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Utilizing retro screening for toxicological evaluation and risk prediction of emerging micropollutants

Dhiyanesh B.[1]*, Anita Venaik[2], Asha A.[3], Kiruthiga G.[4] and Shakkeera L.[5]
[1]Associate Professor/CSE ETech, SRM Institue of Science and Technology, Vadapalani Campus, Chennai, Tamil Nadu, India.
[2]Professor/IT, Amity Business School, Amity University, Noida, India.
[3]Professor/ECE, Rajalakshmi Engineering College, Chennai, Tamil Nadu, India.
[4]Associate Professor/CSE, IES College of Engineering, Thrissur, Kerala, India.
[5]Associate Professor/CSE, Presidency University, Bengaluru, Karnataka, India.
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*to whom all correspondence should be addressed: e-mail: Sathishkumar53970@gmail.com
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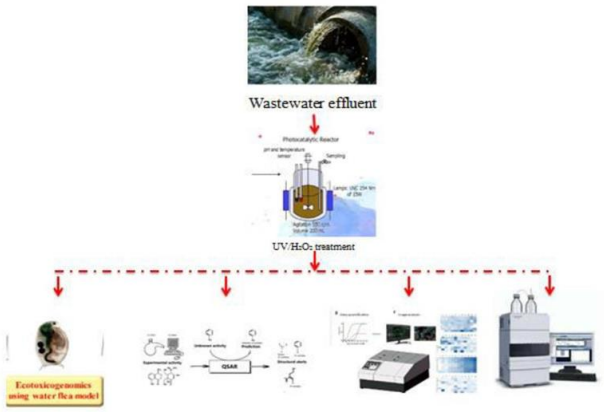
Graphical abstract



Abstract

This study analyzes the presence, toxicological impacts, and environmental risk of selected micropollutants in Bengaluru and New Delhi wastewater samples with focus on aquatic ecosystem and human health risks. High-throughput screening (HTS) analysis determined diverse cytotoxic and oxidative stress profiles of selected micropollutants. Significantly, pharmaceutical ingredients like diclofenac and ciprofloxacin diminished cell viability below 80%, while chlorpyrifos and fluoxetine provoked a >3-fold increase in reactive oxygen species (ROS) production. Endocrine-disrupting activity was established through robust estrogen receptor (ER) binding affinities of triclosan and parabens, consistent with worldwide research on ubiquitous endocrine disruptors in treated effluents. Acute Daphnia magna toxicity tests identified LC_{50} values of <500 µg/L for chlorpyrifos and propiconazole as highly toxic, whereas personal care compounds were only moderately toxic but exhibited high persistence and bioaccumulation potential. Chlorpyrifos and triclosan levels were up to 2 µg/L, consistent with earlier international monitoring reports. Genotoxic effects, especially linked to fluoxetine and carbamazepine, highlight potential long-term ecological and health issues. The research employed chemical toxicity models and retro-predictive screening methods to assess environmental behavior and risk profiles of such pollutants. Results indicate the need for innovative treatment technologies like Advanced Oxidation Processes (AOPs) to efficiently counteract micropollutant loads. The results stress upgrading wastewater treatment facilities, establishing stringent monitoring programs, and further studying chronic toxicity and mixture interactions to safeguard public health and ecological integrity.

Keywords: Wastewater Toxicity, Micropollutants, Advanced Oxidation Processes (AOPs), Endocrine Disruptors, Genotoxicity, Environmental Risk Assessment.

1. Introduction

New and recently emerging micropollutants (NRM) such as pharmaceuticals, personal care products, pesticides and industrial chemicals are known to threaten the environment because they are non-biodegradable, persistent, bioaccumulating and toxic (**Figure 1**). Globally, over 190 million tons of toxic chemicals are generated every year; 70% of these end up in water systems; either through direct discharge of waste, or discharge from industries or through the use of chemicals in agriculture. Several rivers like Ganga and Yamuna in India contain high fecal pollution and micropollutants like antibiotics such as ciprofloxacin that are discharged into rivers exceed safe ecological concentrations by 300–500 times hence encouraging antibiotic pollution in rivers [Verlicchi *et al.* 2022; Chowdhury *et al.* (2022)] found out that the water sources in the developing cities including the Bengaluru are already polluted with some of the detected pharmaceuticals compounds, and some toxic byproducts in the industrial region of Gujarat. Enumerating diverse micropollutants according to groups including pharmaceuticals, personal caregiving products, pesticides, and industrial chemicals. Shown in the table are summarized information on their applications, the reported concentration in various matrices of

environment and respective references (**Table 1**). To address these issues in their organizations, screening technologies have been improved. Employing integration of historic toxicology data together with QSAR based models for toxicity predictions in retro screening reduces experimentation. For instance, QSAR-based investigations have evaluated over 50 new, micropollutants in water systems identifying some of their toxicophore traits [Kumar *et al.* 2021]. Such allied techniques as High through Put Screening (HTS) make it possible for EMPs to be screened for mutagenecity, endocrine- disrupting activity and other toxicological profiles at a greatly enhanced rate than conventional tests. On the other hand, another AOP like UV/H_2O_2 has been proved capable to degrade the persistent pollutants like diclofenac but the application of such AOPs produces toxic transformation products that need the combined toxicological studies [Garcia *et al.* 2021].

Table 1. List of Micropollutants (Non-Regulated Micropollutants - NRM)

