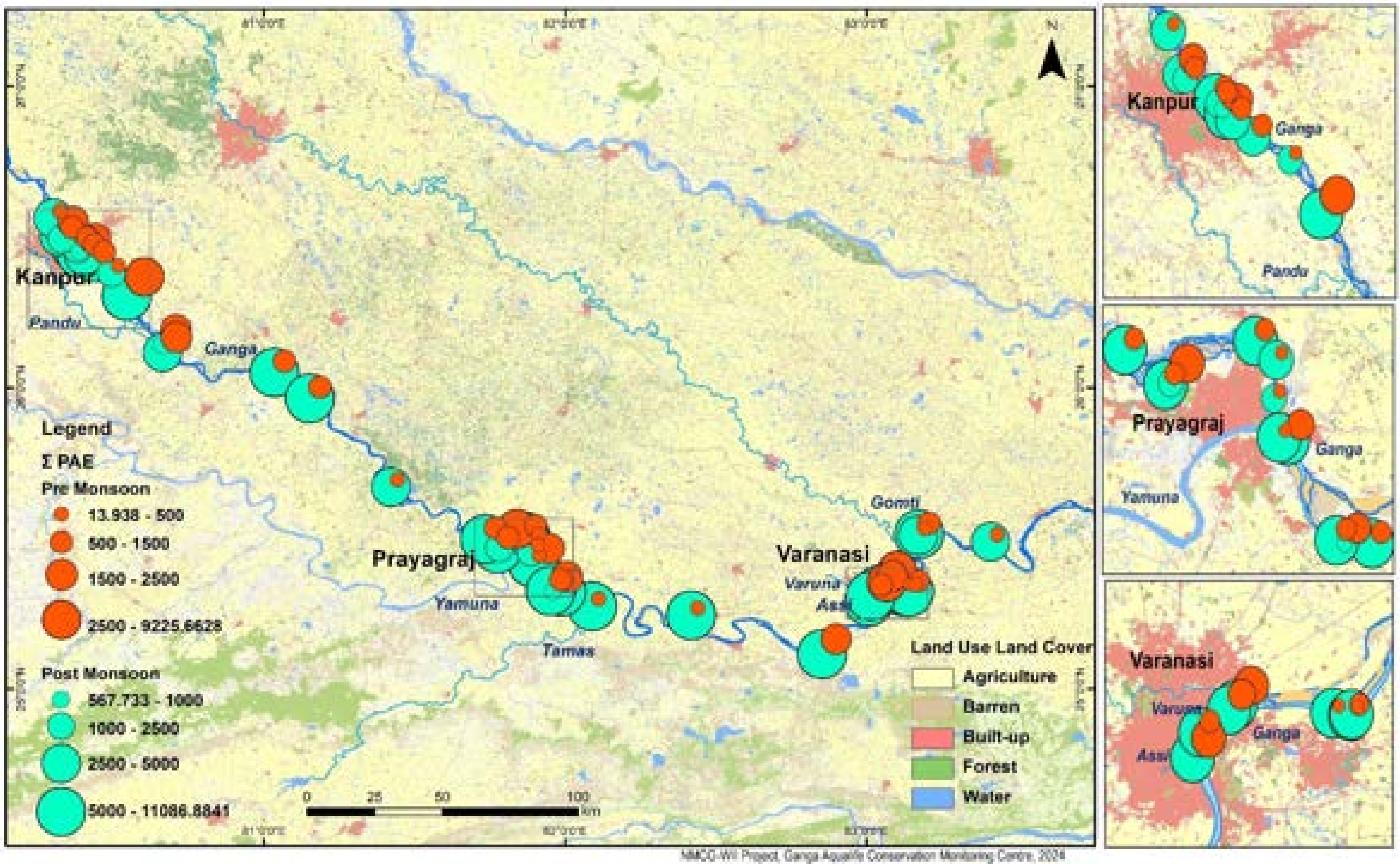


Figure 4-11: Spatiotemporal characteristics of ΣPAEs along the MGR

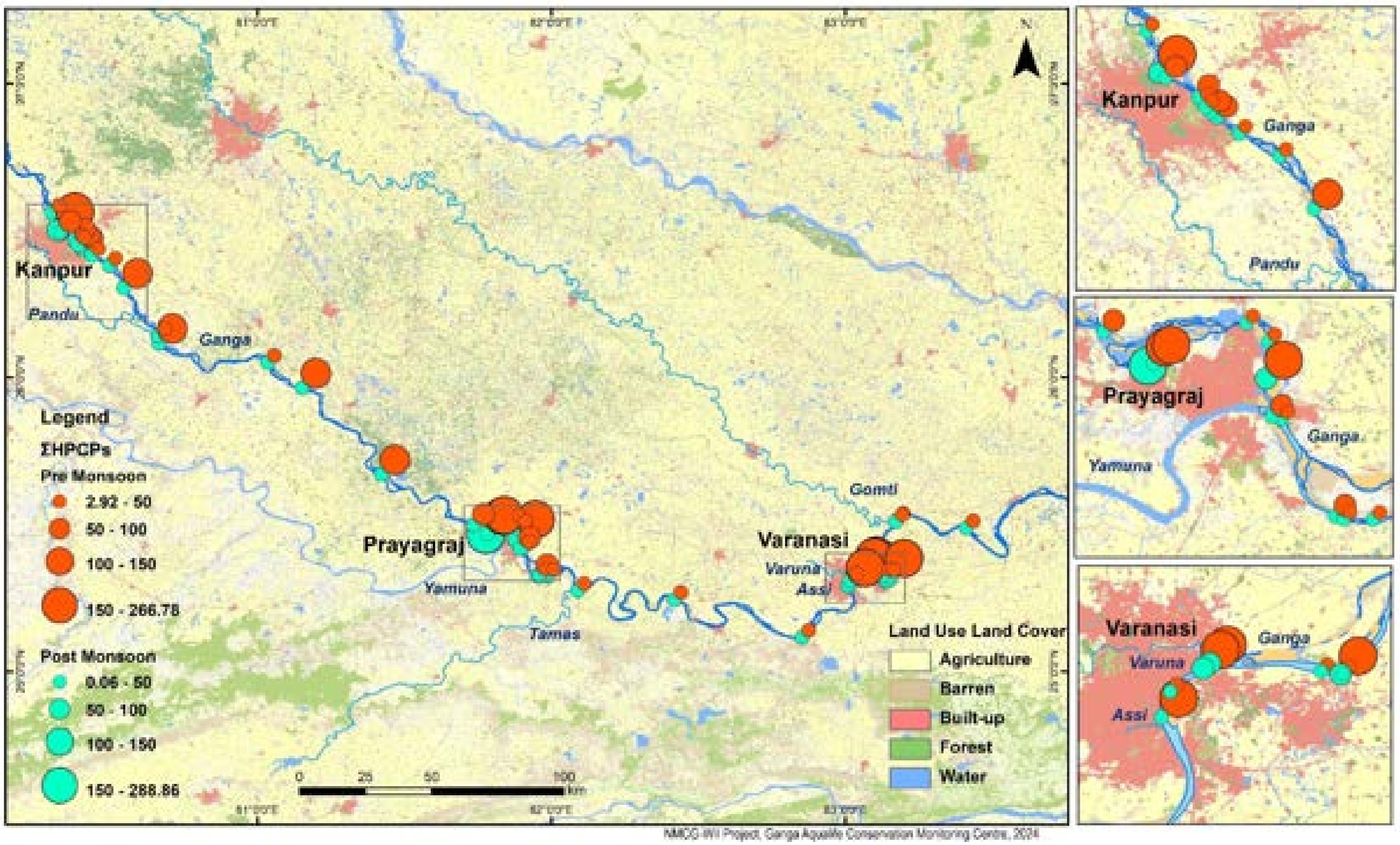


Figure 4-12: Spatiotemporal characteristics of ΣHPCPs along the MGR

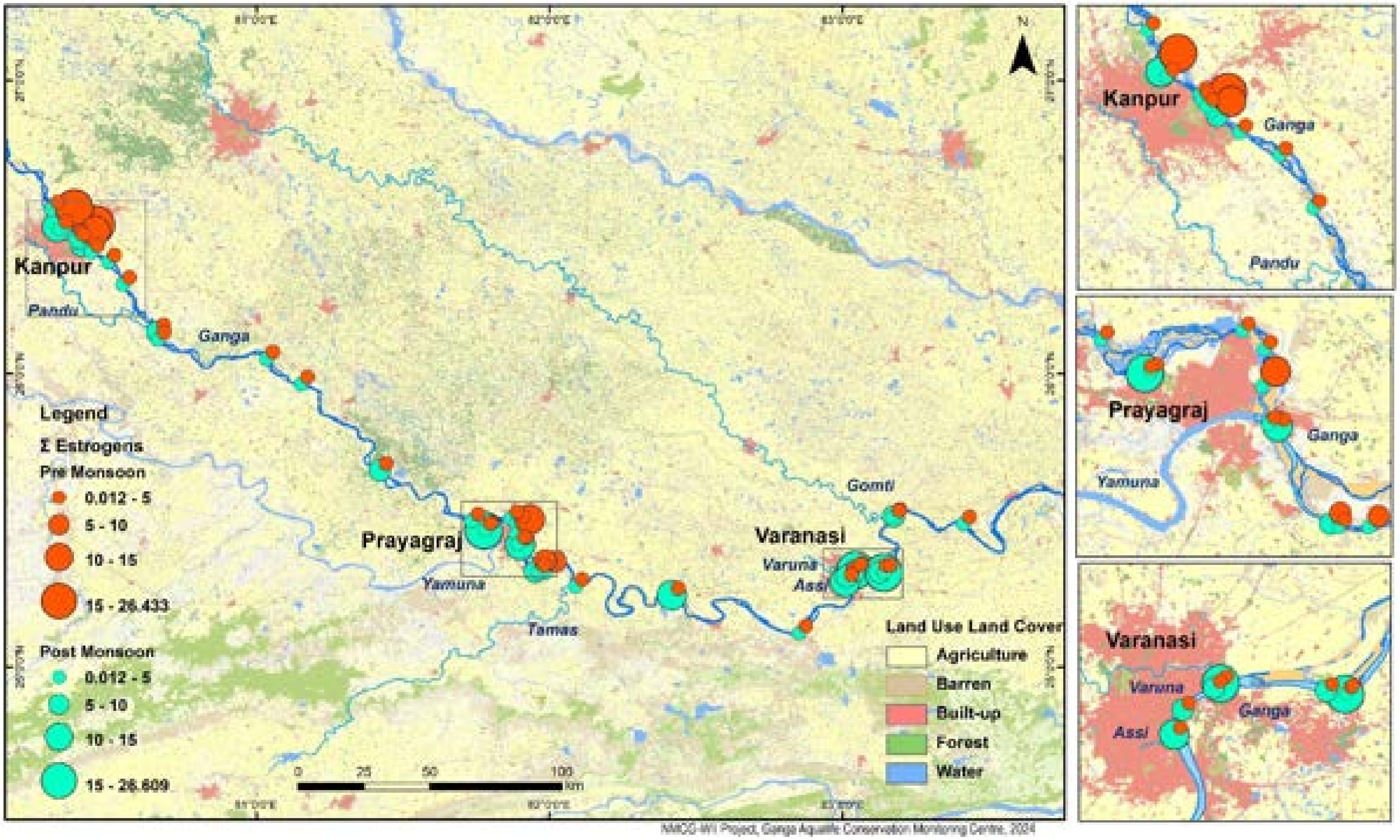


Figure 4-13: Spatiotemporal characteristics of ΣEstrogens along the MGR

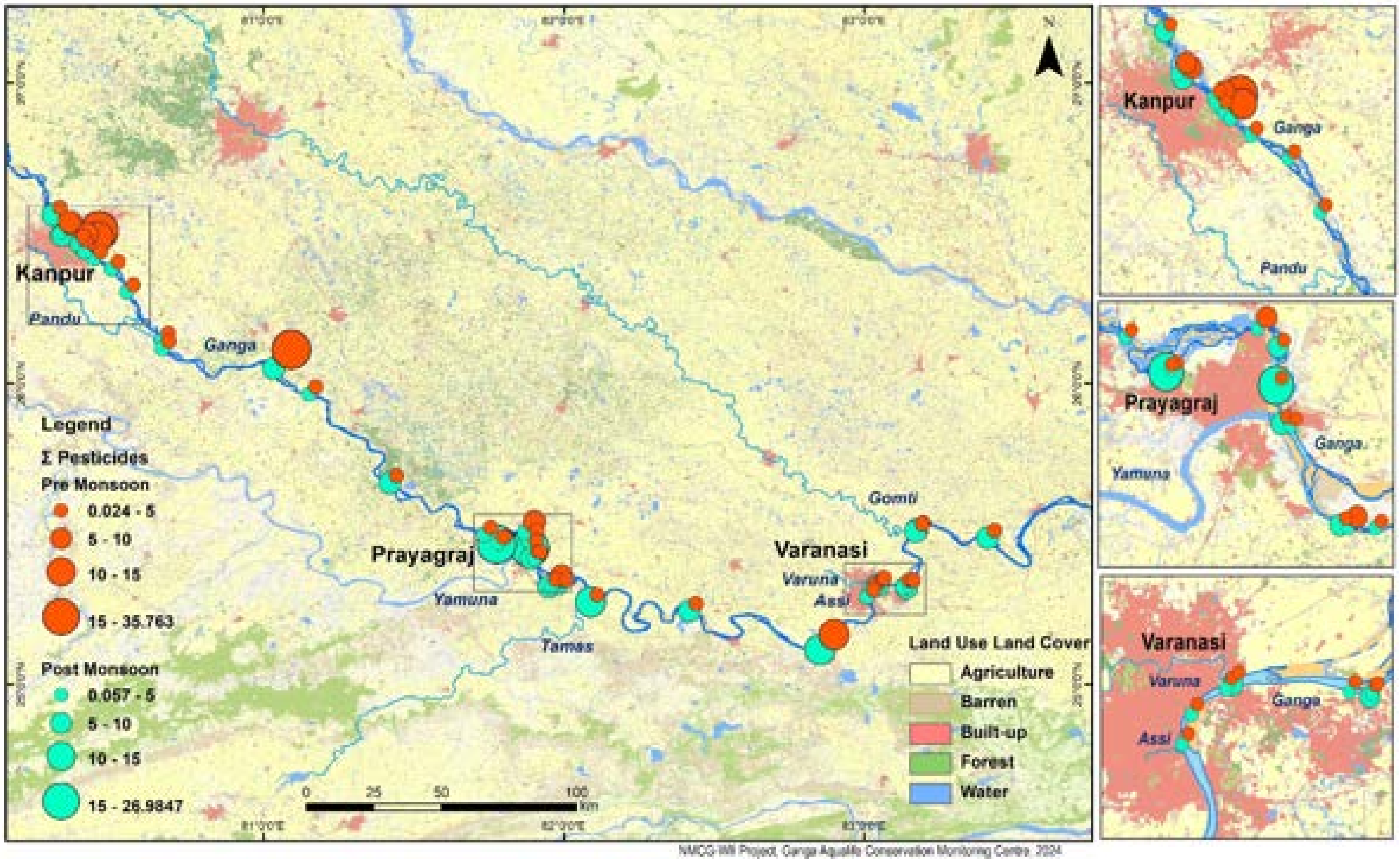


Figure 4-14: Spatiotemporal characteristics of ΣPesticides along the MGR

4.3.2 Comparison with Global and Indian rivers

Plastic Additives

The temporal trend of BPA concentrations in the Ganga River suggests a decline in pollution levels over recent years (Figure 15 a). Previous studies reported significantly higher BPA levels, such as 710 ng/L in 2019 and 803 ng/L in 2016, while the current study from 2021 shows a reduced concentration of 176.745 ng/L. This indicates possible improvements in controlling BPA pollution, likely due to better management practices or changes in industrial activities. However, it's important to consider that the sampling for the present study was conducted during the COVID-19 pandemic, which may have impacted the results. The pandemic led to widespread industrial slowdowns and reduced human activities, potentially resulting in temporarily lower pollutant levels, including BPA. This context suggests that while the reduction in BPA levels is encouraging, ongoing monitoring is essential to determine whether these reductions are sustained as normal activities resume.

Comparatively, the Ganga River’s BPA levels are moderate among Indian rivers—lower than the highly polluted Cooum (692.33 ng/L) but higher than the Sarda (19.9 ng/L). On a global scale, the BPA concentration in the Ganga is lower than those in severely polluted rivers like the New Calabar River in Nigeria (46,264 ng/L) and the Mahakam River in Indonesia (922 ng/L). However, it remains comparable to or slightly higher than levels found in several rivers in China and Japan, positioning the Ganga's BPA contamination as moderate globally (Figure 4-15 b).

The PAEs concentration (Figure 4-16 a) in the Ganga River (3338.94 ng/L) reported in the present study is notably high, with a significant increase compared to earlier findings in 2019 (1600 ng/L) and 2016 (176.10 ng/L). This sharp rise suggests a worsening of phthalate pollution in the Ganga, potentially due to increased industrial discharges and plastic waste, especially considering the challenges posed by the pandemic on waste management practices.

The Ganga River has the highest PAE concentration among Indian rivers, while other rivers such as the Adyar (78.00 ng/L), Vellar (8.26 ng/L), and Thamiraparani (0.56 ng/L) exhibit significantly lower PAE levels, indicating differing levels of industrial influence and waste management effectiveness throughout the country. The high PAE levels in the Ganga River are likely due to significant industrial discharges, urban runoff, inadequate waste management, high population density, and agricultural runoff compared to other Indian rivers.

The Ganga River's PAE concentration is moderate when compared to global rivers (Figure 4-16 b). It is significantly lower than the extremely high levels reported in the Owena River, Nigeria (11,930 ng/L) and the Jiulong River, China (5540 ng/L), but higher than concentrations in many other global rivers, such as the Selangor River Basin in Malaysia (332 ng/L) and the Rhône River in France (315 ng/L).

Household and personal care products (HPCPs)

The Ganga River exhibits moderate HPCP contamination compared to other Indian (Figure 4-17 a). While not as severely polluted as the Mithi or Gomti rivers, the Ganga

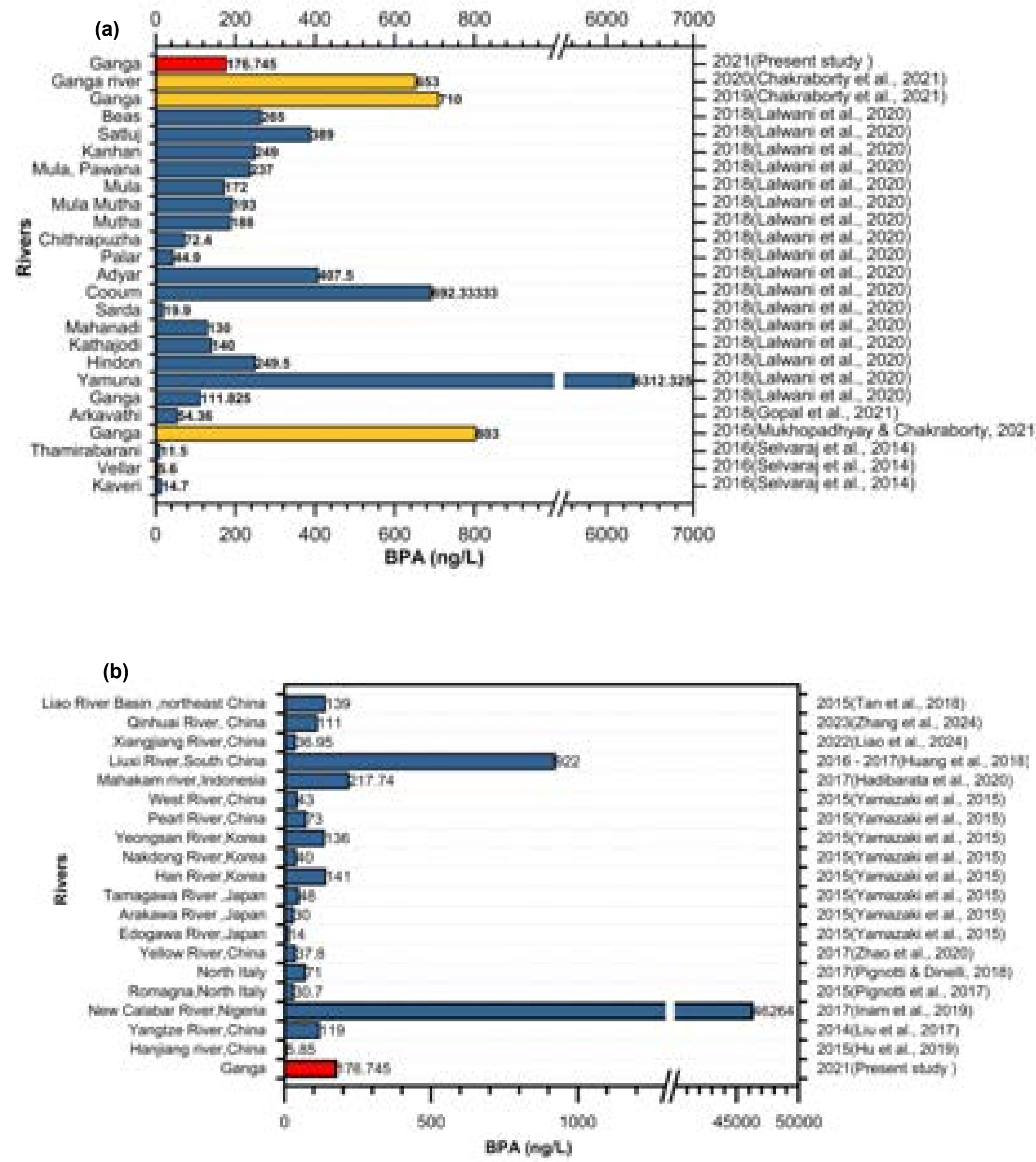


Figure 4-15: Comparative assessment of BPA levels in (a) Indian Rivers and (b) Global Rivers

The red bars represent BPA levels from the current study in the MGR, while the yellow-highlighted bars in Figure 4-15 (a) indicate BPA levels in the Ganga River across different time periods.

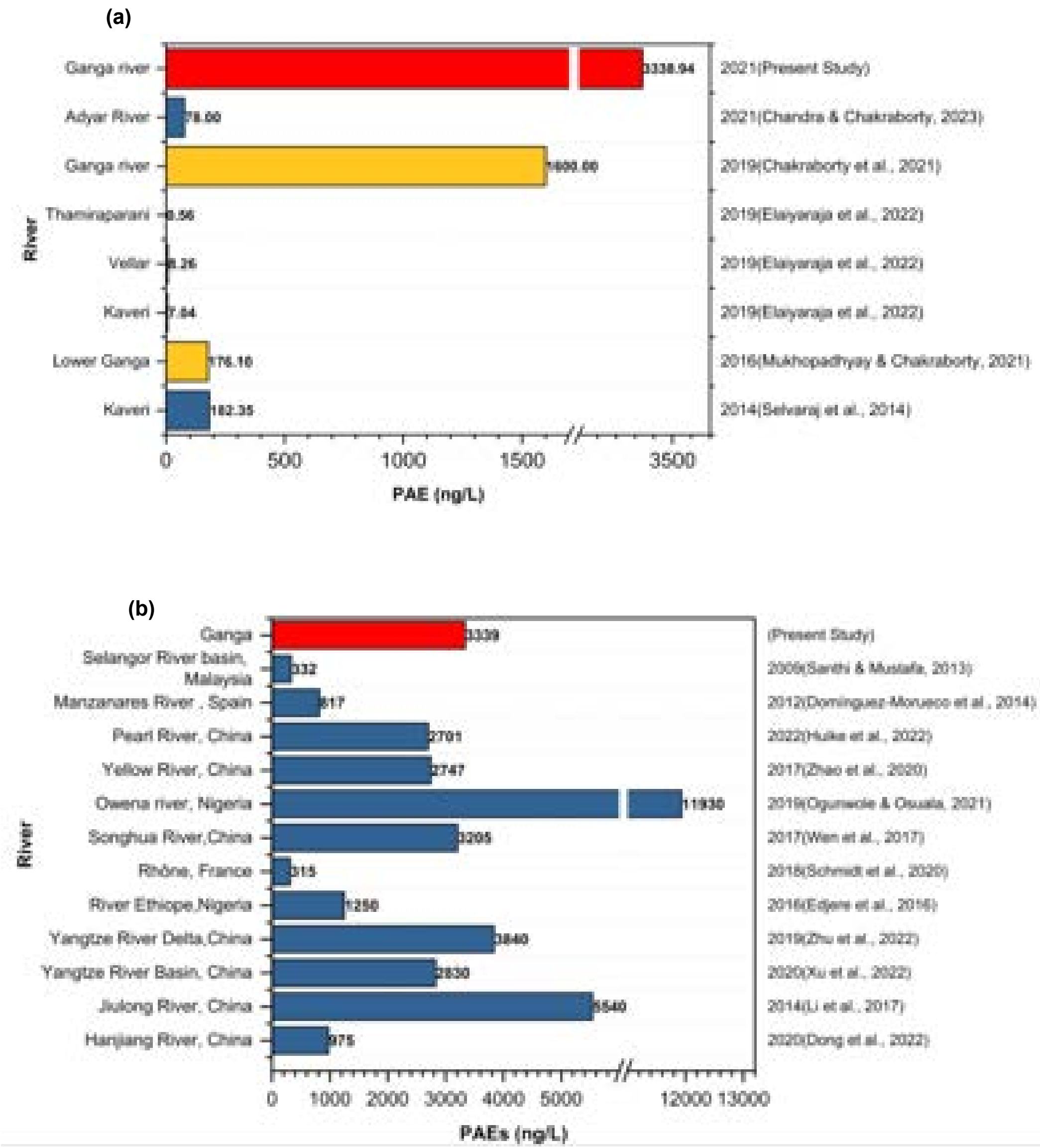


Figure 4-16: Comparative assessment of PAEs levels (a) Indian Rivers and (b) Global Rivers

The red bars represent PAEs levels from the current study in the MGR, while the yellow-highlighted bars in Figure 4-16 (a) indicate PAEs levels in the Ganga River across different time periods.

still faces significant challenges in managing contamination from household and personal care products. The temporal trend of HPCP concentrations in the Ganga River shows that there has been an increase over recent years. Previous studies reported lower HPCP levels, such as 30.6 ng/L in 2020. The current study from 2021, however, reports a higher concentration of 56.04 ng/L. This rise could be attributed to the increased use of personal care and hygiene products during the COVID-19 pandemic, as well as the associated increase in effluents containing these contaminants entering the river. The findings suggest that the pandemic-driven surge in the use of antimicrobial products like hand washes and sanitizers may have temporarily raised HPCP concentrations in the river. This underscores the need for ongoing monitoring to determine if these levels will decrease as the use of these products normalizes post-pandemic. It also highlights the importance of understanding how changes in societal behavior, such as those caused by global events like the pandemic, can impact environmental pollution. On a global scale, the Ganga River's HPCP concentration is moderate (Figure 4-17 b).

It is higher than concentrations found in many rivers across China, Canada, and Spain, such as the Yangtze River (1.90 ng/L), Okanagan River (1.49 ng/L), and Liao River (28.40 ng/L). However, the Ganga's levels are still much lower than those in severely contaminated rivers like the Shijing River in China (400.00 ng/L), the Manzanares River in Spain (125.00 ng/L), and the Lambro River in Italy (90.35 ng/L).

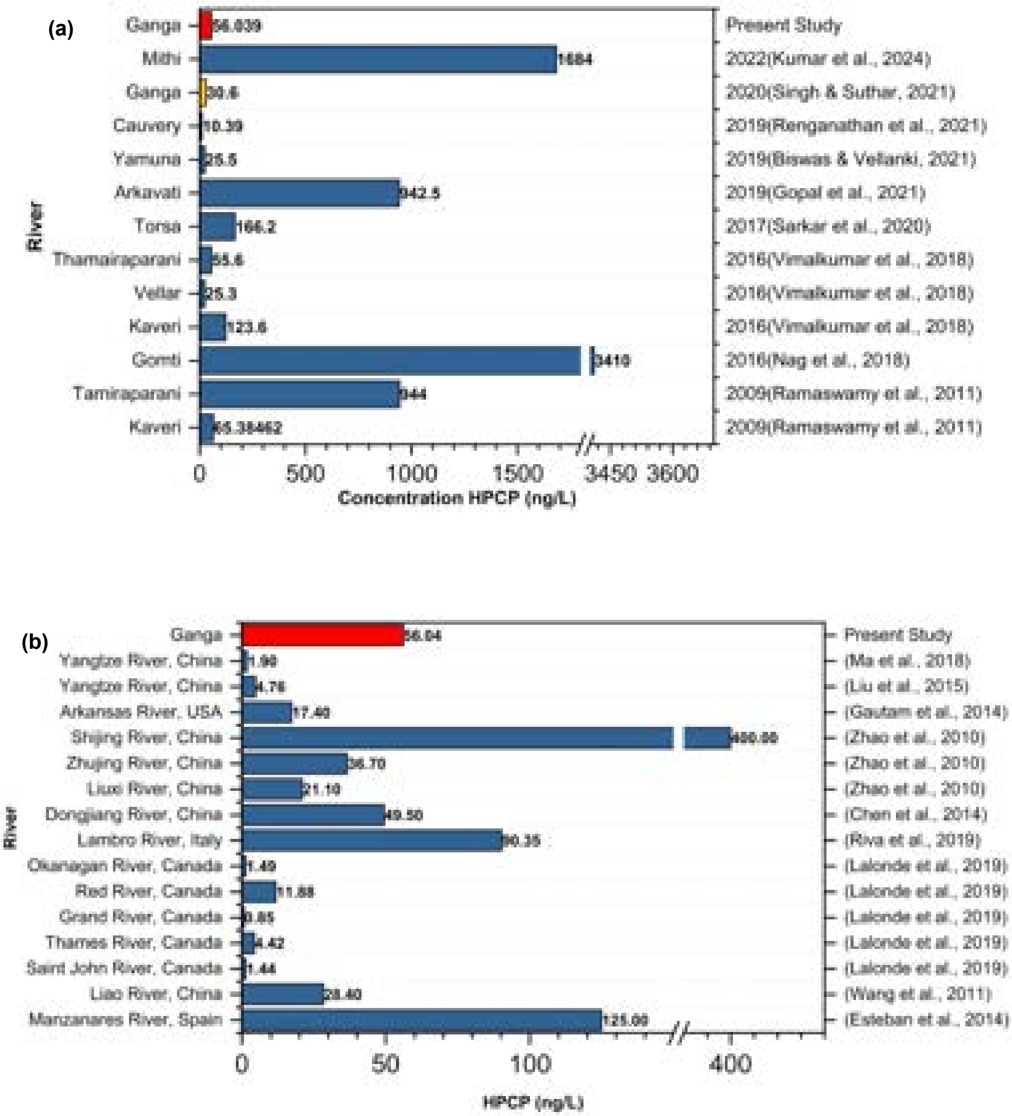
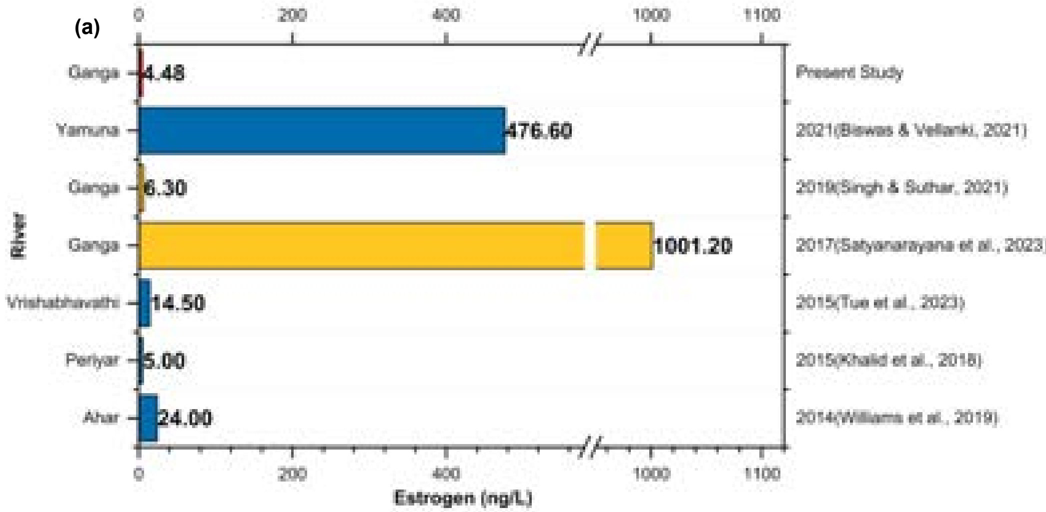


Figure 4-17: Comparative assessment of HPCPs levels in (a) Indian Rivers and (b) Global Rivers

The red bars represent HPCPs levels from the current study in the MGR, while the yellow-highlighted bars in Figure 4-17 (a) indicate HPCPs levels in the Ganga River across different time periods.



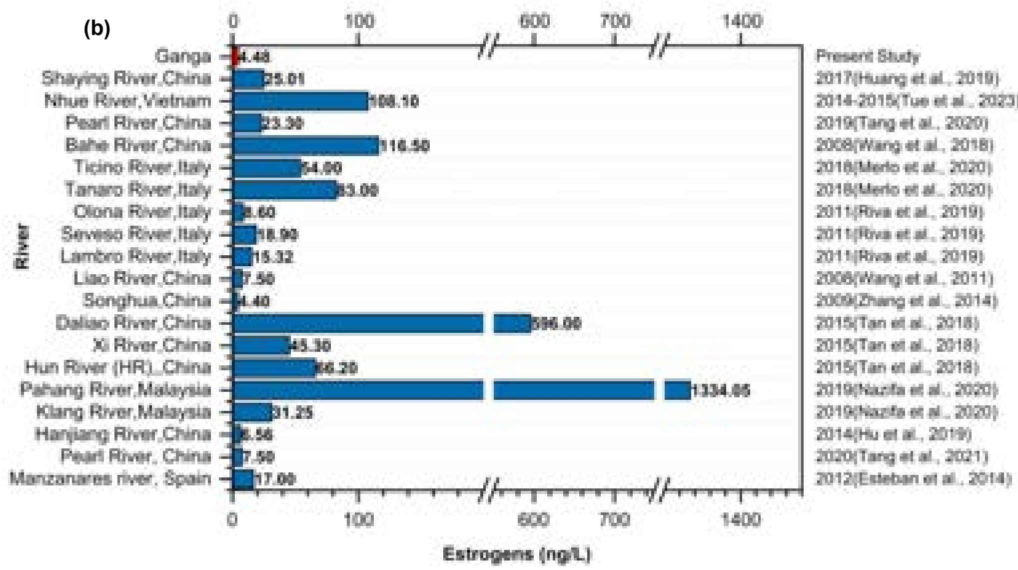


Figure 4-18: Comparative assessment of Estrogens in (a) Indian Rivers and (b) Global Rivers

The red bars represent Estrogens levels from the current study in the MGR, while the yellow-highlighted bars in Figure 4-18 (a) indicate Estrogens levels in the Ganga River across different time periods.

Estrogens

The Ganga River currently shows relatively low estrogen concentrations compared to other Indian rivers, such as the Yamuna (476.60 ng/L) and Ahar (24.00 ng/L), which have much higher levels, indicating ongoing pollution challenges in those regions (Figure 4-18 a). The temporal trend analysis for the Ganga River reveals a significant decrease in estrogen levels over recent years, reflecting progress in pollution control efforts, particularly in wastewater management. Earlier studies reported much higher estrogen levels, such as 1001.20 ng/L in 2017 and 6.30 ng/L in 2019, while the present study from 2021 shows a substantially lower concentration of 4.48 ng/L. On a global scale (Figure 4-18 b), the estrogen concentration in the Ganga River is relatively low, significantly lower than levels found in rivers like the Hun River in China (1334.05 ng/L), the Xi River in China (596.00 ng/L), and the Nhue River in Vietnam (108.10 ng/L). These findings suggest that the Ganga River, at least in this specific stretch, is less contaminated with estrogens compared to many heavily polluted rivers worldwide.

Pesticides

The Ganga River currently shows low pesticide concentrations compared to other Indian rivers, like the Thamirabarani, Hindon, Sabarmati, Tapi and Yamuna, reflecting a positive trend in reducing contamination over recent years (Figure 4-19). Furthermore, the significant reduction in pesticide levels in the Ganga River, from 1267 ng/L in our previous study to 4.85 ng/L in the present study, suggests that regulatory measures and enforcement efforts for banned and regulated pesticides are proving effective. However, the continued presence of even low levels of contamination indicates ongoing challenges, such as illegal use, improper disposal, and legacy pollution.

4.3.1 Key Drivers: Influence of environmental factors on EDCs pollution

The analysis of water quality parameters conducted during the PreM and PostM seasons revealed significant ($p<0.05$) seasonal variations in three key indicators (Figure 4-20): Total Dissolved Solids (TDS), Nitrate (NO3), and Dissolved Oxygen (DO).

A significant difference ($p < 0.05$) was observed in TDS levels between the PreM and PostM seasons, with higher values recorded during the PreM season compared to the PostM season. This variation could be attributed to the reduced dilution and increased evaporation during the dry pre-monsoon period, leading to higher concentrations of dissolved solids in the water. TDS levels exceeded the aquatic life criteria (150-500 mg/L) in 18% and 13% of the sampled sites in PreM and PostM respectively, indicating potential risks to aquatic organisms in those areas. Nitrate levels also exhibited significant seasonal variation ($p < 0.01$), with higher concentrations observed during the PostM season (mean = 4.39 mg/L) compared to the PreM season (mean = 3.20 mg/L). The increase in nitrate levels during the PostM season is likely due to agricultural runoff and nutrient loading following monsoon rains. Despite this variation, nitrate levels exceeded the threshold of 10 mg/L at <5 % of the sites, indicating a low to moderate risk of nutrient pollution.

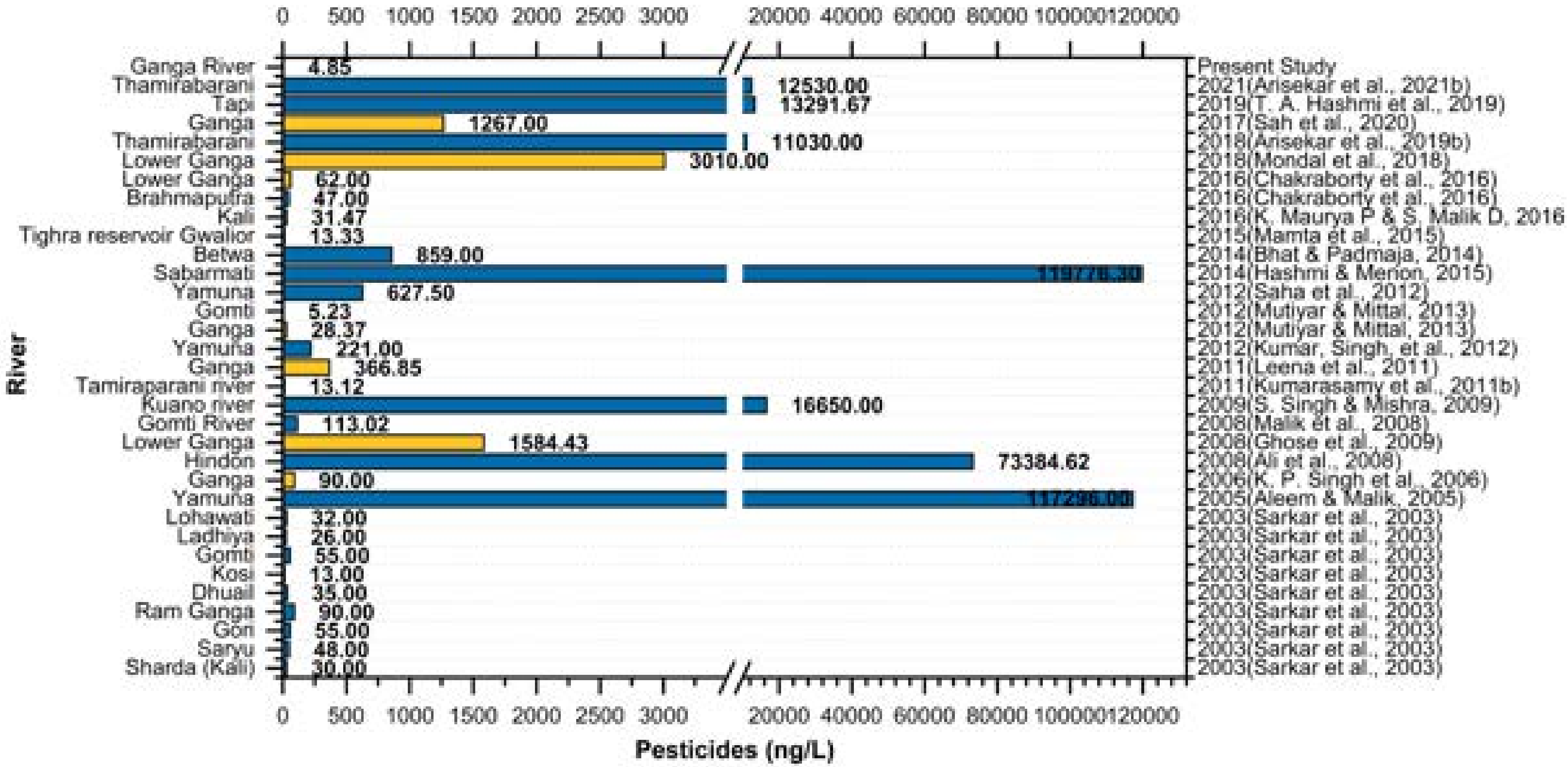


Figure 4-19: Comparative assessment of Pesticides in Indian Rivers

The target pesticides studied, HCH and DDTs, have been banned in many countries globally long before India implemented similar bans. As a result, the available literature on these pesticides is limited. Consequently, the comparison for this class of e-EDCs was made exclusively among Indian rivers.

Similarly, DO levels showed significant seasonal variation ($p < 0.05$), with higher levels observed in the PostM season (mean = 9.70 mg/L) compared to the PreM season (mean = 8.39 mg/L). The increased DO in the PostM season can be attributed to the enhanced oxygenation due to increased water flow and reduced temperature during this period.
DO levels were generally within acceptable limits (>5 mg/L) at all sites in PostM season, with only 13% of the sites showing exceedance in PreM, suggesting that the oxygen levels in MGR are mostly sufficient to support aquatic life.
The conductivity values varied between the two seasons, with a slight decrease ($p>0.05$) observed in the PostM season (mean = 498.80 µS/cm) compared to the PreM season (mean = 723.76 µS/cm). The high conductivity during the PreM season indicates higher ionic content in the water, likely due to concentrated pollutants during the dry season. Notably, 33% in PreM and 35% in PostM of the sites exceeded the recommended conductivity limit of 500 µS/cm, pointing to potential pollution load that could affect aquatic life.

The pH values remained relatively stable ($p>0.05$) across both seasons, with an average of 8.46 during the PreM season and 8.29 during the PostM season. The slight decrease in pH during the PostM season may be due to the influx of fresh water from monsoon rains, which can slightly acidify the water. During the PreM season, 53% of the sites exceeded the recommended pH range of 6.5-8.5, while 30% exceedance was observed in the PostM season. The exceedance in pH, which was predominantly alkaline, could be attributed to discharges from alkaline industrial sectors such as textiles, paper mills, or chemical industries in the MGR, particularly if the effluents are not adequately treated.