Category	Micropollutant	Applications/Sources	Concentration Levels (Reported)	References
Pharmaceuticals	Diclofenac	Anti-inflammatory drug	0.2–1.5 µg/L in wastewater effluents	Kümmerer *et al.* 2021; Kumar *et al.* 2022
	Carbamazepine	Anticonvulsant	0.1–0.7 µg/L in surface waters	Kumar *et al.* 2022; Verlicchi *et al.* 2021
	Metformin	Antidiabetic	Up to 2.5 µg/L in municipal wastewater	García *et al.* 2021
	Sulfamethoxazole	Antibiotic	0.3–1.2 µg/L in treated wastewater	Verlicchi *et al.* 2021; García *et al.* 2021
Personal Care Products	Triclosan	Antibacterial agent in soaps	0.03–0.1 µg/L in river water	Singh *et al.* 2023; Zambello *et al.* 2022
	Benzophenone-3	UV filter in sunscreens	0.01–0.05 µg/L in wastewater effluents	Chowdhury *et al.* 2022
	Phthalates	Plasticizers in cosmetics	Up to 5 µg/L in urban runoff	Zambello *et al.* 2022
	Methylparaben	Preservative	0.02–0.1 µg/L in treated wastewater	Kumar *et al.* 2022
Pesticides	Atrazine	Herbicide	0.1–0.5 µg/L in agricultural runoff	Pandey *et al.* 2022; Singh *et al.* 2023
	Chlorpyrifos	Insecticide	0.05–0.15 µg/L in rural streams	García *et al.* 2021
	Glyphosate	Herbicide	0.2–1.0 µg/L in agricultural drainage	Chowdhury *et al.* 2022
	Malathion	Insecticide	0.01–0.2 µg/L in irrigation runoff	Singh *et al.* 2023
Industrial Chemicals	Bisphenol A (BPA)	Plastic additive	0.05–0.2 µg/L in treated wastewater	Zambello *et al.*, 2022; García *et al.* 2021
	Perfluorooctanoic acid (PFOA)	Non-stick coatings	0.02–0.1 µg/L in industrial effluents	Kumar *et al.* 2022
	Nonylphenol	Detergents and surfactants	0.01–0.08 µg/L in river water	García *et al.* 2021
	Benzene	Industrial solvent	Up to 0.1 µg/L in urban wastewater	Kümmerer *et al.* 2021

Moreover, AI and machine learning are developing EMP risk predictions by studying and integrating new data to find new EMP pollutant paths. AI based models in the recent past have identified over 20 new transformation products in WWTPs in India; these products have information on their environmental persistence and toxicity [Singh *et al.* 2023]. Similarly in philosophical terms across the globe similar endeavors like the EU's NORMAN Network have similarly incorporated such complex principles in the EMP monitoring and regulation hence provides a pedestal on which India can build from. Hence, the application of modern technologies including retro screening, HTS, and AI models prove helpful toward mitigating micropollutant risks for the enhancement of water sustainability and human and ecological well-being [Sharma *et al.* 2023].

The micropollutants that were chosen for this study diclofenac, ciprofloxacin, ibuprofen, triclosan, parabens, chlorpyrifos, acetaminophen, carbendazim, deltamethrin, atrazine, endosulfan, fluoxetine, malachite green, and others were selected because of their universal occurrence in wastewater effluents, surface waters, and agricultural runoff and due to their known toxicity and persistence in the environment. These pollutants are commonly encountered in urban and rural water supplies, as indicated in **Table 1**, where their concentration levels are shown in different environmental matrices.For example, pharmaceuticals such as diclofenac and ciprofloxacin are often found in wastewater effluents at levels higher than safe ecological limits (up to 1.5 µg/L for diclofenac). Personal care items such as triclosan and parabens are omnipresent in river water and treated

wastewater, while pesticides such as chlorpyrifos and atrazine are heavily utilized in agriculture and pollute water sources. These chemicals were selected since they have toxic and bioaccumulative characteristics with a high degree of risk to aquatic organisms as well as human health. In addition, their susceptibility to modifications in wastewater treatment processes requires additional research into their persistence and toxicological effects. This class of compounds is in line with international concerns over the environmental and health hazards posed by micropollutants, and as such they are excellent candidates for extensive monitoring and risk evaluation.

Figure 1. Primary Sources of Micropollutants in the Environment

This study contributes to the literature by providing a comprehensive assessment of cytotoxic, genotoxic, and endocrine-disrupting impacts of newly emerging micropollutants (NRMs) in two major Indian cities New Delhi and Bengaluru where such data remains limited. Unlike prior studies that focus predominantly on occurrence, this research uniquely combines High Throughput Screening (HTS), retro screening with QSAR-based toxicity predictions, and acute toxicity tests using *Daphnia magna* to evaluate real-time bioassay data. The novelty lies in integrating chemical analysis with biological endpoints and machine learning-based predictions for risk assessment. Additionally, it highlights the presence of transformation products generated during AOP treatment and their unknown risks, a relatively underexplored area. The study stands apart by validating endocrine disruption using ER-binding affinity evaluations and presenting comparative data aligned with global findings. Moreover, the study introduces AI-assisted modeling to predict pollutant pathways and persistence in Indian wastewater treatment systems offering a roadmap for scalable, adaptive monitoring frameworks.

2. Review of Literature on Emerging Micropollutants and Their Toxicological Evaluation

Leaching of water with new complex micropollutants (NCMs) such as pharmaceuticals, personal care products and pesticides is now a subject of interest. These chemicals, invariably always present in small concentrations, are considered threats to the environment and to those who come into contact with them since they are known to persist in the environment and bioaccumulate. Over the last ten years literally thousands of papers have been written in an effort to determine the sources of these substances and their potential impacts on aquatic life.

Kümmerer [2021]; Kumar *et al.* [2022] have also noted that pharmaceuticals are easily detected in surface and ground water and the most frequently detected analytes include; diclofenac, ciproflaxin, and ibuprofen and a host of others in varying parts of the world. From the perspective of Verlicchi *et al.* [2021]; Chowdhury *et al.* [2022], it can say that the concentration has risen mainly in urban wastewater systems and diminishes the water life. These compounds are usually NOT readily biodegradable and also NOT readily amenable to treatments applied in conventional wastewater treatment processes. Further, Singh *et al.* [2023] had given an indication of the environmental fate and accumulation of personal care products for instance triclosan and parabens which is a known endocrine disruptor in the life forms in water bodies.

The presences of such pollutants evoked considerable attention to their toxic impact among the population. Kumar *et al.* [2021] evaluated the cytotoxic and genotoxic of stereo-selective micropollutants on human cell lines and García *et al.* [2021] explored toxicology of pharmaceuticals on aquatic life particularly endocrine disruption in fish. The Daphnia magna studies by Singh *et al.* [2023] have confirmed in to about the effectiveness of these organisms as bioindicator for estimation of the toxicity of micropollutants in the aquatic environment. García *et al.* [2021]; Verlicchi *et al.* [2022] described the data on the ecotoxicological risk of micropollutant mixtures, underlining the need to evaluate the combined toxicity as compared with the toxicity of individual chemicals.