Spearman correlation analysis (Figure 4-21) indicates that the relationships between some EDCs and water quality parameters such as conductivity, TDS, pH, and nitrate are generally consistent across pre- and post-monsoon seasons. The significant correlations observed suggest that these water quality parameters can serve as indicators for the presence and distribution of EDCs in aquatic systems, with conductivity and TDS being particularly strong indicators of plasticizer contamination. For single environmental factor, both negative and positive correlations were observed with different EDCs. This may be because EDCs have a wide range of physicochemical properties (Chapter 3) and distinct behaviors impacted by multiple factors.

pH and DO

Lower pH and DO levels, often resulting from industrial discharges, wastewater effluents, and agricultural runoff, are associated with higher concentrations of certain EDCs In the pre-monsoon season, significant positive correlations were observed between pH and BPA ($p<0.05$) and Triclosan ($p<0.05$), while significant negative correlations were found between DO and BBP ($p<0.01$), BPA ($p<0.05$), and DEHP ($p<0.05$). These results suggest that in acidic conditions (low pH), EDCs become more stable and less likely to degrade, leading to their accumulation in water.

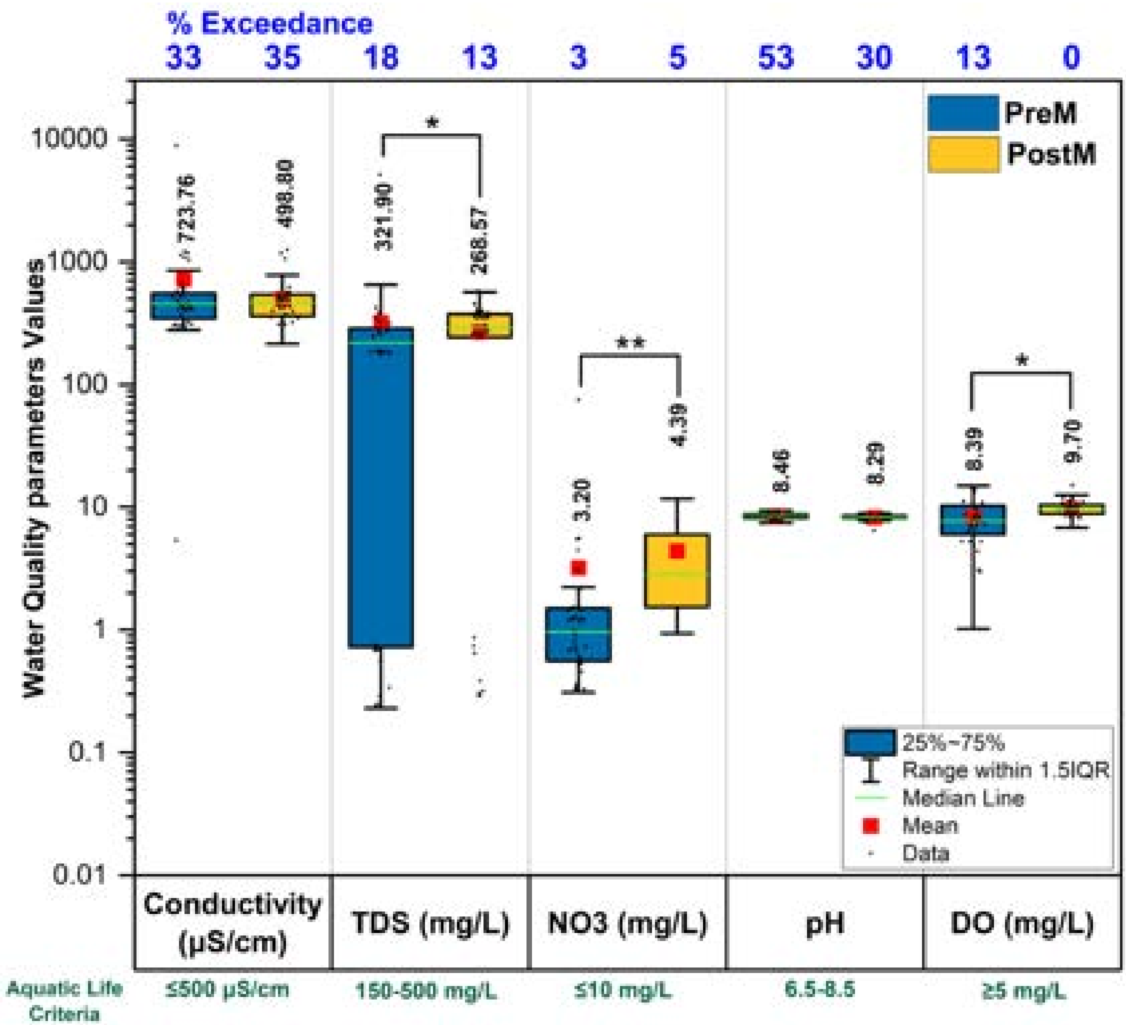


Figure 4-20: Range (Log scale) of key environmental factors in PreM and PostM season.

Values in blue denotes % exceedance of USEPA aquatic life criteria at 40 sites

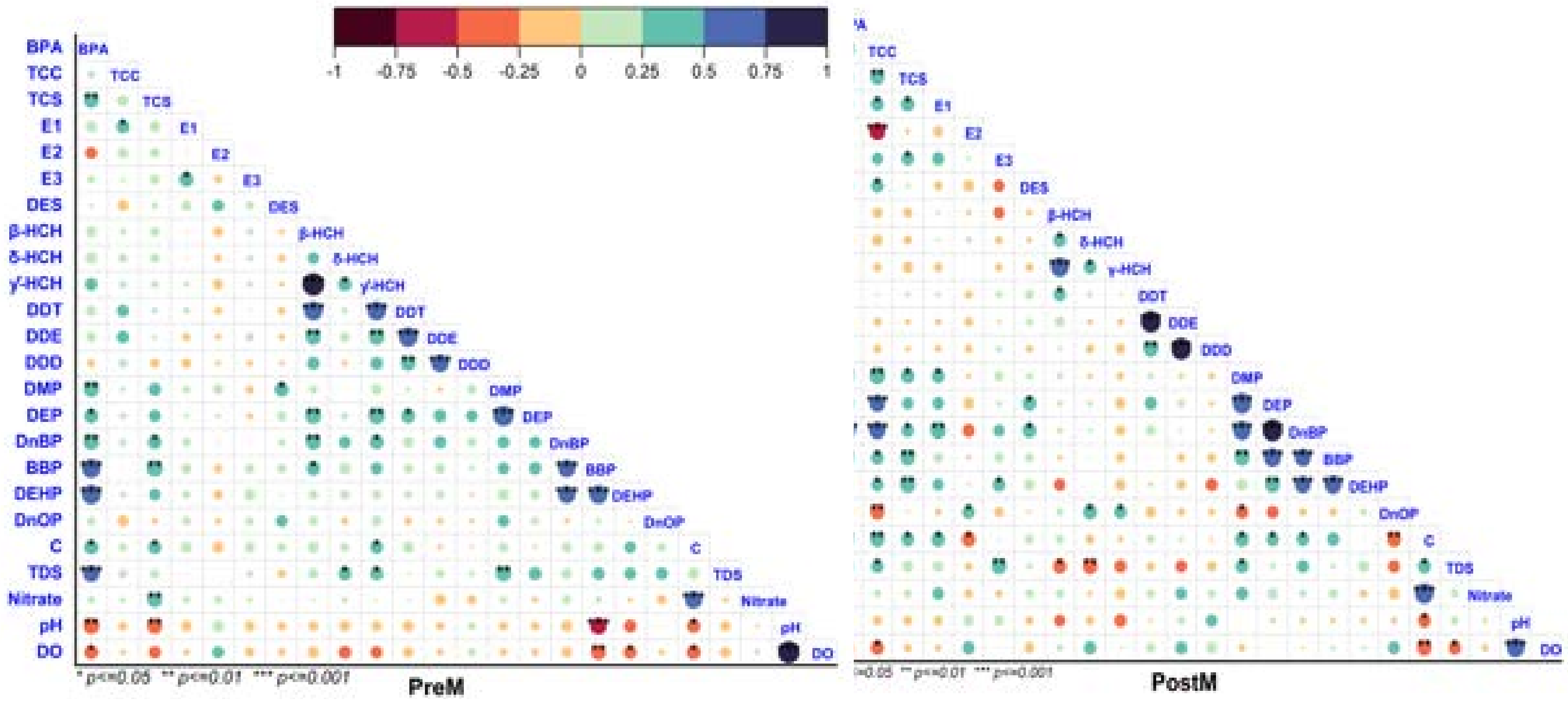


Figure 4-21: Spearman rank correlations among target EDCs and various environmental factors in PreM and PostM

Colored circles represent different levels of correlation as indicated by the legend, while stars within the circles signify the degrees of statistical significance.

Similarly, low DO levels reduce microbial activity, slowing the aerobic degradation of these compounds, which results in their persistence. In the post-monsoon season, no significant associations were found between EDCs and pH, but negative correlations were observed with DO for BPA (p<0.05) and Triclocarban (p<0.05). The same pollution sources that contribute to the presence of EDCs also create conditions that lower pH and DO, explaining these negative correlations and indicating that EDCs are more prevalent in environments with lower pH and oxygen levels.

Conductivity

In the pre-monsoon season, positive correlations (p<0.05) between conductivity and BPA, TCS, and γ-HCH suggest links to municipal/industrial discharges and agricultural runoff, contributing to higher ionic content and conductivity. In the post-monsoon season, a broader range of EDCs, including BPA (p<0.01), TCC (p<0.01), TCS (p<0.05), E1 (p<0.05), DMP (p<0.05), DEP (p<0.05), and DnBP (p<0.05), showed positive correlations with conductivity, indicating that increased runoff mobilizes these substances along with ions. However, E2 (p<0.05) and DnOP (p<0.01) exhibited negative correlations, likely due to their higher hydrophobicity and susceptibility to biodegradation and adsorption onto particulates. These findings emphasize the role of seasonal variations and physico-chemical properties in influencing EDC behavior and their correlations with conductivity, with certain EDCs persisting in the dissolved phase while others degrade or adsorb onto sediments.

TDS

Except for DnOP in the post-monsoon (PostM) season, Plastic additives generally show positive correlations with TDS in both seasons. This suggests common sources like industrial discharges and urban runoff contribute to both. Significant associations were found between TDS and BPA (PreM: $p<0.001$; PostM: $p<0.05$) and DMP (PreM: $p<0.05$; PostM: $p<0.05$), indicating similar source influences.

In the pre-monsoon (PreM) season, HPCPs and Estrogens show weak correlations with TDS due to their distinct sources, biodegradation, and tendency to adsorb onto particulates rather than remain dissolved. However, TCC ($p<0.05$) and E3 ($p<0.01$) show positive correlations with TDS in the PostM season, likely due to their persistence and the increased water flow and runoff, which keep them dissolved. TDS and pesticides like δ-HCH and γ-HCH show a positive correlation ($p<0.05$) in the PreM season, driven by lower water volumes and increased runoff. In contrast, the PostM season exhibits negative correlations between TDS and pesticides like β-HCH ($p<0.05$) and δ-HCH ($p<0.01$), likely due to dilution, adsorption, and degradation, despite TDS potentially increasing from urban runoff and sediment resuspension.

4.3.2 Riverward fluxes of EDCs from drain discharges

Contaminant concentration in water serves as a crucial metric for assessing ecological risk, whereas flux calculation is the primary quantitative measure used to determine the contribution of individual pollution sources and to understand the environmental fate of contaminants (Wang et al., 2022).

Based on spatiotemporal characteristics of EDCs contamination in MGR, the riverward fluxes of EDCs from drain discharges were estimated by multiplying the annual average discharge with the average concentration of EDCs in water during two seasons. The results are presented in Figure 4-22 and Table 4-7.

In the study area, seventeen targeted drains across five districts of Uttar Pradesh discharge into the MGR, each with distinct characteristics. Among these, six drains (D6, D7, D8, D12, D14, and D15) carry a mixture of effluents from both municipal and industrial sources, while the remaining drains exclusively carry domestic effluents. The mass flux of target EDCs discharged from these drains into the MGR ranged from 0.88 to 866.90 kg/year, with a median concentration of 31.82 ng/L and an average annual load of 161.34 ± 262.31 kg/year. As expected, Plastic additives were the predominant contributors, accounting for 97.5% of the total EDC load in the MGR, followed by HPCPs (2.3%), estrogens (0.13%), and pesticides (0.01%) (Figure 4-15 and Table 4-1). The overwhelming presence of plastic additives underscores the widespread nature of plastic pollution Four drains, with the highest flow rates ranging from 103.1 to 157.3 million liters per day (MLD), contributed a significant 81% of the total EDC mass flux into the river. The contributions from these drains were distributed as follows: Khirkia Drain (29%), Assi Drain (19.6%), Varuna Drain (18.8%), and Permiya Drain (13.1%). Of these major contributors, the Khirkia and Varuna drains transport mixed effluents from both domestic and industrial sources, whereas the Assi and Permiya drains are solely domestic in nature. This pattern indicates that both industrial and domestic activities substantially contribute to the EDC load in the MGR.

The significant EDC loads, particularly from plastic additives, discharged by these drains present a serious and long-term threat to the health of the Ganga River's aquatic ecosystem, necessitating immediate and focused intervention.

It is important to note that sludge from treatment plants often contains higher levels of EDCs than the effluent itself and is commonly used for soil amendment (Kasprzyk-Hordern et al., 2009; Inam et al., 2019; Liu et al., 2018; Luo et al., 2019). Agriculture, along with livestock effluent and manure, are also significant sources of EDC contamination (Luo et al., 2019). To better estimate the transport flux and environmental fate of EDCs, future studies should focus on identifying and quantifying these contamination sources.

4.3.1 Estimation of Estrogenic Potency of e-EDCs in MGR

Given the relatively high concentrations of target EDCs, including plastic additives, triclosan, and estrogens, the contribution of these contaminants to the overall estrogenicity of surface water in the MGR was quantified using estradiol equivalents (EEQs).

Table 4-7: Riverward fluxes from in drains across MGR

Target EDCs	Riverward fluxes from drains (Kg/year)		
	Range	Median	Average±SD
TCC	<0.01-5.42	0.09	0.892±1.5
TCS	0.01-20.77	0.74	3.639±5.86
ΣHPCPs	0.86-839.89	29.17	157.45±255.23
E1	<0.01-0.27	0.04	0.06±0.08
E2	<0.01-0.01	-	0.003±0.01
E3	<0.01-1.39	0.04	0.24±0.41
DES	<0.01-0.16	-	0.01±0.04
ΣEstrogen	<0.01-1.67	0.08	0.21±0.46
β-HCH	<0.01-0.07	-	0.01±0.02
δ-HCH	<0.01-0.02	-	0.004±0.01
γ-HCH	<0.01-0.05	-	0.006±0.01
p,p'-DDT	<0.01-0.16	-	0.016±0.05
p,p'-DDE	<0.01-0.01	-	0.001±0
p,p'-DDD	<0.01-0	-	0±0
ΣPesticides	<0.01-0.29	-	0.02±0.08
DMP	<0.01-1.02	0.09	0.221±0.34

DEP	0.05-332.48	19.61	64.575±99.72
DnBP	0.06-202.34	5.07	37.97±58.53
BBP	<0.01-4.27	0.19	0.84±1.26
DEHP	0.26-304.39	16.15	64.993±100.61
DnOP	<0.01-3.31	0.13	0.392±0.81
BPA	0.06-63.13	1.29	11.697±19.72
ΣPlastics Additives	0.01-26.19	0.72	3.66±7.15

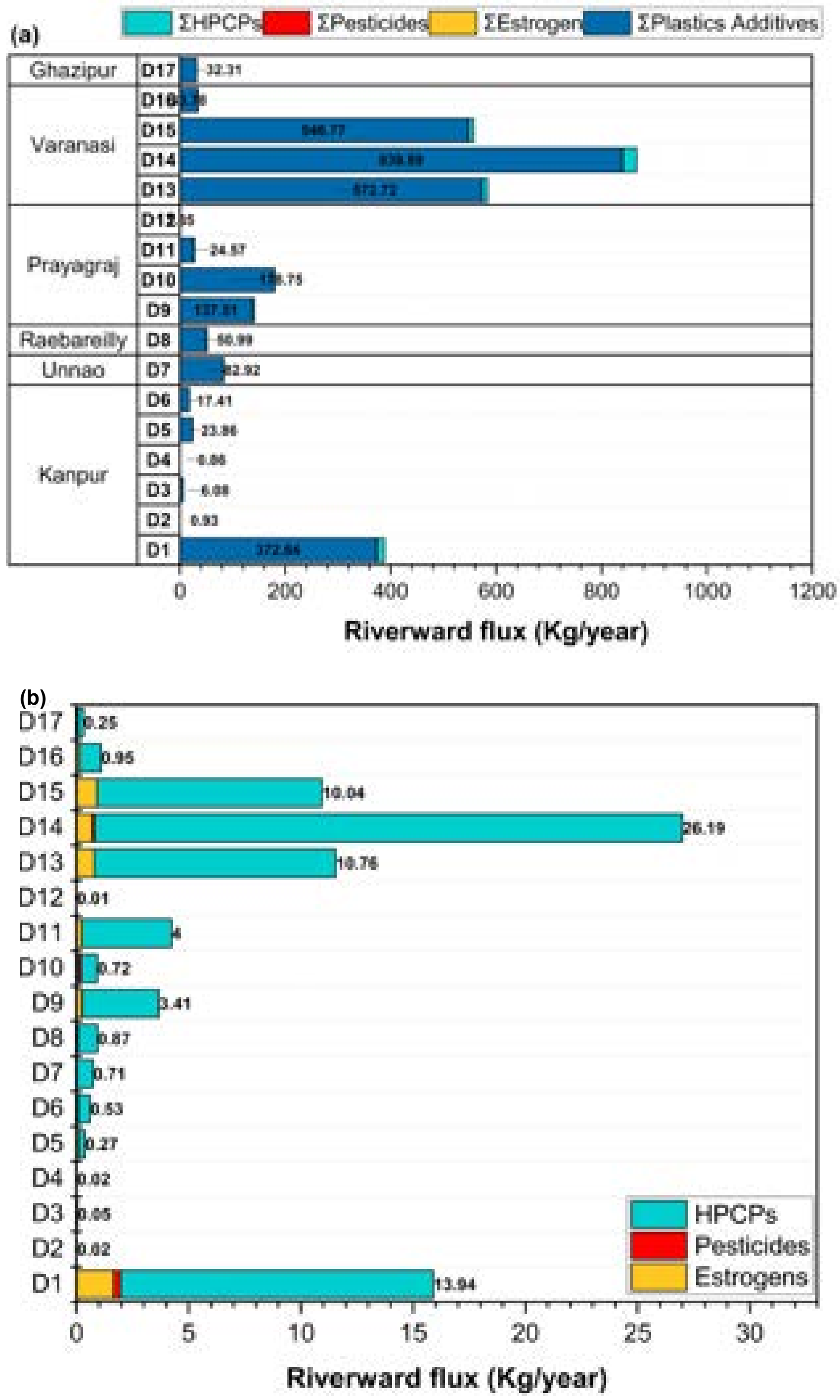


Figure 4-22: (a) Riverward flux (Kg/year) of EDCs from drain discharges (b) Detailed view of riverward flux (Kg/year) of HPCPs, Pesticides, Estrogens from drain discharges in (a)

The estimated EEQs (Figure 4-23) for E1 ranged from <0.01 to 10.64 ng/L (mean ± SD: 1.08 ± 1.87 ng/L), for E3 from <0.01 to 2.99 ng/L (0.58 ± 0.76 ng/L), and for E2 from <0.01 to 3.78 ng/L (0.42 ± 0.87 ng/L). The majority of the estrogenic activity (99%) in the MGR is attributed to E1 (51%), followed by E3 (27.5%) and E2 (19.8%).

The total estrogenic activity (EEQT) of the fifteen target EDCs exceeded the 1 ng E2/L threshold (Caldwell et al., 2012) for adverse effects at 63% of the sampling sites (Figure 4-24). The elevated EEQ values for estrogens (E1, E2, and E3) can be attributed to their high estrogenic equivalency factors (EEFs), in contrast to other EDCs, such as plastic additives, which, despite their prevalence, exhibited lower EEQ values.

Given the ecological risks, E1, and E2 are included on the European Commission's Watch List, and the environmental quality standards for surface water are set at 3.6 ng/L for E1 and 0.4 ng/L for E2 (EC, 2018; Cislak et al., 2023). In the MGR, the average concentrations of E1 and E2 surpassed these standards at 13% and 25% of the sampling sites, respectively, with these exceedances particularly concentrated near point sources, including drain outflows and the confluences of the Pandu and Yamuna Rivers. Thus, it is imperative to implement effective remediation technologies to remove E1, E2, and E3 from these point discharges in the MGR to mitigate their harmful effects on the aquatic environment (Li et al., 2023).

4.3.1 Multi-tier ecological risk assessment

4.3.1.1 Tier-1 risk quotient (RQ): A screening-level risk assessment

The Figure 4-25 illustrates the Risk Quotient (RQ) values for various EDCs, each detected with a frequency (DF) greater than 20%, plotted on a logarithmic scale. These RQ values are calculated based on the maximum concentration and the predicted no effect concentration (PNEC) for the most sensitive species across three trophic levels: algae, invertebrates, and fish. The RQ values are categorized into three risk levels: high risk (RQ ≥ 1), moderate risk (0.1 ≤ RQ < 1), and low risk (RQ < 0.1). The results indicate that several EDCs fall within the high-risk category (RQ ≥ 1), with RQ values exceeding 1, indicating a significant ecological threat. DnBP is identified as the most critical EDC, with an RQ of 518.09 and 100% of sites exceeding the RQ threshold, indicating a widespread and severe threat to aquatic ecosystems. Similarly, E1 and DEHP are of major concern, with RQ values of 181.66 and 94.47, and exceedance rates of 75% and 100%, respectively. These chemicals demonstrate substantial ecological risks across multiple locations. TCS, E3, DEP, BPA, and TCC also exhibit high RQ values of 84.13, 68.1, 41.2, 25.6, 8.57, and 2.74 with exceedance rates of 100%, 55%, 98%, 100%, 83% and 85% respectively, confirms their pervasive presence and high ecological threat across all monitored sites. E2, γ-HCH, and p,p'-DDT, with RQ values of 41.4, 1.42 and 2.74 with exceedance of 28% , 48% and 20% respectively, indicating localized but critical risks.

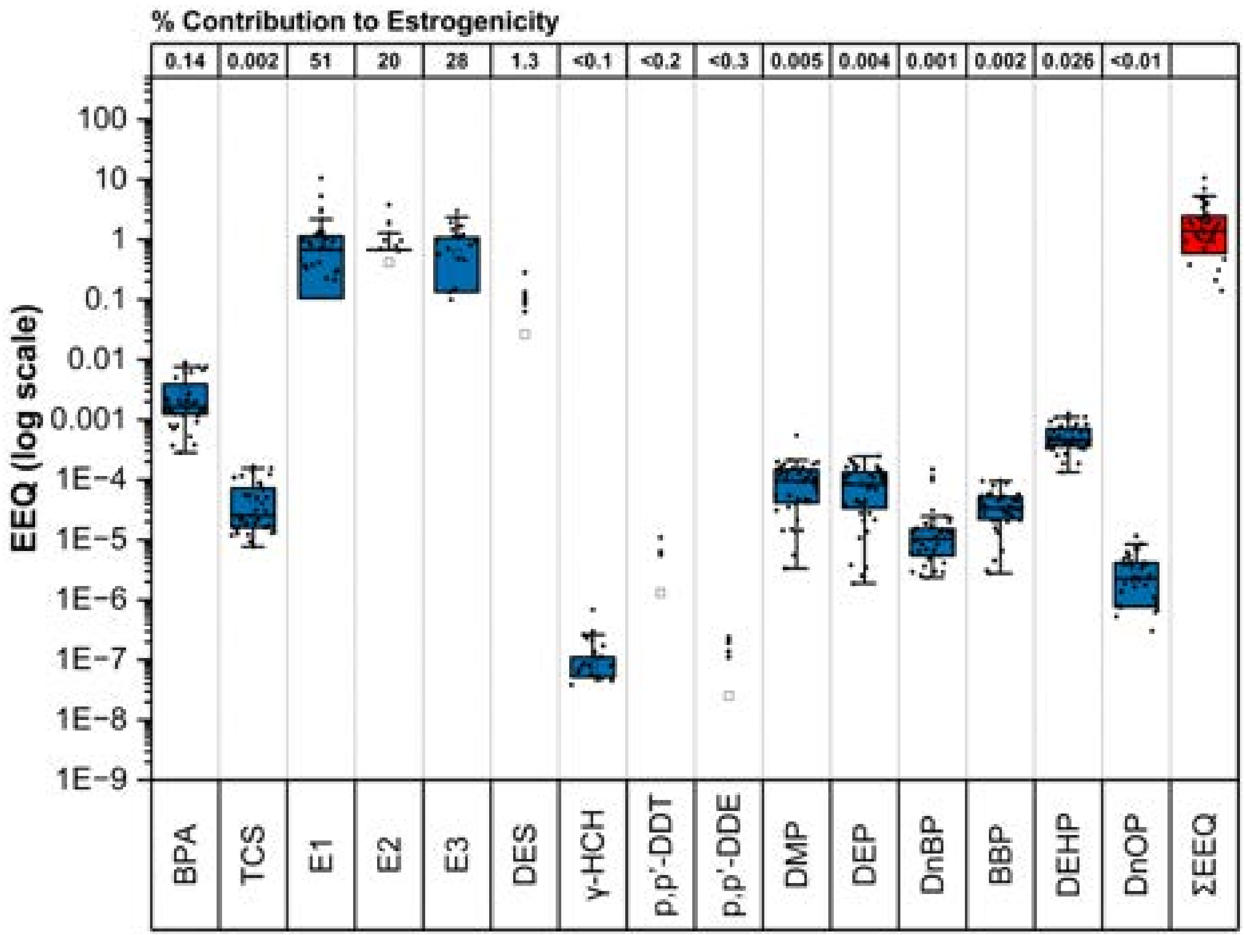


Figure 4-23: Risk (RQ) ranking of target EDCs (with DF >20%), based and the maximum concentrations in surface water of MGR and PNEC for the most sensitive species

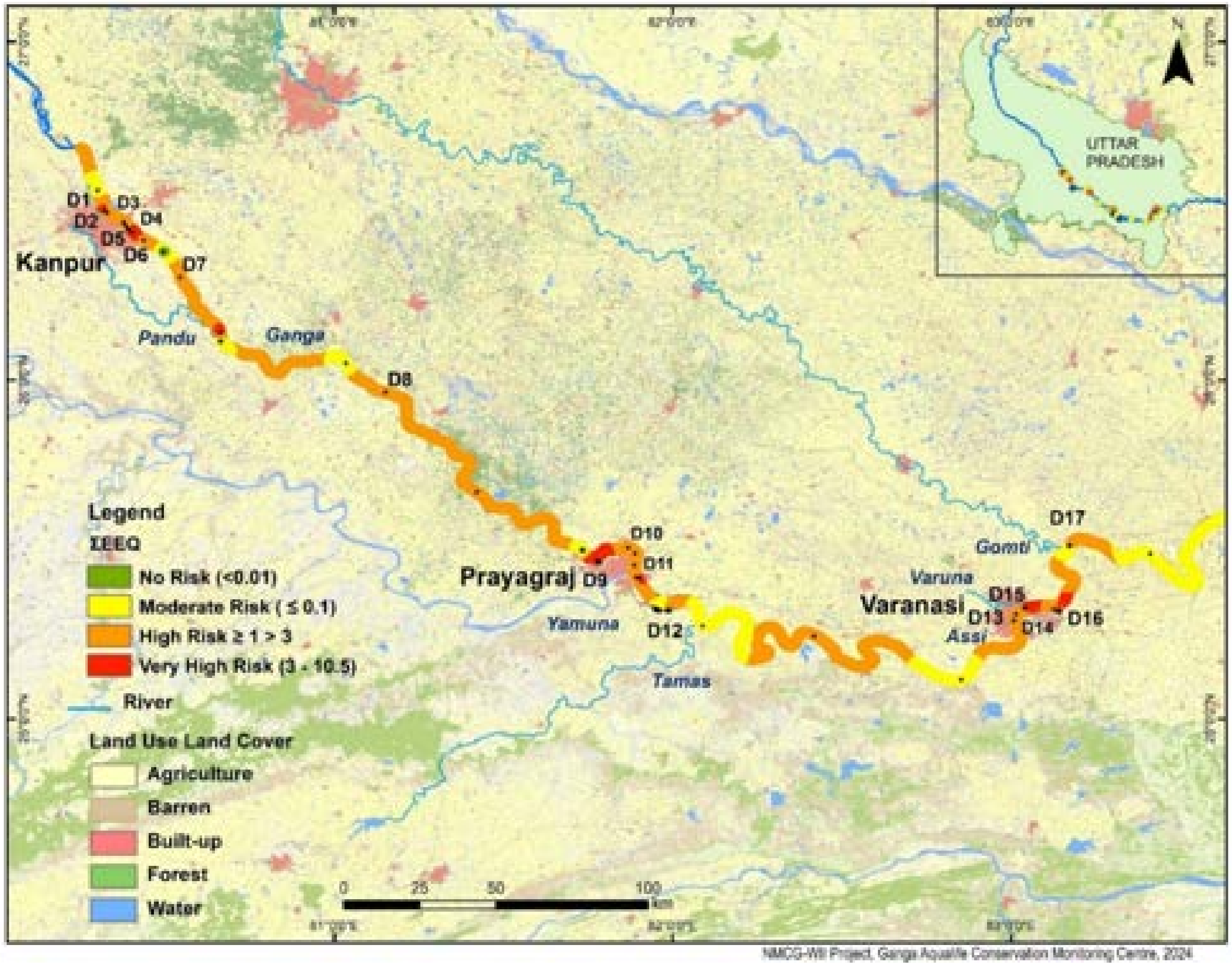


Figure 4-24: Spatial distribution of estrogenic potency in MGR

The high risk associated with these chemicals may have serious health impacts on aquatic species, affecting organisms from primary producers to top predators, including the endangered Gangetic dolphin (Table 2-2 and 2-3). BBP falls into the moderate-risk category with an RQ of 0.28, but with 88% exceedance, indicating that while it is prevalent, it does not exceed the risk threshold at any site. EDCs such as p,p' DDE, DnOP DMP, DnOP, DES and δ-HCH fall within the low-risk category, with RQ values well below 0.1. These chemicals currently pose a lower threat under existing environmental concentrations.

Overall, the data highlights the urgent need for regulatory interventions to address the high-risk EDCs, particularly those with widespread exceedance rates such as DnBP, E1, DEHP, and TCS, to protect aquatic ecosystems from further degradation. Although the moderate- and low-risk e-EDCs present a lower immediate threat, ongoing monitoring is essential, especially for those that are widespread, to mitigate potential future risks and maintain the long-term health of aquatic environments.

4.3.1.2 Tier-2 frequency of PNEC exceedance: A semi-probabilistic approach

Figure 4-26 presents the percentage of samples (F) in which measured environmental concentrations exceeded the respective PNECs. For 11 EDCs, including 4 plastic additives, 3 natural estrogens, 2 HPCPs, and 2 pesticides, this was the case. The highest frequency of exceedance (FTotal) was noted for BPA, DnBP, DEHP, and TCS (100%, exceeded in 40 of 40 samples), followed by DEP (98%), TCC (83%), E1 (75%), E3 (55%) γ-HCH (48%), E2 (28%), and p,p'-DDT (20%). As expected, as plastic additives and TCS are ubiquitous in the environment (Rishikesh Mankidy et al., 2013; Lehmler et al., 2018; Tijana Vasiljevic & Harner, 2021; Li et al., 2021a).

The PNEC exceedance for estrogens was observed to be highest among drain samples compared to river samples, indicating their greater prevalence in areas directly impacted by untreated or partially treated wastewater discharges. This suggests that estrogens are more concentrated in environments with higher inputs of domestic or industrial effluents, underscoring the need for targeted management strategies to address these point-source pollutants and protect downstream aquatic ecosystems. Using median concentrations in deterministic risk assessments can distort the analysis and result in overly conservative conclusions. A more effective approach is to assess the probability of EDC concentrations in surface waters exceeding the PNEC for aquatic organisms. The semi-probabilistic risk assessment identified a potential threat (20–28%) from E2 and p,p' DDT, challenging the RQ method's classification of negligible risk and emphasizing the need to account for concentration distribution in surface waters when assessing the potential risks of EDCs. Conversely, six other EDCs (BBP, p,p' DDE, DnOP, DES, β-HCH, and δ-HCH) did not exceed PNEC values, indicating no substantial ecotoxicological risk to aquatic organisms at present exposure levels.

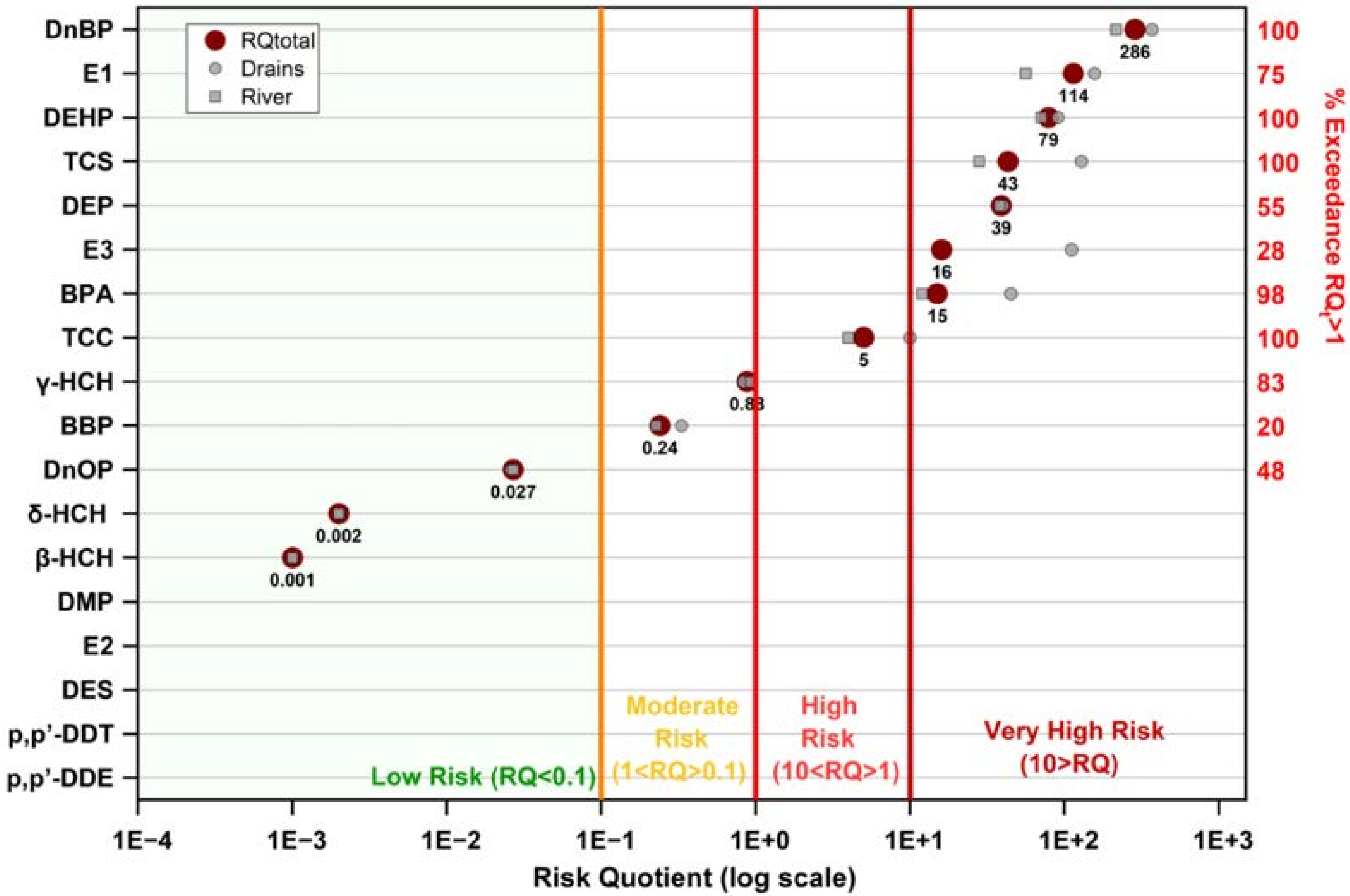


Figure 4-25: Risk (RQ) ranking of target EDCs (with DF >20%), based and the maximum concentrations in surface water of MGR and PNEC for the most sensitive species

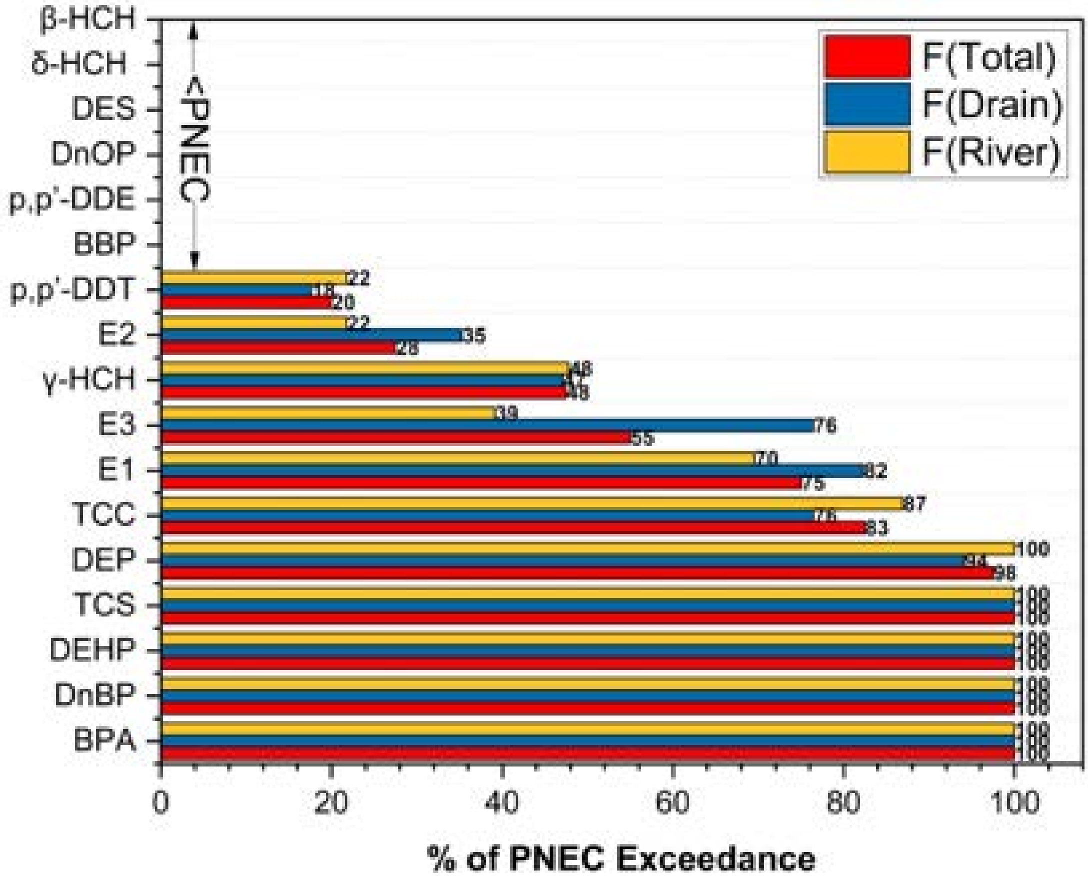


Figure 4-26: Frequency (%) of 11 EDCs (DF>20%) in surface waters of MGR with concentration >PNEC

F_T, F_D, F_R represents frequency exceedance of PNEC in total (drain+river), drains, and river respectively.
RQtotal represent risk values in drain and river

4.3.1.3 Tier-3 prioritization indexes: An optimized risk assessment

Figure 4-27 shows nine prioritized EDCs according to prioritization indexes (PI) in descending order for $River_{total}$ (samples from Drains discharges + river), Drains, and rivers. Prioritization indexes ranged from 286 for DnBP to 0.42 for ɣ-HCH. Compared to the RQ value (Figure 4-15), prioritization indexes showed a greater difference in the potential environmental risks of the estrogens (E1 and E3) that presented a lower frequency of concentrations exceeding PNECs. However, both were still identified as potential risk due to their high frequency of PNEC exceedance of 55 to 75%.

For the EDCs, such DnBP, DEHP, TCS, DEP, BPA, and TCC, those posed high risk with the RQ method were still identified as risk due to their great frequency of exceedance (≥83%). For the BBP, however, class of risk were downgraded from a moderate risk with RQ method to an insignificant risk with PI method, because it presented a no frequency of PNEC exceedance. By accounting for the fluctuations in concentrations that exceed PNECs, the optimized risk assessment approach offers a more efficient means of identifying and prioritizing contaminants for large-scale water resource management.

4.3.2 Identification of e-EDC hot-spots

Based on the multi-tier ecological risk assessment, the entire 600 km stretch of the Middle Ganga Reach has been classified as a "high-risk" zone for at least two or more EDCs at all sampling sites, underscoring pervasive contamination throughout the study area. The EDCs identified include BPA, DnBP, DEHP, and Triclosan. Furthermore, estrogenic potency analysis indicated that 63% of this stretch exhibited high estrogenicity, suggesting a significant potential for endocrine disruption in the aquatic organisms residing in this region. This high-risk designation is particularly alarming because this stretch overlaps with critical habitats of the endangered Gangetic Dolphin (Figure 4-17), a species that is especially vulnerable to chemical pollutants due to its position at the apex of the aquatic food web.

The known toxicity of EDCs like phthalates, BPA, and Triclosan to coastal cetaceans, as evidenced by studies (Hinther et al., 2011; Lunardi et al., 2016; Dziobak et al., 2022; Guo et al., 2023; Xie et al., 2023), combined with their high production volumes and widespread use, raises serious concerns about their impact on freshwater cetaceans like the Gangetic Dolphin. The high ptential risk and frequent exceedance of estrogenic compounds, such as E1 and E3, further exacerbates the risk to aquatic mammals, potentially leading to severe reproductive and developmental disorders.

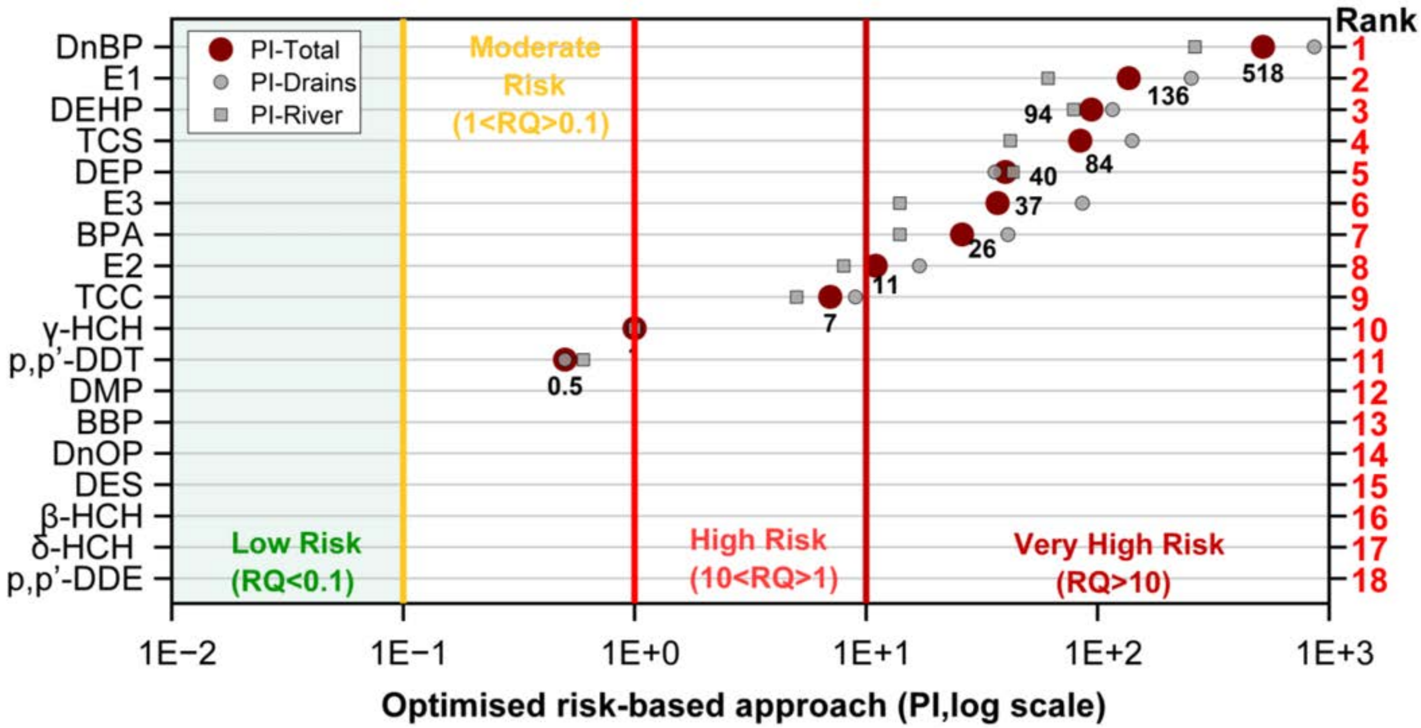


Figure 4-27: Priority EDCs according to prioritization indexes (PI) in descending order

4.4 Conclusion

This study provides the first comprehensive baseline information on the occurrence and spatiotemporal distribution of EDCs in the Middle Ganga Reach (MGR). It also offers the inaugural baseline data on EDCs load from drain discharges into the MGR. By identifying high-risk zones through a multi-tier ecological risk assessment framework, the study offers a strategic direction for screening and prioritizing EDCs based on their ecological risks, thereby guiding targeted conservation and remediation efforts. The key conclusions based on the synthesis of this chapter are:

Key Conclusions

1. **Significant Contamination by EDCs**:

   The study underscores the substantial contamination posed by EDCs, particularly plastic additives, HPCPs, estrogens, and pesticides.

2. **Predominance and Prevalence of Plastic Additives**:

   Plastic additives, Phthalates and BPA, emerged as the most predominant EDCs, reflecting their extensive production and widespread usage in consumer products and industrial applications, coupled with inadequate waste management practices.

3. **Seasonal Dynamics**:

   Pre-monsoon Season (PreM): Higher concentrations of BPA, and HPCPs estrogens were observed during this period. This can be attributed to their increased usage during the peak of the COVID-19 pandemic (March-April 2021), lower water volumes, and reduced dilution capacity. In contrast, lower concentrations of hormones (E1 and E3) were found in the MGR during the PreM season, indicating their vulnerabilities to enhanced degradation due to stronger sunlight and higher temperatures. These conditions accelerate photolytic and microbial degradation processes, reducing the presence of these hormones in the water.

Post-monsoon Season (PostM): This season saw a significant influx of phthalates, primarily due to atmospheric deposition and runoff from rainfall, which facilitated the dispersal and redistribution of these contaminants.

The degradation of EDCs through photolytic and microbial processes, coupled with atmospheric deposition and seasonal rainfall/surface runoff, significantly influences their seasonal variation. These factors play a crucial role in determining the fate and transport of EDCs within the river system.

4. **Temporal Dynamics: Major Contributors**

   Drain discharges have been identified as contributors to the EDC load in the MGR. Significant variations were observed for BPA, TCS, E1 and E3.

5. **Riverward fluxes of EDCs from drains discharges**

   The mass flux of EDCs from drains to the river revealed significant EDC loads entering the MGR from these sources. Notably, three drains—two in Varanasi and one in Kanpur—were identified as contributing 81% of the total EDC load into the MGR.

6. **Influence of Water Quality Parameters**:

   Key water quality parameters such as conductivity, total dissolved solids, nitrate, pH, and dissolved oxygen were found to influence the concentrations of certain EDCs, highlighting the complex interactions between pollutants and environmental factors.

7. **Identification of Priority EDCs**:

   The multi-tier ecological risk assessment identified the top 10 EDCs— DnBP, E1, DEHP, TCS, DEP, E3, BPA, E2, TCC, and γ-HCH—that should be prioritized for routine monitoring. This proactive approach is vital for continuous assessment and management of these contaminants to safeguard the river's ecological health.

8. **Risk Stratification**:

   The risk stratification, based on estrogenicity potential and ecological risk assessment, revealed that 63% of the MGR is classified as high risk and 25% as moderate risk. Additionally, the ecological risk assessment indicated that the entire study area is potentially at risk from more than two EDCs. This stratification is crucial for prioritizing monitoring and remediation efforts. Addressing these contamination hotspots is vital not only for the conservation of the Gangetic Dolphin but also for maintaining the ecological integrity of the Middle Ganga Reach.

In summary, the findings of this study highlight the urgent need for improved wastewater management practices, enhanced monitoring programs, and targeted remediation strategies to mitigate the ecological risks posed by EDCs in the conservation-priority stretch of the Ganga River. Future research should prioritize the investigation of food-web bioaccumulation and trophodynamics of EDCs, particularly for those ranked as priority EDCs.

Chapter 5 Do dietary exposures to multi-class Endocrine Disrupting Chemicals translate into health risks for Gangetic Dolphins?

This chapter is reprinted from Sah, R., Talukdar, G., Megha Khanduri, Chaudhary, P., Ruchi Badola, & Syed Ainul Hussain. (2024). Do dietary exposures to multi-class endocrine disrupting chemicals translate into health risks for Gangetic dolphins? An assessment and way forward. Heliyon, 10(15), e35130–e35130. https://doi.org/10.1016/j.heliyon.2024.e35130

Abstract

This chapter investigates the dietary exposure risks of multi-class e-EDCs to the threatened Gangetic dolphins (*Platanista gangetica*) in a conservation-priority segment of the MGR. Elevated e-EDCs bioaccumulation was observed across prey fish species, with di(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DnBP) significantly contributing to the EDC burden. The concentrations of persistent organochlorines in prey revealed a shift from dioxin-like polychlorinated biphenyls (PCBs) to non-dioxin-like PCBs. The prevalence of regulated p,p' DDT (Dichlorodiphenyltrichloroethane) and γ-HCH (Lindane) residues suggests regional non-compliance with regulatory standards. The concentration of some EDCs is dependent on the habitat, foraging behavior, trophic level and fish growth. The potential drivers of EDCs contamination in catchment includes agriculture, vehicular emissions, poor solid waste management, textile industry, and high

tourist influx. Risk quotients (RQs) based on toxicity reference value were generally below 1, while the RQ derived from the reference dose highlighted a high risk to Gangetic dolphins from DEHP, DDT, DnBP, arsenic, PCBs, mercury, and cadmium, emphasizing the need for their prioritization within monitoring programs. The study also proposes a monitoring framework to provide guidance on monitoring and assessment of chemical contamination in Gangetic dolphin and habitats.

5.1 Introduction

The probable extinction of the Yangtze River Dolphin, a tragedy propelled by a myriad of human-induced pressures, starkly illustrates the precarious situation of riverine dolphins across the globe (Turvey et al., 2007). With only five extant species of river dolphins worldwide, all classified as threatened, the imperative to address their vulnerability to various anthropogenic stressors is more urgent now than ever (IUCN 2024). These vulnerabilities are particularly pronounced for species in densely populated river basins undergoing rapid urban development, such as the Gangetic Dolphin (*Platanista gangetica*) in the Ganga Basin (IUCN, 2024).

As an apex predator, Gangetic Dolphin (henceforth referred to as GD) plays a key role in maintaining the structural and functional integrity of freshwater ecosystems through top-down processes. The GD is often considered an appropriate umbrella species, offering protection not only for itself but also facilitating conservation efforts for other co-occurring species (Hussain et al., 2013; Behera et al., 2014; Das et al., 2024). This makes understanding GD's responses to various anthropogenic stressors pivotal for devising effective conservation and management strategies that benefit the entire freshwater ecosystem and its biodiversity.

Recent estimates in Ganga Basin reveal a concerning 24.37 % contraction in GD range (Das et al., 2022) and a population decline of over 50% since 1957 (Behera et al., 2013; IUCN, 2024).Despite stringent regulatory conservation measures implemented by the Indian government, the species remains highly susceptible to anthropogenic threats, including bycatch mortality, poaching, boat traffic, a compromised (both in quantity and quality) prey base, climate change, flow modification by dams and barrages, and continual exposure to diverse pollutants (IUCN, 2024).

Riverine cetaceans, including the GD, are exposed to various chemical pollutants primarily via dietary intake or offloading to the next generations via gestation and lactation (Tanabe et al., 1991). With their extended lifespans, status as apex predators within local food chains, and high lipid reserves, GD often encounter significant health risks due to the substantial accumulation of various contaminants (Tanabe et al., 1991; Kannan et al., 1994; Senthilkumar et al., 1999). While studies on habitat modification, direct mortality, habitat loss, and overexploitation are frequently addressed (Sinha et al., 2010; Kelkar et al., 2018; WII-GACMC, 2019; Das et al., 2022; Das et al., 2024), there remains a significant gap in understanding how chemical pollutants contribute to the declines of these species' populations, potentially undermining conservation efforts.

Within the broad spectrum of pollutants, Endocrine-Disrupting Chemicals (EDCs) that pose threat to aquatic mammals, have emerged as a critical ecological concern due to their persistent, bioaccumulative, mobile, and toxic properties. EDCs comprise a highly diverse class of compounds, including natural hormones and man-made substances such as pesticides, pharmaceuticals, plasticizers, potentially toxic elements, industrial chemicals, surfactants, and household and personal care products (Tan et al., 2017).

Over the past three decades, our understanding of the hazards and ecological risks associated with EDCs has advanced considerably, notably their correlation with disruptions in reproduction and development, that may result in observable regional population shiftsin some aquatic mammalian species (Delong et al., 1973; Reijnders, 1986; Hyvärinen et al., 1998; Dove, 2022). Although the detrimental impacts of EDCs on aquatic mammals are well-established globally, there is a notable gap in research specifically examining the exposure of GD to EDCs and the resultant effects on their individual health and population dynamics. While research exists on GD's exposure to some EDCs, they only cover the period from 1988 to 1996 (Kannan et al., 1993; Kannan et al., 1994; Kannan et al.,1997; Subramanian et al., 1988; Senthilkumar et al., 1999; Giesy & Kannan, 2001; Senthilkumar et al., 2001; Kumari et al., 2002; Yeung et al., 2009). This gap indicates that recent changes in EDC levels over the past few decades remain undocumented and unanalyzed, resulting in significant gaps in our understanding of current risks and potentially leading to inadequately informed and insufficient conservation efforts.Hence, understanding GD's vulnerabilities to EDCs is crucial for creating targeted interventions to reduce exposure and health impacts, thereby protecting this endangered species and maintaining ecological balance in their riverine habitats.

Biomonitoring of EDCs in GD can reveal health risks, but regulatory, ethical, and technical challenges complicate this traditional method. There is an urgent need for risk assessment methodologies that minimize risk to the species and its populations. In the past two decades, the screening-level ecological risk evaluation (SLERA) approach based on dietary exposures has been successfully used as an indirect and non-invasive alternative to explore the potential health risk posed by contaminants to threatened aquatic mammals (Hung et al., 2006; Mos et al., 2010; Yu et al., 2020).

Given the predominantly piscivorous nature of GD, assessing EDC levels in fish facilitates the evaluation of potential health risks associated with EDC exposure in this species.Furthermore, studies suggest variations in EDC accumulation in fish is driven by biological traits, niche, feeding preferences, and geographical influences, these patterns seldom adhere to consistent trends (Lin et al., 2021; Cordoba-Tovar et al., 2022; Yan et al., 2022). Assessing these factors is vital for understanding biomagnification within the food web; however, limited efforts have been directed toward investigating these dynamics in GD habitats.

Given the above, the study aims to address the following key research questions: (1) what is the current extent of EDC contamination in the prey base of GD? (2) what factors contribute to the observed bioaccumulation patterns? and (3) what risk do EDCs pose to GD through dietary pathways? The overarching objective of this study is to screen for EDCs that pose risk to GD. Additionally, the study offers an issue-based framework for monitoring EDCs within the GD range.

5.2 Materials and methods

5.2.1 Study area and sample collection

The investigated study area is a 60 Km stretch of MGR that lies between north latitudes 26° 33' 40.608" N-25° 34' 56.64" N, and east longitudes, 80° 18' 3.096" E - 83° 36'6.48" E in the Varanasi district of Uttar Pradesh, India (Figure 1). The stretch holds immense religious, economic, and socio-cultural importance and is an important habitat for diverse flora and fauna including GD (IUCN, 2023). Within the Varanasi district, there are 38 towns and 1327 villages, with a population of 3,676,841(urban: 43.44% and rural:56.56%) projected to reach approximately 4,300,000 in 2023 (2011 Census).

Varanasi, one of the oldest continuously inhabited cities globally, confronts obstacles in modernizing its sewage infrastructure owing to spatial limitations and preservation issues tied to its historic design. The river's catchment area, characterized by rapid development and high population density, coupled with poor wastewater management, frequently results in the discharge of untreated wastewater through multiple point-sources (CPCB, 2016). Meanwhile, its vast agricultural landscape, including cultivation on dry riverbeds, contributes as non-point sources of EDCs such as organochlorine pesticides (CPCB, 2013; UPPCB, 2013).The primary industrial sector is textiles, which holds a prominent position in the local economy, after tourism. Tourism plays a central role in Varanasi's economy, with an annual footfall exceeding 9 million from domestic travelers and 1 million from international visitors (CPCB,2016). The study area features three major sewage discharge points: Nagwa Drain (formerly Assi River), the Khirkia Drain, and the Varuna River

(Figure 1). Both Varuna and Assi rivers, often referred to as drains due to their poor water quality, receive significant wastewater and sewage inflow from numerous industrial and municipal drains, ultimately discharging into the Ganga River.

To understand to what extent the patch of the urban settlements and/or cities along the conservation priority habitats pose threats to the health of GD, three sampling sites S1, S2, and S3 (S1 and S3-predominantly agriculture landscape and rural settlements; S3-urban and industrial settlements) were chosen to represent the habitats in the MGR for EDC monitoring (Figures 5-1 and S1, Table S1).

The Platanista genus exhibits a preference for prey based on size rather than species, primarily due to their narrow oesophagi, and species richness (Takahashi and Yamasaki, 1972). Typically, their prey size distribution is dominated by items measuring less than 20-30 cm (Sinha et al., 1993; Kelkar et al., 2018). Consequently, our sampling efforts were focused on collecting specimens falling within these preferred size ranges. Sampling was carried out in March (lean flow) 2021 and a total of 187 freshly-caught fishes of 21 species were obtained. Freshly caught fish samples were obtained on-site from the local fishermen, as part of their routine catch. Ethical approval for the collection of biological samples was obtained from the Institutional Animal Ethical Committee (IAEC), Wildlife Institute of India. The species were identified and their length and weight were recorded on-site. The samples were packed in precleaned aluminum sealed bags and kept in an ice box for transportation to the laboratory, where they were stored in a deep freezer at -20°C until further processing. Individual whole fish of the same species from each site were pooled and homogenized in a customized stainless steel tissue homogenizer. Details of the fish species collected from each site are given in Table 6-1. Data on trophic levels and habitat preference of each species were obtained from the FishBase (Froese and Pauly, 2023).

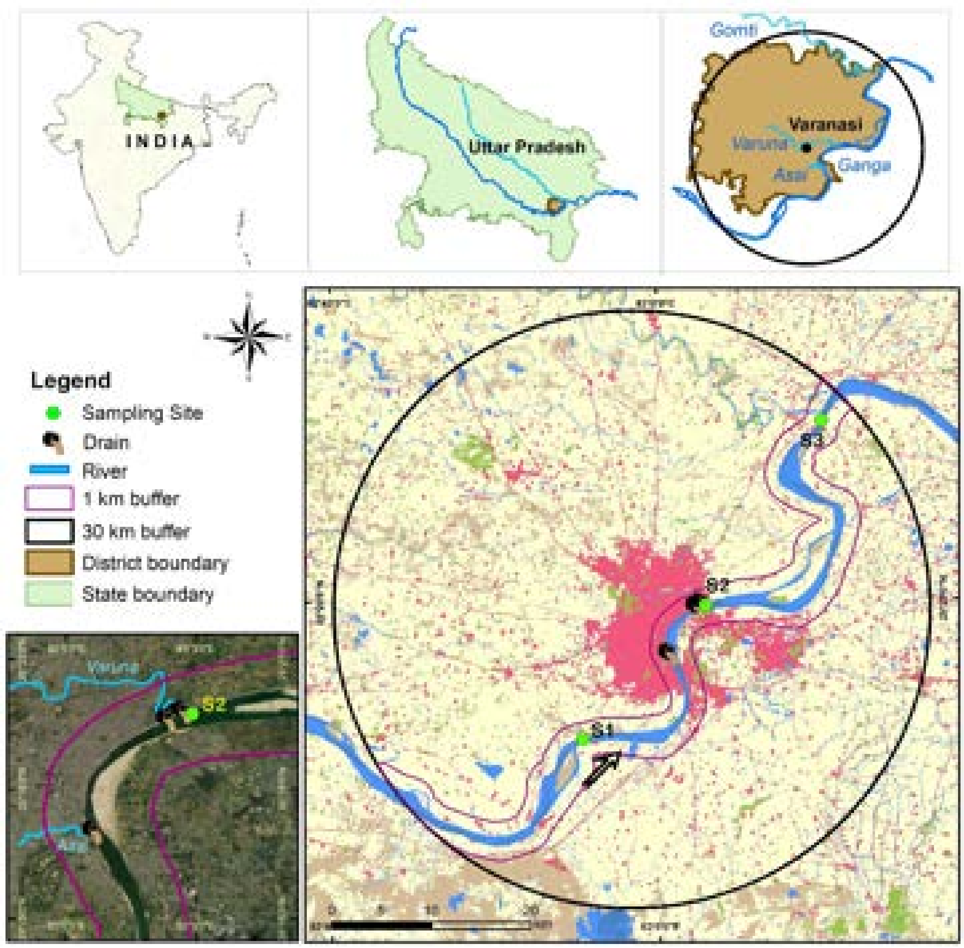


Figure 5-1: Representation of sampling sites along MGR

5.2.2 Materials, extraction, and analyses

EDCs were selected mainly based on their major categories, and potential toxic impacts on aquatic mammals. Thirty-nine EDCs, including seven platics additives - six phthalate (PAEs, the sum expressed as ΣPAEs): Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Di-n-butyl phthalate (DnBP), Butyl benzyl phthalate (BBP), Bis(2-ethylhexyl) phthalate (DEHP), Di-n-octyl phthalate (DnOP) and Bisphenol A; Seven organochlorine pesticides: p,p'-DDT(1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl) ethyl]benzene), p,p'-DDE (1,1-Dichloro-2,2-bis (4-chlorophenyl) ethene dichlorodiphenyldichloroethylene), p,p'-DDD (1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene) -the sum expressed as ΣDDTs; four Hexachlorocyclohexane (HCHs; sum expressed as ΣHCHs) isomers: α-HCH, β-HCH, γ-HCH, δ-HCH; plasticizer: ; two household and personal care products (the sum expressed as ΣHPCPs): Triclosan (TCS), Triclocarban (TCC); Nineteen polychlorinated biphenyls (the sum expressed as ΣPCBs): PCB 8, PCB 28, PCB 44, PCB 52, PCB 77, PCB 81, PCB 101, PCB 105, PCB 114, PCB 118, PCB 126, PCB 138, PCB 153, PCB 156, PCB 167, PCB 169, PCB 180, PCB 189, PCB 209, and four potentially toxic elements (PTEs): Cadmium (Cd), Lead (Pb), Mercury (Hg), Arsenic (As).

Biota samples were spiked with internal standards and allowed to equilibrate for 4 h at room temperature (25 °C). Sample pretreatment was conducted according to previously documented methods for persistent organochlorines (Barni et al., 2016; Cui et al., 2020), BPA and HPCPs (Omar et al., 2019; Fan et al., 2019), and PAEs (Wang et al., 2022), with minor modifications for enhanced recoveries.

Table 6-1: Details of fish species collected, across the three rivers, with average length, average weight, trophic level and habitat preference

Species	**No.of Individuals**	**Niche**	**Feeding habit**	**Trophic Level**	**Average length (cm)**	**Average weight (grams)**
O. niloticus	6	BP	OV	2	22.55±2.55	226.51±77.58
R. corsula	11	P	OV	2.4±0.2	21.5±4.27	32.27±5.18
S. aor	5	D	CV	3.6±0.53	29.25±1.50	145.00±11.37
N. caelata	3	D	CV	4±0.64	23.5±3.56	152.41±8.23
C. latius	8	BP	HV	2.3±0.2	13.45±2.69	26.67±3.82
A. morar	45	BP	OV	3.2±0.4	10.17±1.41	8.78±1.46
R. rita	12	D	CV	3.7±0.57	19.8±3.82	77.54±34.65
L. rohita	5	BP	OV	2.2±0.12	27.7±1.44	301.11±23.48
M. armatus	7	D	CV	2.8±0.27	13.2±2.58	9.08±1.35
J. coitor	*6*	D	CV	3.4±0.5	16.22±1.25	31.88±6.22
S. bacaila	10	BP	CV	3.2±0.4	12.87±1.01	10.77±2.41
L. calbasu	5	D	HV	2	29.75±1.52	311.33±36.51
M. cavasius	14	D	CV	3.4±0.4	12.17±2.76	8.33±2.68
C. reba	7	BP	OV	2.5±0.2	19.39±2.45	53.36±29.92
C. mrigala	5	D	OV	2.3±0.2	28.45±1.24	271.44±8.38
C. catla	5	BP	OV	2.8±0.22	22±2.83	147.65±41.37
L. bata	6	BP	HV	2	28.58±1.73	230±22.08
C. chagunio	4	D	OV	2.8±0.3	19.2±1.63	60±4.24
L. pangusia	6	BP	HV	2	26.17±0.91	166.67±16.44
C. garua	13	D	CV	3.73±0.59	19.1±4.01	43.67±17.93
B. dario	4	D	OV	3.2±0.4	7.5±0.28	8.36±0.36

*OV=Omnivore; HV=Herbivore; CV=Carnivore; BP=Benthopelagic; D=Demersal; P=Pelagic
Trophic level information is retrieved from FishBase (Froese and Pauly, 2023)

The extracts were identified and quantified by Ultra-High Performance Liquid Chromatography–Tandem Mass Spectrometry (UHPLC–MS/MS), Gas Chromatography–Tandem Mass Spectrometry (GC-MS-MS), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Relative recoveries for OCPs and PCBs ranged 81–108% and 91–103% respectively. The average recoveries for PAEs, BPA, and HPCPs were 80-110%, 78-112%, and 81-108% respectively. The percentage recoveries of the PTEs in the SRM ranged from 90.9%-106%. Detailed pretreatment methods, clean-ups, instrument parameters, quality assurance and quality control are detailed in Annexure (Text S1 and Table S2).

5.2.3 Risk assessment for GD

Two dietary tissue guidelines—the reference dose (RfD; mg kg^{-1} ww day^{-1}) and the toxicity reference value (TRV, mg kg^{-1} ww day^{-1}), previously used for humpback dolphins (Hung et al., 2004, 2007)—were adapted and applied to assess potential health hazards to GD from consuming EDC-contaminated prey.

The TRV for EDC in GD was calculated based on the no observable adverse effect dose ($NOAEL_t$) for mammalian test species, and body weight scaling procedure (bodyweight of the dolphins/bodyweight of the test species) (Hung et al., 2004, 2007).

For dose-response assessment, the methodology detailed elsewhere (Hung et al., 2004, 2007) is utilized to derive a maximum allowable concentration (MAC) based on the Reference Dose (MAC_{RfD}) and Toxicity Reference Value (MAC_{TRV}) for a specific EDC in prey fish tissue. The MAC_{RfD} and MAC_{TRV} represents the highest concentration of each toxicant that can occur in the prey without causing harm to the species, and are derived using variables dependent on biological parameters of GD. The calculated values of MAC_{RfD} and MAC_{TRV} for GD is provided in Annexure (Table S3).

The risk quotient (RQ) was determined from the ratio of the observed concentration and the the MAC of a specific EDC in fish for GD consumption. The values of $RQ > 1$, $0.1 < RQ < 1$, and $RQ < 0.01$ indicate high, medium, and low dietary exposure risks of EDCs to the GDs, respectively (Dueñas-Moreno et al., 2024). The limited availability of comprehensive biological data on these dolphins may restrict the accuracy of SLERA. This limitation arises from uncertainties in exposure scenarios, including reliance on standardized parameters such as consumption habits, body weight, exposure frequency, fraction ingested, and exposure duration. Despite these limitations, the SLERA methodology adopted in this study aims to approximate a worst-case scenario, opting for a more conservative approach to ensure a higher level of protection for the threatened dolphins.

5.2.4 Statistical

Chemical concentrations were expressed as the range; average ± standard deviation, and reported as ng/g wet weight (ng/g ww). To prepare data for analysis, data below the LOQ were adjusted to ½ LOQ (Fenske et al., 2002; Lignell et al., 2009). Assumptions were tested with Levene's tests for homogeneity of variance and Shapiro–Wilk tests for normal distribution. Non-parametric equivalents were used as the assumptions were violated. Kruskal-Wallis H-test was conducted to test variations of statistical significance for bioaccumulation patterns among sites, niches, and feeding behavior. Spearman's rank coefficient was conducted to evaluate the role of ecological factors (Trophic level) and biological traits (average weight and length) in bioaccumulation of EDCs. Statistical tests results were considered significant at p-value <0.05, and <0.01. Values were log-transformed for linear model application to assess relationships between tissue contaminant concentrations and species trophic levels.

5.3 Results and Discussion

5.3.1 Bioaccumulation of Endocrine Disrupting Chemical classes in prey

The average concentration and prevalence (detection frequency, DF) of multi-class EDCs in prey of GD is presented in Figure 5-2. The dominant EDCs were DEHP (681.23-174990 ng/g ww) and DnBP (1218.07-146814.29 ng/g ww) accounting for 52.2% and 45.3 % of total EDC burden, respectively.

The concentrations of DEHP and DnBP were followed by Pb, DEP, DDTs, As, Cd, Hg, TCS, BPA, DMP, PCBs (PCB 8, 44, 180, 290), BBP, HCHs, and TCC whereas DnOP and other target PCBs were below the method detection limits (<DL) in all the samples.Among the investigated freshwater fish species, *C.latius* (As), *A.morar* (Cd, Pb), *L.rohita* (TCS), *C.garua* (Hg), *R.corsula* (DDTs, PCBs, HCHs, DEHP), *R.rita* (BPA), and *S.aor* (DnBP) *S.bacaila* (TCC), and *C.reba* (BBP) exhibited the highest contamination for various categories of EDCs.

While high EDC concentrations were generally observed in prey fish from S3, likely owing to the downstream influence of both the point-sources at S2 and non-point sources at S3 itself, a Kruskal-Wallis test revealed no significant ($p > 0.05$) variations across the three locations.

The subsequent section provides group-wise bioaccumulation potential of EDCs in prey of GD.

Persistent Organic Pollutants

Bioaccumulation potential of three persistent organochlorines-DDT, HCH, and PCB on the GD prey were explored in this study (Figure 5-2), with DDT (98.7%) notably showing significant ($p<0.05$) predominance, then ΣPCBs (0.97%) and ΣHCHs (0.32%).

The observed variances in the bioaccumulation patterns are consistent with a previous study on GD and its prey in the Ganga River (Kannan et al., 1994), where DDTs were noted as the highest residue in GDs (~1300 ng g^{-1} wet weight), significantly surpassing PCBs and HCHs by a factor of 16.

The ΣDDTs concentration in the fish, collected from all three locations ranged from 2.877-3129.80 ng/g (368.53±634.67 ng/g ww),with p,p' DDT ($p<0.001$) as the major contributor (76%) towards ΣDDT load, followed by p,p' DDD (16%) and p,p' DDE (8%). Notably, DDT concentrations in GD prey in our study are twice as high as those reported by Kannan et al. (1994), suggesting a potentially high accumulation in this apex predator, particularly considering their limited metabolic capacity for DDTs.

The DDE/DDT ratio were consistently low (0.03-0.4) in prey fish suggesting recent DDT inputs (Yogui et al., 2003). Furthermore, DDD being the second most abundant isomer suggests anaerobic degradation or dechlorination of DDTs in the environment (Hitch et al., 1992). The use of DDT in agriculture has been banned in India since 2006, with its application restricted to public health programs aimed at controlling vectors of malaria, dengue, kala-azar, and similar diseases. Nevertheless, despite restrictions, fresh DDT input persistently appear in various aquatic compartments of Ganga River, prompting apprehensions about potential illicit DDT use in the catchment (Sah et al., 2020).Further, the application of DDT in indoor residual spraying for the control of disease vectors seems to represent another potential source of DDT inputs into freshwater environments (Sah et al.2020). An increased surveillance and regulatory measures are imperative to guarantee the prudent and effective utilization of DDT in disease vector control programs.

The ΣPCBs concentrations in the all the fish samples ranged from <DL-10.47 ng/g ww (3.65±2.20 ng/g ww). Notably, ΣPCBs were detected in prey fish species with concentrations almost 100 orders of magnitude lower ($p < 0.001$) than ΣDDT, and twice as high ($p < 0.001$) as ΣHCHs. In India, PCBs are highly regulated under the Environment Protection Rules (CPCB, 2016), which prohibit the manufacture, import, export, and use of equipment containing PCBs (CPCB, 2016).

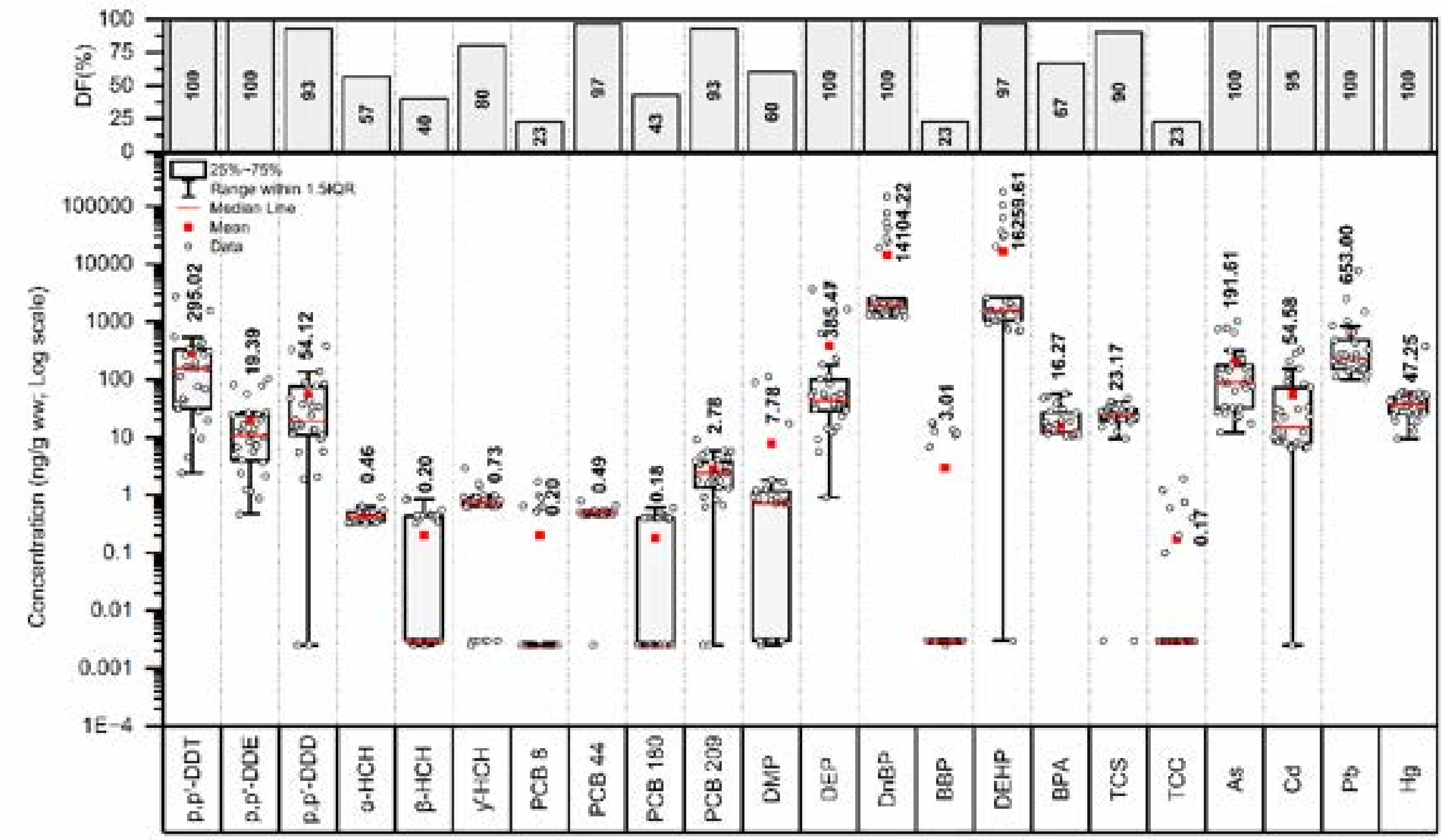


Figure 5-2: Average concentration (ng/g ww; log scale) and detection frequency (DF%) of EDCs in prey of GD

The dominant PCB was PCB-209 (69%) followed by PCB-44 (20%), PCB-8 (6%), and PCB 180 (6%), while all other investigated PCBs were below detection limits (<DL). The potential sources of PCB-209 and other have been reported with the inadvertent or unintentional formation in specific organic pigment categories, including phthalocyanine green, dioxazine, azo, isoindolinone (PCB 8), monoazo (PCB 8), Titanium dioxide (PCB 209, and 180) and polycyclic pigments (Hu et al., 2009; Jahnke & Hornbuckle, 2019; Hannah et al., 2022).The study area, particularly Site 2, is a prominent textile hub in the country, appears to be the primary source of unintentionally produced PCBs (UP-PCBs) attributed to the utilization of pigments in its manufacturing processes. Other potential inadvertent sources for the detected UP-PCBs include paint used on boats, printed material, and newsprint (Stone, 2014). The presence of PCB 44, which undergoes dechlorination in anaerobic conditions (Rodenburg et al.,2015), might be further explained by the high effluent load entering through three drains in S2. A similar pattern for PCB 44 has also been identified in other Indian rivers (Rex & Chakraborty, 2022).

Based on the findings of this study and the low metabolic capacity of GDs towards PCBs (Kannan et al., 1994), it is recommended to consider UP-PCBs (including PCB-11, 52), in routine monitoring programs. Additionally, further confirmation studies in the fields of toxicology, environmental monitoring, and chemistry are needed to comprehensively understand the dynamics and impacts of UP-PCBs in GD and its habitats.

The ΣHCH concentrations, in prey fish ranged from <DL to 4.689 ng/g ww (1.22±0.94 ng/g ww). The γ-HCH (50%) concentration was major contributor towards ΣHCH, followed by α-HCH (25%) and β-HCH (25%), while δ-HCH levels in all fish species were <DL (Figures 5-2).

Compared to DDTs and PCBs, HCH concentrations were notably low, a trend attributed to their low usage, relatively high volatility, lower bioconcentration factors, limited bioaccumulative capacity, and relatively biodegradable nature in the aquatic food chain (Kannan et al., 1994; Yogui et al., 2003). India banned technical HCH in 1997 (a mixture of α-HCH=~70%, β-HCH= 5 to 12%, γ-HCH=10 to 15%, and δ-HCH=6 to 10%), whereas lindane (~99% γ-HCH) was banned for manufacturing, import, or formulation in 2011 and for use in 2013 (ICAR-IISR, 2018).

Furthermore, the α-HCH/γ-HCH ratio, with values typically ranging from 3 to 7 in technical grade HCH, is used to assess the nature of HCH contamination with ratios below 3 indicating recent inputs of Lindane or γ-HCH. Interestingly, compared to previous studies (Kanan et al, 1994; Senthilkumar et al., 1999), the HCH isomeric patterns in the present study were observed to be that of lindane (α-HCH/γ-HCH<3) rather than the technical HCH formulation. This finding aligns with our previous study conducted on the surface waters of Ganga River (Sah et al, 2020), where high concentrations of γ-HCH were observed. It suggests an association with the illicit use of this chemical in paddy and wheat fields during the flowering season, as well as in other dry riverbed cultivation practices (Sah et al, 2020).

Despite lower concentrations of PCBs and HCHs compared to DDTs, the combined effects of these persistent organochlorine mixtures on exposed biota can be more complex than expected, potentially leading to compromised health and population-level consequences (Van Bressem et al., 2009; Ylitalo et al., 2005). Additionally, the high transfer rate (~60%) of organochlorine residues from mother to offspring, a characteristic unique to cetaceans, presents a considerable risk, especially to calves (Tanabe et al., 1991; Subramanian et al., 1988). Hence, despite regulatory measures and improvements in freshwater conditions, the persistence of these organochlorines could lead to enduring multi-generational toxic effects on dolphin populations.

Plastic Additives

The cumulative concentration of 6 PAEs (∑PAEs) in prey of GD ranged from 1581.21 to 254266.28 ng/g ww (30760.09±65887.92 ng/g ww). DEHP and DnBP were the predominant (DEHP-53%; DnBP-46%) and prevalent (DF(DEHP)-100%; DF(DnBP)-100%) phthalates in all the fish samples, driven by their widespread production and usage, with environmental dispersion occurring at every stage of their life cycle. BPA concentrations in prey fishes varied from <DL to 56.66 ng/g ww, (16.26±16.75 ng/g ww; DF:67%). Compared to phthalates, BPA is less prevalent in the environment likely due to its chemical embedment within products, which poses challenges for direct volatilization and leaching (Vasiljević et al., 2021).

Our results are consistent with recent findings by Chakraborty et al. (2021), which reported dominance of DEHP and DnBP in the surface waters of the MGR and the highest concentration of BPA (4.46 µg/L) in Varanasi along the Ganga River.

The densely populated catchment area of the MGR suffers from inadequate solid waste management, which can lead to leaching of plastics additives due to extensive dumping of plastic water bottles, single-use polyethylene bags, and food packaging materials (UPPCB, 2019, Chakraborty et al., 2021; Chakraborty et al., 2022). Additionally, the high tourist influx may inadvertently contribute to littering, either from unawareness or a lack of proper disposal options, further straining the local waste management infrastructure, particularly during peak seasons.

The textile industries in the catchment also appear to be a potential source of phthalates. These industries use various classes of chemicals, including phthalates, throughout their manufacturing processes, potentially leading to the discharge of these chemicals into the MGR (UPPCB, 2019).

Household and Personal Care Products (HPCPs)

Commonly found in a variety of HPCPs, including hand soaps, toothpaste, detergents, plastics, and cosmetics, TCC and TCS are two polychlorinated aromatic antimicrobials that have been in use for decades. The escalating demand and production of these chemicals unavoidably lead to their ubiquitous presence in various environmental compartments (Camino-Sanchez et al., 2016; Chen et al., 2019; Larsson et al., 2017; Vimalkumar et al., 2018).

The ∑HPCPs concentration detected in fish samples ranged from <DL to 47.05 ng/g ww (23.17±10.99 ng/g ww). Interestingly, TCS was recorded to be the predominant (99.26%) and prevalent (90%) HPCP in all the fish samples compared to TCC (DF:23%) as shown in Figure 5-2. The variation in concentrations between the two antimicrobial agents could be linked to their distinct consumption patterns, chemical characteristics, and degradation rates in environmental matrices.

Triclosan is commonly found in liquid soaps, whereas triclocarban is mainly used in solid soap bars, which could account for their varying presence. Additionally, our sampling time (March, 2021) coincided with the peak of the COVID-19 pandemic when enhanced hygiene practices spurred increased demand for liquid soaps, typically high in triclosan (European Commission, 2024). This surge likely led to significant triclosan discharge into aquatic systems, contributing to the observed bioaccumulation and variations.

Potentially Toxic Elements (PTEs)

PTEs such as Pb, Hg, Cd, and As are recognized as EDC in both humans (Zhang et al., 2004; Chattopadhyay & Ghosh, 2010; Sapmaz-Metin et al., 2017), and aquatic mammals (Freeman & Sangalang, 1977; Schaefer et al., 2011; Thomas et al., 2021; Guo et al., 2023).

In the present study, Pb was the most dominant PTE with concentrations ranging from 100-7550 ng/g ww. In order of decreasing rank, concentrations of Pb were followed by As (12-1007 ng/g ww), Cd (0.25-322 ng/g ww), and Hg (9.4-374 ng/g ww) as presented in Figure 5-3. Similar bioaccumulation patterns of these PTEs have also been noted in the tissue of the GD (Kannan et al., 1993).

The accumulation of Cd, and Pb in fish samples points towards their industrial origins, including industries like textile dyeing, electroplating, metallurgy, etc. Additionally, the presence of busy national highways and heavy boat traffic at all monitoring stations, along long-range atmospheric transport of Pb, suggests contributions of lead emissions from both land-based and river activities (Das et al., 2018).

PTEs such as Cd, Cu, and Pb are also present in small quantities within chemical additives used in plastics, to enhance the functional and aesthetic attributes of these materials (Hahladakis et al., 2018). The incorporation of PTEs in these additives, coupled with the improper utilization, recycling, and disposal of plastics, raises concerns about the potential unintentional release of PTEs into freshwater environments (Hahladakis et al., 2018). The accumulation of Cd, and Pb in fish samples points towards their industrial origins, including industries like textile dyeing, electroplating, metallurgy, etc. Additionally, the presence of busy national highways and heavy boat traffic at all monitoring stations, along long-range atmospheric transport of Pb, suggests contributions of lead emissions from both land-based and river activities (Das et al., 2018).

5.3.2 Relationships of EDCs concentrations with ecological, and biological factors

A Spearman's correlation analysis indicates a significant direct relationship between DDTs ($p<0.01$) and Hg ($p<0.05$) accumulation with trophic level (Figure 5-3). In contrast, As ($p < 0.05$) show an inverse relationship with the trophic level of the species. These results are further supported by linear modelling (Figure S4) that revealed significant effect of trophic levels on the accumulation of DDTs ($R^2 = 0.24$), As ($R^2 =0.22$) and Hg ($R^2 = 0.14$) in fish tissues, indicating biomagnification for DDTs and Hg, but trophic dilution for As. Biomagnification of DDT and mercury in the riverine food web of the present study, is consistent with observations by other authors (Deribe et al., 2013; Ikemoto et al., 2008). As has been reported to dilute through the food chain, owing to the ease of oxidation of As(III) to As(V) compared to the methylation of accumulated As in organisms with increased trophic levels (Huang, 2016). No significant relationships were observed for PCBs, DEP, Cd, and, Pb, whereas non-linear relationship between trophic levels of the species and HCHs, BPA, DnBP and DEHP. The findings suggests that contaminant dynamics may not always be simple enough to be explained by linear regressions and simplification by process selection, and assumptions of equilibrium in predictive models can considerably affect the reliability and predictive power of models (Borgå et al., 2004).