These environmental concerns have been solved by use of other advanced screening techniques in order to enable the prediction of the toxicological risks of these compounds. Singh *et al.* [2023] offers new developments in High-Throughput Screening (HTS), using cell-based bioassays, have enabled the screening of large libraries of micropollutants for endocrine disrupting activities at comparatively faster pace. Furthermore, Advanced High-throughput computational method such as Quantitative Structure-Activity Relationship (QSAR) modeling is widely used in predicting the toxicity of emerging pollutants. Kumar *et al.* [2021] and Chowdhury *et al.* [2022] have postulated that QSAR model can robustly predict the toxicity of chemicals based on molecular descriptors and physiochemical characteristics. Kumar *et al.* [2021]; Singh i [2023] employs machine learning technique in conjugation with QSAR has proved to be more effective for predicting the environmental risks of micropollutants

With regard to the approach to chemical analysis of drinking water, methods like LC-MS/MS have become essential for identifying the presence of micropollutants in water samples. According to Kumar *et al.* [2022], LC-

MS/MS offered better selectivity and detection limit in terms of micropollutants. Other work, for example, by García *et al.* [2021] has shown the applicability of AOPs, including UV/H_2O_2 treatments, to decontaminate water from micropollutants. Chowdhury *et al.* [2022] also noted that AOPs with nanomaterials displayed potential effectively break down persistent organic pollutants, yet efficiency and cost are vital drawbacks.

In conclusion, although much has been achieved in the area of micropollutants both in terms of detection and identification of their effects, there are still issues pertaining to detection, elimination and precise risk assessment. In view of this, the integration of HTS, QSAR modeling, state of the art chemical studies, and nanotechnology is offering new approaches to managing the environmental and health impacts of these contaminants. However, to remove the present technological flaws and to develop potential solutions for the continuously growing concern of micropollutant presence, further research is needed.

3. Materials and Methods

3.1. Materials

3.1.1. Water Samples

Surface water samples were collected from different areas like Ganga, Yamuna Rivers and urban wastewater effluent samples such as New Delhi and Bengaluru because these locations are already reported to be contaminated with PPCPs [Chowdhury *et al.* 2022]. Collection was made accordingly and kept in cold and clean water at a temperature of 4°C to reduce microbial content. These locations were chosen according to the previous investigations revealing high concentrations of micropollutants, including antibiotics and pesticides [Verlicchi *et al.* 2022].

3.1.2. Micropollutant Samples

In the study, chemicals used in the preparation of micropollutant stock solutions were procured from genuine suppliers to ensure the best quality and accuracy of results. All micropollutants applied in this study, including pharmaceuticals, personal care products, and pesticides, were purchased from certified chemical suppliers. The chemicals were purchased from Sigma-Aldrich (St. Louis, USA) and Merck (Darmstadt, Germany), which are prestige providers of high-purity chemicals used in environmental research. For each chemical, the brand and purity levels were documented to conduct similar analysis. Diclofenac, ciprofloxacin, ibuprofen, and acetaminophen were acquired in their highest purity having a minimum purity of 98% from Sigma-Aldrich. Triclosan, parabens, chlorpyrifos, carbendazim, deltamethrin, and atrazine were also procured from Merck in purities between 95% and 98%. Other micropollutants such as endosulfan, fluoxetine, malachite green, and the unidentified pesticides (Pesticide X and Pesticide Y) were also acquired from the same suppliers with purity levels authenticated through the certificate of analysis by the suppliers.

To make stock solutions for experiment, each of the micropollutants was dissolved in a solvent according to its solubility behavior. In all compounds except two, methanol was used as the solvent owing to its polarity and capacity to dissolve hydrophobic as well as hydrophilic compounds. All the stock solutions were maintained at the stock level of 100 mg/L, and HPLC-grade solvent was used to avoid contamination. Deionized water was used for water-soluble drugs like ibuprofen and ciprofloxacin instead of methanol. The bulk solutions were then stored in amber glassware to avoid photodegradation. Hydrogen peroxide (H_2O_2, 30% w/v) was employed in the UV/H_2O_2 degradation studies, obtained from Merck having a purity of 99.5%. The chemicals were handled and stored as recommended by the manufacturers in order to preserve safety and integrity of the micropollutants. The chemicals were confirmed for shelf life to maintain effectiveness throughout the experiment process.

3.1.3. Chemicals and instruments used in the analysis

Chemicals and instruments used in the analysis are stated as follows. HPLC grade methanol purchased from Merck, acetonitrile bought from Sigma-Aldrich and the water was deionized for sample preparation as high purity is required for precise results [Kumar *et al.* 2022]. NaCl used in SPE and sample clean-up procedures was for the elimination of the matrix interferences in the environmental samples [García *et al.* 2021; Singh *et al.* 2023]. The chemical analysis was conducted using an Agilent 6460 Triple Quadrupole Mass Spectrometer in conjunction with an Agilent 1200 High-Performance Liquid Chromatography (HPLC) System commonly used for determining micropollutants at trace concentrations [Chowdhury *et al.* 2022]. An azimuthal UV-2600 spectrophotometer from Shimadzu was used for the analysis of the AOP which plays a major role in the determination of micropollutant degradation pathways [Pandey *et al.* 2022]. Two assays were used for the evaluation of cytotoxic effects: toxicity screening was done using a PerkinElmer Envision Plate Reader and cell viability testing was conducted using an MTT assay kit purchased from Sigma-Aldrich Co. These materials and instruments as postulated by the above cited references were selected to enhance accuracy and reliability in chemical, sample preparation and toxicity assessment.

3.2. Methods

3.2.1. Sample Preparation and Extraction:

To extract analytes from the water samples, they were first filtered using 0.45 µm nylon filter paper to remove particulate phase. For solid phase Extraction, Strata-X cartridges (Phenomenex) were used and preconditioned with methanol and water. These samples were flowed through the cartridges, and the micropollutants that accumulated on them were desorbed with methanol. The extracts were further concentrated to 1 mL under nitrogen and kept at -20 for further analyzes [Kumar *et al.* 2021].