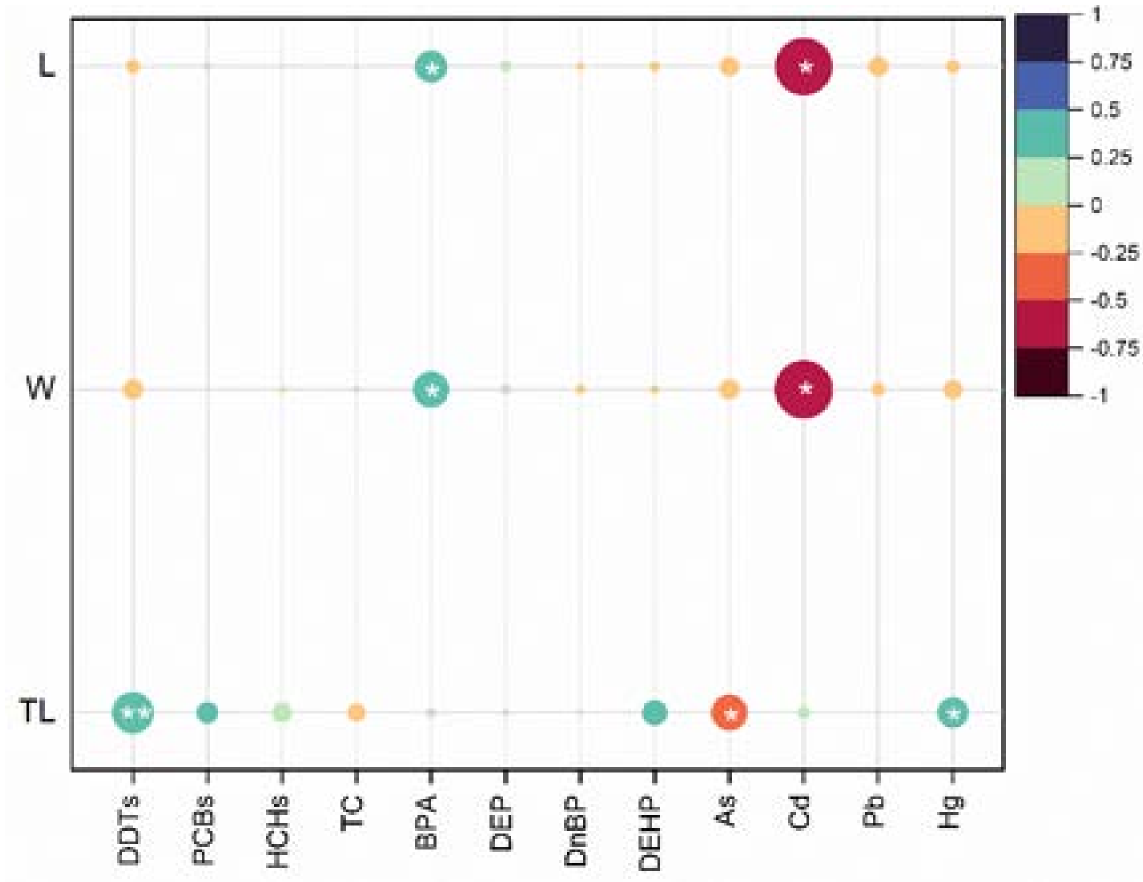


Figure 5-3: Correlation of tissue EDCs concentrations with ecological factor

(TL:Trophic level) and biological traits (W: Average weight; L: Average Length)
*Correlation significant at *=p<0.05; **=p<0.01*

Additionally, as bioaccumulation and biodilution depend on various factors including local food webs and environmental factors, thus further investigations are necessary for effective modeling, prediction, and risk assessment. A Spearman's correlation (Figure 3) analysis indicates a significant correlation with BPA and Cd. BPA accumulation shows positive relationship with average length and weight, consistent with the results Zhou et al. (2018). Knowledge gaps pertaining to the relationship of BPA accumulation and size of the species, however, need to be addressed to get a clearer picture of its risk to piscivores with specific prey size preferences. Conversely, studies on other phenolic compounds show decreasing toxicity with increasing fish size (Gupta et al., 1982). Cd shows an inverse relationship with both length and weight of the species. Similar relationships have been observed in previous studies, and especially in smaller individuals (Cutshall and Pearcy, 1977). The higher significance of smaller individuals for the accumulation and toxicity of these compounds could potentially increase the contamination risk to GD, whose prey preference is limited by size.

It is important to consider that both length and weight may serve as proxies for various factors such as species, sexual maturity, health, diet, and habitat quality, thus a thorough investigation is warranted to understand such dynamics (Richter et al., 2000; Moutopoulos and Stergiou, 2002).

5.3.3 Relationships of EDCs concentrations with niche and feeding behaviour

A significant difference ($p<0.05$) in bioaccumulation of DEP and DEHP was observed between pelagic and benthopelagic fish (Figure S2). A significant variation was also observed in As accumulation between pelagic and benthic, and benthopelagic and benthic species. For all three of these contaminants, pelagic fish species showed the highest bioaccumulation, indicating their uptake from organisms of the pelagic food web such as algae and zooplankton (Mackintosh et al., 2004; Soto-Jiménez et al., 2023; Liu et al., 2024).

With regards to feeding behaviour, As was the only EDC that showed a significant ($p<0.05$) variation in accumulation between herbivorous, carnivorous and omnivorous species (Figure S3). Omnivorous species accumulated higher concentrations of As on average, whereas carnivores reported the lowest concentrations2. As discussed in the previous section, this may be owed to the ease of oxidation compared to methylation of As in carnivores (Huang, 2016).

5.3.4 Risk assessment of EDCs exposure to GD

The current study relies on the dietary tissue residue guidelines method to assess the health risks that GD may encounter due to exposure to EDCs. As toxicological data were only available for a select group of EDCs, risk assessments were not carried out for those lacking such data.

A summary of the calculated Risk Quotients (RQs), based on MAC_{RfD} and MAC_{TRV}, that selected categories of EDCs in prey-fish species may pose to GD, is provided in in Figure 5-4 a-d.

In general, the average risks associated with EDCs, as assessed by MAC_{TRV}, was consistently less than 1, with all RQs falling within the range of <0.001 to 0.51. The average risk associated with dietary exposure to DEHP and As was noted to be moderate (Figure 4a), with data from the 95th percentile indicating high risk for both EDCs (Figure 4c).

The RfD, commonly utilized in human health risk assessment, offers a more robust and conservative evaluation of potential adverse health effects associated with exposure to environmental contaminants, incorporating higher safety factors for added protection to threatened species.

Based on the MAC_{RfD}, DEHP > DDT > DnBP > As> PCBs > Hg > Cd, demonstrated a high average potential risk (RQ = 2.72-30.84) to GD through dietary intake whereas HCHs revealed moderate risk (RQ=0.17). The substantially high RQs can be attributed to both the high toxicity of the chemicals and their widespread usage within densely populated, agriculturally dominated and industrially active catchment area. In the present study, the RQ values for TCS, BPA, and DEP remained below 1, indicating their low potential to pose a risk to GD.

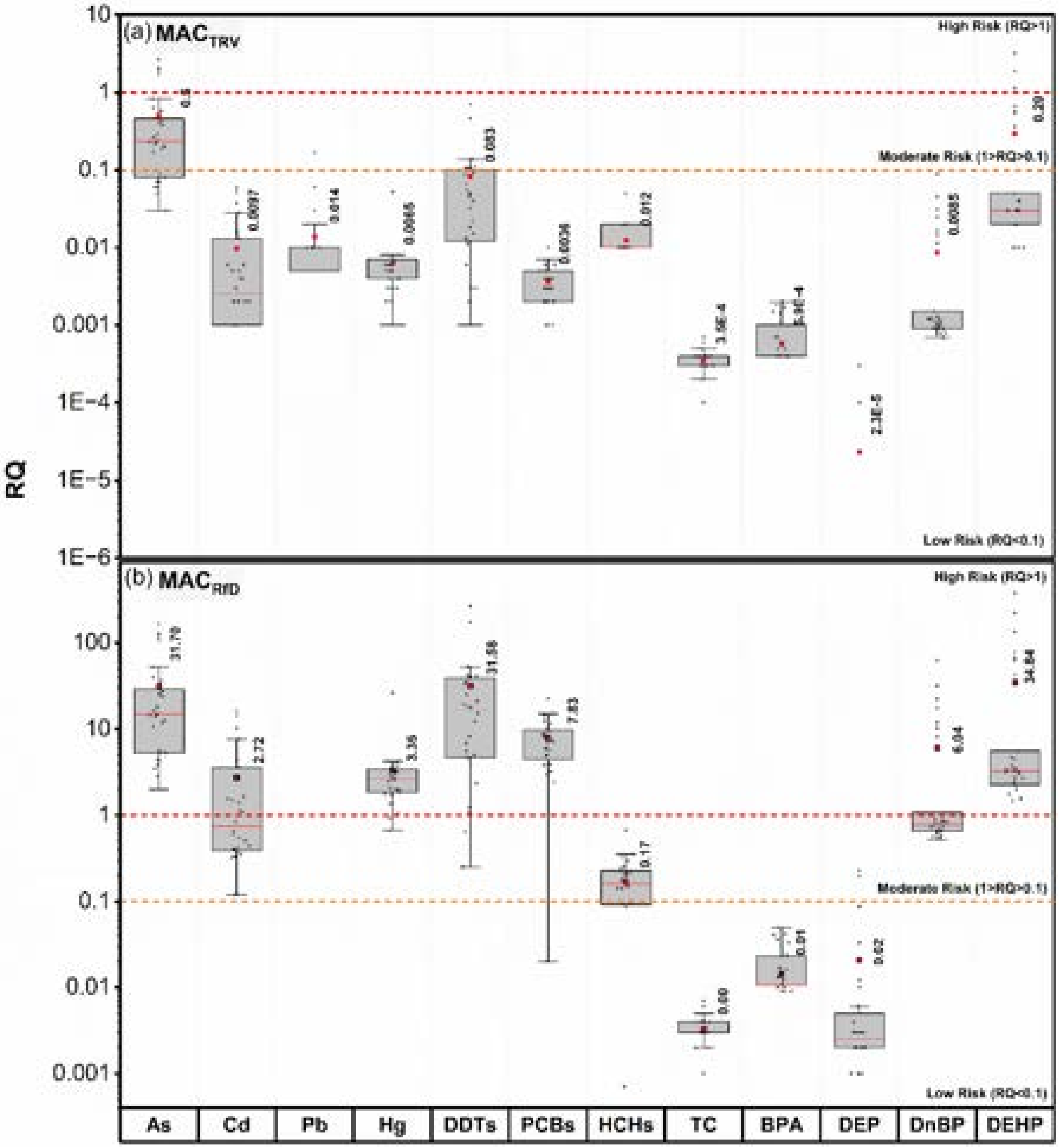
Considering a worst-case scenario based on 95th percentile data, the RfD-based RQs for DEHP, DDT, and As exceeded 100, while ranging from 12.41 to 27.65 for DnBP, PCBs, and Cd.

To date, there are no known studies specifically investigating the dose-response relationships of contaminants on Gangetic dolphins. Hence, given the known health impacts of EDCs in other aquatic mammals including cetaceans, we can anticipate potential impacts on Gangetic dolphins.

The substantially high RQ_{RfD} for DEHP and DnBP may pose an elevated risk to these species, as phthalates and their metabolites can potentially affect thyroid function (Dziobak et al., 2022) and cause lipid disruption (Xie et al., 2023) in cetaceans.

Similarly, persistent organochlorines such as DDTs and PCBs have serious implications for cetacean health and populations around the world through endocrine disruption, carcinogenicity, cytotoxicity, reproductive impairments and immunosuppression (Jepson et al., 2016; Galligan et al., 2019; Yu et al., 2019; Simond et al., 2019; Guo et al., 2023). PCBs and their alternatives have been recorded to significantly raise tetraiodothyronine, testosterone, and cortisol levels of the Indo-Pacific finless porpoise (Guo et al., 2023) and have been also associated with the population collapse of the common seal (Reijnders, 1986), and Killer whales (Orcinus orca) (Hickie et al., 2007; Hall et al., 2018; Desforges et al., 2018). Additionally, the high tendency of these organochlorines for transplacental transfer raises further concerns about their potential to exert toxic effects on foetal growth and development (Borrell and Aguilar, 2005).

PTEs such as Hg, Cd, As and Pb have been associated with various immunotoxic and neurotoxic effects in aquatic mammals (Desforges et al., 2016; López-Berenguer et al., 2020). In St. Lawrence Beluga Whales, individuals with high Hg and Pb concentrations were also observed to have chronic lesions and reproductive impairment (Béland et al., 1992). Although the current study indicates a low risk associated with TCS, TCC, and BPA, their known toxicity to coastal cetaceans (Hinther et al., 2011; Lunardi et al., 2016; Guo et al., 2023), along with their high production and widespread use, justifies their inclusion in regular monitoring programs as a proactive measure.



(a) MAC_TRV
High Risk (RQ>1)
Moderate Risk (1>RQ>0.1)
Low Risk (RQ<0.1)
(b) MAC_RfD
High Risk (RQ>1)
Moderate Risk (1>RQ>0.1)
Low Risk (RQ<0.1)
RQ
As
Cd
Pb
Hg
DDTs
PCBs
HCHs
TC
BPA
DEP
DnBP
DEHP

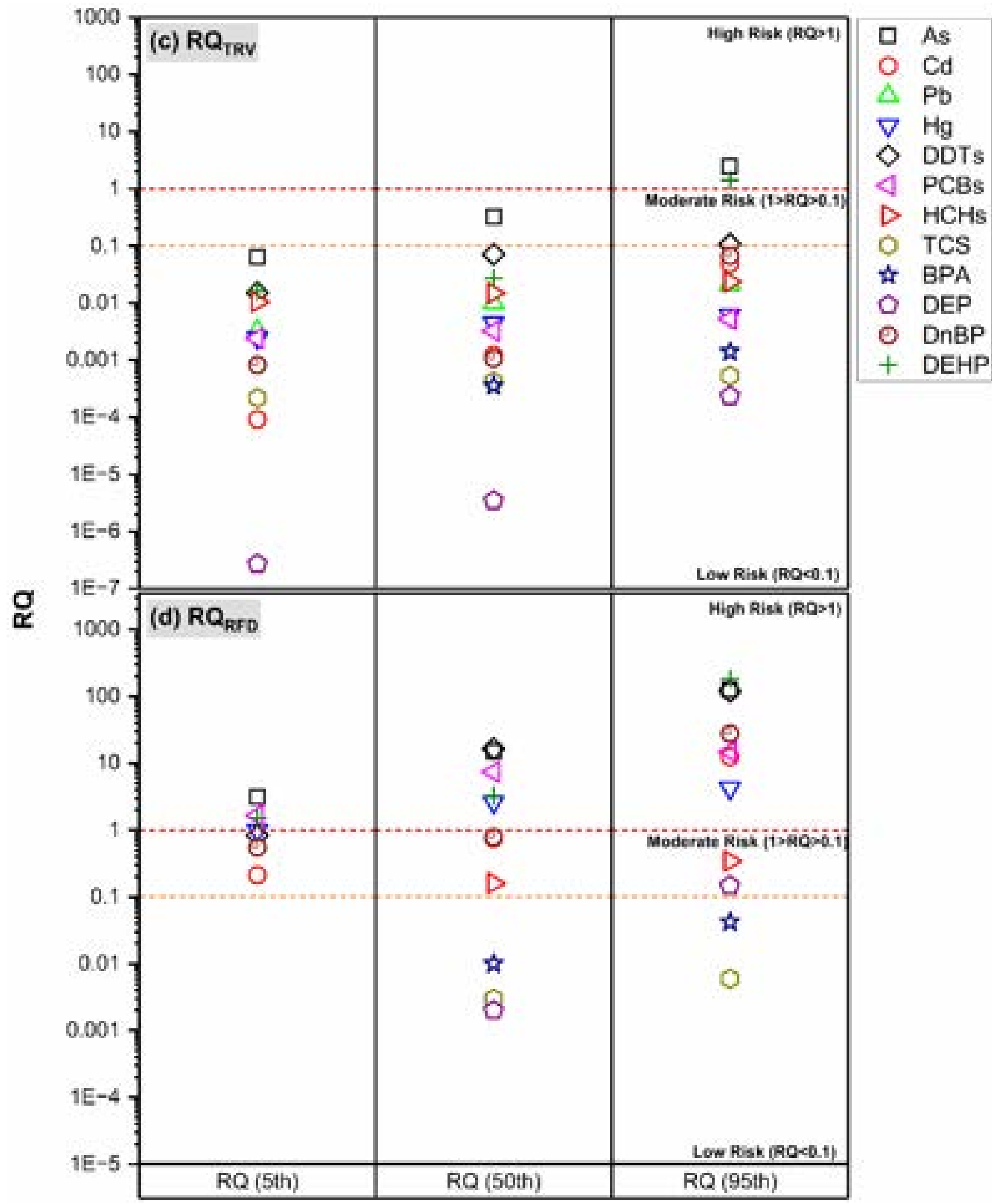


Figure 5-4: Risk assessment (RQ-log scale) of EDCs to GD MAC$_{TRV}$ and MAC$_{RFD}$ based on average concentrations (a-b) and 5th, 50th, and 95th percentile data (c-d)

Considering the significant risks that these EDCs pose to GD, as identified in this study, alongside their adverse effects on other aquatic mammals, it is imperative to deepen our understanding of these threats to GD for effective conservation.

A pivotal first step in addressing the threats from these EDCs could involve establishing a robust monitoring program to assess the levels and effects of these EDCs in GD and their habitat. Such a program would lay the groundwork for informed, effective conservation actions and policy development aimed at reducing pollution and protecting this umbrella species and its ecosystem.

5.4 Way Forward: Issue-based monitoring recommendations to strengthen existing GD conservation program

While marine cetaceans have greatly benefited from monitoring programs targeting chemical pollution threats (Kuiken & Hartmann, 1991; Ijsseldijk et al., 2019; Fossi & Panti, 2021), there is a marked lack of such monitoring program for GD highlighting a significant oversight in current conservation efforts. This gap in monitoring is particularly concerning given that a high extinction risk often coincides with low availability of data, especially those pertaining to dose and response (Schaap et al., 2023). Addressing this research gap is paramount for devising comprehensive conservation strategies that effectively mitigate chemical pollution threats to species, thereby enhancing their chances for survival and stability.

To address this, we identified the issues with the current approach for monitoring chemical contaminants in GD and its habitats (Figure 5). Based on the identified issues, we propose an issue-based framework for monitoring and assessing chemical stressors affecting Gangetic dolphins and their habitats (Figure 6). This framework is designed to provide relevant data on exposure and risk assessments, which are imperative for formulating informed conservation strategies and policy interventions. Expanding on our earlier GD conservation guidelines (WII-GACMC, 2019), the proposed framework is a detailed strategy devised in collaboration with interdisciplinary experts. This tailored approach focuses on meeting the specific monitoring requirements of Gangetic dolphins and their habitats in response to the challenges posed by chemical pollution.

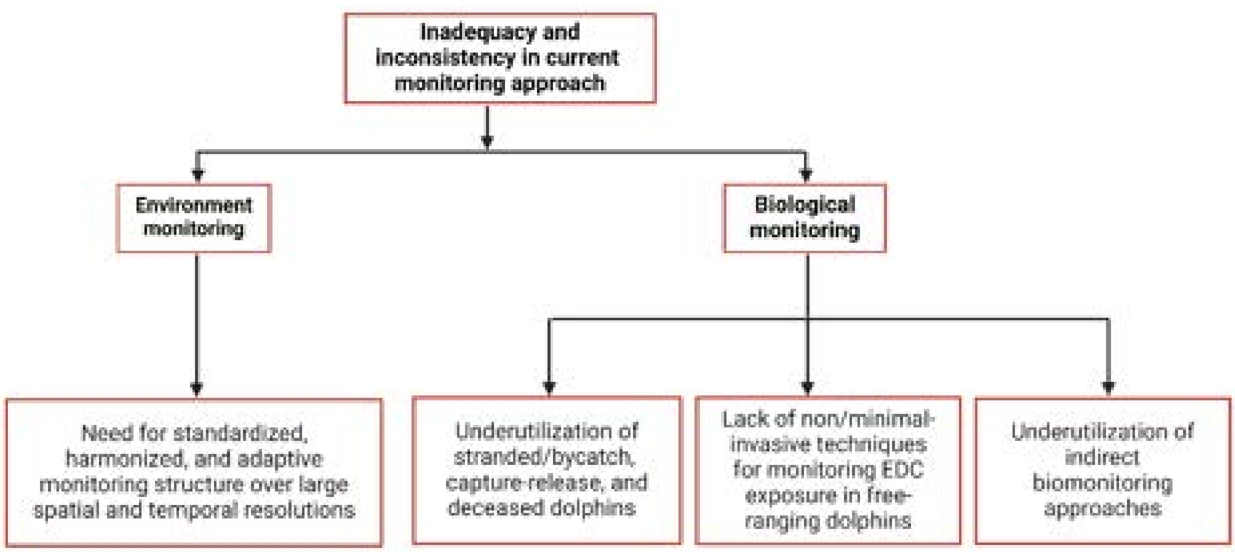


Figure 5-5: Identified issues in the current approach for monitoring chemical contaminants in Gangetic Dolphin and its habitats

Furthermore, the proposed framework's development is informed by valuable insights from successful monitoring programs, reports, and publications globally that focus on the threats posed by chemical pollution to aquatic mammals, particularly cetaceans (Benjamins et al., 2014; Nelms et al., 2021; Evans, 2011; Bevan, and Schneider, 2021; UK Cetacean Strandings Investigation Programme, 2023; Best Practice on Cetacean Post-Mortem Investigation and Tissue Sampling | ASCOBANS; Fossi & Panti, 2021; Sigmund et al., 2023; Hollender et al., 2023; Crimmins & Holsen, 2019;).

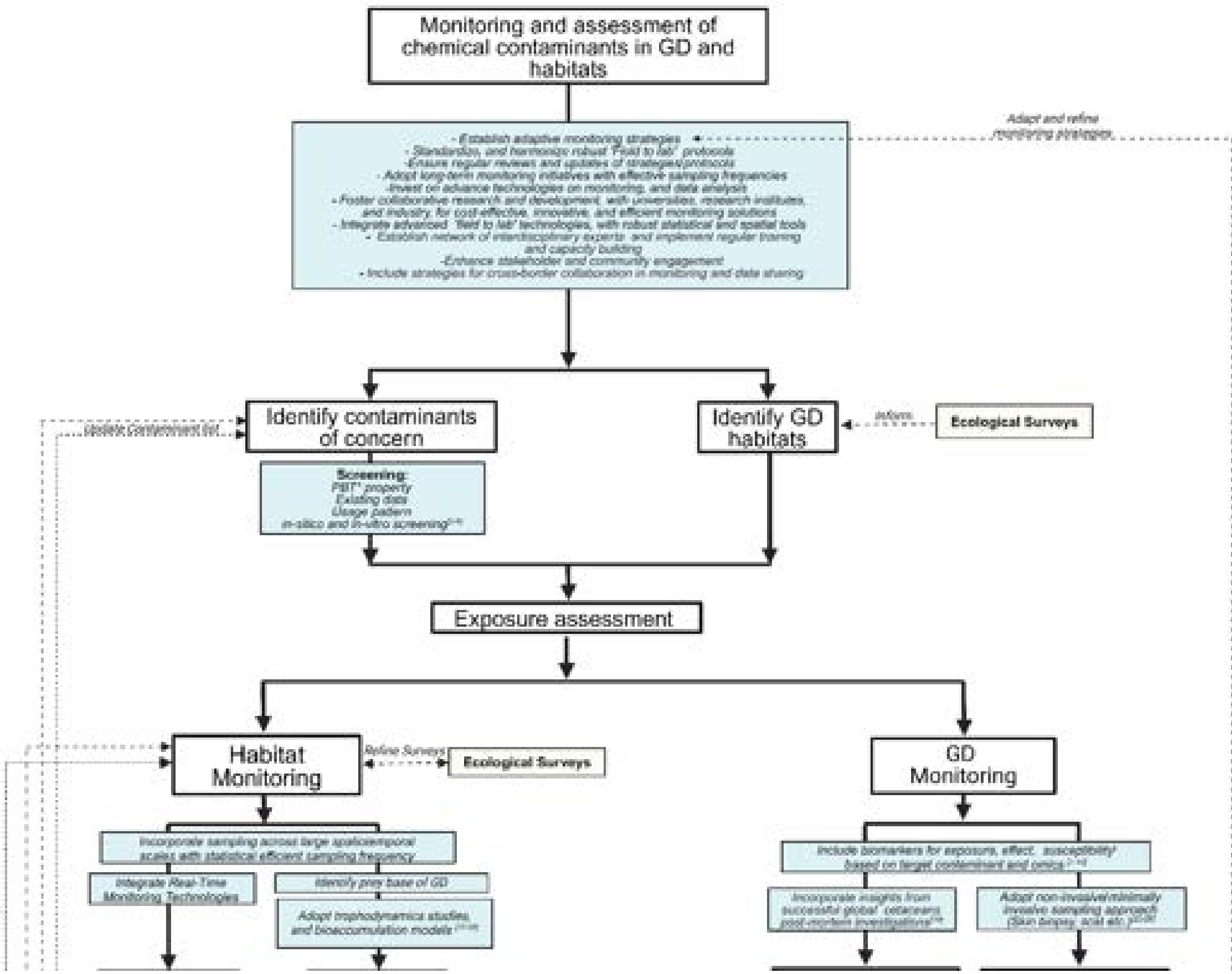
At each stage of this framework, specific prerequisites are identified and deemed necessary for the successful implementation of the framework. It is noteworthy that the framework is designed in such a way that its scope extends beyond riverine cetacean species other than those covered in the present study.

5.5 Conclusion

This study presents the first investigation into the health risks posed to Gangetic dolphins by multi-class EDCs.

Plastic additives, DEHP and DnBP, were the dominant EDCs in the prey fishes of GD. The study highlights the prevalence of unintentionally produced PCBs in the dolphin prey. Fresh inputs of p,p’ DDT, and γ-HCH in GD prey indicates the ineffectual compliance with regulatory standards and policies at the regional level.

Bioaccumulation of some EDCs in prey fish were found to be dependent on foraging behavior (As), niche (DEP, DEHP, As), trophic level (DDT, As, and Hg) and size (Cd and BPA). The trophic magnification, displayed by DDT and Hg, is particularly concerning due to its potential for severe impacts on apex predators like GD. Agricultural settlements, vehicular emissions, improper plastic disposal, textile industry, and high tourist influx are identified as the primary drivers of EDCs contamination in MGR. Implementation of stricter regulations on industrial discharges, improving wastewater treatment infrastructure, and promoting sustainable practices in agriculture and industry are essential to mitigate the sources of these contaminants and minimize their impact on GD and their habitats.


Monitoring and assessment of chemical contaminants in GD and habitats
Adapt and refine monitoring strategies
- Establish adaptive monitoring strategies
- Adopt long-term monitoring initiatives with effective sampling frequencies
- Integrate advanced "field to lab" technologies, with robust statistical and spatial tools
-Enhance stakeholder and community engagement
- Include strategies for cross-border collaboration in monitoring and data sharing
Identify contaminants of concern
Screening:
Existing data
Usage pattern
Identify GD habitats
Ecological Surveys
Exposure assessment
Habitat Monitoring
Refine Surveys
Ecological Surveys
Incorporate sampling across large spatiotemporal scales with statistical efficient sampling frequency
Integrate Real-Time Monitoring Technologies
Identify prey base of GD
Adopt trophodynamics studies, and bioaccumulation models
GD Monitoring
Include biomarkers for exposure, effect, susceptibility based on target contaminant and omics
Incorporate insights from successful global cetaceans post-mortem investigations
Adopt non-invasive/minimally invasive sampling approach

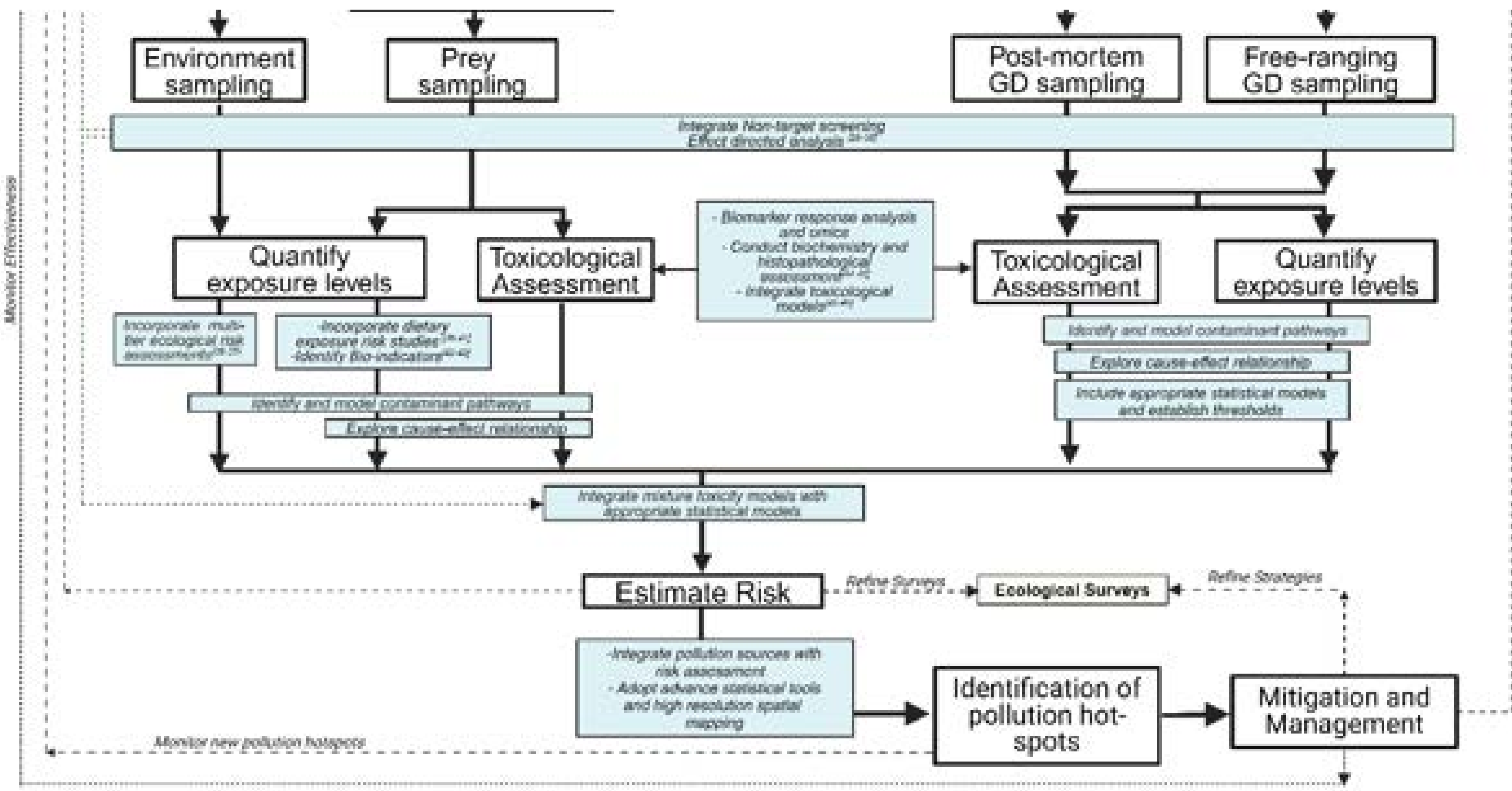


Figure 5-6: Proposed framework for monitoring chemical contaminants in Gangetic Dolphin and its habitats.

Green box represents the pre-requisite deemed necessary for the successful implementation of the framework; yellow box represents contributions from ecological survey (habitats, population dynamics, distribution range, biology, and behaviours)

References 1-49 are provided in Annexure.

A screening-level ecological risk assessment, utilizing both TRV and RfD, reveal varying levels of risk to GD through dietary exposure. While TRV-based risk for most EDCs were low, RfD-based RQ showed high risk to GD from DEHP, DDTs, DnBP, As, PCBs, Hg, and Cd. Given their known adverse effects on other mammalian species, especially cetaceans, these threats should not be overlooked. While the current study suggests a low risk associated with BPA, and TCS, TCC, considering their high production, widespread usage, and known toxicities, it is recommended to include these EDCs in regular, comprehensive monitoring initiatives as a proactive measure.

Efforts should focus on understanding the mechanisms of exposure, bioaccumulation, and toxicological impacts of EDCs on GDs and their habitats. Additionally, holistic investigations of EDCs and other contaminants of concern, across large temporal and spatial scales in GD and its habitats are also recommended. Furthermore, the framework proposed in this study has the potential to enhance GD conservation efforts.

Chapter 6 Assessing the potential of a gastropod (*F. bengalensis*) and bivalves (*L. marginalis*) as potential biomonitor of EDC contamination in effluent-impacted aquatic environments

Abstract

This chapter investigates the bioaccumulation of e-EDCs in two mollusc species, Filopaludina bengalensis and Lamellidens marginalis, from both upstream and downstream locations of an effluent-impacted stretch of the Ganga River. The results highlight the prevalence of plastic additives, such as DnBP, DEHP, and DEP, in the river, likely stemming from urban runoff, industrial discharges, and inadequate plastics waste disposal practices. The study reveals that both species exhibited significant bioaccumulation of EDCs, particularly downstream of a wastewater discharge site, indicating the influence of effluents on contaminant levels. *F. bengalensis* accumulated more sediment-bound contaminants, while *L. marginalis* primarily captured waterborne pollutants.

Additionally, the study emphasizes the importance of standardizing biomonitoring protocols to include these mollusk species in long-term environmental assessments. Expanding the geographic scope of future research and assessing the toxicological impacts of EDCs will provide deeper insights into the bioaccumulation dynamics in

freshwater ecosystems and help formulate better conservation strategies for species inhabiting polluted environments like the Ganga River.

6.1 Introduction

The concentrations of contaminants in aquatic environments can be quantified using direct analysis of both water and sediment samples (Superville et al., 2014).

However, this approach offers limited insight into the concentrations of bioavailable contaminants—those that can be absorbed and accumulated by living organisms. From an ecotoxicological viewpoint, bioavailable contaminants are particularly significant because they represent the portion that could ultimately pose health risks to aquatic organisms including apex predators (Rainbow, 1995; Soto et al., 1995). Biological monitoring complement assessments of water and sediment to provide a more accurate estimation of the risk contaminants pose to the habitat's biodiversity and ecosystem function (Li et al., 2009). However, the selection of an organisms for effective biomonitoring involves certain criteria, including their capacity to accumulate contaminants reflecting environmental contamination and bioavailability, a sedentary lifestyle reflecting geographical variations in contamination, high tolerance to pollutants allowing studies over wide ranges of concentrations, metabolic passiveness to preserve the form of the contaminants for ease of analysis, and ubiquity to enable studies and comparisons over large geographical scales (Sericano, 2000; Perez et al., 2019).

Molluscs, particularly gastropods and bivalves, are highly effective as bioindicators or biomonitors due to a unique combination of characteristics, making them ideal for assessing environmental quality including contaminant exposure and effects (Markert et al., 1999). Molluscs are also fairly large and easy to manage, making them well-suited for field and laboratory research. They are widely distributed across marine, freshwater, and terrestrial ecosystems, enabling broad geographical studies. Many species, are cosmopolitan, making them particularly useful in pollution studies. As keystone species, their health often reflects broader ecological impacts. Molluscs typically have limited mobility or are sessile, allowing for precise assessment of localized contamination. Their diverse reproductive strategies enable monitoring of specific contamination effects on reproduction. Some species have long lifespans, integrating environmental contamination over extended periods (Markert et al., 1999). Due to their direct contact with the environment and limited detoxification abilities, they bioaccumulate pollutants effectively, serving as sensitive early indicators. Additionally, their well-documented biology and ecology make them reliable for ecotoxicological studies and environmental monitoring.

Among the seven molluscan classes, gastropods account for over 80% of the species, while bivalves represent the majority of the remaining 15% (Oehlmann & Schulte-Oehlmann, 2003). In freshwater ecosystems, gastropods can make up to 50% of the benthic invertebrate biomass (Cummings et al., 2015), and are valuable for monitoring pollution in aquatic environments due to their widespread presence and significant role.

As primary consumers, they serve a crucial role in the food chain, functioning as grazers and detritus feeders (Côte et al., 2015) whereas their limited mobility allows site-specific pollution assessment (Smitha & Mustak, 2017). Additionally, their ability to bioaccumulate persistent pollutants provides insight into the behavior and potential risks of these contaminants in the ecosystem (Benali et al., 2022). These characteristics make gastropods promising candidates for use as early warning indicators of environmental degradation in freshwater habitats (Benali et al., 2022).

Similarly, bivalves are widely recognized as effective bioindicators in environmental monitoring due to their unique ability to bioaccumulate pollutants from their surroundings (Goldberg et al., 1978; Bayne et al., 1979; Zuykov et al., 2013). Their soft tissues and shells store contaminants over time, providing a comprehensive record of environmental pollution rather than just a brief snapshot. This characteristic is particularly valuable compared to direct environmental measurements, which may only capture temporary or localized conditions. The sedentary nature of bivalves allows them to reflect the specific environmental conditions of their location accurately, making them ideal for localized assessments (Goldberg et al., 1978; Sericano, 2000). Additionally, their high bioaccumulation capacity and broad distribution across various aquatic environments make them both easy to analyze chemically and accessible for widespread monitoring efforts (Tavares et al., 1988; Schöne & Krause, 2016). The Mussel Watch program exemplifies the value of bivalves as biomonitors. This program has effectively utilized bivalve mollusks to monitor a range of contaminants, including potentially toxic elements

(PTEs), pharmaceuticals, flame retardants, and surfactants (Apeti et al., 2018; Sericano, 2000; NCCOS, 2023).

Both bivalve and gastropod molluscs have also been successfully used to monitor contamination of Indian aquatic environments, from marine to freshwater habitats, including the Cochin backwaters (George et al., 2013), the Sunderban wetland (Sarkar et al., 2008), Kole wetland (Menon et al., 2023), and the Gomti river (Gupta et al., 2014), although most of these studies have been restricted to potentially toxic elements (PTEs). This constitutes a knowledge gap regarding the application of molluscs for monitoring contamination by EDCs in Indian aquatic systems.

The molluscs *Lamellidens marginalis* (a bivalve) and *Filopaludina bengalensis* (a gastropod) are extensively distributed across the Ganga River Basin (GRB). Their wide geographical presence, sedentary habits, and ability to accumulate pollutants position them as strong candidates for monitoring the spatial distribution of contaminants. However, there is a significant gap in research concerning the bioaccumulation of endocrine-disrupting chemicals (EDCs) in these species, as well as their potential use as biomonitors, particularly in river stretches affected by effluent discharge. This highlights the need to evaluate their effectiveness in tracking EDC contamination in such impacted environments.

Considering the above facts, the present chapter aims to investigate the potential of two benthic molluscs - *Lamellidens marginalis* and *Filopaludina bengalensis*, for biological monitoring of endocrine-disrupting chemical (EDC) contamination in an effluent-impacted stretch of the MGR.

6.2 Materials and methods

6.2.1 Study area, sampling sites, and sampling methodology

Sampling was conducted 1 km upstream (25°16'31.6560", 083°00'59.0400") and downstream (25°19'44.6880", 083°03'37.2240") of three drain discharges—Assi, Khirkia, and Varuna—within Varanasi city during March-April 2021 (Figure 6-1). A total of 70 freshly-caught *Lamellidens marginalis* (N=43) and *Filopaludina bengalensis* (N=26) were collected as part of routine fishing practices by local fishermen. The length and weight of the collected samples were recorded on-site. The samples were packed in precleaned aluminum-sealed bags and kept in an ice box for transportation to the laboratory, where they were stored in a deep freezer at -20°C until further processing. The identification of the samples was achieved by sending them to the Zoological Survey of India, Kolkata. The shells of the individual macrobenthos were removed, and the whole soft tissues of the same species from each site were pooled and homogenized in a customized stainless steel tissue homogenizer.

The target analytes, sample treatment and instrument analysis are same as studied in Chapter 5.

6.2.2 Statistical

Chemical concentrations were expressed as the range; average ± standard deviation, and reported as ng/g wet weight (ng/g ww). To prepare data for analysis, data below the LOQ were adjusted to ½ LOQ (Fenske et al., 2002; Lignell et al., 2009).

6.3 Results and Discussion

The results indicate significant variations in the concentration of several EDCs between the two species and across the sampling sites, reflecting the differential bioaccumulation capacities and the influence of effluent discharge on contaminant levels (Figure 6-2 and Table 6-1). p,p'-DDT, BBP, E2 and TCS were not detected in the gastropod species in the upstream site, while BBP, E1, E2, E3, TCS and TCC were not detected in the bivalve species at the same site, indicating an inter-species variation in accumulation of these contaminants. All compounds, except for BBP and E2, were detected in *F. bengalensis* at the downstream site, whereas E1 remained undetected in *L. marginalis* at the same site.

Filopaludina bengalensis, a gastropod species, exhibited substantial bioaccumulation of various EDCs at both sites. Upstream of the drain, p,p' DDT was below detection limits (<DL), whereas downstream, it was detected at a concentration of 7.78 ± 0.22 ng/g. p,p'-DDE was present at 0.84 ± 0.12 ng/g upstream and increased to 1.967 ± 5.64 ng/g downstream.

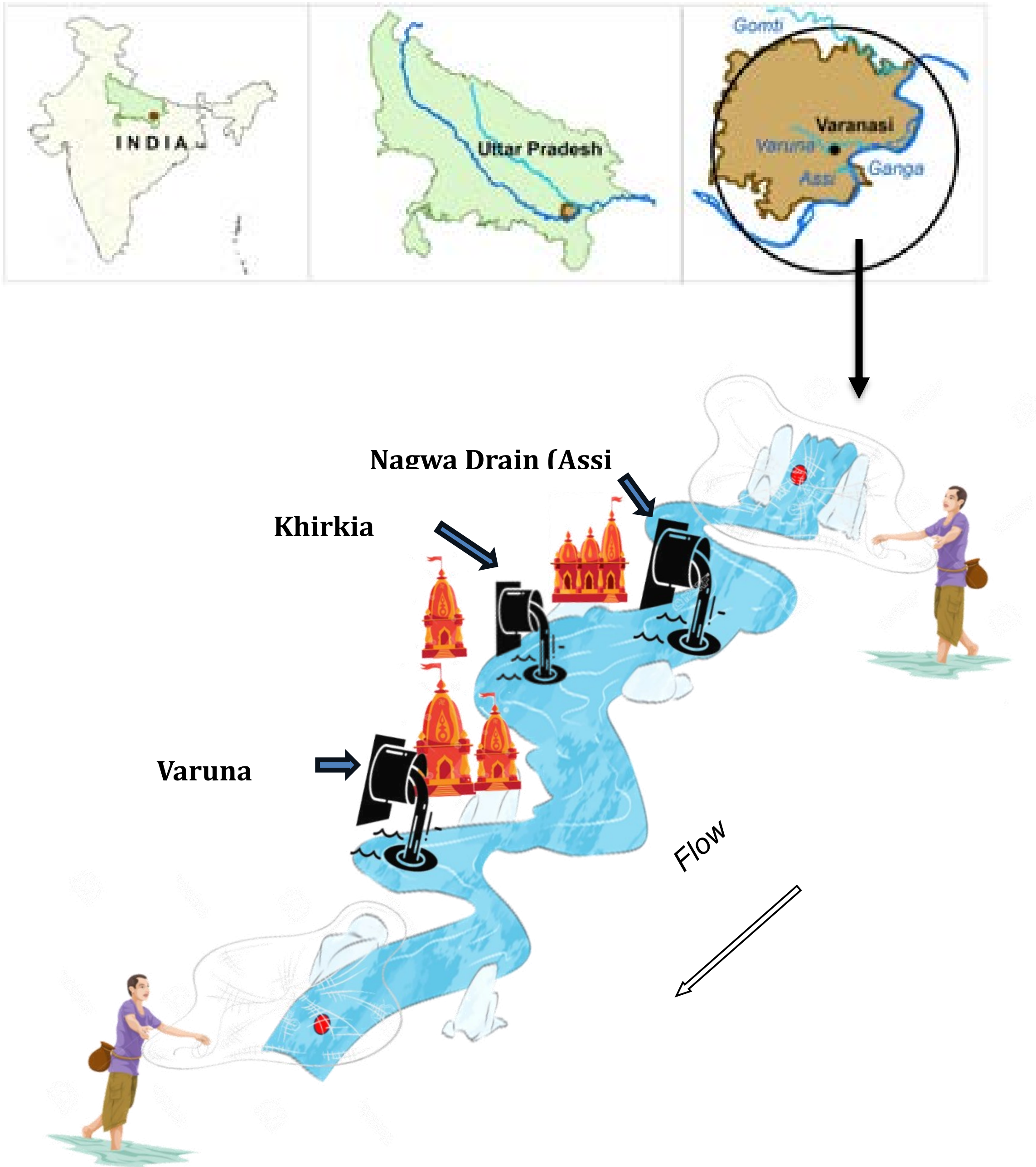


Figure 6-1: Study area and sampling sites (red dots)

Plasticizers such as DMP and DEP were detected at significantly higher concentrations, with DMP at 275.09 ± 2.45 ng/g upstream and decreasing to 101.4 ± 10.11 ng/g downstream, while DEP increased from 8469.32 ± 457 ng/g to 11796.64 ± 1156 ng/g downstream. Notably, the phthalate DnBP showed a substantial increase from 49451.76 ± 789 ng/g upstream to 280599.02 ± 4557 ng/g downstream, highlighting the impact of effluent discharge on contaminant levels.

DEHP, another significant plasticizer, was recorded at 21345.96 ± 1449 ng/g upstream and showed a notable increase to 193380.44 ± 1089 ng/g downstream. BPA, a common component in plastics, showed a moderate increase from 1.76 ± 0.45 ng/g upstream to 2.89 ± 0.84 ng/g downstream. Estrogens, including E1, E2, and E3, showed varying detection patterns, with E1 detected at low levels (0.58 ± 0.05 ng/g upstream and 0.685 ± 0.13 ng/g downstream), while E2 was undetected upstream but appeared at 1.88 ± 0.22 ng/g downstream. E3 increased from 1.53 ± 0.15 ng/g upstream to 2.42 ± 1.31 ng/g downstream. Triclosan (TCS) and Triclocarban (TCC) showed significant increases downstream, with TCS increasing to 3.54 ± 0.99 ng/g and TCC from 0.52 ± 0.11 ng/g upstream to 9.19 ± 2.12 ng/g downstream. Polychlorinated biphenyls (PCBs) such as PCB 8, PCB 44, and PCB 209 were also detected at higher concentrations downstream, reflecting the accumulation of these persistent organic pollutants in the aquatic environment.

The bivalve species *Lamellidens marginalis* also demonstrated varying levels of EDC accumulation between upstream and downstream sites. p,p' -DDT was detected at 1.838 ± 0.51 ng/g upstream, increasing to 6.97 ± 1.08 ng/g downstream, while p,p'-DDE remained relatively stable, with a slight increase from 0.945 ± 0.11 ng/g upstream to 1.721 ± 0.6 ng/g downstream. DMP and DEP showed substantial differences, with DMP increasing from 0.332 ± 0.09 ng/g upstream to 49.513 ± 3.33 ng/g downstream, and DEP from 27.069 ± 3.22 ng/g upstream to 2352.292 ± 12.56 ng/g downstream. DnBP, a major phthalate, exhibited a dramatic increase from 1870.052 ± 25 ng/g upstream to 353543.513 ± 255 ng/g downstream, underscoring the influence of effluent discharge on contaminant bioavailability.

DEHP concentrations were notably higher downstream (102311.907 ± 559 ng/g) compared to upstream (1096.291 ± 58 ng/g). The levels of BPA, a known estrogen mimic, showed a minor increase from 1.14 ng/g upstream to 1.26 ± 0.09 ng/g downstream. Estrogenic compounds displayed varying accumulation patterns, with E1 and E2 undetected upstream but present downstream at 0.685 ± 0.13 ng/g and 1.854 ± 0.48 ng/g, respectively. E3 was detected only downstream at 3.1 ± 0.86 ng/g. TCS and TCC showed increased levels downstream, with TCS at 2.83 ± 0.19 ng/g and TCC at 6.16 ± 0.49 ng/g, reflecting the increased input of these compounds from the effluent. PCBs also followed a similar pattern, with higher concentrations detected downstream.

The results from this study clearly demonstrate that both *Filopaludina bengalensis* and *Lamellidens marginalis* accumulated higher concentrations of most endocrine-disrupting chemicals (EDCs) downstream of the drainage site, underscoring the significant impact of effluent discharge on contaminant levels in the Ganga River. This finding aligns with previous research, which has shown that effluent discharge from industrial and urban sources is a major contributor to the presence of EDCs in aquatic environments (Kolpin et al., 2002; Li et al., 2010).

Table 6-1: Concentration profiles (ng/g) of EDCs in *Filopaludina bengalensis* and *Lamellidens marginalis*

Detected EDCs	Concentration ng/g			
	F.Bangalensis		***L.marginalis***	
	Upstream	Downstream	Upstream	Downstream
DDT-p,p'	<DL	7.776±0.22	1.838±0.51	6.97±1.08
DDE-p,p'	0.84±0.12	1.967±5.64	0.945±0.11	1.721±0.6
DMP	275.09±2.45	101.4±10.11	0.332±0.09	49.513±3.33
DEP	8469.32±457	11796.64±1156	27.069±3.22	2352.292±12.56
DnBP	49451.76±789	280599.02±4557	1870.052±25	353543.513±255
BBP	<DL	<DL	<DL	226.011±11.65
DEHP	21345.96±1449	193380.44±1089	1096.291±58	102311.907±559
BPA	1.76±0.45	2.89±0.84	1.14	1.26±0.09
E1	0.58±0.05	0.685±0.13	<DL	<DL
E2	<DL	1.88±0.22	<DL	1.854±0.48
E3	1.53±0.15	2.42±1.31	<DL	3.1±0.86
TCS	<DL	3.54±0.99	<DL	2.83±0.19
TCC	0.52±0.11	9.19±2.12	<DL	6.16±0.49
PCB 8	2.455	6.54±1.82	0.44±0.06	1.11±0.07
PCB 44	4.473	7.54±2.56	0.87±0.09	2.55±0.65
PCB 209	2.518	4.65±0.83	1.53±0.56	1.88±0.99

The distinct differences in bioaccumulation between *F. bengalensis* and *L. marginalis* can be attributed to their differing feeding strategies and ecological niches. Lamellidens filter-feed on planktonic organisms (Mandal et al., 2007), and are known to assimilate high concentrations of contaminants from their environment. These and other bivalves have been widely used as bioindicators of toxicants owing to their sedentary nature allowing them to represent geographical variations, sensitivity to pollutants, and capability to detoxify when pollution ceases (Oertel & János Salánki, 2003).

F. bengalensis, on the other hand, is a grazing gastropod and also a bioengineer, feeding on organic detritus along with its herbivorous diet (Chakraborty et al., 2023). Therefore, this species is expected to accumulate higher concentrations of detritus- and sediment-bound contaminants as compared to the filter feeder *L. marginalis,* which primarily obtains both its food and the contaminants from water. In addition to feeding strategies, variations in the species' biotransformation availability may also be a possible reason for the variation in bioaccumulation (La Guardia et al., 2012). Marine bivalves have been observed to limit xenobiotic accumulation by closing their shells, and efficient depuration of retained residues to limit toxic effects.

Gastropods, in comparison, have been observed to rely solely on their limited biotransformation abilities to eliminate such compounds, leading to a difference in the tissue concentrations for both groups (Shofer et al., 1993). Therefore, *F. bengalensis* is an effective indicator of contamination of benthic sediments from anthropogenic sources.

Nevertheless, the differences in bioaccumulation between these two species underscore the importance of selecting appropriate bioindicator species based on the specific contaminants of interest and the environmental compartments they inhabit.

The most significant concentrations of contaminants found in both mollusks species were phthalates, specifically DnBP, DEHP, and DEP. This trend is indicative of the widespread use of these plasticizers in consumer products, such as packaging materials, cosmetics, personal care products, and various industrial. Phthalates are known to be persistent in the environment and have a strong tendency to adsorb onto particulate matter, making them prevalent in both water and sediment. The slightly higher levels of these hydrophobic phthalates in *F. bengalensis* are consistent with findings that organisms feeding on benthic material or living in close association with sediments tend to accumulate higher concentrations of lipophilic contaminants (with high $\log_{Kow}$), such as DnBP (4.27) and DEHP (7.33) (Liu et al., 2024). This is because benthic environments are reservoirs for these persistent pollutants, which adsorb strongly to sediment particles. In contrast, *L. marginalis*, which filters waterborne particulates, accumulates these phthalates to a lesser extent, reflecting its different exposure pathway.

Moreover, the trophic dilution of phthalates (Hu et al., 2016), further supports the observed bioaccumulation patterns. As phthalates undergo metabolic transformation at higher trophic levels, their concentrations tend to decrease, implying that *F. bengalensis*, being lower on the food chain and more directly exposed to sediment-bound contaminants, retains higher levels of DnBP and DEHP compared to species at higher trophic levels. This reinforces the idea that the specific ecological niches and feeding strategies of these molluscs are critical factors in determining the extent of contaminant accumulation, with gastropods like *F. bengalensis* serving as more sensitive indicators of sediment-associated EDCs in aquatic ecosystems.

The difference in bioaccumulation patterns between the two species also underscores the importance of using multiple bioindicators to gain a comprehensive understanding of the contamination in both water and sediment.

The presence of other EDCs like BPA and TCS can be traced back to urban runoff, including the discharge of untreated or partially treated wastewater. These compounds are commonly found in products used in daily human activities, such as bathing, washing, and industrial processes. The increase in EDC concentrations downstream of the drain indicates an influx of these contaminants with the wastewater, from where they partition into the sediment and are taken up by these benthic grazers. High concentrations of these contaminants were recorded upstream of the drain as well, indicating contamination from other sources, such as direct disposal of plastic waste into the river including abandoned and lost fishing gear (Raha et al., 2020; Nelms et al., 2021).

The city's large resident population as well as high tourist footfall could potentially be a reason for higher consumption of such products and their entry into the MGR, through various activities, such as bathing, washing, or general neglect. Total estrogen concentrations (E1, E2, and E3) in *F. bengalensis* remained more or less similar at both the sites, indicating their entry through sources other than municipal wastewater, such as bathing. The detection of DDT and metabolites (p,p'-DDE) suggests ongoing inputs from agricultural activities, despite the ban on DDT in many regions. These inputs likely originate from the runoff of older, persistent pesticide residues still present in soils.

6.4 Conclusion

This chapter assessed the bioaccumulation of EDCs in two mollusc species, *Filopaludina bengalensis* and *Lamellidens marginalis*, collected from both upstream and downstream locations in an effluent-impacted stretch of the Ganga River. The findings provide compelling evidence of the significant bioaccumulation of EDCs in two mollusc species *F. bengalensis* and *L. marginalis* in an effluent-impacted segment of the Ganga River. The study reveals that both species accumulated higher concentrations of EDCs downstream of the drainage site, indicating the impact of wastewater discharge on contaminant levels. This underscores the urgent need for continued monitoring and targeted mitigation strategies to protect the river's ecological health and integrity.

The distinct bioaccumulation patterns observed between the two species could be attributed to their differing ecological roles and feeding strategies, with *F. bengalensis* accumulating higher levels of sediment-bound contaminants and *L. marginalis* more effectively capturing waterborne pollutants. The predominance of phthalates, particularly DnBP, DEHP, and DEP, suggests the pervasive presence of plasticizers in the river, likely originating from urban runoff, industrial discharges, and improper waste disposal. The results underscore the need to standardize biomonitoring protocols and integrate these mollusc species into long-term environmental monitoring programs. Additionally, future research should expand on these findings by increasing geographic coverage, assessing toxicological impacts, and exploring environmental factors that influence the bioaccumulation of EDCs and other contaminants in these species.

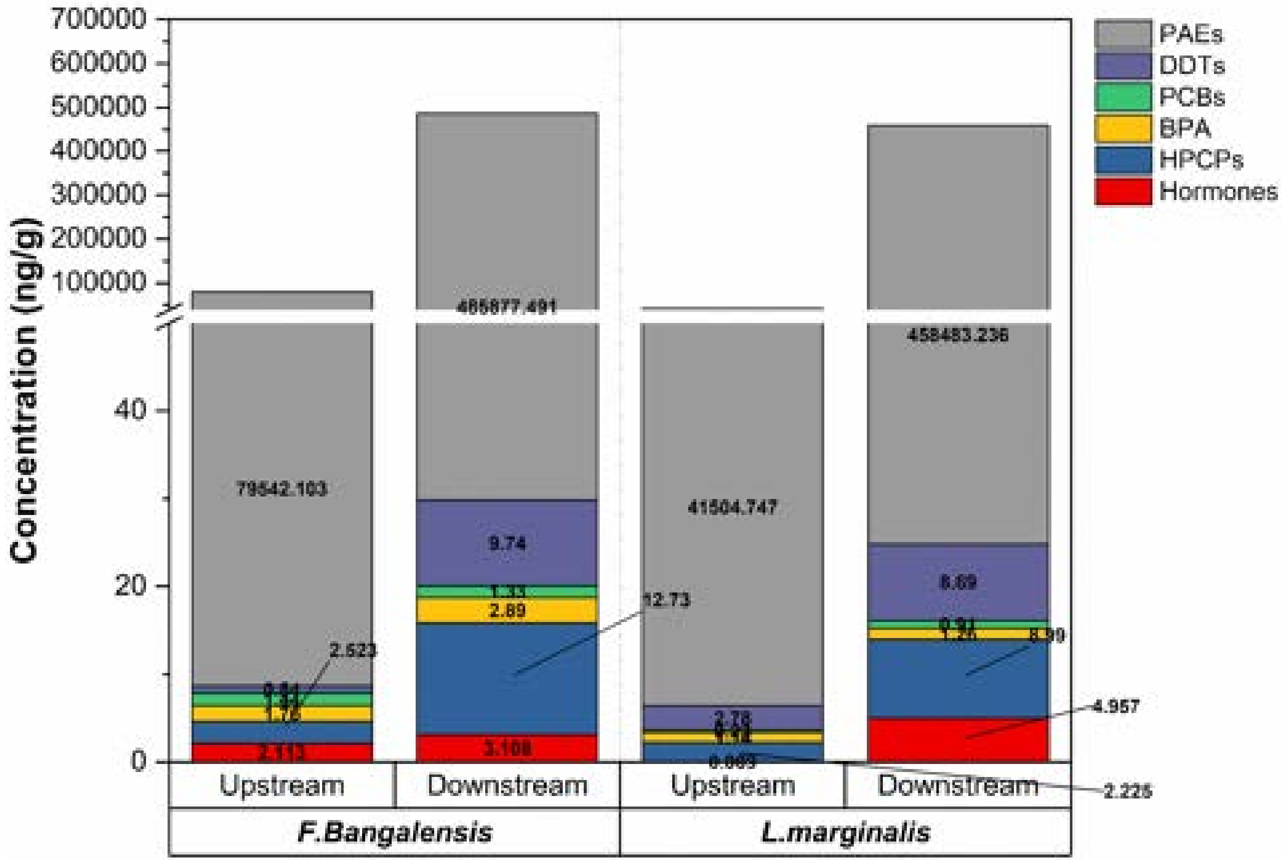


Figure 6-2: Bioaccumulation Patterns of Endocrine-Disrupting Compounds (EDCs) in *F. bengalensis* and *L. marginalis* along the Upstream and Downstream regions of Varanasi

Chapter 7 Summary, and Way Forward

7.1 Summary

In the title of this thesis, **"Ecotoxicological and Spatial Risk Assessment of Estrogenic Endocrine Disrupting Compounds (e-EDCs) from the Middle Ganga**", the focus is on assessing the ecological threats posed by e-EDCs in the Gangetic dolphin conservation priority section of the MGR. This focus is explored by addressing the research objectives and questions through a range of methodologies, including spatiotemporal analysis, bioaccumulation studies, and ecotoxicological risk assessments.

As outlined in the introduction, e-EDCs—primarily originating from industrial, municipal, and agricultural sources—disrupt endocrine functions, leading to adverse individual effects, population-level declines, and transgenerational impacts, particularly in aquatic mammals. Therefore, it is imperative to comprehensively understand the occurrence, spatiotemporal dynamics, bioaccumulation patterns, and ecological risks posed by these contaminants.

This thesis advances our understanding of these issues in the MGR, which is crucial for identifying high-risk areas, implementing targeted mitigation efforts, and informing regulatory frameworks to reduce e-EDC inputs and exposure risks to aquatic organisms, particularly the endangered Gangetic dolphin.

Objective I, II, and III

1 Spatiotemporal distribution of multi-class e-EDCs in MGR

This study is the first to report the spatiotemporal dynamics of target emerging e-EDCs in the MGR. High loads of e-EDCs (range), particularly PAs (range) and HPCPs (range), are prevalent in the MGR. Seasonal variations play an important role, with degradation processes such as photolytic and microbial breakdown, atmospheric deposition, and rainfall/surface runoff significantly influencing e-EDC concentrations in MGR. The potential sources of e-EDC contamination include high population, rapid urbanization, extensive agricultural settlements, improper plastic disposal, textile industries, and poor wastewater management practices.

The significant temporal variability in e-EDC concentrations, reveals periods of peak contamination that may overlap with critical ecological periods, such as the breeding seasons of aquatic species. This information is vital for informing the timing of targeted mitigation interventions, ensuring that vulnerable species are protected during these critical periods. Insights into the influence of seasonal dynamics also allow for more precise identification of contamination hotspots, facilitating more effective management of pollution peaks. Understanding the potential sources of e-EDCs informs environmental policies aimed at minimizing their release during high-risk periods, thereby contributing to the protection of biodiversity and the overall ecosystem health of the MGR.

2 Contribution of Drain Discharges to the e-EDC Load in the MGR

This study is the first to report the spatiotemporal dynamics of target e-EDCs from drain discharges into the MGR. The contribution of drain discharges to the e-EDC load in the MGR is substantial. The e-EDC concentrations in drain samples are generally higher than in river samples, indicating that drains are a major source of these contaminants. The persistent presence of e-EDCs in discharge-affected areas creates localized contamination zones that may serve as critical ecological pressure points, reducing habitat suitability for a wide range of aquatic species. The downstream transport of these contaminants further extends the risk beyond the immediate area, impacting broader riverine ecosystems. A statistically significant difference was observed for certain down-the-drain e-EDC, such as BPA, TCC, TCS, E1, and E3. However, for other e-EDC, the lack of statistical significance suggests that both point sources (such as drains) and non-point sources (such as rainfall offs, agricultural runoff and atmospheric deposition) contribute to their presence in the river. A substantial riverward fluxes of e-EDC from drain discharges were noted with highest contribution from drains.

This study highlights the urgent need for targeted interventions, such as advanced wastewater treatment (with secondary and tertiary treatment processes) and stricter regulatory oversight of drain discharges, to protect the ecological integrity of the MGR. By mitigating e-EDC inputs, it is possible to prevent long-term biodiversity loss and ensure the survival of key species like the Gangetic dolphin, maintaining the balance of the river ecosystem. In addition, while the National Mission for Clean Ganga under the

Ministry of Jal Shakti has implemented measures to tap and remediate drain outfalls into the Ganga River, regular assessments and systematic monitoring are essential to evaluate the effectiveness of these interventions and optimize strategies for further pollution mitigation in the river.

3 Influence of water quality parameters on distributions of EDCs

Key environmental factors such as pH (), conductivity (), TDS (), and DO () levels influence the fate of some e-EDCs in MGR. These findings underscore the critical role that water quality parameters play in influencing the concentrations and behaviors of EDCs in aquatic environments. By understanding these relationships, we can better predict the fate of EDCs in river systems and develop targeted strategies for monitoring and mitigating their impact on aquatic ecosystems.

4 Estrogenic Potency and Ecological Risks

The study reveals that 85% of the river stretch exhibits moderate to high (63%) estrogenic potency, posing significant ecological risks to aquatic organisms across various trophic levels. Estrogenic potency is a critical concern because it can disrupt endocrine functions in wildlife, leading to adverse reproductive and developmental effects. These disruptions can cascade through the food web, affecting species diversity, population dynamics, and ecosystem stability. A multi-tier ecological risk assessment identifies ten priority EDCs, belonging to PAs, HPCPs, and Estrogens, that require regular monitoring due to their high-risk profiles. By concentrating on the identified priority EDCs and employing comprehensive monitoring and regulatory strategies, we can significantly enhance the protection of aquatic ecosystems and safeguard the health of the species that rely on them.

Objective IV a and IV b

5 Bioaccumulation profiles of EDCs in prey fish and health risks to Gangetic Dolphins

The study reveals concerning bioaccumulation trends of EDCs, notably plastic additives, triclosan, and p,p' DDT, in the prey species of Gangetic dolphins. The presence of regulated p,p' DDT and γ-HCH residues suggests that regional compliance with environmental standards is lacking. EDC levels are influenced by factors such as habitat, foraging habits, trophic levels, and fish growth. Major sources of EDC contamination in the catchment area include agricultural practices, vehicular emissions, inadequate solid waste management, the textile industry, and an influx of tourists.

This study is the first to apply tissue dietary guideline values (toxicity reference value and reference dose) to estimate dietary exposure and risk of EDCs to dolphins, offering a vital tool for ongoing and future monitoring initiatives. The findings indicate a significant risk to Gangetic dolphins from consuming prey fish contaminated with DEHP, DDT, DnBP, and PCBs, based on reference dose analysis, highlighting the importance of prioritizing these EDCs in routine monitoring efforts. The proposed issue-based framework for monitoring chemical pollution in Gangetic dolphins and their habitat, shows great potential for improving conservation efforts for this endangered riverine cetacean. Moreover, this framework could be applied to other apex freshwater mammals, broadening its impact on freshwater ecosystem conservation.

Objective IV c

6 Potential Bioindicators for EDC Contamination

F. bengalensis and L. marginalis have shown promise as biomonitor for EDC contamination. The widespread distribution and sedentary nature and bioaccumulation capabilities of *L. marginalis* make it ideal for site-specific monitoring, especially in the effluent-impacted stretches of the Ganga River.

7.2 Future Research Directions for e-EDC Studies in the Ganga River

This thesis advanced the understanding of the spatiotemporal dynamics and ecological risks of e-EDCs in the MGR. However, several key areas warrant further exploration to deepen insights and enhance mitigation and management strategies. Future research on e-EDCs in the Ganga River should prioritize a comprehensive, multi-faceted approach that integrates advanced scientific methodologies with robust community engagement and policy development. Key areas include:

1. Expanded Spatiotemporal Monitoring

Future research should focus on implementing large-scale spatiotemporal studies across various segments of the Ganga River. Continuous monitoring systems with improved temporal resolution (e.g., seasonal or monthly) are essential to detect fluctuations in e-EDC concentrations, particularly during high-risk periods such as the monsoon season. Expanding geographical coverage to include areas beyond the Middle Ganga Reach would provide a broader understanding of contamination hotspots across the river system.

2. Expanded Chemical Analysis

While this study focused on specific classes of e-EDCs, it may have overlooked other emerging contaminants. Future research could develop comprehensive screening methods for newly emerging EDCs, investigate transformation products and metabolites of existing e-EDCs, and explore non-targeted screening approaches to identify unknown contaminants. Additionally, studying the synergistic effects of multiple e-EDCs in mixture scenarios would provide a more holistic understanding of their impact.

3. Advanced Biomonitoring Techniques

The study primarily utilized traditional biomonitoring species such as *F. bengalensis* and *L. marginalis*. Future research should also focus on developing and validating novel biomarkers for early detection of e-EDC exposure, explore the use of environmental DNA (eDNA) for monitoring the impacts of e-EDCs, and investigate microbiome changes as indicators of contamination.

4. Enhanced Source Tracking and Mitigation

While point sources like industrial effluents and wastewater discharges are well-known contributors, more research is needed to quantify contributions from non-point sources, including agricultural runoff, surface runoff, and atmospheric deposition. Advanced source apportionment techniques, such as isotope tracing or chemical fingerprinting, should be developed to differentiate between these sources. Additionally, evaluating the effectiveness of different wastewater treatment technologies, including advanced filtration and degradation methods, will provide insights into reducing e-EDC inputs at the source.

5. Long-term Ecological and Transgenerational Impact Assessment

The long-term ecological consequences of e-EDC exposure, particularly on reproductive health and population dynamics, remain underexplored. Multi-year studies are required to assess the transgenerational effects of e-EDCs on species such as the Gangetic dolphin and other aquatic organisms. Multi-year studies are required to assess the transgenerational effects of e-EDCs on species such as the Gangetic dolphin and other aquatic organisms. Utilizing tissue samples from stranded or deceased dolphins can provide valuable insights into the bioaccumulation of e-EDCs in top predators. These samples, when analyzed for contaminant loads, can reveal the long-term impacts of e-EDCs on the health of vulnerable species. Additionally, future research should incorporate non-invasive methods, such as using fecal samples, skin biopsies, or environmental DNA (eDNA), to monitor e-EDC exposure in live dolphins. These approaches minimize harm while allowing for continuous monitoring of dolphin populations and their exposure to contaminants. Developing predictive models to anticipate long-term ecological changes, including shifts in species populations and food web dynamics, will improve conservation strategies. A comprehensive set of guidelines for monitoring chemical pollutants in the Gangetic dolphin and its habitat is proposed in Chapter 5.

6. Climate Change Interactions

Future research should incorporate climate change factors, such as rising temperatures and altered precipitation patterns, into e-EDC fate and transport models. Climate change can affect the degradation, mobility, and bioavailability of e-EDCs, potentially exacerbating contamination. Modelling future scenarios that consider combined stressors—e-EDCs and climate-induced changes—will provide a more accurate picture of their impact on the Ganga River ecosystem.

7. Community Engagement and Traditional Ecological Knowledge

Integrating traditional ecological knowledge (TEK) with scientific monitoring can provide valuable historical insights into contamination patterns and support more localized conservation efforts. Citizen science initiatives, where local communities are involved in data collection and monitoring, should be prioritized to gather broader and more frequent data. Collaborative research frameworks with multi-stakeholder participation, including local communities, researchers, and policymakers, will ensure that research findings are translated into actionable policies that benefit both ecosystems and the people dependent on the Ganga River.

8. Improved Risk Assessment Models

Current ecological risk assessment models should be adapted to incorporate multiple stressor scenarios, including the combined effects of e-EDCs, habitat degradation, and climate change. Developing more sophisticated, region-specific risk assessment models tailored to the unique ecological and socio-economic conditions of the Ganga River system is essential. These models should account for cumulative risks and provide guidance on threshold levels for e-EDCs that are safe for both aquatic ecosystems and human populations.

9. Policy and Governance Integration

The findings from future research should directly inform regulatory frameworks and governance strategies. Establishing evidence-based policies, including setting threshold values for e-EDCs specific to the Ganga River ecosystem, will enhance regulatory compliance and reduce contamination. Strengthening wastewater management systems and implementing stricter regulations for both point and non-point sources will play a critical role in reducing e-EDC inputs. Furthermore, collaboration between scientific communities and policymakers is crucial to ensure that mitigation strategies are effectively implemented and continuously monitored.

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Annexure I

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Table S1: Sampling sites with observed Area of concerns (AOCs)

River	GPS Coord.	AOCs
Site 1	25.20572 N, 82.92295 E	FA, WC, CB, CR, AR, MB, PW, DM, SW, NH
Site 2	25.32903 N, 83.05533E	FA, WC, CB, RA, CR, MB, PW, DM, NH, B, SW
Site 3	25.49852 N, 83.16764 E	FA, WC, CB, CR, AR, MB, PW, DM, SW, NH

FA: Fishing Activities, CB- community bathing; CR- cremation and post cremation activity (e.g. disposing of ashes etc.); DM- domestic effluents (point and nonpoint); AR-agricultural runoff, RA -recreational activities; WC: Washing Clothes; B-Bridge; NH-National Highway; SW- Solid Waste; PW-Plastic Waste MB-Motor Boat; IE: Industrial Effluents

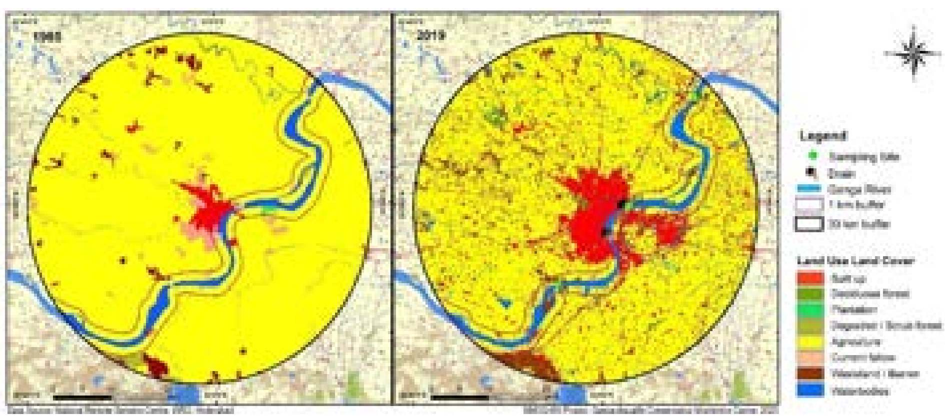

Figure S1: Land-use change in the catchment from 1985 to 2019

Text S1

Organochlorines

Samples were extracted for organochlorines according to previously documented methods (Barni et al., 2016; Cui et al., 2020) with minor modifications (time for Soxhlet extraction modified and increased from 8h to 16 h).

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2.5 g whole fish tissue → Homogenized with sodium sulfate (anhydrous) → 25 ng of Tetrachloro-m-xylene (TCMX), 13C12 PCB 52, 13C12 PCB 138, and 13C12 PCB 209 spiked → Soxhlet extracted for 16 h with a mixture of 200 mL of n-hexane-dichloromethane (1:1 v/v) → Concentrated to 1 mL under vacuum and nitrogen flow → Lipid content removed by gel permeation chromatography → Extract concentrated to 1 mL → Purification with a silica gel column chromatography→ (First Elution) 10 mL n-Hex→ (Second Elution) 7 mL of n-hexane-dichloromethane (1:1, v/v) → Eluent dried under nitrogen gas and redissolved in 100 µL of iso-octane, and internal standards added for targeted OCPs and PCBs analysis.

Target OCPs and PCBs quantification was performed using an Agilent 6850 GC system coupled to 7010B QQQ with an Electron Impact (70 eV) source (source temperature-300°C) operating in dynamic multiple reaction monitoring mode. Chromatographic separation, is achieved in two 15 m Agilent HP-5MS capillary column (15 m × 0.25 mm 0.25 µm film thickness), and injector temperature was set to 125°C and the injection volume was 1 µL. High purity helium (99.999%) was used as the carrier gas (Column 1: 1 mL/min; and Column 2: 1.2 mL/min). The temperature program was as follows: initial temperature of 60 °C (Hold 1 min), → 40°C/min to 120 °C → 5°C/min to a final temperature of 310°C.

Phthalates

Samples were extracted for PAEs according to methods by Wang et al., 2022 with minor modifications (time for Soxhlet extraction increased from 16h to 24 h).

2.5 g whole fish tissue → Homogenized with sodium sulfate (anhydrous) → 200 ng of DnBP-D4 and DEHP-D4 spiked → Soxhlet extracted for 24 h with a mixture of 200 mL of Acetone:n-hexane:dichloromethane (1:1:1 v/v) → Concentrated to 1 mL under vacuum and nitrogen flow → Solvent exchange in 10 mL n-hexane →Extract concentrated to 1 mL → Purification with Florisil column clean-up as per Standard Method 3620C (USEPA, 2014) → Elution with 100 mL ethyl ether/n-hexane (1:4, v/v) → Eluent concentrated under nitrogen gas to 1 mL, and internal standards added for PAEs chemical analysis.

Target Phthalates quantification was performed using an Agilent 6850 GC system coupled to 7010B QQQ with an Electron Impact (70 eV) source operating in dynamic multiple reaction monitoring mode. Chromatographic separation, is achieved in two identical 15 m Agilent HP-5MS capillary column (15 m × 0.25 mm 0.25 μm film thickness) joined by pneumatic switching device (PSD). Injector temperature was set to 125°C and the injection volume was 1 μL. High purity helium (99.999%) was used as the carrier gas (Column 1: 1 mL/min; and Column 2: 1.2 mL/min). The temperature program was as follows: initial temperature of 60 °C (Hold 1 min), → 40 °C/min to 170 °C → 10 °C/min to a final temperature of 310°C (Hold-3 min).

Alkyl Phenol and HPCP

QuEChERS method

Samples were extracted for Alkyl Phenol and HPCPs according to methods by Omar et al., 2019 and Fan et al., 2019 with minor modifications (centrifugation and vortex time increased).

0.5 g whole fish tissue → Homogenized with sodium sulfate (anhydrous) → 50 ng of bisphenol A-d_{16},13C6 Triclocarban, and Triclosan 13C12, spiked → 5 mL of acidified acetonitrile (5% acetic acid) + 1 mL ultra-pure water → vortexed for 1 min → 400 mg of MgSO4 + 100 mg of NaCl added→ Tubes shaken vigorously in vortex shaker for 6 min → Centrifuge at 4000 rpm (7 min at 25 °C)→ Supernatant transferred to dispersive-SPE tubes → 600 mg MgSO4 + 200 mg PSA, 200 mg C18+ 200 mg of Florisil→ Vortexed (1 min) → Centrifuged (6 min) at 4000 rpm → Extract dried under nitrogen → Dissolved in 200 µL mobile phase → Filtered using a 0.22 µm PTFE filter into a 2-mL amber glass with a 250 µL glass insert.

Target Alkyl phenol and HPCP quantification was performed using Liquid chromatography tandem mass spectrometry (LC-MS/MS) an Agilent 1290 infinity II UHPLC system coupled to 6470 QQQ with an ESI+Agilent Jet Stream source operating in dynamic multiple reaction monitoring mode. Chromatographic separation, is achieved in an Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm column at a constant temperature of 50°C and flow-rate of 0.4 mL/min, and the injection volume was 20 µL. Ultrapure water with 5 mM Ammonium Fluoride (A) and Acetonitrile (B) were used as mobile phase. The gradient program is as follows: 0 min: 98% (A), 2 % (B); 0-6 min: 0%(A), 100% (B); 6-11 min; 0% (A), 100 % (B); 11-11.10 min: 98% (A), 2 % (B).

Potentially Toxic Elements

Grounded 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 minutes, ramped to 200°C in 15 minutes and digested at 200°C for 30 minutes. After cooling, the digested sample was diluted to with ultrapure water. Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent ICP-MS-7850) was utilised to identify and quantify Cadmium (Cd), Lead (Pb), Mercury (Hg), Arsenic (As), in both the habitat and 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). Internal standards for As, Cd, Pb, and Hg were used for calibration.

Quality Control and assurance (QA/QC)

All glassware underwent a thorough precleaning process with deionized water and acetone, followed by baking at 200 °C for 5 hours. For PAEs analysis, plastic products were excluded during both sample collection and analysis. A method blank, and two solvent blanks, were processed and analyzed for every 7 samples checking for any potential carryover and background contamination during sample preparation and injection. The linear calibration curve for each compound demonstrated a high level of precision, with an R2 value exceeding 0.992. Lipid was determined gravimetrically. Due to lack of correlation between %lipid and EDC concentration, lipid normalization was not taken into account (Hebert and Keenleyside, 1995). The detection limits of the method for OCPs, PCBs, PAEs, BPA, and HPCP ranges from 0.01 – 0.025 ng g−1, 0.012-0.022 ng/g, 2-9 ng/g, 0.52 ng/g, 0.10-0.16 ng/g respectively. For PTEs the accuracy and precision of the analytical procedure was tested by recovery measurements using SRM (ERM-CE278k- Mussel tissue) from European Reference Materials. The precision of the analytical procedures, expressed as the relative standard deviation (RSD), ranged from 5 to 10%. Two blanks one SRM samples 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 <±5%. Contamination was significantly reduced at each step of sample preparation and analysis, and for any detected contaminants in the method blanks, the samples were corrected accordingly.

OCPs, PCBs, PAEs, BPA, and HPCP ranges from 0.01 – 0.025 ng g−1, 0.012-0.022 ng/g, 2-9 ng/g, 0.52 ng/g, 0.10-0.16 ng/g

Table S2: LOD and LOQ of target analytes:

Contaminant	LOD (ng/g)	LOQ (ng/g)
p,p'-DDT	0.018	0.06
p,p'-DDE	0.015	0.05
p,p'-DDD	0.025	0.08
α-HCH	0.008	0.27
β-HCH	0..012	0.04
γ-HCH	0.015	0.05
δ-HCH	0.01	0.03
TCS	0.16	0.53
TCC	0.10	0.33
BPA	0.52	1.73
DEP	6	20
DnBP	9	30
DEHP	7	23.3
DMP	2	6.6
BBP	2.5	8.33
DnOP	2	6.6
PCBs	0.012-0.022	0.04-0.07
Arsenic	16	47
Cadmium	7	23.3
Lead	16	47
Mercury	2.5	8.3

Table S3: MACRfD and MACTRV values for GD

Contaminant	RFD (mg/kgwwday)	MACrfd. (mg/kgww)	TRV (mg/kgww/day	MACtrv (mg/kgww)
Arsenic	0.0003	0.01	0.02	0.38
Cadmium	0.001	0.02	0.24	5.57
Lead	NA	NA	1.91	44.55
Mercury	0.001	0.01	0.31	7.24
DDTs	0.001	0.01	0.19	4.45
PCBs	0.00002	0.0005	0.04	1.01
HCHs	0.0003	0.01	0.004	0.10
TC	0.3	7.00	2.86	66.82
BPA	0.05	1.17	1.19	27.84
DEP	0.8	18.67	592	13808
DnBP	0.1	2.33	71.00	1657
DEHP	0.02	0.47	2.36	55.13

The equations used to derive the MAC_{RfD} and MAC_{TRV} can be found in Hung et al.,2004
Rfd values Obtained from Hung et al.,2004, Integrated risk Information System USEPA (IRIS) (https://www.epa.gov/iris); Rowland 1998
No observable adverse effect level (NOAEL) values for terrestrial mammals were obtained from Sample et al., 1996;
MACRfD and MACTRV values depend on biological parameters and the relevant information for GD and SCO were obtained from Kasuya 1972, Weigl, 2005, Choudhary et al., 2006; IUCN/SSC, 2008, Sinha & Kannan, 2014, Bihar Envis Centre - Environment Information System, 2015, EQMS India Pvt Ltd. 2020, and Braulik et al., 2021. Body weights for GD was taken 108 kg. Ingestion rate of the species, was selected at 5% of bodyweight and exposure duration was considered 40 years.
NA-Not available

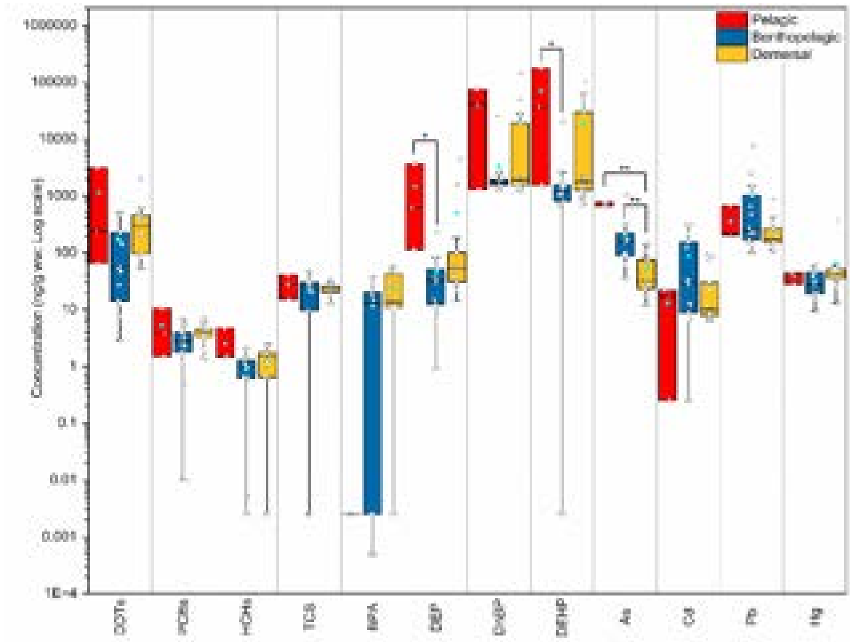

Figure S2: EDCs concentrations (ng/g ww) across different habitats

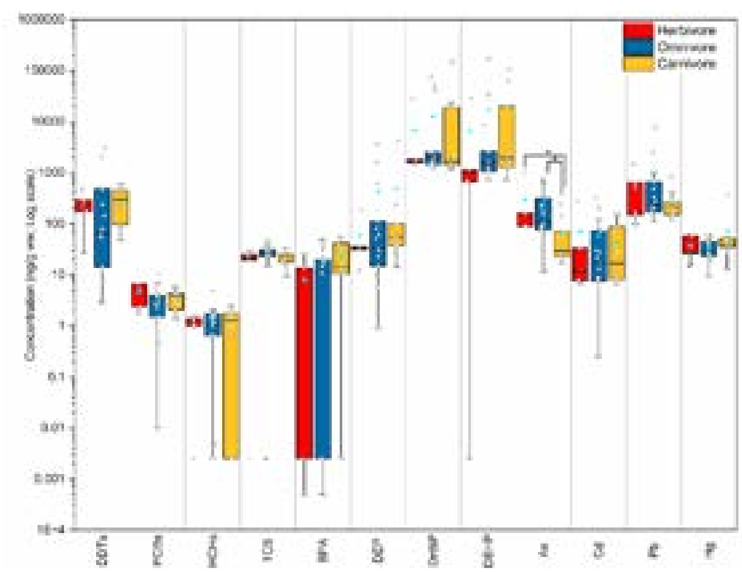

Figure S3: EDCs concentrations (ng/g ww) across different foraging behaviour

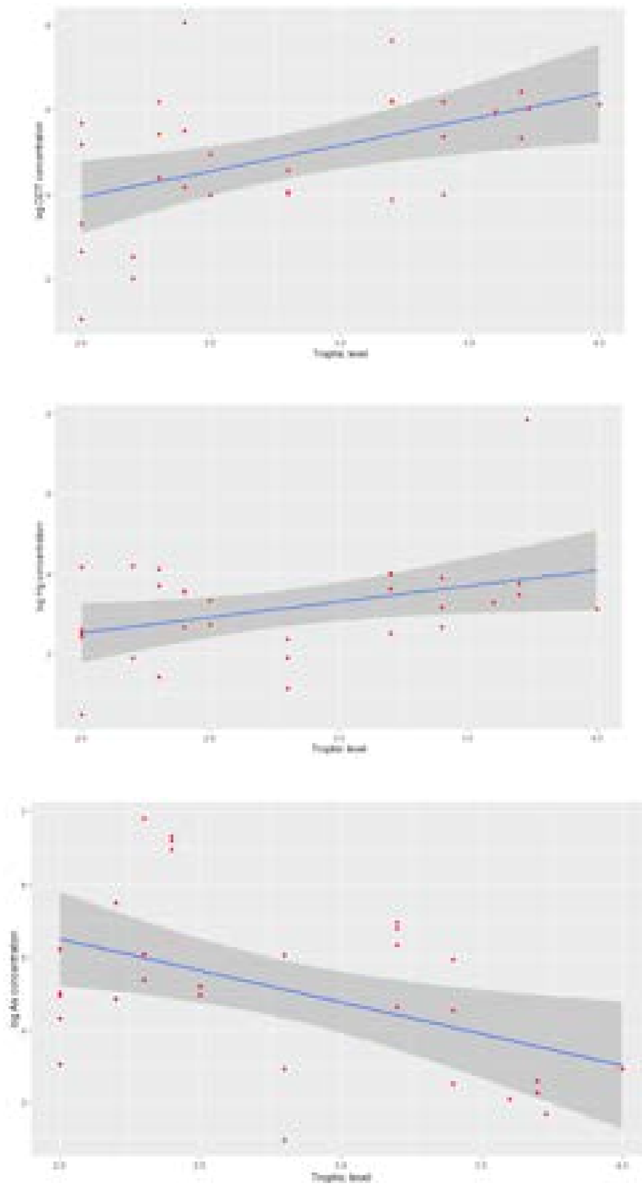

Figure S4: Impact of Trophic level on the accumulation of DDT, Hg and As

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Annexure II

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Research article

Do dietary exposures to multi-class endocrine disrupting chemicals translate into health risks for Gangetic dolphins? An assessment and way forward

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A B S T R A C T

Dietary exposure risks of 39 multi-class Endocrine Disrupting Chemicals (EDCs) to the threatened Gangetic dolphins (*Platanista gangetica*) were investigated in a conservation-priority segment of the Ganga River. Elevated EDCs bioaccumulation was observed across prey fish species, with di(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DnBP) significantly contributing to the EDC burden. The concentrations of persistent organochlorines in prey revealed a shift from dioxin-like polychlorinated biphenyls (PCBs) to non-dioxin-like PCBs. The prevalence of regulated p,p′ DDT (Dichlorodiphenyltrichloroethane) and γ-HCH (Lindane) residues suggests regional non-compliance with regulatory standards. The concentration of some EDCs is dependent on the habitat, foraging behavior, trophic level and fish growth. The potential drivers of EDCs contamination in catchment includes agriculture, vehicular emissions, poor solid waste management, textile industry, and high tourist influx. Risk quotients (RQs) based on toxicity reference value were generally below 1, while the RQ derived from the reference dose highlighted a high risk to Gangetic dolphins from DEHP, DDT, DnBP, arsenic, PCBs, mercury, and cadmium, emphasizing the need for their prioritization within monitoring programs. The study also proposes a monitoring framework to provide guidance on monitoring and assessment of chemical contamination in Gangetic dolphin and habitats.