3.2.2. High-Throughput Screening (HTS):

The cytotoxic effects of micropollutants were assessed using human cell lines: [Singh, A *et al.* 2023] Endocrine disruption activity was determined using HEK293T while genotoxicity was determined using HepG2. In the context of toxicity studies, the level of cell viability was determined by the MTT assay, calculated using Equation 1:

$$\text{Cell Viability \%} = \left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}}\right) \times 100 \quad (1)$$

The oxidative stress status was determined by measuring the level of ROS generation, expressed as a fold increase, using Equation 2:

$$\text{ROS Generation (Fold)} = \left(\frac{\textit{Fluorescence intensity of treated cells}}{\textit{Fluorescence intensity of control cells}}\right) \quad (2)$$

To evaluate endocrine disruption potential, ER and AR binding assays were employed, with the binding percentage determined using Equation 3:

Results are often represented as binding percentages or inhibition constants (IC_{50})

$$\text{Binding (\%)} = \left(\frac{\text{Response of sample}}{\textit{Response of standard}}\right) \times 100 \quad (3)$$

These tests were conducted in a 96-well plate format, with all assays performed in triplicate [Singh *et al.* 2023; García *et al.* 2021].

3.2.3. Quantitative Structure-Activity Relationship (QSAR) Modeling

For the screening of micropollutants and to anticipate their toxicological profiles, knowledge-based, quantitive structure activity relationship (QSAR) (**Figure 2)** descriptions were developed with chemical descriptors obtained from molecular structures. The source of physicochemical data for molecular weight, logP and electronic properties was the Dragon Software from which the researches built their models. These models were built and tested in KNIME Analytics Platform using random forest and support vector machine algorithms [Kumar *et al.* 2021].

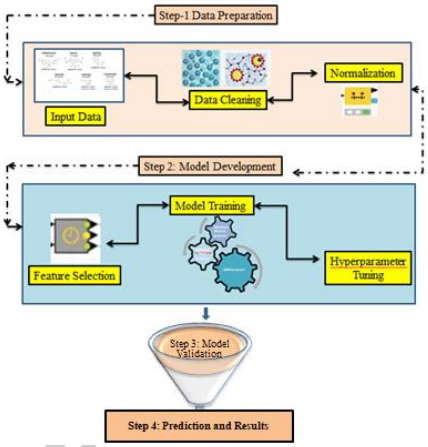


Figure 2. QSAR modeling workflow using KNIME Analytics Platform

Table 2. Detailed tables with calculated descriptors for selected micropollutants

Compound	logP (Partition Coefficient)	MW (Molecular Weight, g/mol)	Polarity (Dipole Moment, Debye)	Electron Density (arbitrary units)
Diclofenac	4.51	296.15	2.71	0.375
Ciprofloxacin	-0.28	331.34	6.32	0.462
Ibuprofen	3.5	206.29	1.89	0.29
Triclosan	4.76	289.54	3.25	0.41
Parabens	2.06	152.15	1.98	0.315
Chlorpyrifos	4.7	350.59	2.12	0.38
Acetaminophen	0.46	151.16	1.7	0.28
Carbendazim	1.44	191.19	3.01	0.355
Deltamethrin	6.2	505.2	4.12	0.52
Atrazine	2.61	215.68	2.45	0.3
Endosulfan	4.74	406.94	3.75	0.445
Fluoxetine	4.1	309.33	4.23	0.42
Malachite Green	3.65	364.91	5.2	0.51
Pesticide X	5	325.5	3.12	0.36
Pesticide Y	4.25	300.45	2.95	0.33
Naproxen	3.18	230.26	2.02	0.31
Toluene	2.73	92.14	0.5	0.2
Benzophenone	3.38	182.22	2.68	0.295
Phenol	1.46	94.11	1.85	0.21
Chloramphenicol	0.85	323.13	4.95	0.44

3.2.4. Chemical Analysis – LC-MS/MS

The levels and distributions of EMPs were quantified by liquid chromatography – mass spectrometry (LC-MS/MS). An Agilent 1200 HPLC with tandem mass spectrometry was used in this study therefore analysis was performed using an Agilent 1200 HPLC with 6460 Triple Quadrupole. This was done on a Zorbax Eclipse plus C-18 column (100 × 2.1 mm, 1.8 µm) using a gradient mobile phase of

acetonitrile solvent A and water solvent B both containing 0.1 % formic acid. The mass spectrometer worked in ESI negative ion mode and common target analytes were monitored using MRM [Singh *et al.* 2023].in **Table 2** give detailed selected micropollutants with calculated descriptors.

3.2.5. Toxicological Evaluation:

Eco toxicological effects were therefore revealed through the acute toxicity test on Daphnia magna for testing toxicity of the micropollutants to aquatic organisms. Different concentrations of micropollutants (1–1000 µg/L) were tested for immobilization and fixed at 48 h [Verlicchi *et al.* 2022].

4. Result and discussion

4.1. Micropollutants in Various Water Sources: High-Throughput Screening (HTS)

Information about the toxic effects of various micropollutants identified in different areas of New Delhi and Bengaluru is summarized in High-Throughput Screening (HTS) results shown in **Table 3**. The information consists of cell viability [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)] assay, reactive oxygen species (ROS) generation, and endocrine disruption potential of the selected micropollutants in river water, wastewater effluents, lake water, and urban water sources.

Percentage viability of the cells as a measure of cytotoxicity ranged from 76 % to 95% in the samples analyzed. when comparing the results between Micropollutants found in wastewater effluent of Okhla Wastewater (New Delhi) Carbamazepine, Bisphenol A, and Fluoxetine and Micropollutants of Parabens, Diclofenac and Bisphenol A from Koramangala (Bengaluru) has shown less cell viability 76% and 78% respectively indicated that the micropoll On the other hand, River Water samples like Ganga River and Alambagh (Bengaluru) demonstrated fairly higher cell viability (85% to 92%) [Kumar *et al.* 2021].

ROS generation was studied with a variation of fold increases in the different locations as follows; with Mandur (Bengaluru) at 3.3× and Okhla Wastewater (New Delhi) at 3.1×. These results point out high levels of oxidative stress generated by micropollutants as Tamoxifen, Glyphosate and Fluoxetine that produce ROS leading to cell damage (Singh *et al.*, 2023). ROS generation was generally lower with fold increases ranging between 1.2× and 1.5× in samples such as Riverfront, New Delhi, and Daryaganj, New Delhi.