1. Introduction

The probable extinction of the Yangtze River Dolphin, a tragedy propelled by a myriad of human-induced pressures, starkly illustrates the precarious situation of riverine dolphins across the globe [1]. With only five extant species of river dolphins worldwide, all classified as threatened, the imperative to address their vulnerability to various anthropogenic stressors is more urgent now than ever [2]. These vulnerabilities are particularly pronounced for species in densely populated river basins undergoing rapid urban development, such as the Gangetic Dolphin (*Platanista gangetica*) in the Ganga Basin [2].

As an apex predator, Gangetic Dolphin (henceforth referred to as GD) plays a key role in maintaining the structural and functional integrity of freshwater ecosystems through top-down processes. The GD is often considered an appropriate umbrella species, offering protection not only for itself but also facilitating conservation efforts for other co-occuring species [3–5]. This makes understanding

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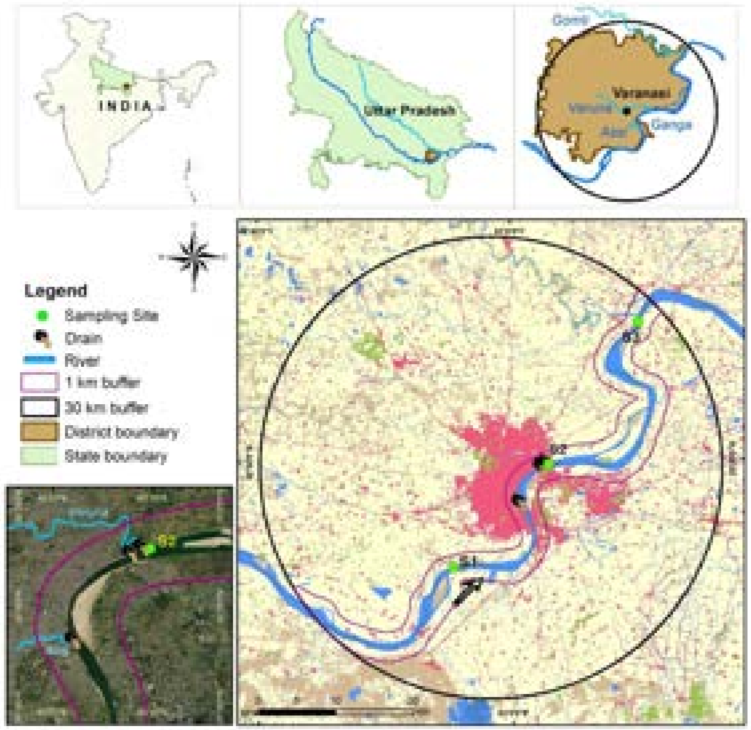

Fig. 1. Representation of sampling sites along MGR.

GD's responses to various anthropogenic stressors pivotal for devising effective conservation and management strategies that benefit the entire freshwater ecosystem and its biodiversity.

Recent estimates in Ganga Basin reveal a concerning 24.37 % contraction in GD range [6] and a population decline of over 50 % since 1957 [2,7]. Despite stringent regulatory conservation measures implemented by the Indian government, the species remains highly susceptible to anthropogenic threats, including bycatch mortality, poaching, boat traffic, a compromised (both in quantity and quality) prey base, climate change, flow modification by dams and barrages, and continual exposure to diverse pollutants [2].

Riverine cetaceans, including the GD, are exposed to various chemical pollutants primarily via dietary intake or offloading to the next generations via gestation and lactation [8]. With their extended lifespans, status as apex predators within local food chains, and high lipid reserves, GD often encounter significant health risks due to the substantial accumulation of various contaminants [8–10]. While studies on habitat modification, direct mortality, habitat loss, and overexploitation are frequently addressed [5,6,11–13], there remains a significant gap in understanding how chemical pollutants contribute to the decline of GD population, potentially undermining conservation efforts.

Within the broad spectrum of pollutants, Endocrine Disrupting Chemicals (EDCs) have emerged as a critical ecological concern due to their persistent, bioaccumulative, mobile, and toxic properties. EDCs comprise a highly diverse class of compounds, including natural hormones and man-made substances such as pesticides, pharmaceuticals, plasticizers, potentially toxic elements, industrial chemicals, surfactants, and household and personal care products [14].

Over the past three decades, our understanding of the hazards and ecological risks associated with EDCs has advanced considerably, notably their correlation with disruptions in reproduction and development, that may result in observable regional population shifts in some aquatic mammalian species [15–18].

Despite global evidence on EDCs impact on aquatic mammals' health, there has been no assessment of their risk to GD, potentially compromising the efficacy of ongoing conservation efforts. While research exists on GD's exposure to some EDCs, they only cover the period from 1988 to 1996 [9,10,19–25]. This gap indicates that recent changes in EDC levels over the past few decades remain undocumented and unanalysed, resulting in significant gaps in our understanding of current risks and potentially leading to inadequately informed and insufficient conservation efforts. To develop targeted conservation interventions that protect this endangered species

Table 1
Details of fish species collected, across the three sites, with average length, average weight, trophic level and habitat preference.

Species	No of Individuals	Niche	Feeding habit	Trophic Level	Average length (cm)	Average weight (grams)
O. niloticus	6	Benthopelagic	OV	2	22.55 ± 2.55	226.51 ± 77.58
R. corsula	11	Pelagic	OV	2.4 ± 0.2	21.5 ± 4.27	32.27 ± 5.18
S. aor	5	Demersal	CV	3.6 ± 0.53	29.25 ± 1.50	145.00 ± 11.37
N. chitala	3	Demersal	CV	4 ± 0.64	23.5 ± 3.56	152.41 ± 8.23
C. latius	8	Benthopelagic	HV	2.3 ± 0.2	13.45 ± 2.69	26.67 ± 3.82
A. morar	45	Benthopelagic	OV	3.2 ± 0.4	10.17 ± 1.41	8.78 ± 1.46
R. rita	12	Demersal	CV	3.7 ± 0.57	19.8 ± 3.82	77.54 ± 34.65
L. rohita	5	Benthopelagic	OV	2.2 ± 0.12	27.7 ± 1.44	301.11 ± 23.48
M. armatus	7	Demersal	CV	2.8 ± 0.27	13.2 ± 2.58	9.08 ± 1.35
J. coitor	6	Demersal	CV	3.4 ± 0.5	16.22 ± 1.25	31.08 ± 6.22
S. bacaila	10	Benthopelagic	CV	3.2 ± 0.4	12.07 ± 1.01	10.77 ± 2.41
L. calbasu	5	Demersal	HV	2	29.75 ± 1.52	311.33 ± 36.51
M. cavasius	14	Demersal	CV	3.4 ± 0.4	12.17 ± 2.76	8.33 ± 2.60
C. reba	7	Benthopelagic	OV	2.5 ± 0.2	19.39 ± 2.45	53.36 ± 29.92
C. mrigala	5	Demersal	OV	2.3 ± 0.2	28.45 ± 1.24	271.44 ± 8.38
C. catla	5	Benthopelagic	OV	2.8 ± 0.22	22 ± 2.83	147.65 ± 41.37
L. bata	6	Benthopelagic	HV	2	28.58 ± 1.73	230 ± 22.08
C. chagunio	4	Demersal	OV	2.8 ± 0.3	19.2 ± 1.83	60 ± 4.24
L. pangusia	6	Benthopelagic	HV	2	26.17 ± 0.91	166.67 ± 16.44
C. garua	13	Demersal	CV	3.73 ± 0.59	19.1 ± 4.01	43.67 ± 17.93
B. dario	4	Demersal	OV	3.2 ± 0.4	7.5 ± 0.28	8.36 ± 0.36

*OV–Omnivore; HV–Herbivore; CV–Carnivore.
Trophic level information is retrieved from FishBase [30].

and maintain ecological balance in their riverine habitats, it is crucial to urgently understand the health risks posed by EDC exposures to GD and its populations.

Biomonitoring of EDCs in GD can reveal health risks, but regulatory, ethical, and technical challenges complicate this traditional method. In the past two decades, the screening-level ecological risk evaluation (SLERA) approach based on dietary exposures has been successfully used as an indirect and non-invasive alternative to explore the potential health risk posed by contaminants to threatened aquatic mammals [26–28].

Given the predominantly piscivorous nature of GD, assessing EDC levels in fish facilitates the evaluation of potential health risks associated with EDC exposure in this species. Furthermore, studies suggest variations in EDC accumulation in fish is driven by biological traits, niche, feeding preferences, and geographical influences, these patterns seldom adhere to consistent trends [29–31]. Assessing these factors is vital for understanding biomagnification within the food web; however, limited efforts have been directed toward investigating these dynamics in GD habitats.

Given the above, the study aims to address the following key research questions: (1) what is the current extent of EDC contamination in the prey base of GD? (2) what factors contribute to the observed bioaccumulation patterns? and (3) what risk do EDCs pose to GD through dietary pathways? The overarching objective of this study is to screen for EDCs that pose risk to GD. Additionally, the study proposes an issue-based framework for monitoring and assessment of chemical pollution in GD and their habitats.

2. Study area

The investigated study area is a 60 Km stretch of Middle Ganga Reach (MGR) that lies between north latitudes 26° 33′ 40.608″ N-25° 34′ 56.64″ N, and east longitudes, 80° 18′ 3.096″ E – 83° 36′6.48″ E in the Varanasi district of Uttar Pradesh, India (Fig. 1). The stretch holds immense religious, economic, and socio-cultural importance and is an important habitat for diverse flora and fauna including GD [2]. Within the Varanasi district, there are 38 towns and 1327 villages, with a population of 3,676,841 (urban: 43.44 % and rural: 56.56 %) projected to reach approximately 4,300,000 in 2023 [32].

Varanasi, one of the oldest continuously inhabited cities globally, confronts obstacles in modernizing its sewage infrastructure owing to spatial limitations and preservation issues tied to its historic design. The river's catchment area, characterized by rapid development and high population density, coupled with poor wastewater management, frequently results in the discharge of untreated wastewater through multiple point-sources [33]. Meanwhile, its vast agricultural landscape, including cultivation on dry riverbeds, contributes as non-point sources of EDCs such as organochlorine pesticides [34,35].The primary industrial sector is textiles, which holds a prominent position in the local economy, after tourism. Tourism also plays a central role in Varanasi's economy, with an annual footfall exceeding 9 million from domestic travelers and 1 million from international visitors [35]. The study area features three major sewage discharge points: Nagwa Drain (formerly Assi River), the Khirkia Drain, and the Varuna River (Fig. 1). Both Varuna and Assi rivers, often referred to as drains due to their poor water quality, receive significant wastewater and sewage inflow from numerous industrial and municipal drains, ultimately discharging into the Ganga River.

2.1. Sample collection

To assess the impact of urban settlements on the health of GD within conservation priority habitats, three sampling sites (S1, S2, and S3) were selected for EDC monitoring in the MGR. S1 and S3 predominantly represent agricultural landscapes and rural settlements, while S2 is characterized by urban and industrial settlements (Fig. 1, Fig. S1 and Table S1).

The Platanista genus exhibits a preference for prey based on size rather than species, primarily due to their narrow oesophagi, and species richness [36]. Typically, their prey size distribution is dominated by items measuring 20–30 cm [12,37]. Consequently, our sampling efforts were focused on collecting specimens falling within these preferred size ranges. Sampling was carried out in March 2021 and a total of 187 prey fishes of 21 species were obtained. Prey fish samples were obtained onsite from local fishermen as part of their routine catch for commercial purpose. Review and/or approval by Institutional Animal Ethical Committee (IAEC), Wildlife Institute of India was not needed for the study design.

The species were identified and their length and weight were recorded on-site.

The samples were packed in precleaned aluminum sealed bags and kept in an ice box for transportation to the laboratory, where they were stored in a deep freezer at −20 °C until further processing. Individual whole fish of the same species from each site were pooled and homogenized in a customized stainless steel tissue homogenizer. Details of the fish species collected from each site are given in Table 1. Data on trophic levels and habitat preference of each species were obtained from the FishBase [38].

3. Materials, extraction, and analyses

3.1. Chemicals and reagents

EDCs were selected mainly based on their major categories, and potential toxic impacts on aquatic mammals. Thirty-nine EDCs, including seven plastics additives - six phthalate (PAEs, the sum expressed as ΣPAEs): Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Di-n-butyl phthalate (DnBP), Butyl benzyl phthalate (BBP), Bis(2-ethylhexyl) phthalate (DEHP), Di-n-octyl phthalate (DnOP) and Bisphenol A; Seven organochlorine pesticides: p,p′-DDT(1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl) ethyl]benzene), p,p′-DDE (1,1-Dichloro-2,2-bis (4-chlorophenyl) ethene dichloro diphenyl dichloroethylene), p,p′-DDD (1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene) -the sum expressed as ΣDDTs; four Hexachlorocyclohexane (HCHs; sum expressed as ΣHCHs) isomers: α-HCH, β-HCH, γ-HCH, δ-HCH; plasticizer: ; two household and personal care products (the sum expressed as ΣHPCPs): Triclosan (TCS), Triclocarban (TCC); Nineteen polychlorinated biphenyls (the sum expressed as ΣPCBs): PCB 8, PCB 28, PCB 44, PCB 52, PCB 77, PCB 81, PCB 101, PCB 105, PCB 114, PCB 118, PCB 126, PCB 138, PCB 153, PCB 156, PCB 167, PCB 169, PCB 180, PCB 189, PCB 209, and four potentially toxic elements (PTEs): Cadmium (Cd), Lead (Pb), Mercury (Hg), Arsenic (As) were selected for present study.

Biota samples were spiked with internal standards and allowed to equilibrate for 4 h at room temperature (25 °C). Sample pretreatment was conducted according to previously documented methods for persistent organochlorines [39,40], BPA and HPCPs [41,42], and PAEs [43], with minor modifications for enhanced recoveries. The extracts were identified and quantified by Ultra-High Performance Liquid Chromatography–Tandem Mass Spectrometry (UHPLC–MS/MS), Gas Chromatography–Tandem Mass Spectrometry (GC-MS-MS), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Relative recoveries for OCPs and PCBs ranged 81–100 % and 91–103 % respectively. The average recoveries for PAEs, BPA, and HPCPs were 80–110 %, 78–112 %, and 81–108 % respectively. The percentage recoveries of the PTEs in the SRM ranged from 90.9% to 106 %. Detailed pretreatment methods, clean-ups, instrument parameters, quality assurance and quality control are detailed in Text S1 and Table S2.

3.2. Risk assessment for GD

Two dietary tissue guidelines—the reference dose (RfD; mg kg^{-1} ww day^{-1}) and the toxicity reference value (TRV, mg kg^{-1} ww day^{-1}), previously used for humpback dolphins [44,45]—were adopted and applied to assess potential health hazards to GD from consuming EDC-contaminated prey.

The TRV for EDC in GD was calculated based on the no observable adverse effect dose ($NOAEL_t$) for mammalian test species, and body weight scaling procedure (bodyweight of the dolphins/bodyweight of the test species) [44,45].

For dose-response assessment, the methodology detailed elsewhere [44,45] is utilized to derive a maximum allowable concentration (MAC) based on the Reference Dose (MAC_{RfD}) and Toxicity Reference Value (MAC_{TRV}) for a specific EDC in prey fish tissue. The MAC_{RfD} and MAC_{TRV} represents the highest concentration of each toxicant that can occur in the prey without causing harm to the species, and are derived using variables dependent on biological parameters of GD. The calculated values of MAC_{RfD} and MAC_{TRV} for GD is provided in Table S3.

The risk quotient (RQ) was determined from the ratio of the observed concentration and the MAC of a specific EDC in fish for GD consumption. The values of $RQ > 1$, $0.1 < RQ < 1$, and $RQ < 0.01$ indicate high, medium, and low dietary exposure risks of EDCs to the GDs, respectively [46]. The limited availability of comprehensive biological data on these dolphins may restrict the accuracy of SLERA. This limitation arises from uncertainties in exposure scenarios, including reliance on standardized parameters such as consumption habits, body weight, exposure frequency, fraction ingested, and exposure duration. Despite these limitations, the SLERA methodology adopted in this study aims to approximate a worst-case scenario, opting for a more conservative approach to ensure a higher level of protection for the threatened dolphins.

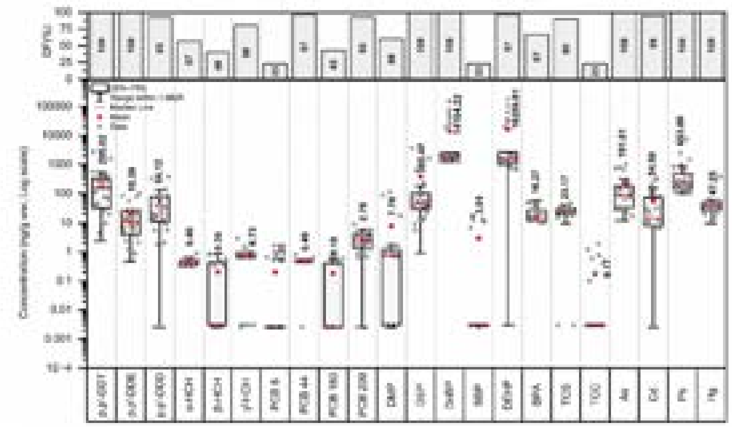

Fig. 2. Average concentration (ng/g ww; log scale) and detection frequency (DF%) of EDCs in prey of GD.

3.3. Statistical

Chemical concentrations were expressed as the range; average ± standard deviation, and reported as ng/g wet weight (ng/g ww). To prepare data for analysis, data below the LOQ were adjusted to ½ LOQ [47,48]. Assumptions were tested with Levene's tests for homogeneity of variance and Shapiro–Wilk tests for normal distribution. Non-parametric equivalents were used as the assumptions were violated. Kruskal-Wallis H-test was conducted to test variations of statistical significance for bioaccumulation patterns among sites, niches, and feeding behavior. Spearman's rank coefficient was conducted to evaluate the role of ecological factors (Trophic level) and biological traits (average weight and length) in bioaccumulation of EDCs. Statistical tests results were considered significant at p-value <0.05, and <0.01. Values were log-transformed for linear model application to assess relationships between tissue contaminant concentrations and species trophic levels.

4. Results and discussion

4.1. Bioaccumulation of multi-class EDCs in prey of GD

The average concentration and prevalence (detection frequency, DF) of multi-class EDCs in prey of GD is presented in Fig. 2. The dominant EDCs were DEHP (681.23–174990 ng/g ww) and DnBP (1218.07–146814.29 ng/g ww) accounting for 52.2 % and 45.3 % of total EDC burden, respectively.

The concentrations of DEHP and DnBP were followed by Pb, DEP, DDTs, As, Cd, Hg, TCS, BPA, DMP, PCBs (PCB 8, 44, 180, 290), BBP, HCHs, and TCC whereas DnOP and other target PCBs were below the method detection limits (<DL) in all the samples. Among the investigated freshwater fish species, *C.fatius* (As), *A.morar* (Cd, Pb), *L.rohita* (TCS), *C.garua* (Hg), *R.corsula* (DDTs, PCBs, HCHs, DEHP), *R.rita* (BPA), and *S.aor* (DnBP) *S.bacaila* (TCC), and *C.reba* (BBP) exhibited the highest contamination for various categories of EDCs.

While high EDC concentrations were generally observed in prey fish from S3, likely owing to the downstream influence of both the point-sources at S2 and non-point sources at S3 itself, a Kruskal-Wallis test revealed no significant ($p > 0.05$) variations across the three locations.

The subsequent section provides group-wise bioaccumulation profiles of EDCs in prey of GD.

4.1.1. Persistent organochlorines

Bioaccumulation potential of three persistent organochlorines-DDT, HCH, and PCB on the GD prey were explored in this study (Fig. 2), with DDT (98.7 %) notably showing significant ($p < 0.05$) predominance, then ΣPCBs (0.97 %) and ΣHCHs (0.32 %).

The observed variances in the bioaccumulation patterns are consistent with a previous study on GD and its prey in the Ganga River [9], where DDTs were noted as the highest residue in GDs (~1300 ng/g wet weight), significantly surpassing PCBs and HCHs by a factor of 16.

The ΣDDTs concentration in the fish, collected from all three locations ranged from 2.87 to 3129.80 ng/g (368.53 ± 634.67 ng/g ww),with p,p′ DDT ($p < 0.001$) as the major contributor (76 %) towards ΣDDT load, followed by p,p′ DDD (16 %) and p,p′ DDE (8 %). Kannan et al. [9] found significant DDT concentrations in the prey fish from the gut of GD, indicating considerable exposure to DDT through their diet. Notably, our study reveals that DDT concentrations in GD prey are twice as high as those reported by Kannan et al. [9]. This suggests a potentially high accumulation in GD, particularly given their apex predator status and limited capacity to metabolize DDTs.

The presence of DDD as second mostabundant metabolite indicates anaerobic degradation or dechlorination of DDT in the environment [49]. In the present study, the DDE/DDT ratio were consistently low (0.03–0.4) in prey fish suggesting recent DDT inputs [50]. The use of DDT in agriculture has been banned in India since 2006, with its application restricted to public health programs aimed at controlling vectors of malaria, dengue, kala-azar, and similar diseases [51].

Nevertheless, despite restrictions, fresh DDT inputs persistently appear in various aquatic compartments of Ganga River, prompting apprehensions about their illicit use in the catchment [52]. Further, the application of DDT in indoor residual spraying for the control of disease vectors seems to represent another potential source of DDT inputs into freshwater environments [52]. An increased surveillance and regulatory measures are imperative to guarantee the prudent and effective utilization of DDT in disease vector control programs.

The ΣPCBs concentrations in the all the fish samples ranged from <DL-10.47 ng/g ww (3.65 ± 2.20 ng/g ww). Notably, ΣPCBs were detected in prey fish species with concentrations almost 100 orders of magnitude lower ($p < 0.001$) than ΣDDT, and twice as high ($p < 0.001$) as ΣHCHs. In India, PCBs are highly regulated under the Environment Protection Rules [33], which prohibit the manufacture, import, export, and use of equipment containing PCBs [33].

The dominant PCB was PCB-209 (69 %) followed by PCB-44 (20 %), PCB-8 (6 %), and PCB 180 (6 %), while all other investigated PCBs were <DL. The potential sources of PCB-209 and other have been reported with the inadvertent or unintentional formation in specific organic pigment categories, including phthalocyanine green, dioxazine, azo, isoindolinone (PCB 8), monoazo (PCB 8), Titanium dioxide (PCB 209, and 180) and polycyclic pigments [53] [54,55]. The study area, particularly Site 2, is a prominent textile hub in the country, appears to be the primary source of unintentionally produced PCBs (UP-PCBs) attributed to the utilization of pigments in its manufacturing processes. Other potential inadvertent sources for the detected UP-PCBs include paint used on boats, printed material, and newsprint [56]. The presence of PCB 44, which undergoes dechlorination in anaerobic conditions [57], might be further explained by the high effluent load entering through three drains in S2. A similar pattern for PCB 44 has also been identified in other effluent-impacted Indian rivers [58].

Based on the findings of this study and the low metabolic capacity of GDs towards PCBs [9], it is recommended to consider UP-PCBs (including PCB-11, 52), in routine monitoring programs. Additionally, further confirmation studies in the fields of toxicology, environmental monitoring, and chemistry are needed to comprehensively understand the dynamics and impacts of UP-PCBs in GD and its habitats.

The ΣHCH concentrations, in prey fish ranged from <DL to 4.689 ng/g ww (1.22 ± 0.94 ng/g ww). The γ-HCH (50 %) concentration was major contributor towards ΣHCH, followed by α-HCH (25 %) and β-HCH (25 %), while δ-HCH levels in all fish species were <DL (Fig. 2).

Compared to DDTs and PCBs, HCH concentrations were notably low, a trend attributed to their low usage, relatively high volatility, lower bioconcentration factors, limited bioaccumulative capacity, and relatively biodegradable nature in the aquatic food chain [9, 50]. India banned technical HCH in 1997 (a mixture of α-HCH = ~70 %, β-HCH = 5–12 %, γ-HCH = 10–15 %, and δ-HCH = 6–10 %), whereas lindane (~99 % γ-HCH) was banned for manufacturing, import, or formulation in 2011 and for use in 2013 [51].

Furthermore, the α-HCH/γ-HCH ratio, with values typically ranging from 3 to 7 in technical grade HCH, is used to assess the nature of HCH contamination with ratios below 3 indicating recent inputs of Lindane or γ-HCH. Interestingly, compared to previous studies [9,10], the HCH isomeric patterns in the present study were observed to be that of lindane (α-HCH/γ-HCH<3) rather than the technical HCH formulation. This finding aligns with our previous study conducted on the surface waters of Ganga River [52], where high concentrations of γ-HCH were observed. It suggests an association with the illicit use of this chemical in paddy and wheat fields during the flowering season, as well as in dry riverbed cultivation practices [52].

Despite lower concentrations of PCBs and HCHs compared to DDTs, the combined effects of these persistent organochlorine mixtures on exposed biota can be more complex than expected, potentially leading to compromised health and population-level consequences [59,60]. Additionally, the high transfer rate (~60 %) of organochlorine residues from mother to offspring, a characteristic unique to cetaceans, presents a considerable risk, especially to calves [8,21]. Notwithstanding regulatory measures and improvements in freshwater conditions, the persistence of these organochlorines could lead to enduring multi-generational toxic effects on dolphin populations.

4.1.2. Plastic additives

The cumulative concentration of 6 PAEs (∑PAEs) in prey of GD ranged from 1501.21 to 254266.20 ng/g ww (30760.09 ± 65887.92 ng/g ww). DEHP and DnBP were the predominant (DEHP-53 %; DnBP-46 %) and prevalent (DF(DEHP)-100 %; DF(DnBP)-100 %)phthalates in all the fish samples, driven by their widespread production and usage, with environmental dispersion occurring at every stage of their life cycle [61]. BPA concentrations in prey fishes varied from <DL to 56.66 ng/g ww, (16.26 ± 16.75 ng/g ww; DF:67 %). Compared to phthalates, BPA is less prevalent in the environment likely due to its chemical embedment within products, which poses challenges for direct volatilization and leaching [62] Fig. 2.

Our results are consistent with recent findings by Chakraborty et al. [61], which reported dominance of DEHP and DnBP in the surface waters of the MGR and high concentration of BPA (4.46 μg/L) in Varanasi.

The densely populated catchment area of the MGR suffers from inadequate solid waste management, which can lead to leaching of plastics additives due to extensive dumping of plastic water bottles, single-use polyethylene bags, and food packaging materials [35,61, 63]. Additionally, the high tourist influx may inadvertently contribute to littering, either from unawareness or a lack of proper disposal options, further straining the local waste management infrastructure, particularly during peak seasons. The textile industries in the catchment also appear to be a potential source of phthalates. These industries use various classes of chemicals, including phthalates,

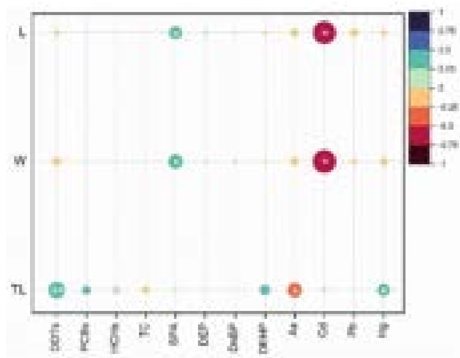

Fig. 3. Correlation of tissue EDCs concentrations with ecological factor (TL:Trophic level) and biological traits (W: Average weight; L: Average Length) Correlation significant at * = $p < 0.05$; ** = $p < 0.01$.

throughout their manufacturing processes, potentially leading to the discharge of these chemicals into the MGR [35].

4.1.3. Household and personal care products (HPCPs)

Commonly found in a variety of HPCPs, including hand soaps, toothpaste, detergents, plastics, and cosmetics, TCC and TCS are two polychlorinated aromatic antimicrobials that have been in use for decades. The escalating demand and production of these chemicals unavoidably lead to their ubiquitous presence in various environmental compartments [64–66].

The ∑HPCPs concentration detected in fish samples ranged from <DL to 47.05 ng/g ww (23.17 ± 10.99 ng/g ww). Interestingly, TCS was recorded to be the predominant (99.26 %) and prevalent (90 %) HPCP in all the fish samples compared to TCC (DF:23 %) as shown in Fig. 2. The variation in concentrations between the two antimicrobial agents could be linked to their distinct consumption patterns, chemical characteristics, and degradation rates in environmental matrices.

Triclosan is commonly found in liquid soaps, whereas triclocarban is mainly used in solid soap bars, which could account for their varying presence. Additionally, our sampling time (March 2021) coincided with the peak of the COVID-19 pandemic when enhanced hygiene practices spurred increased demand for liquid soaps, typically high in triclosan [67]. This surge likely led to significant triclosan discharge into aquatic systems, contributing to the observed bioaccumulation and variations.

4.1.4. Potentially toxic elements (PTEs)

PTEs such as Pb, Hg, Cd, and As are recognized as EDC in both humans [68–70], and aquatic mammals [71–74].

In the present study, Pb was the most dominant PTE with concentrations ranging from 100 to 7550 ng/g ww. In order of decreasing rank, concentrations of Pb were followed by As (12–1007 ng/g ww), Cd (0.25–322 ng/g ww), and Hg (9.4–374 ng/g ww) as presented in Fig. 2.

Similar bioaccumulation patterns of these PTEs have also been noted in the tissue of the GD [19].

The accumulation of Cd, and Pb in fish samples points towards their industrial origins, including industries like textile, dyeing, electroplating, metallurgy, etc. Additionally, the presence of busy national highways and heavy boat traffic at all monitoring stations, along with long-range atmospheric transport of Pb, suggests contributions of Pb emissions from both land-based and river activities [75].

PTEs such as Cd, and Pb are also present in small quantities within chemical additives used in plastics, to enhance the functional and aesthetic attributes of these materials [76]. The incorporation of PTEs in these additives, coupled with the improper utilization, recycling, and disposal of plastics, raises concerns about the potential unintentional release of PTEs into freshwater environments [76]. The accumulation of arsenic (As) in fish species may be associated with the application of phosphate fertilizers containing high levels of As [77]. These fertilizers, characterized by elevated arsenic concentrations, could potentially enter the river during flood events.

4.2. Relationships of EDCs concentrations with ecological, and biological factors

A Spearman's correlation analysis indicates a significant direct relationship between DDTs ($p < 0.01$) and Hg ($p < 0.05$) accumulation with trophic level (Fig. 3). In contrast, As ($p < 0.05$) shows an inverse relationship with the trophic level of the species. These results are further supported by linear modelling (Fig. S4) that revealed significant effect of trophic levels on the accumulation of DDTs ($R^2 = 0.24$), As ($R^2 = 0.22$) and Hg ($R^2 = 0.14$) in fish tissues, indicating biomagnification for DDTs and Hg, but trophic dilution for As. Biomagnification of DDT and mercury in the riverine food web of the present study, is consistent with observations by other authors [78,79]. As has been reported to dilute through the food chain, owing to the ease of oxidation of As(III) to As(V) compared to the methylation of accumulated As in organisms with increased trophic levels [80]. No significant relationships were observed for PCBs,

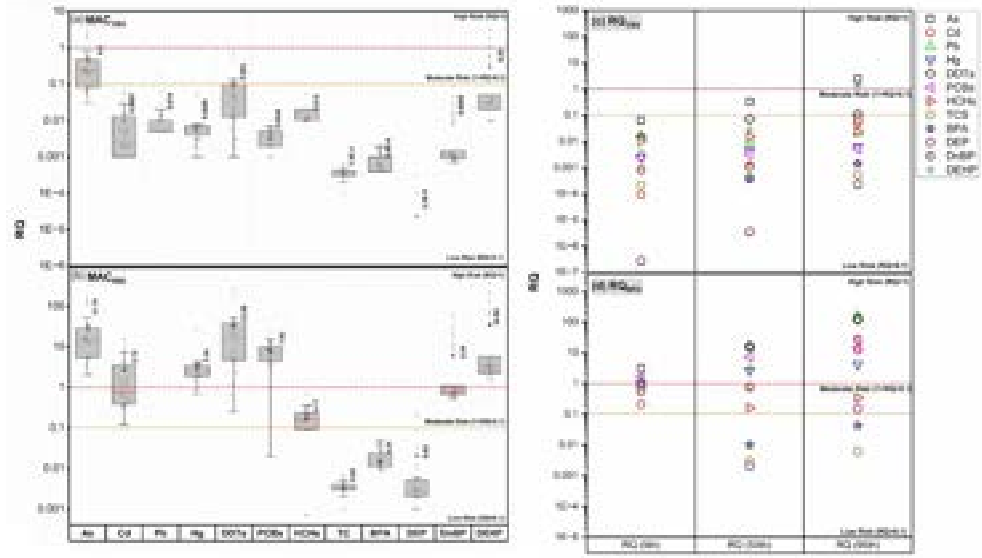

Fig. 4. Risk assessment (RQ-log scale) of EDCs to GD MAC_{TRV} and MAC_{RFD} based on average concentrations (a–b) and 5th, 50th, and 95th percentile data (c–d).

DEP, Cd, and, Pb, whereas non-linear relationship between trophic levels of the species and HCHs, BPA, DnBP and DEHP were noted. These findings suggests that contaminant dynamics may not always be simple enough to be explained by linear regressions and simplification by process selection, and assumptions of equilibrium in predictive models can considerably affect the reliability and predictive power of models [81]. Additionally, as bioaccumulation and biodilution depend on various factors including local food webs and environmental factors, thus further investigations are necessary for effective modeling, prediction, and risk assessment.

A Spearman's correlation (Fig. 3) analysis indicates a significant correlation of biological factors +with BPA and Cd. BPA accumulation shows positive relationship with average length and weight, consistent with the results Zhou et al. [82]. Studies on other phenolic compounds show decreasing toxicity with increasing fish size [83], however, knowledge gaps pertaining to the relationship of BPA accumulation and size of the species, need to be addressed to understand its risk to piscivores with specific prey size preferences. An inverse relationship of Cd with both length and weight of the species is observed. Similar relationships have been observed in previous studies, and especially in smaller individuals [84]. The higher significance of smaller individuals for the accumulation and toxicity of these compounds could potentially increase the contamination risk to GD, whose prey preference is limited by size.

Nevertheless, it is important to consider that both length and weight may serve as proxies for various factors such as species, sexual maturity, health, diet, and habitat quality, thus a thorough investigation is warranted to understand such dynamics [85,86].

4.3. *Relationships of EDCs concentrations with niche and feeding behaviour*

A significant difference ($p < 0.05$) in bioaccumulation of DEP and DEHP was observed between pelagic and benthopelagic fish (Fig. S2). A significant variation was also observed in As accumulation between pelagic and benthic, and benthopelagic and benthic species. For all three of these contaminants, pelagic fish species showed the highest bioaccumulation, indicating their uptake from organisms of the pelagic food web such as algae and zooplankton [87–89].

With regards to feeding behaviour, As was the only EDC that showed a significant ($p < 0.05$) variation in accumulation between herbivorous, carnivorous and omnivorous species (Fig. S3). Omnivorous species accumulated higher concentrations of As on average, whereas carnivores reported the lowest concentrations2. As discussed in the previous section, this may be owed to the ease of oxidation compared to methylation of As in carnivores [90].

4.4. *Risk assessment of EDCs exposure to GD*

The current study relies on the dietary tissue residue guidelines method to assess the health risks that GD may encounter due to exposure to EDCs. As toxicological data were only available for a select group of EDCs, risk assessments were not carried out for those lacking such data.

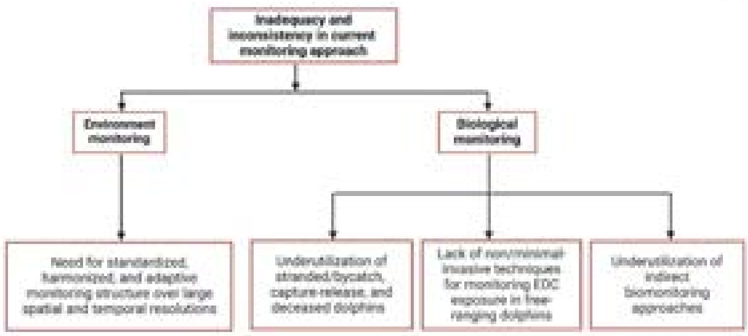

Fig. 5. Identified issues in the current approach for monitoring chemical contaminants in Gangetic Dolphin and its habitats Color should be used for figure in print. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

A summary of the calculated Risk Quotients (RQs), based on MAC_{RfD} and MAC_{TRV}, that selected categories of EDCs in prey-fish species may pose to GD, is provided in in Fig. 4a-d.

In general, the average risks associated with EDCs, as assessed by MAC_{TRV}, was consistently less than 1, with all RQs falling within the range of <0.001 to 0.51. The average risk associated with dietary exposure to DEHP and As was noted to be moderate (Fig. 4a), with data from the 95th percentile indicating high risk for both EDCs (Fig. 4c).

The RfD, commonly utilized in human health risk assessment, offers a more robust and conservative evaluation of potential adverse health effects associated with exposure to environmental contaminants, incorporating higher safety factors for added protection to threatened species.

Based on the MAC_{RfD}, DEHP > DDT > DnBP > As > PCBs > Hg > Cd, demonstrated a high average potential risk (RQ = 2.72–30.84) to GD through dietary intake whereas HCHs revealed moderate risk (RQ = 0.17). The substantially high RQs can be attributed to both the high toxicity of the chemicals and their widespread usage within densely populated, agriculturally dominated and industrially active catchment area. In the present study, the RQ values for TCS, BPA, and DEP remained below 1, indicating their low potential to pose a risk to GD.

Considering a worst-case scenario based on 95th percentile data, the RfD-based RQs for DEHP, DDT, and As exceeded 100, while ranging from 12.41 to 27.65 for DnBP, PCBs, and Cd.

To date, there are no known studies specifically investigating the dose-response relationships of contaminants on Gangetic dolphins. Hence, given the known health impacts of EDCs in other aquatic mammals including cetaceans, we can anticipate potential impacts on Gangetic dolphins.

The substantially high RQ_{RfD} for DEHP and DnBP may pose an elevated risk to these species, as phthalates and their metabolites can potentially affect thyroid function [90] and cause lipid disruption [91] in cetaceans.

Similarly, persistent organochlorines such as DDTs and PCBs have serious implications for cetacean health and populations around the world through endocrine disruption, carcinogenicity, cytotoxicity, reproductive impairments and immunosuppression [74,92–95]. PCBs and their alternatives have been recorded to significantly raise tetraiodothyronine, testosterone, and cortisol levels of the Indo-Pacific finless porpoise [74] and have been also associated with the population collapse of the common seal [16], and Killer whales (Orcinus orca) [96–98]. Additionally, the high tendency of these organochlorines for transplacental transfer raises further concerns about their potential to exert toxic effects on foetal growth and development [99].

PTEs such as Hg, Cd, As and Pb have been associated with various immunotoxic and neurotoxic effects in aquatic mammals [100, 101]. In St. Lawrence Beluga Whales, individuals with high Hg and Pb concentrations were also observed to have chronic lesions and reproductive impairment [102].

Although the current study indicates a low risk associated with TCS, TCC, and BPA, their known toxicity to coastal cetaceans [74, 103,104], along with their high production and widespread usage, justifies their inclusion in regular monitoring programs as a proactive measure.

Considering the significant risks that these EDCs pose to GD, as identified in this study, alongside their adverse effects on other aquatic mammals, it is imperative to deepen our understanding of these threats to GD for effective conservation.

A pivotal first step in addressing the threats from these EDCs could involve establishing a robust monitoring program to assess the levels and effects of these EDCs in GD and their habitat. Such a program would lay the groundwork for informed, effective conservation actions and policy development aimed at reducing pollution and protecting this umbrella species and its ecosystem.