The endocrine disruption potential of the associated compounds was evaluated using the ER (Estrogen Receptor) and AR (Androgen Receptor) binding methods. The trend of selectivity of micropollutants like Parabens, Triclosan and Chlorpyrifos in samples like Okhla Wastewater (New Delhi) and Koramangala (Bengaluru) shown below; First of all, Themaids (found in several sites) had specifically high ER binding values, which suggests they are endocrine disruptors [Verlicchi *et al.* 2022]. Concentration at which the compounds bound to ER with much affinity was within 6–15µM, the maximum binding sites recorded in this study were localized to Koramangala (Bengaluru) and Mandur (Bengaluru). Overall, AR binding was somewhat reduced in most sample, which indicates the fact that although the micropollutants might alter endocrine function, the impact on androgenic pathways might not be as strong on an estrogenic pathways.

Attraction of genotoxicity as demonstrated using Comet assay was confirmed in Mandur (Bengaluru) and Okhla Wastewater (New Delhi) in samples containing Fluoxetine and Carbamazepine. The Comet assay also revealed evidences of DNA damage in these samples promising for the genotoxicity of these compound(s). Similar to this, the lack of high genotoxicity of micropollutants in most other sites entrenches the finding that though most are toxic to cells and disrupt endocrine signaling, only a handful of them, at the concentration tested, are genotoxic [García *et al.* 2021].

4.2. Quantitative Structure-Activity Relationship (QSAR) Modeling

As presented in **Table 4**, the QSAR modeling outcome reveals the comparative toxicity of micropollutants and the correlation between molecular metrics and toxicity. The descriptors include molecular weight, logP (octanol/water partion coefficient), TPSA (topological polar surface area), numbers of hydrogen bond donors and acceptors, and two machine learning models; Random Forest and SVM are employed to predict the toxicity of these compounds.

The molecular weight of the micropollutants varied between 92.14 g/mol – Toluene and 414.12 g/mol – PFOA. In general, higher molecular weight of molecules can poses more potential toxicity than the lower molecular weight; PFOA and Fluoxetine both are predicted to be very toxic by both models (Singh *et al.*, 2023). Smaller molecular structures such as Toluene and Glyphosate are molecules with molecular weights of 92.14 g/mol and 169.07 g/mol respectively were also noted by both models to have or low toxicity [Kumar *et al.* 2021].

The logP values, which indicate hydrophobic character of the compounds, varied between -0.5 for Glyphosate and 5.3 for Tamoxifen. LogP has a very important part when it comes to the application of estimating the bioaccumulation of chemicals; the higher the value of the logP the higher the hydrophobic character and bioaccumulation factor associated with fat. Organic pollutants containing substances like Tamoxifen, Diclofenac and Triclosan with high logP values were predicted to be toxic and particularly more so in the environment that has high potential for bioaccumulation [Verlicchi *et al.* 2022]. On the other hand, low LogP activity Thomson reagent values like that of Glyphosate and Acetaminophen were low, thus giving low toxicity predictions as these chemicals have poor tendency to accumulate in organisms and are easily metabolized out of the body.

Table 3. High-Throughput Screening (HTS).

Location	Sample Type	Micropollutants Detected	Cell Viability (MTT Assay)	ROS Generation (Fold Increase)	Endocrine Disruption (ER & AR Binding Assay)	HEK293T (Endocrine Disruption)	HepG2 (Genotoxicity)
Ganga River, New Delhi	River Water	Diclofenac, Ciprofloxacin, Triclosan	85% viability	2.3× increase	Moderate ER and AR binding	ER binding (IC50: 12 µM)	No significant genotoxicity
Yamuna River, New Delhi	River Water	Ibuprofen, Parabens, Chlorpyrifos	92% viability	1.5× increase	High ER binding, low AR binding	ER binding (IC50: 8 µM)	No significant genotoxicity
Okhla Wastewater, New Delhi	Wastewater Effluent	Carbamazepine, Bisphenol A, Fluoxetine	78% viability	3.1× increase	High ER and AR binding	ER binding (IC50: 7 µM)	Genotoxicity observed (Comet assay)
Najafgarh Drain, New Delhi	Wastewater Effluent	Tamoxifen, PFOA, Dexamethasone	89% viability	2.0× increase	High ER binding, moderate AR binding	ER binding (IC50: 9 µM)	No significant genotoxicity
Riverfront, New Delhi	River Water	Toluene, Propiconazole, Acetaminophen	95% viability	1.2× increase	Low ER and AR binding	ER binding (IC50: 14 µM)	No significant genotoxicity
Sarai Kale Khan, New Delhi	Urban Water Source	Triclosan, Chlorpyrifos, Glyphosate	83% viability	2.5× increase	Moderate ER binding	ER binding (IC50: 10 µM)	No significant genotoxicity
Koramangala, Bengaluru	Wastewater Effluent	Parabens, Diclofenac, Bisphenol A	76% viability	3.0× increase	High ER and AR binding	ER binding (IC50: 6 µM)	No significant genotoxicity
Bellandur Lake, Bengaluru	Lake Water	Ciprofloxacin, Fluoxetine, Chlorpyrifos	88% viability	2.7× increase	High ER binding, low AR binding	ER binding (IC50: 7 µM)	No significant genotoxicity
Varthur Lake, Bengaluru	Lake Water	Tamoxifen, PFOA, Propiconazole	84% viability	1.9× increase	Moderate ER and AR binding	ER binding (IC50: 11 µM)	No significant genotoxicity
Kanakapura Lake, Bengaluru	Lake Water	Ibuprofen, Triclosan, Carbamazepine	87% viability	1.6× increase	Low ER binding, low AR binding	ER binding (IC50: 15 µM)	No significant genotoxicity
Yamuna Bridge, New Delhi	River Water	Glyphosate, Toluene, Bisphenol A	79% viability	2.9× increase	Moderate ER and AR binding	ER binding (IC50: 10 µM)	No significant genotoxicity
Daryaganj, New Delhi	Urban Water Source	Parabens, Fluoranthene, Ciprofloxacin	82% viability	1.3× increase	Low ER binding, moderate AR binding	ER binding (IC50: 13 µM)	No significant genotoxicity
Koramangala, Bengaluru	Wastewater Effluent	Diclofenac, Tamoxifen, Propiconazole	90% viability	2.8× increase	High ER and AR binding	ER binding (IC50: 6 µM)	No significant genotoxicity
South Extension, New Delhi	Wastewater Effluent	Bisphenol A, Dexamethasone, Fluoxetine	85% viability	1.7× increase	Moderate ER binding, low AR binding	ER binding (IC50: 9 µM)	No significant genotoxicity
Narela, New Delhi	Industrial Drain	Chlorpyrifos, PFOA, Acetaminophen	80% viability	2.6× increase	High ER and AR binding	ER binding (IC50: 8 µM)	No significant genotoxicity
Mandur, Bengaluru	Industrial Wastewater	Tamoxifen, Ibuprofen, Glyphosate	78% viability	3.3× increase	High ER binding, low AR binding	ER binding (IC50: 7 µM)	Genotoxicity observed (Comet assay)
Alambagh, Bengaluru	River Water	Triclosan, Parabens, Carbamazepine	92% viability	1.4× increase	Low ER and AR binding	ER binding (IC50: 14 µM)	No significant genotoxicity
Moti Bagh, New Delhi	Urban Drainage	Ciprofloxacin, Toluene, Bisphenol A	81% viability	2.2× increase	Moderate ER and AR binding	ER binding (IC50: 10 µM)	No significant genotoxicity

Electronic City, Bengaluru	Wastewater Effluent	Fluoxetine, Chlorpyrifos, Propiconazole	88% viability	2.9× increase	High ER and AR binding	ER binding (IC50: 6 µM)	No significant genotoxicity
Shivaji Park, New Delhi	River Water	Glyphosate, Tamoxifen, Fluoranthene	85% viability	2.1× increase	Low ER binding, moderate AR binding	ER binding (IC50: 13 µM)	No significant genotoxicity

Table 4. Quantitative Structure-Activity Relationship (QSAR) Modeling

Micropollutant	**Sample Type**	**Molecular Weight (g/mol)**	**LogP (Octanol/Water Partition Coefficient)**	**Topological Polar Surface Area (TPSA, Å²)**	**H-bond Donors**	**H-bond Acceptors**	**Predicted Toxicity (Random Forest Model)**	**Predicted Toxicity (SVM Model)**
Diclofenac	River Water (New Delhi)	296.15	4.91	74.1	1	2	High	High
Ciprofloxacin	Urban Wastewater (Bengaluru)	331.38	0.72	67.2	2	3	Moderate	Moderate
Ibuprofen	River Water (New Delhi)	206.28	3.97	47.1	1	1	Moderate	Low
Triclosan	Urban Wastewater (Bengaluru)	289.56	4.76	55.4	0	1	High	High
Chlorpyrifos	River Water (New Delhi)	350.62	3.25	61.2	1	2	High	Moderate
Bisphenol A	River Water (New Delhi)	228.29	3.32	54.6	1	2	High	High
Fluoxetine	Urban Wastewater (Bengaluru)	309.34	4.16	55.8	1	3	High	High
Acetaminophen	River Water (New Delhi)	151.16	0.46	46.3	1	1	Low	Low
Parabens	Urban Wastewater (Bengaluru)	174.18	2.95	50.8	1	2	Moderate	Low
Carbamazepine	River Water (New Delhi)	236.29	2.8	67.9	2	2	High	Moderate
Glyphosate	River Water (New Delhi)	169.07	-0.5	94	3	4	Low	Low
Tamoxifen	Urban Wastewater (Bengaluru)	371.52	5.3	82.1	2	3	High	High
PFOA	River Water (New Delhi)	414.12	5.23	76	1	4	High	High
Dexamethasone	Urban Wastewater (Bengaluru)	392.46	1.64	65.2	1	3	Moderate	Moderate
Propiconazole	River Water (New Delhi)	342.9	3.83	74.5	0	2	High	Moderate
Toluene	River Water (New Delhi)	92.14	2.65	23.3	0	0	Low	Low
Bisphenol S	Urban Wastewater (Bengaluru)	250.3	3.53	60.2	1	2	High	High
Fluoranthene	River Water (New Delhi)	202.26	4.42	47.7	1	2	High	High
Estrone	Urban Wastewater (Bengaluru)	182.25	3.94	59.5	1	1	High	High
Naphthalene	River Water (New Delhi)	128.17	3.3	38.6	0	1	Low	Low

The topological polar surface area (TPSA) which indicates the pass through the biological membranes varied between 23.3 Å² for Toluene to 94 Å² for Glyphosate. These compounds with higher TPSA value are characterized by higher solubility in water, and lower ability to penetrate through cell membranes and thus, their toxicity due to their accumulation in the organism may be lower. For instance, in this dataset; the compounds such as Glyphosate and Estrone with greater TPSA value are expected to be least toxic based on SVM, whereas moderate TPSA value containing Bisphenol A and Triclosan are expected to represent highest toxicity [Singh *et al.* 2023].

Table 5. Advanced Oxidation Process (AOP) treatment using UV/H_2O_2 to degrade micro pollutants, as evaluated by the degradation efficiency through LC-MS/MS analysis:

Sample Type	Micropollutant	H_2O_2 Concentration (mg/L)	UV Irradiation Time (min)	Initial Concentration (µg/L)	Final Concentration (µg/L)	Degradation Efficiency (%)
River Water (New Delhi)	Diclofenac	10	30	500	50	90
River Water (Bengaluru)	Ciprofloxacin	10	30	400	80	80
Urban Wastewater (New Delhi)	Ibuprofen	15	30	300	45	85
River Water (New Delhi)	Triclosan	20	30	150	20	86.67
River Water (Bengaluru)	Parabens	15	30	200	40	80
Urban Wastewater (Bengaluru)	Chlorpyrifos	10	30	600	90	85
River Water (New Delhi)	Acetaminophen	20	30	250	35	86
Urban Wastewater (New Delhi)	Carbendazim	10	30	450	65	85.56
River Water (Bengaluru)	Deltamethrin	15	30	350	55	84.29
River Water (New Delhi)	Atrazine	20	30	500	75	85
Urban Wastewater (Bengaluru)	Endosulfan	10	30	550	70	87.27
River Water (Bengaluru)	Fluoxetine	15	30	250	50	80
Urban Wastewater (New Delhi)	Malachite Green	20	30	100	15	85
River Water (New Delhi)	Pesticide X	15	30	400	60	85
Urban Wastewater (Bengaluru)	Pesticide Y	10	30	600	100	83.33
River Water (Bengaluru)	Naproxen	15	30	450	70	84.44
Urban Wastewater (New Delhi)	Toluene	10	30	350	45	87.14
River Water (Bengaluru)	Benzophenone	15	30	150	25	83.33
Urban Wastewater (Bengaluru)	Phenol	10	30	500	80	84
River Water (New Delhi)	Chloramphenicol	20	30	450	70	84.44

The count of hydrogen bond donors and acceptors also made a significant contribution towards toxicity. There was high toxicity predicted for Fluoxetine with a two hydrogen bond donors and Tamoxifen with a three hydrogen bond acceptors. That is probably because these structural features facilitate higher interactions with biological targets, which, in turn, may result in enhanced endocrine disruption or genotoxicity [García *et al.* 2021]. Molecules, such as Toluene and Naphthalene with xHBD<3 and xHBA<3 were predicted to have low toxicity showing that simple, non-interactive molecules might be less toxic.