4.5. Way Forward: Issue-based monitoring recommendations to strengthen existing GD conservation program

While marine cetaceans have greatly benefited from monitoring programs targeting chemical pollution threats [105–107], there is a marked lack of such monitoring program for GD highlighting a significant oversight in current conservation efforts. This gap in

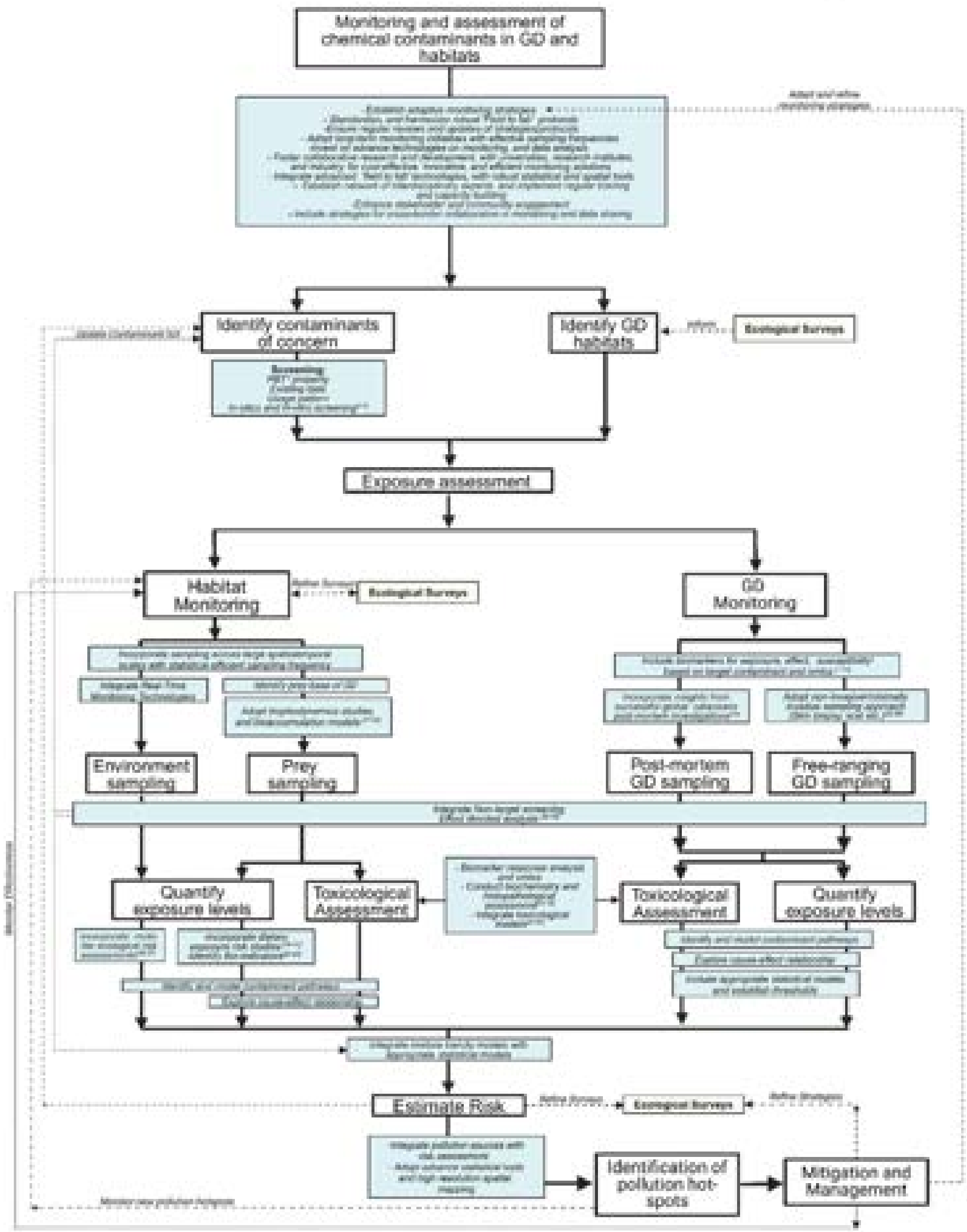

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Fig. 6. Proposed framework for monitoring chemical contaminants in Gangetic Dolphin and its habitats. Green box represents the pre-requisite deemed necessary for the successful implementation of the framework; yellow box represents contributions from ecological survey (such as habitats, population dynamics, distribution range, biology, and behaviours). Dashed lines represent secondary interactions between different components of framework. References 1–49 are provided in Supplementary Information. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

monitoring is particularly concerning given that a high extinction risk often coincides with low availability of data, especially those pertaining to dose and response [100]. Addressing this research gap is paramount for devising comprehensive conservation strategies that effectively mitigate chemical pollution threats to species, thereby enhancing their chances for survival and stability.

To address this, we identified the issues with the current approach for monitoring chemical contaminants in GD and its habitats (Fig. 5). Based on the identified issues, we propose an issue-based framework for monitoring and assessing chemical stressors affecting Gangetic dolphins and their habitats (Fig. 6). This framework is designed to provide relevant data on exposure and risk assessments, which are imperative for formulating informed conservation strategies and policy interventions.

Expanding on our earlier GD conservation guidelines [13], the proposed framework is a detailed strategy devised in collaboration with interdisciplinary experts. This tailored approach focuses on meeting the specific monitoring requirements of GDs and their habitats in response to the challenges posed by chemical pollution.

Furthermore, the proposed framework's development is informed by valuable insights from successful monitoring programs, reports, and publications globally that focus on the threats posed by chemical pollution to aquatic mammals, particularly cetaceans ([107,109–117]).

At each stage of this framework, specific prerequisites are identified and deemed necessary for the successful implementation of the framework. It is noteworthy that the framework is designed in such a way that its scope extends beyond riverine cetacean species other than those covered in the present study.

5. Conclusion

This study presents the first investigation into the health risks posed to Gangetic dolphins by multi-class EDCs.

Plastic additives, DEHP and DnBP, were the dominant EDCs in the prey fishes of GD. The study highlights the prevalence of unintentionally produced PCBs in the dolphin prey. Fresh inputs of p,p′ DDT, and γ-HCH in GD prey indicates the ineffectual compliance with regulatory standards and policies at the regional level.

Bioaccumulation of some EDCs in prey fish were found to be dependent on foraging behavior (As), niche (DEP, DEHP, As), trophic level (DDT, As, and Hg) and size (Cd and BPA). The trophic magnification, displayed by DDT and Hg, is particularly concerning due to its potential for severe impacts on apex predators like GD. Agricultural settlements, vehicular emissions, improper plastic disposal, textile industry, and high tourist influx are identified as the primary drivers of EDCs contamination in MGR. Implementation of stricter regulations on industrial discharges, improving wastewater treatment infrastructure, and promoting sustainable practices in agriculture and industry are essential to mitigate the sources of these contaminants and minimize their impact on GD and their habitats.

A screening-level ecological risk assessment, utilizing both TRV and RfD, reveal varying levels of risk to GD through dietary exposure. While TRV-based risk for most EDCs were low, RfD-based RQ showed high risk to GD from DEHP, DDTs, DnBP, As, PCBs, Hg, and Cd. Given their known adverse effects on other mammalian species, especially cetaceans, these threats should not be overlooked. While the current study suggests a low risk associated with BPA, and TCS, TCC, considering their high production, widespread usage, and known toxicities, it is recommended to include these EDCs in regular, comprehensive monitoring initiatives as a proactive measure.

Efforts should focus on understanding the mechanisms of exposure, bioaccumulation, and toxicological impacts of EDCs on GDs and their habitats. Holistic investigations of EDCs and other contaminants of concern, across large temporal and spatial scales in GD and its habitats are also recommended.

Furthermore, the framework proposed in this study has the potential to enhance GD conservation efforts.

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CRediT authorship contribution statement

Ruchika Sah: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Gautam Talukdar:** Supervision, Writing – review & editing. **Megha Khanduri:** Formal analysis. **Pooja Chaudhary:** Data curation. **Ruchi Badola:** Funding acquisition, Project administration, Resources, Supervision. **Syed Ainul Hussain:** Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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.

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Appendix A. Supplementary data

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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*) occurring 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 Cu 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; Cassola Gatti, 2016; Kenn 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; Ghosh et al., 2022).

The occurrence 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 from (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; Basnyal 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, 2019). PTEs have been documented to be associated with neurotoxicity, immunotoxicity, cytotoxicity and genotoxicity in aquatic mammals (Wise et al., 2009; Meuzo 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 (Liu 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.

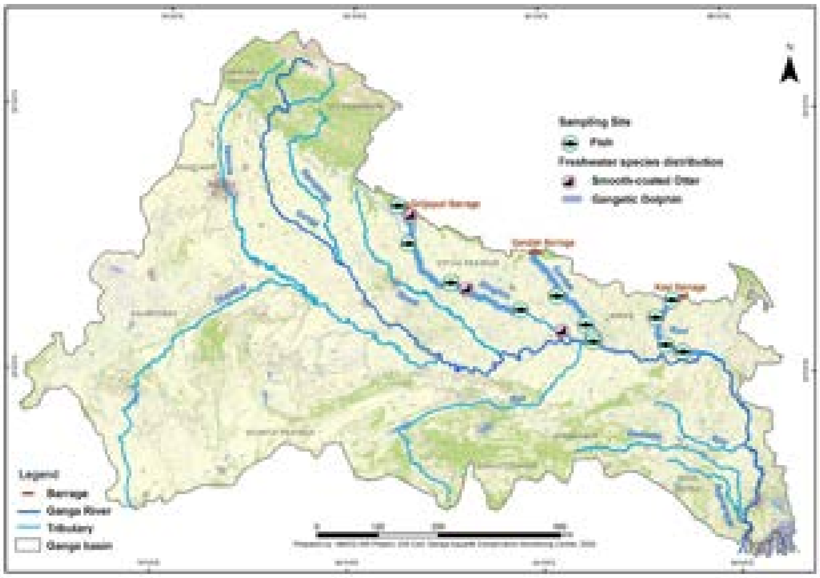

Fig. 1. Study Area representing the sampling sites and GD and SCO habitat in Kosi, Gandak, and Ghaghra River of Ganga River Basin, India.

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

Species	Trophic level	Feeding habit	Habitat preference	Number of individuals
Ailia coila[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
Clupisoma garua	3.7 ± 0.39	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 ± 0.6	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 108%.

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 $<\pm5\%$. 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 analyzed 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; Dos 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

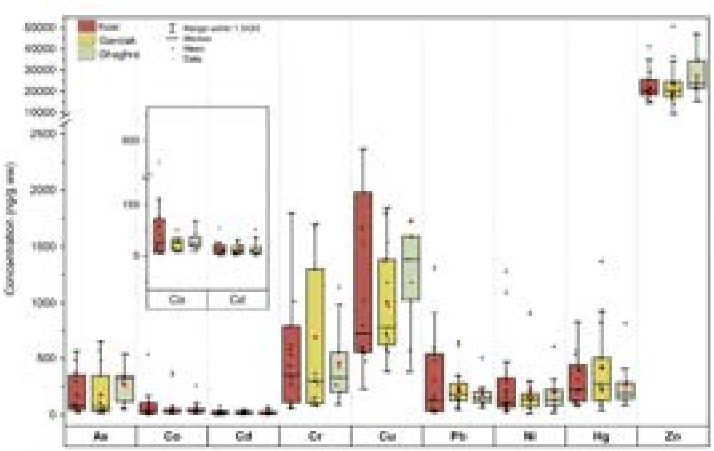

Fig. 2. River-wise concentrations of PTEs (ng/g ww) in fish tissues collected from Gandak, Kosi and Ghaghra Rivers, India.

growth in the built-up area, the catchment lacks heavily industrialized zones, distinguishing it from other rivers, like the Ganga, where evidence of PTE pollution is prevalent (Singh et al., 2020; Parveen 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, 2022). 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; Sinnam 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; Liu 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, 2019; 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 (Liu 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 (Wang et al., 1997; Zhou and Wong, 2000; Hosseini et al., 2013). The recovery of sediments from Hg contamination is known to be slow (Dianne Koper et al., 2015), 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, 2003; 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

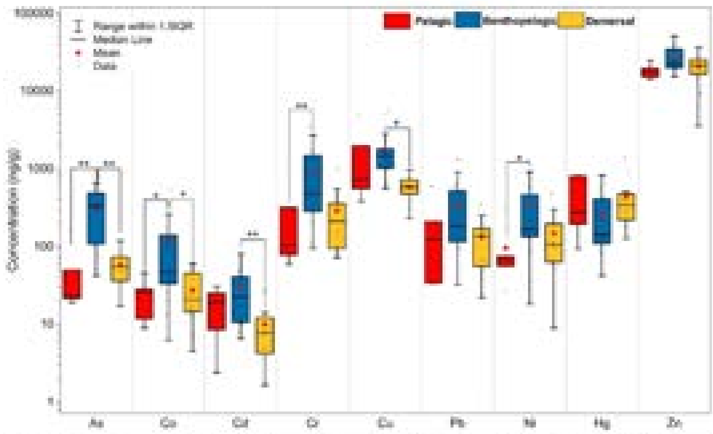

Fig. 3. Variations in tissue PTE concentrations (ng/g ww) in tissues of fish species collected from Gandak, Kosi and Ghaghra Rivers, India, with habitats.

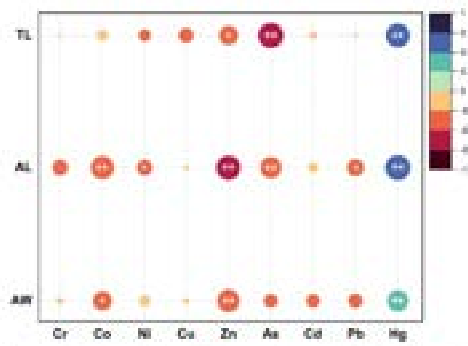

Fig. 4. Correlation of tissue PTE concentrations with ecological factor (TL: Trophic level) and biological traits (AW: Average weight; AL: Average Length) Correlation significant at * = $p < 0.05$; ** = $p < 0.01$.

(Richter et al., 2000; Mountopoulos and Stergiou, 2002; Romanuk et al., 2011; Morais et al., 2015; Zhang et al., 2020; Hoang 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 SCD. 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; Means 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 carcinogenicity and toxicity (Neff, 1997). However, other authors report its potential for carcinogenicity 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

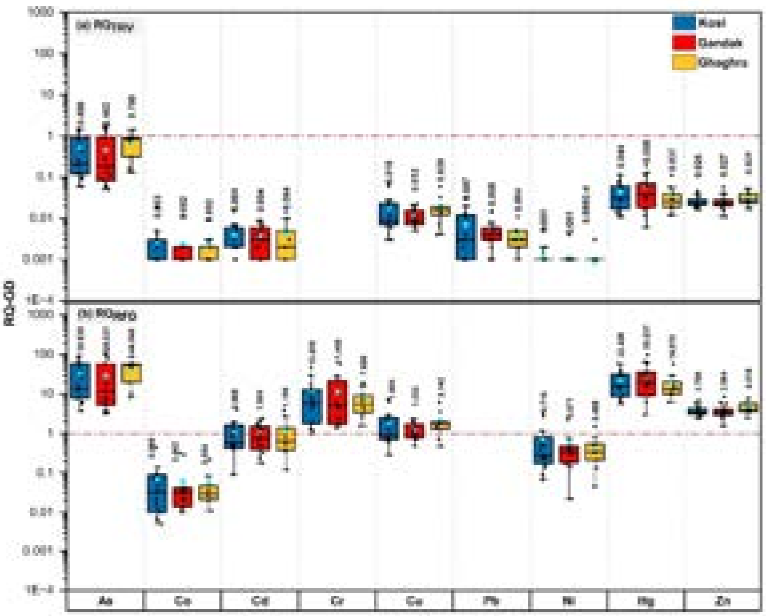

Fig. 5. Mean Risk Quotient (RQ; Log scale) for each PTE in the three rivers calculated for Gangetic dolphin from (a) Toxicity Reference Values (TRV), and (b) Reference Dose (RfD).

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 major 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; Baos et al., 2005; Crotone 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; Hyvärinen et al., 2003; Stroin, 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 (Fruschetti, 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.

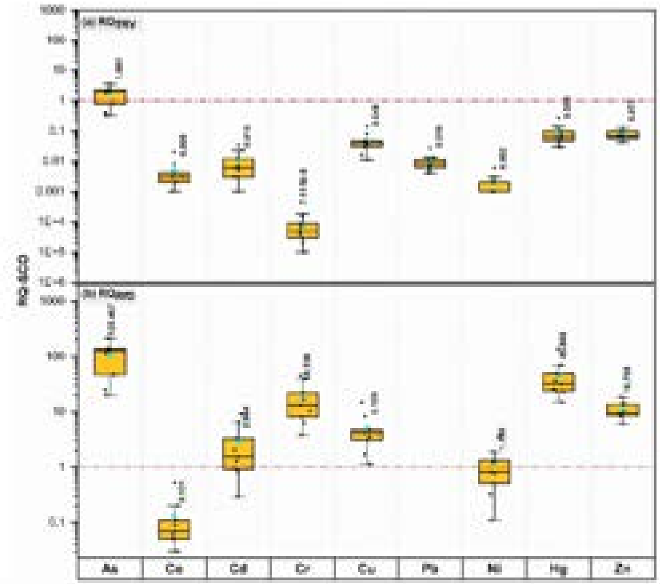

Fig. 6. Mean RQ (Log scale) for each PTE in the three rivers calculated for Smooth-coated otter from (a) Toxicity Reference Values (TRV), and (b) Reference Dose (RfD).

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 Khandurí:** Writing – original draft, Formal analysis, Data curation. **Pooja Chaudhary:** Formal analysis, Data curation. **K. Thomas Paul:** Formal analysis. **Samridhi Gurwani:** 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.2024.123920.

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First account of spatio-temporal analysis, historical trends, source apportionment and ecological risk assessment of banned organochlorine pesticides along the Ganga River☆

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Ecological risk assessment

A B S T R A C T

We conducted the first comprehensive assessment of the presence, source, and ecotoxicological implication of 13 banned and restricted organochlorine pesticides (OCPs) in the surface water along the Ganga River for two different seasons. Surface water samples were collected along the 2525 km stretch of the Ganga through 43 sites representing five zones of diverse land-use pattern, pesticide consumption rate, and varied flow. The mean concentrations of ΣOCPs were significantly higher (~2–5 times) in the post-monsoon or wet season [range: 0.126 to 10.402 μg/L (mean: 2.482 μg/L ± 3.589 and median: 1.433)] than in the post-winter or dry season [range: 0.053 to 3.010 μg/L (mean: 0.765 μg/L ± 1.033 and median: 0.399)]. Lindane (γ-HCH) was the dominant and most frequently detected pesticide at all the sites, indicating possible continued use of this banned pesticide in agricultural practices. The spatial distribution of OCPs revealed non-significant difference amongst different zones and indicate that point source pollution from the open drains along the Ganga could be responsible for observed trend. Ratio diagnostic analysis highlighted the fresh inputs and potential illegal use of lindane and chlordane at all the zones whereas, historical use of DDT was revealed at the majority of sites. Interestingly, fresh inputs of DDT were observed in the relatively pristine high altitude Upper zone (UZ) suggesting long-range atmospheric transfer and its continued use in the zone. Risk quotient (RQ) analysis revealed high ecotoxicological risks (>1), at all the studied sites for p, p′ DDE. The lower zone (LZ) emerged as a high ecological risk zone. The study highlights that though the OCPs analysed in this study are banned/restricted in India, still the implementation of the ban is poor and delayed and the country requires stricter adherence to its National Implementation Plan (NIP) on pesticides.

1. Introduction

Agriculture, along with its associated sectors, is the primary source of livelihood for 58% of the population in India and plays a central role in ensuring the steady growth of the Indian economy. One of the pivotal contributors toward food security and accelerated agricultural yields is the use and application of pesticides and other chemicals. Consequently, the agrochemical industry in India has witnessed an outstanding growth. However, high pesticide applications have also led to the accumulation of these chemicals in the environment through several routes (Schulz, 2001; Gavrilescu, 2005; Holvoet et al., 2007; Tanaka and Katagi, 2008), which has the potential to affect ecosystem and its services" (Tanaka and Katagi, 2008; Carriquiriborde et al., 2014).

Among pesticides, organochlorines pesticides (OCPs) cause serious concern owing to their persistence, toxicity, long-range transmission, and bio-accumulative nature (Naqvi and Vaishnavi, 1993; Willett et al., 1998; Contreras López, 2003 Briz et al., 2011; Gao et al., 2013). In human beings, studies have indicated possible relationships between OCPs and various cancers (Mathur et al., 2002; Rathore et al., 2002; Abdo et al., 2013; Louis, 2019; Ennour-Idrissi et al., 2019), teratogenicity (Kalra et al., 2016; Kim et al., 2017; Ramakrishnan and Jayaraman, 2019), endocrine disruption (Younglai et al., 2004; Fowler et al., 2007; Frye et al., 2012), neurotoxicity (Shinomiya and Shinomiya, 2003; Sharma et al., 2010; Song et al., 2012; Heusinkveld and Westerink, 2012), and genotoxicity (Pandey et al., 1990; Ramirez and Cuenca, 2002; Poli

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et al., 2003; Ennaceur et al., 2008). Similarly, wildlife species exposed to OCPs have shown high rates of malformed genitalia (Sonne et al., 2006), aberrant mating behavior (Fry, 1995), sterility, cancer, egg-shell thinning, and immune system and thyroid dysfunction (Colborn and Smolen, 1996; Briz et al., 2011; Bergman et al., 2012; Newton, 2013; WHO, 2013; Godfray et al., 2019).

Until the year 2006, when India ratified the Stockholm Convention, large amounts of OCPs banned by other countries were still manufactured, used, and exported. Post-ratification, India banned the manufacturing, import, export, and use of all of the 12 persistent organic pollutants (POPs) listed in the convention, except for dichlorodiphenyltrichloroethane (DDT). India is the only major country worldwide to still manufacture and use DDT for vector control and is allowed exemption until 2022. Specific exemption (up to the year 2019) was also allowed for its use as an intermediate in the production of Dicofol (another OCP used against mites). The country also exports DDT to several countries either as a pesticide or as a chemical intermediate (Toxics Link, 2018). However, the production of DDT has declined in the last decade, from 3310 MT in 2008–2009 to 1370 MT in 2018–2019 (Annual Reports, Dept. of Chemicals and Petrochemicals, 2011–2019). Among hexachlorocyclohexanes (HCHs), only lindane (γ-HCH) is banned for production, import, formulation (since March 2011), and use (since March 2013). However, India does not have any restriction on α-, β-, and δ-HCHs (PPQS, 2019). The manufacture, sale, use, and export of endosulfan has been banned since May 2011; however, data suggest exportation of endosulfan from India still occurs. (Dept. of Commerce, India, 2019). The supreme court of India had allowed export of stockpiles of endosulfan, which is lying in stock unsold as an inventory, to other countries that were interested (Interim report, Joint expert Committee, 2011). A summary of other OCPs listed as POPs under the Stockholm Convention and thus banned in India is given in Table S1.

Despite the imposed ban, over the last two decades, high levels and distributions of OCPs have frequently been detected across many rivers in India (Table 3 & Table S10), including the large rivers (Table: S11). Hence, the investigation of OCPs in aquatic environments especially large rivers is the primary source of information to assess the actual status of the pesticide ban policy in the country. The concentrations serve as an indicator of contaminant load and anthropogenic impact on the environment.

For our study, we chose the Ganges River (Ganga), the second-largest river in India supporting 36.1% of the country's population (438.2 million), about 70% of which is dependent on agriculture (Census, 2011). The Ganga has exceptional value in the country owing to its religious, economic, and ecological significance. During its course, from origin to outfall, the Ganga is joined by several tributaries to form the vast and highly fertile Indo-Gangetic plains, which has given rise to intensive agriculture practices along the river (Karnick, 2011). The cumulative consumption of pesticides in Ganga states between 2012 and 2017 was 72,741 metric tons, accounting for 27% of the total pesticide consumption in the country (PPQS, 2017).

Previous studies on the Ganga have shown OCPs to be the dominant group of pesticides detected in selected stretches of the river (Rehana et al., 1995; Nayak et al., 1995; Sankararamakrishnan et al., 2005; Ghose et al., 2009; Singh et al., 2012; Mutiyar and Mittal, 2013; Raghuvanshi et al., 2014; Chakraborty et al., 2016; and Mondal et al., 2018). These studies have mostly focused on DDT, HCH, and endosulfan occurrence in selected stretches without considering the status of other banned pesticides or the full spatial coverage of the river. Besides, assessment of the ecological risks to aquatic species, temporal trends, and possible sources, are also lacking in these studies. The lack of a comprehensive study has affected biodiversity conservation plans and environmental monitoring efforts for the Ganga. Consequently, further research is needed to improve the current information on OCP pollution in the Ganga and create baseline data on the status of banned OCPs across all five Ganga states.

In this paper, we aim to investigate the occurrence, spatiotemporal distribution, potential sources, and ecological risks of persistent and banned OCPs to aquatic species along all the five Ganga states. To the best of our knowledge, this is the first comprehensive study on Ganga to include the above four criteria. This study is a part of the project "Biodiversity Conservation and Ganga Rejuvenation" aimed at developing comprehensive measures to conserve and safeguard the aquatic biodiversity of the Ganga. The results from this study will be helpful to the policymakers in identifying the status and ecological risks of banned pesticide usage at the state level. This in turn will help in designing effective monitoring strategies and formulating appropriate pesticide mitigation efforts that would be directed toward sustaining the healthy ecological diversity of the Ganga.

2. Material and methods

2.1. Study area

The Ganga lies between north latitudes 22°1′43.284″–30°49′59.99″ and east longitudes 79°09′60.00″–88°12′37.584″. The drainage in the Ganga is a combination of precipitation, tributaries, and snowmelt water from the Himalayas. It covers a distance of 2525 km as it flows through five states from the north to the east of the country (Fig. 1).

The catchment of the Ganga is an area of intensive agriculture activity. The Ganga states has a high net irrigation to sown ratio (62.6%) compared to that of the entire country (44%) ESMF (2011). The major crops cultivated in the five Ganga states are either Rabi or Kharif. The Kharif crops such as rice, maize, and sugarcane are sown in June and July and are harvested in September and October, whereas the Rabi crops such as wheat, pulses, and peas are sown in the period between October and December and harvested in April and May. During the dry season (January to May), the riverbed cultivation of seasonal vegetables is a common practice in the dry riverbeds of the Ganga (Uttar Pradesh State Biodiversity Board, 2013; Kumari et al., 2018). The farmers of the Ganga belt practice multiple cropping pattern; hence, the fields are irrigated more than once a year.

Ganga is also home to a rich and diverse fauna, including endangered species, such as the dolphin *Platanista gangetica*; the otters *Lutrogale perspicillata*, *Lutra lutra*, and *Aonyx cinereus*; the gharial *Gavialis gangeticus*; the crocodiles *Crocodylus palustris* and *Crocodylus porosus*; the turtle *Batagur kachuga*; and the fishes *Tor putitora* and *Tenualosa ilisha* (WII, 2018).

Along its course, the Ganga receives 139 drains, discharging 6087 MLD (million litre per day) of municipal and industrial waste daily and it is reported that there is a gap of 80% between generated wastewater and treatment capacity (CPCB, 2013). Both point source (drains) and non-point source (agriculture) of pollution could severely affect the water quality, as well as the life and health of the species—including humans—depending on it.

2.2. Sampling sites

Among the five Ganga states, there is a significant difference in the environmental flows, demographics, average pesticide consumption rates, and land-use patterns (Table 1 & Table S8). Therefore, based on the key features presented in Table 1, we demarcated the 2525 km stretch of the Ganga into five homogenous zones (Fig. 1 & Table 1), to gain a better understanding of the

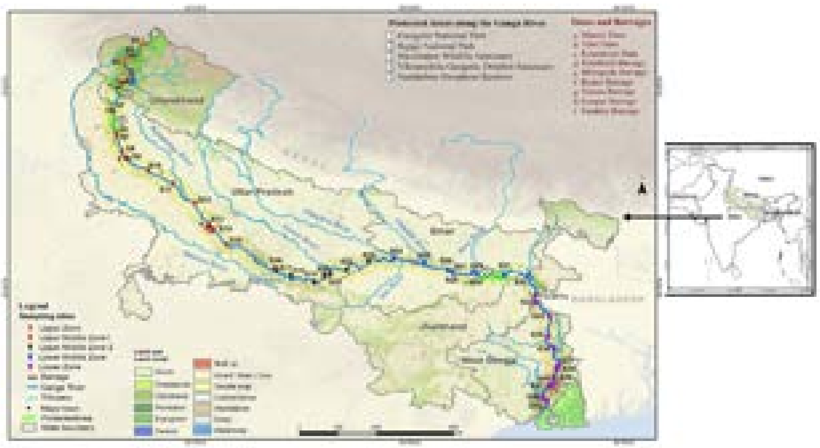

Fig. 1. Representation of sampling sites along Ganga River and segregation into different zones.

Table 1
Demarcation of zones based on demographics, cultivable land, pesticide consumption, and local precipitation in the states of the Ganga.

States	River length (Km)	Geographical Area (Sq Kms)	Demography (Billions) Census (2011) [a]	Total cropped Area (000 Thousand Hectares, 2013–2014) [b]	Cropping Intensity[#] (%) (2013–2014) [b]	Total pesticide consumption (%) 2016–2017 [c]	Annual precipitation 2016	Annual precipitation 2017 [d]	Zone Demarcation
Uttarakhand	450	53483	0.01	1099	146	0.92	1308.6	1476	UZ
Uttar Pradesh	1000	240928	0.2	25896	145	71.03	692.8*	585.2*	UMZ1+ UMZ2++
Bihar	400	94163	0.1	7580	123	5.88	1158	1112	LMZ
Jharkhand	45	79716	0.03	1672	59	3.79	1264	1165.8	
West Bengal**	540	88752	0.09	9618	172	18.38	1427	1568.6	LZ

*Average of total annual rainfall in East Uttar Pradesh and West Uttar Pradesh. **Ganga River in this state/zone is also known as Hooghly River.
+UMZ1 = Before Confluence with Yamuna River.
++UMZ1 = After Confluence with Yamuna River.
#Cropping Intensity = Gross Cropped Area x 100/Net Sown Area.
[a] Census of India Office.
[b] Directorate of Economics and Statistics.
[c] PPQS (2017).
[d] data.gov.in/catalog/rainfall-india.

factors influencing the distribution of OCPs in its different stretches. These five zones are identified as the Upper Zone (UZ), Upper Middle Zone 1 (UMZ1: before the confluence with the Yamuna River), Upper Middle Zone 2 (UMZ2: after the confluence), Lower Middle Zone (LMZ), and Lower Zone (LZ).

Within these five zones, 43 sampling sites (Table S2) were selected for detailed characterization of the OCP pollution dynamics. These sites were selected based on the presence/absence of agricultural and industrial belts, identifying gaps through detailed literature references from previous studies and considering the existing monitoring stations prescribed by the Central Pollution Control Board of India. Taking the above criteria into account, we conducted a rapid reconnaissance survey between May and June, 2017, to finalize the sampling sites. Subsequently, samples were collected throughout two seasons (post-monsoon or wet season [October to November 2017] and post-winter or dry season [April to May 2018]) to characterize the influence of seasonal variation on the water quality.

2.3. *Pesticide sampling method and analysis*

At each sampling site (n = 43), three surface water samples were collected randomly, at an average depth of 30 cm, in amber-colored pre-cleaned high-density polyethylene (HDPE) bottles, which were bulked together to form a composite sample. After collection, samples were stored in an ice box for shipping to the laboratory and kept at 4 °C until analysis (USGS, 2006; CPCB, 2007).

2.3.1. *Target pesticides*

Thirteen OCPs investigated in this study are selected on the basis of their high historic usage, detection frequency reported in previous studies (Table 3), pesticides included in the CPCB- India monitoring network (CPCB, 2013), OCPs listed as POP under Stockholm convention (UNEP (2012)), and, OCPs with endocrine disrupting properties (Ratcliffe, 1970; Schreiber and Risebrough, 1972; Gress et al., 1973; Helle, 1976; Fry and Toone, 1981; Cranmer et al., 1984; Cummings and Gray, 1987; Roy Chowdhury et al., 1987; Gray et al., 1989; Guillette et al., 1994; Willingham and Crews, 1999; Guillette et al., 2000; Huang et al., 2004a,b; Bergman et al., 2012). The target analytes are:

p,p′-dichlorodiphenyltrichloroethane(p,p′-DDT), p,p′-dichlorodiphenyldichloroethane (p,p′-DDD), p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE), alpha-hexachlorocyclohexane (α-HCH), beta-hexachlorocyclohexane (β-HCH), gamma-hexachlorocyclohexane (γ-HCH) or lindane, delta hexachlorocyclohexane (δ-HCH), Chlordanes (CHLs) namely cis-chlordane (c-CHL), and trans-chlordane (t-CHL), Methoxychlor (M-CHLR), Endosulfan (ES) namely α-endosulfan (α-ES), and β-endosulfan (β-ES) and, endosulfan sulfate (ESS).

2.3.2. Chemicals and instruments

The OCP standard mixture solution containing 13 target analytes with >99% purity was purchased from Sigma-Aldrich (USA). A standard OCP stock solution (500 mg/l) was prepared with hexane and stored in dark at 2 °C before use. All the solvents and chemicals used in the extraction process were chromatographically pure grade (Merck, USA). A Shimadzu Model 2010 Gas Chromatograph (GC) equipped with 63Ni Electron Capture detector (ECD) was used for detection and quantification of OCP residues.

2.3.3. OCP extraction and analysis

Following flow diagram shows the extraction method, performed as recommended by EPA method 1699 (EPA, 2007) with slight modifications:

1 L Water → Filtration (0.45 μm filter)→ Liquid-Liquid Extraction ([twice with DCM − 35 ml) & (final Hexane −25 ml & 7.5 ml NaCl])→ Anhydrous Na2SO4(5 gm) → Drying → Reconstitution in 1 ml n-Hexane → GC-ECD (Rtx-5 (30 m × 0.25 mm × 0.25 μm, Restek)

The GC programme was set as follows:

Injector and detector temperature - 200 °C and 320 °C respectively; Oven temperature (Initial) 100 °C (1 min) → 180 °C at 25 °C/min (2 min), ramp 5 °C/min → 240 °C (1 min), and at 4.5 °C/min to 260 °C (2 min) → at 10 °C/min to 280 °C (5 min).

Furthermore, 20% of samples were validated and confirmed by GC-MS/MS results with RSD ≤ 10%. The samples were send to the Shimadzu Analytical Lab, India for GC MS/MS analysis.

2.4. Quality assurance and quality control (QA/QC)

QA/QC was performed according to the requirements of ISO/IEC 17025 and US EPA guidelines. Field, laboratory blank, and solvent blank samples were run routinely to check for interferences and cross-contamination. The blank concentrations were less than the method detection limit (<MDL). Linear calibration curves were obtained with the r^2 value of 0.999 and calibration verification (standard deviation) was less than 5%. The target compound concentrations were quantified by an external standard method using the peak height and area of the standards at five level calibration curves. A matrix spike recovery study was performed by spiking the samples with known working standard solutions of OCPs and extracted and analysed in the same way as the real samples. The matrix spike recoveries were in the range of 82%–124% for the studied compounds. After every 10 samples, a standard quality check was performed. The results of the analysis are reported in μg/L and Schimadzu Lab Solution Software was used for data acquisition (Tables S3 and S4). MDLs were assessed based on US Environmental Protection Agency (EPA) guidelines (USEPA, 2016) and the minimum levels of quantification were determined based on concentrations 10 times the detected MDL. Any target analyte detected below the MDL was considered as below detection limits (<DL).

2.5. Potential ecological risk assessment

An ecological risk assessment across the stretch of the Ganga was conducted using the ecological risk quotient (RQ) model. RQ is established based on Eq. (1).

$$RQ = \frac{MEC}{PNEC} \tag{1}$$

where, MEC is the mean or maximum measured environmental concentration and PNEC is the predicted no-effect concentration. PNEC is derived from the lowest toxicity value (i.e., no-observed effect concentration (NOEC) value) observed for the most sensitive species. When NOEC values were not available, we used LC50 or EC50 values after correction by an assessment factor intended to extrapolate from acute to chronic toxicity and for removing the uncertainty arising from the extrapolation from intra- and inter-species variability in sensitivity (Table S6), (European Commission, 2000) based on Eq. (2).

In this assessment, the respective NOEC, LC50 or EC 50 values for three trophic levels (primary producers i.e., algae; primary consumers i.e., aquatic invertebrates; secondary consumers i.e., fish) were used to determine the PNEC.

$$PNEC = \frac{NOEC\ or\ LC50\ orEC50}{Assessment\ Factor} \tag{2}$$

For most pesticides, NOEC/LC50/EC50 values were obtained from the Pesticides Properties DataBase (PPDB) whereas others were taken from US EPA ECOTOXicology knowledgebase (ECOTOX). Furthermore, to identify the high-risk zones, the average and maximum detected concentrations at each zone were used for determination of the general (RQm) and worst-case (RQex) scenarios, respectively.

The risk to aquatic species, based on risk ratios, was subsequently classified into four risk levels comprising high, medium, low, and negligible ecological risks, corresponding to RQ values ≥ 1, 0.1–1, 0.01–0.1, and <0.01, respectively (Sánchez-Bayo et al., 2002; Palma et al., 2014; Zhang et al., 2016).

2.6. Statistical analysis and Softwares

The sampling data were tested for normality using the Kolmogorov-Smirnov test. As the distribution was non-normal therefore, Kruskal-Wallis (KW) with Dunn's post-hoc was applied to test significant differences among zones whereas Mann-Whitney U test (MWU) was conducted to test the temporal differences. Differences were considered significant at $p < 0.05$. Statistical analyses were performed using the statistical software SPSS (window version 23.0.).

Spatial distribution of OCPs along the Ganga River was analysed with the help of ArcGIS 10.6 (Esri).

3. Results and discussions

3.1. Occurrence and seasonal distribution of OCPs

A summary of the descriptive analysis of the 13 target OCPs detected in the Ganga is presented in Table 1. The total OCP concentrations (ΣOCPs) in water samples ranged from 0.126 to 10.402 μg/L (mean: 2.482 μg/L; median: 1.433 μg/L) in the post-monsoon season, and 0.051–3.010 g/L (mean: 0.765 μg/L; median: 0.399 μg/L) in the post-winter season. The mean concentrations of ΣOCPs were significantly (MWU test, $U = 621$, $p = 0.009$) higher (~2–5 times) in the post-monsoon season than in the post-winter season (Fig. 2). This could be explained by the high atmospheric

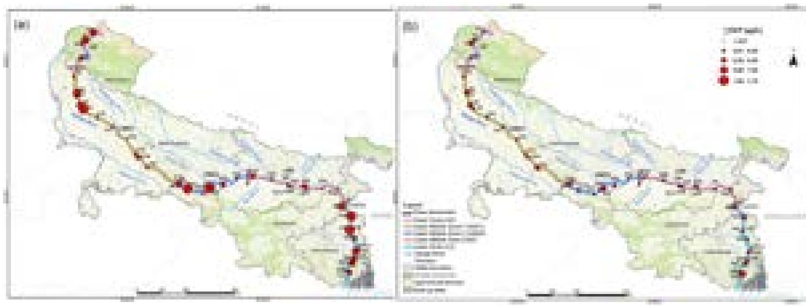

Fig. 2. Seasonal and spatial distribution of total OCPs in surface water samples of Ganga River in (a) Post-Monsoon season (b) Post-Winter season.

precipitation and subsequently higher agricultural surface runoff in the monsoon season, which facilitates the entry of pesticides into nearby waterbodies. This view is further confirmed by previous studies conducted in different river bodies of India (Kumarasamy et al., 2012; Singh et al., 2012; Alam et al., 2015; Maurya and Malik, 2016) and worldwide (Tanabe et al., 2001; Zhang et al., 2004; Yang et al., 2004; Li et al., 2010; Toan et al., 2013; Chen et al., 2018; Sun et al., 2018; Wang et al., 2018). In addition, just before the monsoon, approximately 5 km of land on both sides of the river bank in all the zones except for UZ is utilized extensively for the dry riverbed cultivation of seasonal vegetables, with possible high pesticide applications. These pesticides will likely enter surface water as runoff because of the high flooding and precipitation in the monsoon season, thus increasing their levels in the surface water of the river.

The most frequently detected OCPs (Detection Frequency (DF > 60%) in both the seasons were γ-HCH (DF 74%–95%) and α-HCH (DF 63%–95%). The lowest detection rate in both seasons was observed for isomers of endosulfan (α and β), with frequencies ranging between 9% and 20%. The groupwise mean concentration of OCPs in Ganga River for post-monsoon season followed the order ΣHCHs > ΣCHLs > ΣDDTs > ΣESs > M-CHLR > ΣESS. In the post-winter season, the OCP concentration followed the order ΣHCHs > ΣDDTs > ΣCHLs > ΣESs > M-CHLR > ΣESS. The lower detection rate and relatively low concentrations of M-CHLR and ESS could be attributed to their low usage in the past and relatively fast degradation in the water compartment (Table S1). The mean concentration of ΣHCHs in the Ganga River was approximately five times higher (*MWU test*, $U = 584$, $p = 0.003$) and that of CHL 1.4× higher (*MWU test*, $U = 907$, $p = 0.88$) in the post-monsoon season than in the post-winter season. The mean concentration of DDTs was found to be 1.5 × higher (*MWU test*, $U = 696.5$, $p = 0.003$) while ΣESs (*MWU test*, $U = 715.5$, $p = 0.03$), M-CHLR (*MWU test*, $U = 670.5$, $p = 0.08$), and ESS (*MWU test*, $U = 598$, $p = 0.007$) was 2.5–3x higher in the post-winter season than in the post-monsoon season. The high concentration levels of these OCPs during the post-winter season may be attributed to higher evaporation (low water level) and lower flow volume conditions in this season. Our findings are consistent with the results of previous studies conducted in various rivers of India (Malik et al., 2009; Raghuvanshi et al., 2014; Alam et al., 2015; Mondal et al., 2018).

3.1.1. *Seasonal composition of OCPs*

The compositional analysis of HCHs, DDTs, CHLs, and ESs for the post-monsoon and post-winter seasons are presented in Fig. S1. The compositional profile of HCHs revealed the dominance of the γ-HCH isomer with 71% in the post-monsoon and 43% in the post-winter season, followed by α-HCH (~23% for both seasons). The contribution (%) of two other HCH isomers, namely β-HCH and δ-HCH, toward ΣHCHs was found to be in the range of 1.03%–5% (post-monsoon) and 13%–17% (post-winter). Despite the ban, the widespread occurrence and elevated concentrations of γ-HCH residues could be attributed to their high historic usage (because of their low cost and broad spectrum insecticidal property), strong persistence, and potential fresh inputs (possibly through illegal sources) to the river. In addition, compared to other target OCPs that were banned years ago, γ-HCH was banned relatively recently (2013) in India (PPQS, 2019).

The percentage composition of DDTs revealed the dominance of *p,p'*-DDE (post-monsoon: 73%; post-winter: 48%), a breakdown product of *p,p'*-DDT, in the surface water of the Ganga that could be attributed to their slow degradation (Table S1) in water. *p',p'*-DDD was found to be 11% in the post-monsoon season and 27% in the post-winter season whereas *p',p'*-DDT contributed 16% in the post-monsoon and 25% in the post-winter season. The relative higher detection frequency (Table 2) of *p,p'*-DDD and *p,p'*-DDT in the post-winter season as compared to the post-monsoon season could be because of low dilution during the post-winter season resulting in their high concentration. Further, DDT is still being used in India during the post-winter season for malaria vector control. Therefore, higher concentration of *p,p'*-DDT could also be attributed to the fresh sprays of DDT.

Among the isomers of chlordane, t-CHL was dominant during both seasons (post-monsoon: 82%; post-winter: 54%), whereas among endosulfans, β-ES was identified as the dominant isomer (post-monsoon: 60%; post-winter: 84%).

3.2. *Zone wise distribution of OCPs*

Fig. 3 show the spatiotemporal dynamics and zone-wise comparison of ΣOCPs in the Ganga.

The zone-wise pollution gradient observed in the post-monsoon season was LZ > UMZ2 > UZ > UMZ1 > LMZ (Table S7).

The highest concentration of OCPs in the LZ (representing the state of West Bengal), in the post-monsoon season could be explained by the high cropping intensities in this catchment (Table 1), reduction in river flow (because of the diversion of river water to Bangladesh, and abstraction of water for irrigation) at Farakka Barrage (Table S8) and emergence of estuarine zone before the river enters the bay of bengal.

In the post-winter season, the OCPs followed the distribution pattern UMZ1 > LZ > UMZ2 > UZ > LMZ (Table S7). In the UMZ1, a substantial amount of water is diverted from the river to support agricultural activities through a system of canals, which may

Table 2
Concentration (μg/L) and detection frequency (%) rate of OCPs in water samples from the post-monsoon (n = 43) and the post-winter season (n = 43) for the Ganga.