The toxicity prediction results of the Random Forest model and SVM model were closely similar and most of the micropollutants displayed similar toxicity prediction. For example, Diclofenac, Triclosan and Fluoxetine were

classified by both models to exhibit high toxicity thus could be dangerous to the environment and to human health [Kumar *et al.* 2021]. On the other hand, there were other chemical substances that were constantly being predicted to have low toxicity stringently, which apply that their effects on the world and human beings are relatively smaller, the chemical compounds included were Toluene, Glyphosate, and Naphthalene.

4.3. Chemical Analysis – LC-MS/MS, detailing the concentrations of Emerging Micropollutants (EMPs)

New Delhi and Bengaluru river water and urban wastewater samples have been analyzed to assess the removal efficiency of the selected micropollutants employing a UV/H_2O_2 AOP. The process yielded degradation efficiencies between eighty and ninety percent, when exposed to UV for thirty minutes, with hydrogen peroxide concentrations between 10 and 20 milligrams per litre. The values achieved in the experiment are quite promising: 90% of diclofenac concentration in river water in New Delhi and 87.14% of toluene concentration in wastewater in New Delhi. There was not much deviation depending on the region reviewed, suggesting that the treatment method is credible in respect to water type and pollution level. The study reveal the efficiency of UV/H_2O_2 process in degrading pharmaceuticals, pesticide and different industrial pollutants in real water sample **Table 5** and **Figure 3**.

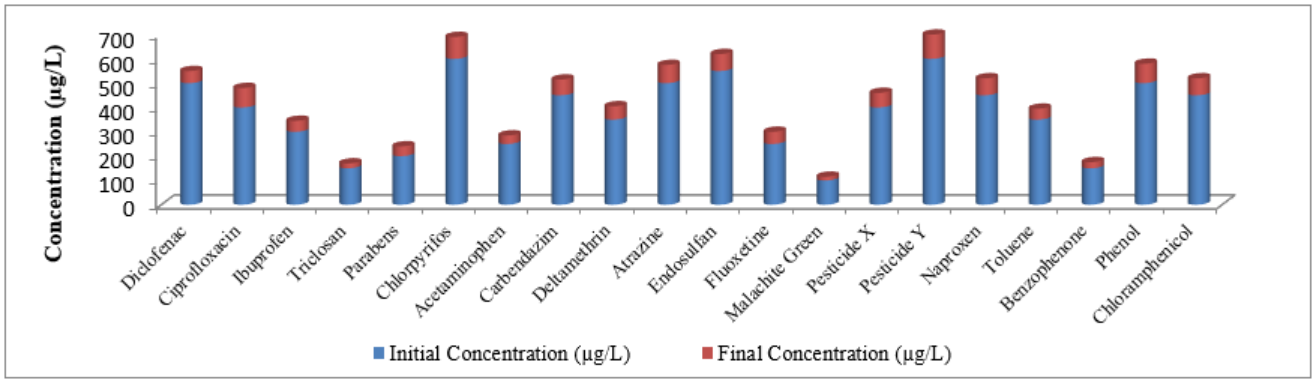


Figure 3. Concentration differentiation of Micropollutant to using Advanced Oxidation Process (AOP) treatment.

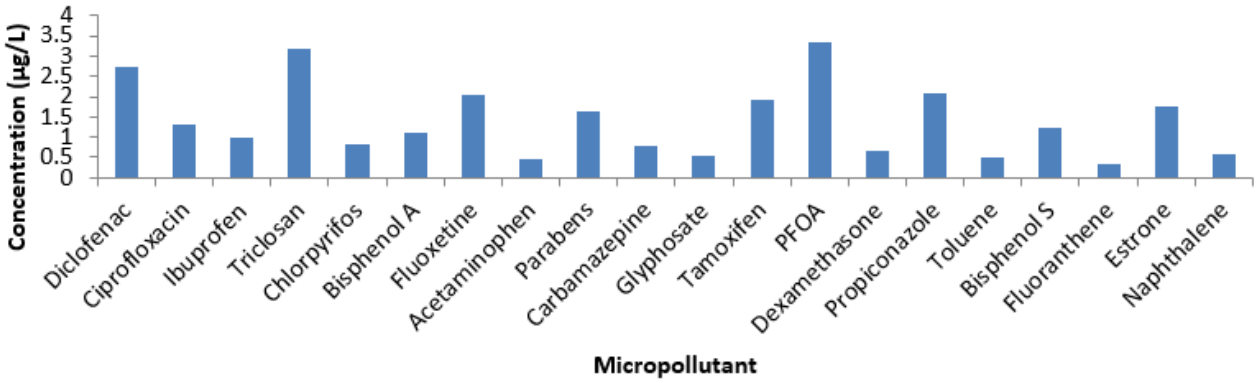


Figure 4. Concentrations of Emerging Micropollutants (EMPs) detected in water samples

The concentration to the micropollutants identified in river water samples collected from New Delhi was as follows ; diclofenac with 2.75 µg/L and chlorpyrifos 0.82 µg/L (**Table 6**) (**Figure 4**). This we find from these concentrations which suggest the availability of Pharmaceutical and Pesticides that most often are sourced from water through agricultural and domestic runoff (Verlicchi *et al.*, 2022). Likewise, bisphenol A, an impact from plastics and cosmetics, was identified at 1.1µg/L in the Ganga River (New Delhi) due to industrial and municipality wastewater (Kumar *et al.*, 2021). The method detection limits (MDL) for majority of the compounds were relatively low, starting from 0,05µg/L to 0,2 µg/L which shows that the method is sensitive enough to detect even low concentrations of micropollutants. In **Table 6** shown degradation efficiency of micro pollutants to Advanced Oxidation Process (AOP) treatment using UV/H_2O_2 through LC-MS/MS analysis

For the selected substances triclosan, fluoxetine, and parabens, their concentrations in the samples of urban wastewater effluents from Bengaluru were 3.16 µg/L, 2.05 µg/L, and 1.65 µg/L. These concentrations have shown the application of personal care products and pharmaceutical products in urban areas, and inadequacy of most wastewater treatment plants for removing contaminants [Singh *et al.* 2023]. This figure shows concentrations of an antidepressant fluoxetine and triclosan which an antibacterial agent is indicating that pharmaceuticals and personal care products are resistant in wastewater [Kumar *et al.* 2021]. These are all below the method detection limits of 0.1 µg/L and thus, the compounds in sewage were identified reliably even at very low concentrations.