Analyte	Post-Monsoon, October to November 2017 (n = 43)					Post-Winter, March to April 2017 (n = 43)				
	Range	Median	Mean	SD	DF%	Range	Median	Mean	SD	DF%
α-HCH	0.006–3.860	0.091	0.432	1.172	63	0.017–0.255	0.087	0.108	0.088	95
β- HCH	0.102–0.847	0.465	0.024	0.036	33	0.015–0.231	0.082	0.098	0.080	91
γ- HCH	0.004–4.346	0.812	1.722	1.888	74	0.016–1.137	0.111	0.233	0.367	95
δ - HCH	<DL -0.252	0.004	0.088	0.110	21	0.001–0.253	0.027	0.058	0.089	58
ΣHCH	**0.112–9.306**	**1.375**	**2.266**	**3.206**		**0.050–1.877**	**0.307**	**0.497**	**0.623**	
p,p′-DDT	<DL -0.091	<DL	0.014	0.032	14	0.002–0.147	0.024	0.042	0.054	20
p,p′-DDD	<DL-0.088	<DL	0.009	0.028	12	<DL-0.207	0.015	0.034	0.063	24
p,p′-DDE	0.004–0.286	0.025	0.070	0.105	47	<DL-0.215	0.018	0.062	0.084	28
ΣDDT	**0.004–0.465**	**0.025**	**0.093**	**0.165**		**0.002–0.568**	**0.057**	**0.138**	**0.201**	
t-Chlordane	0.010–0.345	0.008	0.086	0.116	53	0.001–0.126	0.015	0.036	0.044	34
c-Chlordane	<DL-0.141	<DL	0.018	0.046	16	<DL-0.172	0.002	0.036	0.070	19
ΣChlordane	**0.010–0.486**	**0.008**	**0.104**	**0.162**		**0.001–0.298**	**0.017**	**0.072**	**0.113**	
Methoxychlor	**<DL-0.034**	**0.003**	**0.007**	**0.013**	19	**<DL-0.066**	**0.001**	**0.015**	**0.024**	22
α-Endosulfan	<DL-0.033	<DL	0.005	0.012	12	<DL-0.027	<DL	0.004	0.010	9
β-Endosulfan	<DL-0.051	0.004	0.008	0.018	9	<DL-0.117	0.012	0.025	0.041	20
ΣEndosulfan	**<DL-0.084**	**0.004**	**0.013**	**0.030**		**<DL-0.144**	**0.012**	**0.029**	**0.050**	
Endosulfan Sulfate	**<DL-0.028**	**<DL**	**0.007**	**0.013**	12	**<DL-0.057**	**0.005**	**0.013**	**0.021**	24
ΣOCPs	**0.126–10.402**	**1.433**	**2.482**	**3.509**		**0.053–3.010**	**0.399**	**0.765**	**1.013**	

<DL = below detection limit.

Table 3
Comparison of the present results with other studies on the Ganga.

Sampling Time	Zone	Unit	Σ-HCH	Σ-DDT	Σ-ES	Reference
2014–2016	LZ	ng/mL	ND-2.940(↑)	ND - 1.313(↓)		Mondal et al. (2018)
Jun 2012	LZ	ng/L	ND- 114(↓)	ND - 5(↓)	ND - 10(↓)	Chakraborty et al. (2016)
2013–2014	UMZ1	μg/L	BDL-24.5(↑)	BDL-20.6(↑)	BDL(↓)	Raghuvanshi et al. (2014)
	UMZ2		BDL-13.6(↑)	BDL-18.6(↑)	BDL(↓)	
Dec 2010–Jan 2011	LZ	ng/L	5.5–7.74(↓)	ND-1.01(↓)	ND-0.92(↓)	Mutiyar and Mittal (2013)
Jun–Jul 2011	UMZ1		0.1–1(↓)	0.05–0.2(↓)	0.8–33.6(↑)	
Jun–Jul 2011	UMZ2		0.2–3.5(↓)	0.08–2.21(↓)	ND-85.4(↓)	
Jul–Aug 2011	LMZ		0.1–17.8(↓)	ND-12.3(↓)	ND-85.4(↑)	
2007	LMZ	ng/L	ND (summer)(↓) ND-48.3 (monsoon)(↓)		ND-157.3(↑)	Singh et al. (2012)
2008	LZ	μg/L	ND-6.65 (except lindane)(↓)	0.01–0.65(↑)		Ghose et al. (2009)
2003 (June to December)	UMZ1	μg/L	ND-0.260	ND(↓)		Shankaranamakrishnan et al. (2005)
March 1989 and March 1990	UMZ1	μg/L	ND-1.38(↑)	N.D(↓)	ND(↓)	Rehana et al. (1996)
March 1989 and March 1990	UMZ1	μg/L	ND-3.01(↑)	0.88–5.13(↑)	ND-0.75(↑)	Rehana et al. (1995)
August1992 (monsoon)	UMZ2	μg/L	ND-36.354(↑)	ND-79.818(↑)	ND-48.828(↑)	Nayak et al. (1995)

(↑) – Values higher than present study.
(↓) – Values lower than present study.
ND-Not Detected; BDL- Below Detection Limit – The values are reported as mentioned in the original paper/study.

considerably reduce the volume of water and concentrate the OCPs in the dry season. Additionally, the tributaries joining the Ganga in this zone, particularly the Ramganga and Kali, also bring significant domestic, industrial, and agricultural pollution loads, thereby increasing the pesticide burden in this zone (CPCB, 2016).

The lowest pesticide pollution gradient in the LMZ representing the states of Bihar and Jharkhand, in both seasons could be explained by the high drainage from major tributaries like the Ghaghra (94.4 billion cumecs), Gandak (52.2 billion cumecs), and Son (22.42 billion cumecs). These tributaries contribute significantly to the environmental flow (~5 times high as compared to other zones) and increased dilution factor (Table S9). In addition, the cropping intensity in this catchment is also low (Table 1) compared to that in other zones.

However, statistical analysis for difference between the zones was non-significant ($p > 0.05$) in both post-monsoon (*KW test, H (4), p = 0.34*) as well as post-winter (*KW test, H (4), p = 0.34*) seasons.

The non-significant difference among the zones could be attributed to the large number of point-sources (such as municipal and industrial waste water treatment drains) distributed all along the Ganga. A total of 139 drains carrying industrial and domestic sewage effluent join the Ganga directly. The storm water drains designed to flood-out the storm water during rainy season, are used for disposal of sewage and trade effluents, which ultimately joins the Ganga. These drains discharge pollution load of 361.2 tons per day (CPCB, 2016a) into the Ganga, having presence of pesticides that they may receive from the catchment area. A recent report from CPCB indicates that most of the drains joining Ganga had presence of organochlorine pesticides (CPCB, 2016b).

These factors may have eventually lead to the statistically non-significant difference between the zones despite the highest concentration of pesticide in the LZ and UMZ1.

3.2.1. Group wise abundance of OCPs in each zone

When assessing the zones for most abundant group of pesticides (Table S7), it was revealed that HCH isomers were found to be dominant (highest conc. and detection) in all zones for both seasons; however, in the dry season (post-winter) presence of other OCPs in also highlighted due to low dilution (Figs. 3b & 4).

The distribution of ΣHCHs across zones showed the order LZ > UMZ1 > UMZ2 > UZ > LMZ in the post-monsoon season and UMZ1 = LZ > UMZ2 > UZ > LMZ in the post-winter season. The zone-wise statistical comparison (*KW test, H (4), p = 0.05*), in post-

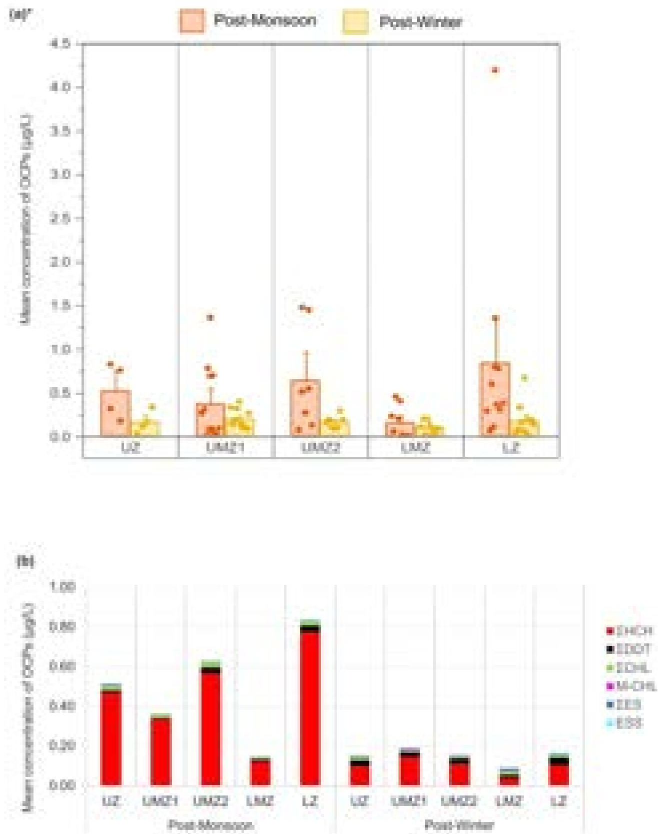

Fig. 3. Seasonal variation and zone-wise distribution of (a) total OCPs (b) Group-wise OCPs in Ganga River. *The bar represents mean Σ OCP and circles show conc. at each site in that zone.

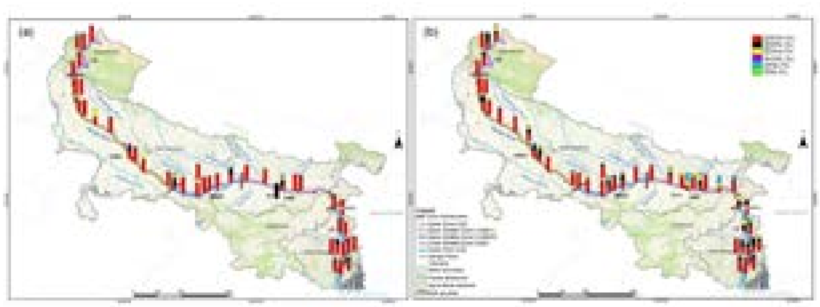

Fig. 4. Relative seasonal and spatial distribution of total OCPs in surface water samples of Ganga River in (a) Post-Monsoon season (b) Post-Winter season.

monsoon revealed no significant differences ($p > 0.05$) in ΣHCHs concentrations. However, significant differences (*KW test, H (4), p = 0.02*) were observed between UMZ1 and LMZ in the post-winter season.

For rest of the zones the difference was statistically insignificant. The environmental flow and contribution of tributaries were considered as major factors influencing the significant difference, in ΣHCHs concentrations, between UMZ1 and LMZ. In the UMZ1, a substantial amount of water is diverted from the river to support agricultural activities through a system of canals, which may considerably reduce the volume of water and concentrate the OCPs in the dry season (post-winter season). Further, the small tributaries joining the Ganga in this zone, particularly the Ramganga and Kali, also bring significant domestic, industrial, and agricultural pollution loads, thereby increasing the pesticide burden in this zone (CPCB, 2016a). In addition, in a recent study from CPCB, a large number of drains on the Ganga (17 out of 33) were found to contain HCHs (range 0.1–7.03 ng/l) as the dominant pesticide. Whereas in the LMZ, the factors of high environmental flow and low cropping intensity, as mentioned in the earlier section, are the contributing factors for lower concentration of ΣHCHs.

The ΣDDT distribution among the five zones followed the order LZ > UMZ2 > LMZ > UMZ1 > UZ in the post-monsoon season (*KW test, H (4), p = 0.10*) and LZ > UMZ1 > UMZ2 > LMZ > UZ in the post-winter season (*KW test, H (4), p = 0.21*). Comparison of the mean concentration of ΣCHLs among the five zones revealed the distribution pattern as LZ > UMZ1 > UMZ2 > LMZ > UZ in the post-monsoon season (*KW test, H (4), p = 0.24*) and LMZ = LZ > UMZ1 > UMZ2 > UZ in the post-winter season (*KW test, H (4), p = 0.62*). The spatial distribution pattern of ΣESs was LZ > LMZ > UZ > UMZ1 > UMZ2 in the post-monsoon season (*KW test, H (4), p = 0.41*) and LMZ > LZ ≫ UMZ1 > UZ = UMZ2 in the post-winter season (*KW test, H (4), p = 0.23*). For M-CHLR, the distribution in the post-monsoon (*KW test, H (4), p = 0.37*) and post-winter season (*KW test, H (4), p = 0.67*) was in the order UZ > UMZ1 > LMZ > UMZ2 > LZ and LMZ > LZ > UMZ1 > UZ = UMZ2, respectively. For the ESS distribution trend in the post-monsoon season (*KW test, H (4), p = 0.20*) was UMZ2 > LZ > UZ and in the post-winter season (*KW test, H (4), p = 0.47*) was LZ > UMZ1 > LMZ > UMZ2 > UZ. However, the zone-wise statistical comparison for both seasons revealed no significant differences ($p > 0.05$) in the concentrations of any of the above pesticide groups.

3.3. Source apportionment of OCPs by ratio analysis

The ratio diagnostic method has been extensively used in the past to identify the potential sources of OCP contamination (Syed and Malik, 2011; Mahmood et al., 2014; Sultana et al., 2014; Yu et al., 2014; Baqar et al., 2018). All the target OCPs investigated in this study are either banned or restricted. Therefore, it is important to ascertain the source (ongoing or historic) of these pesticides. Composition differences between target analytes in surface water environment provide useful information to determine the different pollution sources.

3.3.1. HCHs

Technical-grade HCHs contain a mixture of 60%–70% α-HCH, 5%–12% β-HCH, 10%–15% γ-HCH, 6%–10% δ-HCH and 3%–4% ε-HCH, whereas technical-grade lindane contains ~99% γ-HCH (Iwata et al., 1993; Qiu et al., 2004; Gao et al., 2013; Yu et al., 2014; Mahmood et al., 2014). To identify the potential source of HCH contamination, the α-HCH/γ-HCH ratio is used. In general, the α-HCH/γ-HCH ratio in technical-grade HCHs usually ranges between 3 and 7. A ratio value < 3 indicates fresh inputs of γ-HCH. An α-HCH/γ-HCH ratio >7 indicates long-range transport or the photochemical breakdown of γ-HCH into α-HCH (Barrie et al., 1992; Willett et al., 1998; Wang et al., 2018). The α-HCH/γ-HCH ratio in our study was found to be < 3 in all five zones for both seasons, thus indicating the possible ongoing illegal usage of HCH in the study area. Additionally, relatively low prevalence of β-HCH (Law et al., 2001; Tan et al., 2009), the most persistent HCH isomer, also indicates fresh inputs of HCH (Fig. 5a). We anticipate that the reason for the detection of fresh inputs of technical-grade HCH (particularly lindane) could be linked to their application in paddy fields. In the UMZ1, UMZ2, LMZ, and LZ, rice is generally grown under rainfed conditions along the Ganga basin; these zones (and the corresponding states) are the top cultivators/producers of rice in the country. The elevated concentrations of HCH detected during the wet season in this study indicates that this insecticide is largely applied during the flowering season of paddy (Ramesh et al., 1991).

3.3.2. DDTs

Technical-grade DDT usually contains 77.1% *p,p′*-DDT, 14.9% *o,p′*-DDT, and 4% *p,p′*-DDE (Yu et al., 2014; Baqar et al., 2018). However, with time, under favorable aerobic and anaerobic conditions, the parent compound DDT breakdowns to its metabolites DDE and DDD respectively (Heberer and Dünnbier, 1999; Hitch and Day, 1992). Therefore, the ratios of DDD/DDE could be used to indicate the aerobic (DDD/DDE < 1) or anaerobic (DDD/DDE > 1) degradation (DDD/DDE>1) of DDT. Similarly, the ratio of DDT/(DDE + DDD) is generally used to estimate the age of DDTs; with time, DDT will degrade into DDE and DDD, resulting in a gradual decrease in the level of DDT and subsequent increase in the levels of DDE and DDD (Harner et al., 1999). Therefore, a ratio of DDT/(DDD + DDE) < 1 indicates past usage and historic contamination, whereas a ratio > 1 indicates that there are fresh inputs of DDTs (Gao et al., 2013; Wang et al., 2018). For this study, the ratios of DDD/DDE and DDT/(DDE + DDD) are shown in Fig. 5b. As shown in the figure, at the majority of the sites, the ratio of DDD/DDE was <1, indicating that biodegradation of DDT occurs predominantly under aerobic conditions. Furthermore, in this study, the calculated ratio of DDT/(DDD + DDE) was <1 at the majority of sites (in all zones) for both seasons, indicating that DDT contamination in the Ganga was mainly from its metabolites and historic usage. However, during the post-winter season, the ratio analysis revealed fresh inputs of DDTs at some sampling points in the UZ, UMZ1, LMZ, and LZ. As stated in the previous section (Section 4.2), this could be because of the current use of DDT for the control of malaria vectors under the public health program.

3.3.3. Chlordane

Technical-grade chlordane consists of the major components t-CHL (13%), c-CHL (11%), trans-nonachlor (5%), heptachlor (5%), and over 30 less abundant chlordanes, nonachlors, and chlordenes (Bidleman et al., 2002). Trans-chlordane, owing to its lower half-life (Table S1), degrades relatively faster in the environment than its cis counterpart (Yamada et al., 2008). Therefore, a ratio of cis-chlordane to trans-chlordane < 1 specifies the fresh usage of chlordane (Bidleman et al., 2002; Yu et al., 2014). In the current study (Fig. 5 c), the ratios calculated for cis/trans-chlordane were <1 at all sampling zones, indicating the ongoing usage of chlordane in the study area.

3.3.4. Endosulfan

The endosulfan isomers α-endosulfan, and β-endosulfan constitute up to 70% and 30% of the composition of the technical-grade endosulfan mixture, with the α-isomer being relatively more degradable (Table S1) in the environment than the β-endosulfan (Jiang et al., 2009). The ratio of α-endosulfan/β-endosulfan is used to calculate the age and source of endosulfan in the

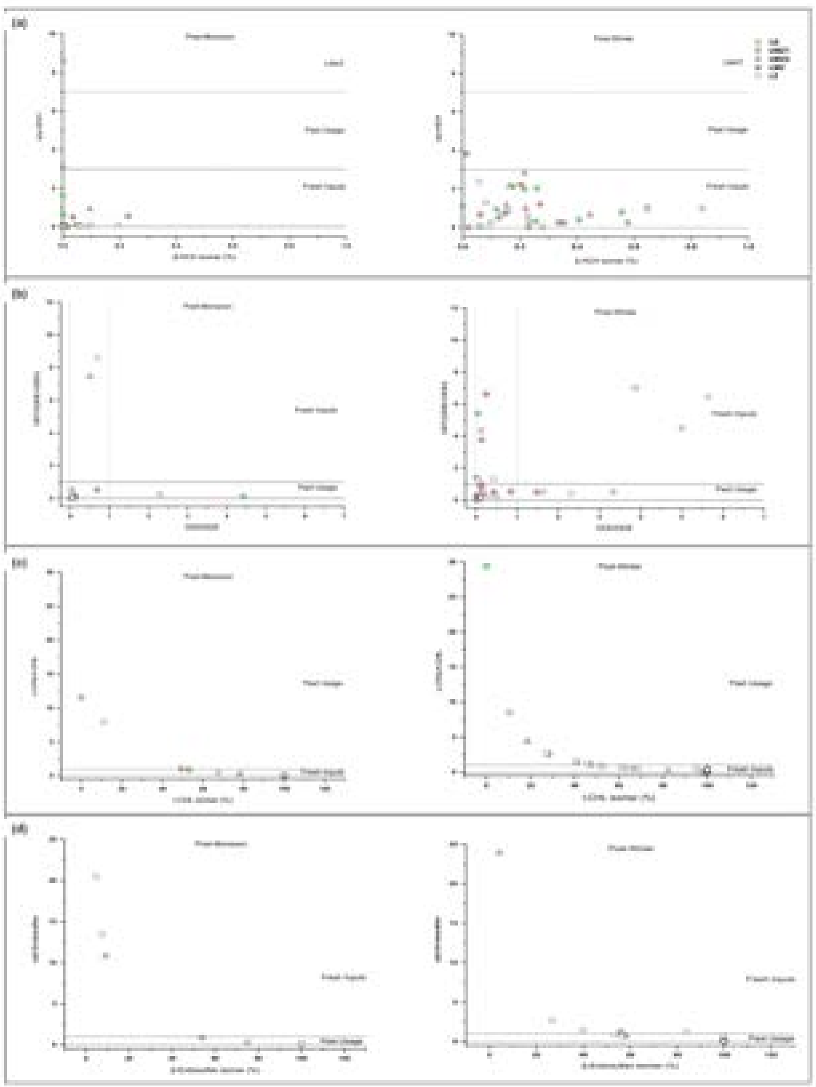

Fig. 5. Source apportionment analysis of OCPs for Post-Monsoon and Post-Winter (a) HCH (b) DDT (c) Chlordane (CHL) (d) Endosulfan (ES).

environment. In general, a value < 1 indicates past usage (Yu et al., 2014). In our study, the ratios of α-endosulfan/β-endosulfan at almost all zones for both seasons were found to be < 1, indicating past usage in these areas (Fig. 5d). However, at some sampling sites in the UMZ2 (post-winter) and LZ (post-monsoon), the ratios were >1, indicating fresh inputs of technical-grade endosulfan.

4. Comparative assessment of present results with other rivers

4.1. Comparison with other studies on the Ganga

To assess trends and patterns regarding OCPs in the Ganga, the results of this study were compared against OCP contamination data from previous studies on the Ganga (Table 3). A limited number of studies were available on OCP contamination in the Ganga from the past two decades, and the majority of these focused on specific small stretches of the river, giving valuable yet incomplete information on the OCP contamination status of river as a whole. In addition, data on temporal trends of OCPs in the Ganga are also very limited. The OCP levels reported in this study point toward a mixed trend where some studies showed the OCP contamination lower than our study while a few reported higher than this study. The ∑HCHs in our study was lower than 60% of the previous studies, whereas for ∑DDTs and ∑ESs, we found that our results were lower than 50% of the previous studies. The results highlight both the success and failure of the government's efforts to ban the target OCPs in India. After the ratification of the Stockholm Convention in 2006, India executed its first National Implementation Plan (NIP) in 2011, wherein the government developed its strategy to deal with these chemicals and subsequently implemented regulations. However, there has been substantial delay in implementing the initiatives to mitigate POPs in the country. The results of our study highlight that the situation has improved compared to that in the past; however, to see 100% success and a complete downward trend, a stricter implementation of the NIP, as well as more time is required to observe a greater reduction in OCP distribution.

4.2. *Comparisons with other Indian and global rivers*

When comparing the results of present study to similar studies on other Indian rivers, we observed that in almost all studies, with the exception of a few, the levels of target pesticides were lower than the levels reported in this study (Table S11). As with any large river, the issues of constructing dams and barrages are known. Additionally, a large number of industrial and sewage drains also release toxic wastes into the water. The Ganga has three major dams and six barrages, which reduces >80% of its flow till it completes half of its journey into the UMZ1. Afterwards, the additional water input from its tributaries does help to dilute the pollution load for a few hundred kilometers, but a major barrage in the LZ (Farrakka) again diverts substantial water to Bangladesh. The water is also abstracted through several feeder canals and diverted for local irrigation needs. Further the Ganga has 139 drains and 767 grossly polluting industries releasing its wastes (both treated and untreated) into the already flow-stressed river, thereby increasing the pollution load (CPCB, 2016a, b). Finally, the use of a considerably large catchment area of the Ganga (compared to other small rivers) for agriculture adds high quantities of pesticides to the river. Hence, the higher concentration of OCPs reported in the Ganga compared to that in other Indian rivers is valid given its extent and other reasons specified above.

Similarly, we compared the results from this study with studies on OCP contamination in global freshwater bodies from Asia, Africa, and developed nations. It was observed that barring few studies, most of the rivers had lower levels of ΣHCHs, ΣDDTs, and ΣCHL than our study, pointing toward the late ban and poor implementation of the regulations in India. A summary of the comparison is presented in Table S12.

5. Potential ecological risk assessment

For this study, we compared the distribution of OCPs in the surface water of the Ganga against the guideline values (GVs) established by USEPA (Table S15). We found that the concentrations for most of the pesticide groups exceeded these GVs for aquatic life criteria at multiple sites across all the zones. The concentrations of ΣHCHs were above the USEPA GV at 12% sites in post monsoon whereas in post-winter it was within the permissible limits at all the sites. Similarly, ΣDDTs exceeded maximum criteria threshold at 51% sites in post-monsoon and 72% sites in post-winter. For ΣCHL, the concentrations were observed above the GVs at 56% sites in post-monsoon and 51% sites in post-winter. The concentrations of methoxychlor were higher than the GVs at 2% sites in both the seasons. For ΣESs, all the sites were within the permissible GVs in both the seasons.

Further, we calculated the risk quotients (RQs) associated with exposure to individual OCPs for three representative trophic levels, viz., algae, aquatic invertebrates, and fish. To identify the high-risk zones, the mean and maximum detected concentrations at each zone were used to determine the general (RQm) and worst-case (RQex) scenarios, respectively. The PNECs, RQs and values corresponding to the maximum and mean concentrations of individual OCPs are presented in Tables S13, S14, and S15 and Fig. 6, respectively.

The RQm values derived from the seasonal data revealed a high ecological risk in the LZ, whereas the RQex values showed ecological risks at the majority of the zones. Among all pesticides, p,p′-DDE showed a RQ > 1 at all monitored sites followed by β-endosulfan, p,p′-DDT and p,p′-DDD, which also showed high ecological risks at the majority of sites, particularly in the post-winter season. The high risk posed by these contaminants is mainly because of their relatively high toxicity to fish, algae, or aquatic invertebrates, hence producing quite low PNEC values. Likewise, β-HCH showed a high risk in the LZ whereas α-ES showed a high risk in the UMZ2, LMZ, and LZ in both seasons. The ecotoxicological risk assessment highlights that these pesticides (RQ > 1) should be prioritized for risk management, particularly because the Ganga is also home to diverse fauna and endangered species. High and continuous exposure to OCPs can compromise the normal function of key physiological processes, which can ultimately affect the survival of these aquatic species.

Furthermore, chlordane (trans and cis), methoxychlor, and γ-HCH showed medium to negligible risks in all zones in both seasons. However, we suggest that the estimated ecological risks should not be overlooked in the current scenario, considering the high concentration of these pesticides detected in the LZ of the Ganga, and their ability to pose negative impacts on aquatic ecosystems.

In the long term, the high aquatic risk caused by pesticides may lead to changes in the fish and invertebrate communities, leading to decreases in the most sensitive species and increases in the most resistant ones, with consequent loss of biodiversity (Palma et al., 2014; Kuzmanovic et al., 2015; Zhang et al., 2016).

6. Conclusion

Thirteen OCPs, their source, and the ecological risk were investigated in surface water of Ganga along 43 sites for two seasons for the first time. A significant difference ($p < 0.05$) in OCP concentrations was found between wet and dry seasons. γ-HCH (lindane) was the most frequently detected compound and found to have fresh inputs across all the zones. Another pesticide chlordane also had fresh inputs across all the zones. The results also point toward the consequences of continued use of DDT in health programs in India. Long-range atmospheric transport and fresh inputs are factors that contribute toward high levels of HCH and DDT in the relatively pristine UZ and are of serious concern. Though different zones showed high concentration of pesticides during different seasons, however the statistical difference amongst the zones was insignificant indicating the contribution of point source pollution from the open drains. The ecotoxicology risk assessment showed that the concentrations of most of the OCPs exceeded international GVs for the protection of aquatic life at several sites. A

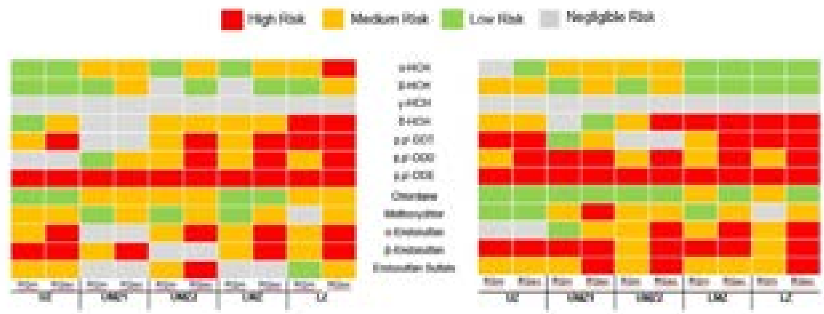

Fig. 6. RQm and RQex of individual OCPs in surface water of the Ganga River (a) Post-Monsoon (b) Post-Winter.

possible high risk to aquatic biota from DDE, DDT, δ-HCH, and α-ES at all or most of the sites was pointed out by RQ model. Pesticides in aquatic ecosystems usually occur as mixture of multiple pesticides than individually, therefore, further research on the potential combined eco-toxicological effects (synergistic, additive, or antagonistic) of OCPs mixture is required.

This study highlights that the Ganga is subjected to contamination by banned and restricted OCPs, and spatial variations have indicated different sources, hydrological differences in the system, and different land-use patterns. Our study validated our hypothesis that there is continued use of banned OCPs at a sizeable spatial level in the Ganga states; this poses a potential risk to the aquatic biodiversity of the river. Since this is the first study to include the spatial and temporal coverage along the length of the river, the present findings could be effectively used in devising a state-wise policy and measures to tackle the illegal usage of OCPs. High levels of OCPs in the post-winter season emphasize the need to maintain minimum environmental flow in the river that is currently compromised due to water abstraction and construction of several dams and barrages on the Ganga. Further, monitoring of drains along the river need to be taken up for the presence of pesticide concentrations and loads. In light of the results presented in this study, we suggest that awareness campaigns and training programs for farmers must be taken up on a priority basis to eliminate the use of banned pesticides. Besides, adoption of integrated pest management, mixed intercropping systems, and organic agroforestry systems must be encouraged. Stricter implementations of the NIP on POPs and timely updates for the next phase is also recommended. We also propose a continuous monitoring campaign to follow up on the occurrence of pesticide residues in the Ganga. Finally, to ensure a healthy ecosystem for the biodiversity of the Ganga, we advocate a holistic ecological risk assessment that includes population-level risk assessment at each critical habitat to evaluate the long-term effects of multiple stressors on aquatic populations.

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.

CRediT authorship contribution statement

Ruchika Sah: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft. **Anju Baroth:** Conceptualization, Validation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Syed Ainul Hussain:** Resources, Supervision, Funding acquisition.

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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.2020.114229.

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BEYOND THE SURFACE: ASSESSING BIOACCUMULATION POTENTIAL OF ENDOCRINE DISRUPTORS IN A MACROBENTHOS (LAMELLIDENS MARGINALIS) INHABITING AN EFFLUENT-IMPACTED URBAN RIVER STRETCH (GANGA RIVER)

[826]

Ruchika Sah, Gautam Talukdar, Gautam Talukdar, Syed Ainul Hussain, Megha Khanduri, Pooja Choudhary, Ruchi Badola

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Globally, freshwater biodiversity faces an array of anthropogenic threats, including contamination by endocrine disruptors (EDs). This category of emerging contaminants has gained vast scientific attention owing to their persistence, bioaccumulative, and toxic properties. Even at very low concentrations, EDs can induce endocrine disruption in the aquatic ecosystem and impact river health.

This study provides the occurrence and bioaccumulation potential of selected EDs such as estrone, 17-beta-estradiol, estriol, 17-alpha-ethinylestradiol, diethylstilbestrol, bisphenol A, triclosan, DDT, vinclozolin, and atrazine in freshwater bivalve Lamellidens marginalis (Lamarck, 1819) and sediment of an effluent-impacted habitat of the Ganga River (Varanasi, Uttar Pradesh). Samples were collected upstream and downstream of effluent discharges, and EDs quantification was done using ultra-performance liquid chromatography-tandem mass spectrometry and Gas Chromatography-tandem mass spectrometry. Key sediment quality parameters were assessed to explore the potential correlations with EDs bioaccumulation in biota and sediment samples.

Downstream sites, generally, showed elevated EDs concentrations in Lamellidens marginalis and sediment samples, except for DDT and BPA. The observed trend for BPA and DDT implies their widespread usage as well as the influence of sources other than effluent discharges in the upstream site. Significant correlations were found between sediment EDs concentrations with total organic carbon and organic matter, yet no such correlations were observed in biota.

The higher accumulation of EDs in Lamellidens marginalis, along with their wide distribution and ease of sampling, indicate that opting for this species in biomonitoring programs could be a cost-effective and efficient method for assessing the EDs contamination, particularly in effluent-impacted riverine habitats. To the best of our knowledge, the present study is the first to investigate this class of emerging contaminants in the wild macrobenthos of the Ganga River. The findings hold the potential to guide comprehensive restoration strategies for enhancing the Ganga River's water quality and preserving its rich biodiversity.

Keywords: Endocrine Disruptors, emerging contaminants, effluent discharge, River Health, Ganga River, Macrobenthos, Biomonitoring

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Ms. Ruchika Sah, Wildlife Institute of India

has done the poster presentation entitled

Beyond the Surface: Assessing Bioaccumulation Potential of Endocrine Disruptors in A Macrobenthos (Lamellidens Marginalis) inhabiting an Effluent-Impacted Urban River Stretch (Ganga River) [826]

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Sah, R., Baroth, A., Hussain, S.A., Johnson, J.A., Ahada, C.P.S, Bhola, S. (2018). Pesticide Pollution and Surface Water Quality Across River Ganga: Spatial Distribution and Ecological Risk Assessment. **International Conference on Water: From Pollution to Purification**. School of Environmental Sciences and Advanced Centre of Environmental Studies and Sustainable Development (ACESSD), Mahatma Gandhi University, Kottayam, Kerala Varanasi, India. December 07-10, 2018.

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PESTICIDE POLLUTION AND SURFACE WATER QUALITY ACROSS RIVER GANGA: SPATIAL DISTRIBUTION AND ECOLOGICAL RISK ASSESSMENT

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Key Words: OCP, OPP, Risk Quotient, water quality

The water quality of river Ganga is deteriorating at a faster pace due to occurrence of various organic and inorganic anthropogenic stressors that can severely jeopardize the survival and biodiversity fitness of its freshwater ecosystem. The excessive discharge of regulated and banned pesticides is one of the key contributors of organic contamination in Ganga that subject the aquatic communities to high risk due to their persistent, and bioaccumulative and toxic nature. Therefore, it becomes highly imperative to quantify the pesticide loads and monitor surface water quality parameters in order to improve the entire aquatic ecosystem and develop effective mitigation measures.

In the present study spatial patterns of surface water quality and degree of pesticide contamination was assessed through recording variation in physico-chemical characteristics and analysing load of organochlorine as well as organophosphate pesticides (OCP and OPP respectively) across river Ganga as it traverses through five states viz., Uttarakhand, Uttar Pradesh, Bihar, Jharkhand and West Bengal.

Post monsoon water samples (Oct-Nov, 2017) were collected from 56 sites covering urban, industrial and agricultural belts across the entire length (~2525 Km) of the river. The surface water quality was analysed through testing pH, water temperature, Total dissolved solids, Dissolved oxygen, Nitrates, and Specific conductivity, which were recorded using digitally calibrated probe meters in the field. Pesticides residues were detected and quantified using Gas Chromatograph equipped with Electron capture/Nitrogen-Phosphorous detector. The environmental risk posed by the selected pesticides was calculated using risk quotient methods.

The detailed results of the study will be reported, interpreted and discussed in the poster.

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This is to certify that ~~Prof./Dr./Mr.~~/Ms. Ruchika Sah
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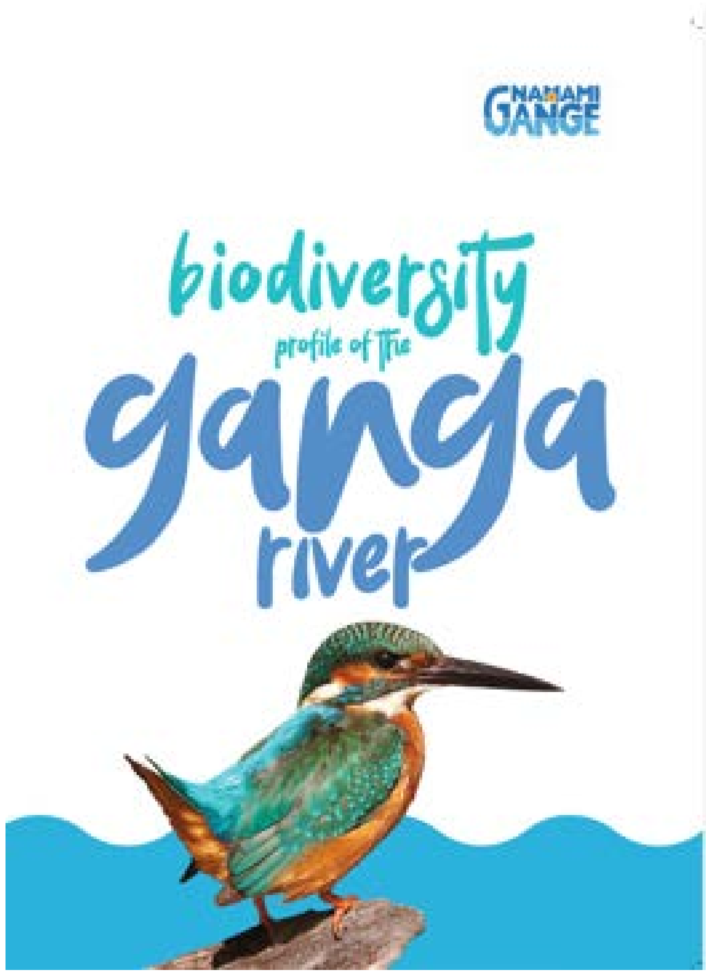

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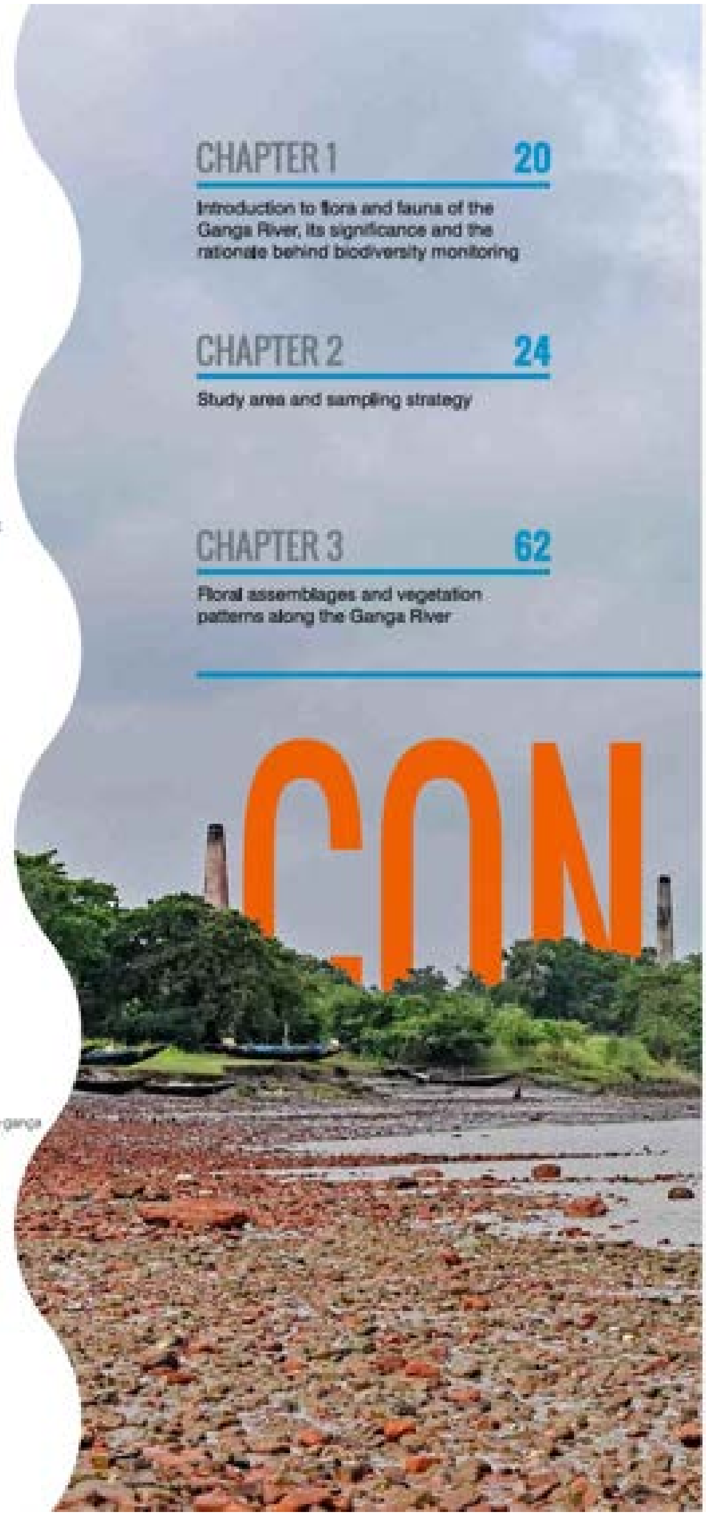

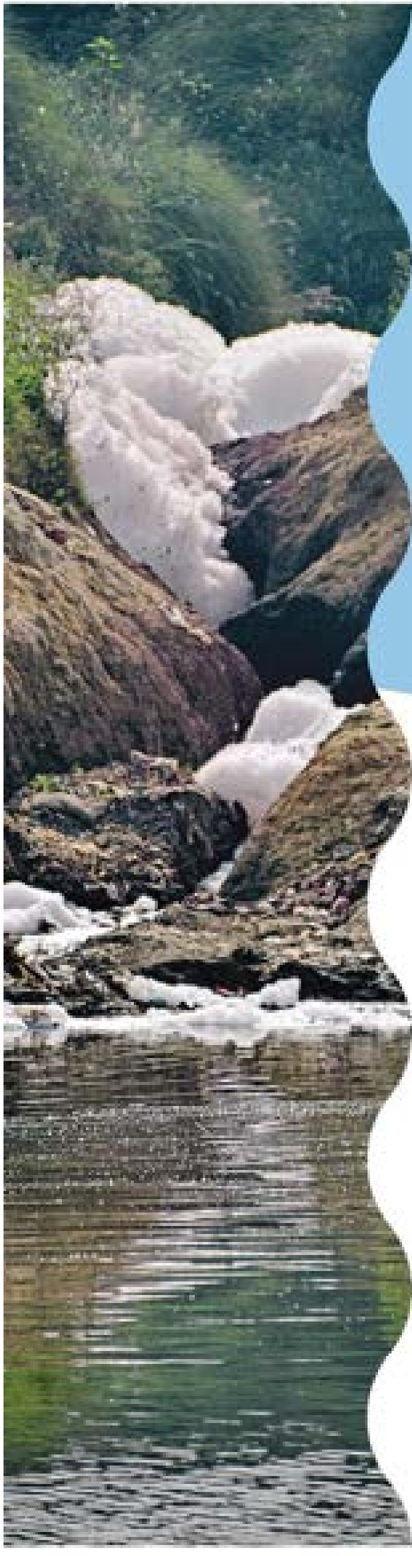

ASSESSMENT OF PHYSICO-CHEMICAL PARAMETERS AND ORGANOCHLORINE PESTICIDES IN WATER AND SEDIMENT OF THE GANGA RIVER: PRELIMINARY STATUS AND TRENDS

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ABSTRACT

The water quality of the Ganga River is deteriorating at a faster pace due to occurrence of various organic and inorganic anthropogenic stressors. These stressors are severely affecting the freshwater ecosystem as its biodiversity. One of the key contributors of organic contamination in the Ganga is the excessive discharge of banned and regulated pesticides. In the present phase of the study, water quality (physico-chemical parameters) and pesticide load (Organochlorine Pesticides) were assessed in the Ganga River as a first step to facilitate clean Ganga mission.

Among Physico-Chemical Parameters (PCPs) pH, water temperature, dissolved solids, dissolved oxygen, nitrates, ammonium and specific conductivity were monitored, whereas pesticide