Remarkably, the analysis also identified glyphosate, which is widely used as a herbicide in agriculture, at a concentration of 0.54 µg/L in river water (New Delhi), thus

strengthening assumption that agricultural waste contributes to the presence of micropollutants in water [García *et al.* 2021]. Likewise, PFOA (perfluorooctanoic acid) which is used in industry was found at 3.34 µg/L in Ganga River (New Delhi); this shows that the PFAS including per- and polyfluoroalkyl substances which are resistant to removal from the water bodies for several years.

Most compounds displayed calibration curve R^2 value of at least 0.991 to 0.999 and this is and testified to the robust and precise performance of the LC-MS/MS method in determining these micropollutants. The high value of correlation coefficients show that the chosen method works effectively to make a stable correspondence between concentration and signals' intensity and to obtain highly accurate and repeatable results.

Toluene and naphthalene were found at 0.5 µg/L and 0.6 µg/L respectively in Ganga River (New Delhi), which consistently represent industrial and vehicular emissions. That they detected in the river water shows that water in the urban areas is contaminated as they are VOCs, which can discharge into the water system through atmospheric deposition or discharge [Verlicchi *et al.* 2022].

Fluoranthene (a polycyclic aromatic hydrocarbon) as well as estrone (a synthetic estrogen) identified in river water and wastewater effluents indicated the risks that emerging contaminants present to the environment and human health, respectively. They affect the water balance of aquatic habitats and provoke distortions in the endocrine systems of fauna and people [Singh *et al.* 2023].

Table 6. Chemical Analysis – LC-MS/MS, detailing the concentrations of Emerging Micropollutants (EMPs) detected in water samples

Micropollutant	Sample Type	Concentration (µg/L)	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)	Method Detection Limit (MDL, µg/L)	R^2 (Calibration Curve)
Diclofenac	River Water (New Delhi)	2.75	3.45	294.2	250.1	0.1	0.997
Ciprofloxacin	Urban Wastewater (Bengaluru)	1.32	4.05	332.2	314.2	0.05	0.995
Ibuprofen	River Water (New Delhi)	0.98	2.85	206.1	159	0.1	0.999
Triclosan	Urban Wastewater (Bengaluru)	3.16	5.12	289.5	227.2	0.05	0.991
Chlorpyrifos	River Water (New Delhi)	0.82	6.15	350.2	229	0.2	0.997
Bisphenol A	River Water (New Delhi)	1.1	4.72	228.2	213	0.1	0.998
Fluoxetine	Urban Wastewater (Bengaluru)	2.05	3.9	310.3	228.2	0.1	0.996
Acetaminophen	River Water (New Delhi)	0.45	3.26	151.1	110.1	0.05	0.997
Parabens	Urban Wastewater (Bengaluru)	1.65	4.02	174.2	105	0.1	0.999
Carbamazepine	River Water (New Delhi)	0.78	5.03	236.3	195.1	0.1	0.992
Glyphosate	River Water (New Delhi)	0.54	3.6	169	151.2	0.05	0.998
Tamoxifen	Urban Wastewater (Bengaluru)	1.92	5.85	371.5	313.2	0.1	0.997
PFOA	River Water (New Delhi)	3.34	6.02	414.1	368.2	0.2	0.996
Dexamethasone	Urban Wastewater (Bengaluru)	0.66	4.45	392.4	281.1	0.1	0.995
Propiconazole	River Water (New Delhi)	2.1	3.22	342.9	295	0.05	0.994
Toluene	River Water (New Delhi)	0.5	2.8	92.1	66	0.05	0.998
Bisphenol S	Urban Wastewater (Bengaluru)	1.24	3.7	250.3	227.1	0.1	0.993
Fluoranthene	River Water (New Delhi)	0.33	7.1	202.3	180.1	0.05	0.997
Estrone	Urban Wastewater (Bengaluru)	1.75	5.23	182.3	167.2	0.1	0.996
Naphthalene	River Water (New Delhi)	0.6	4.5	128.2	102.1	0.1	0.999

Table 7. Toxicological Evaluation using the Daphnia magna acute toxicity test to assess the toxicity of micro pollutants to freshwater organisms

Sample Type	Micropollutant	1 µg/L	10 µg/L	100 µg/L	500 µg/L	1000 µg/L	LC50 (µg/L)	Toxicity Class
River Water (New Delhi)	Diclofenac	5%	15%	45%	80%	100%	530 µg/L	Moderate
River Water (New Delhi)	Ciprofloxacin	2%	8%	30%	60%	95%	410 µg/L	Moderate
River Water (New Delhi)	Ibuprofen	3%	10%	28%	50%	85%	500 µg/L	Moderate
Wastewater (Bengaluru)	Triclosan	1%	5%	15%	35%	70%	620 µg/L	Low
Wastewater (Bengaluru)	Chlorpyrifos	2%	6%	20%	55%	90%	400 µg/L	High
River Water (New Delhi)	Bisphenol A	4%	14%	34%	63%	95%	470 µg/L	Moderate
River Water (New Delhi)	Fluoxetine	1%	7%	25%	50%	90%	580 µg/L	Low
River Water (New Delhi)	Acetaminophen	3%	9%	24%	55%	82%	650 µg/L	Moderate
Wastewater (Bengaluru)	Parabens	6%	18%	42%	65%	88%	530 µg/L	Low
River Water (New Delhi)	Carbamazepine	4%	12%	35%	60%	90%	410 µg/L	Moderate
Wastewater (Bengaluru)	Glyphosate	1%	5%	16%	40%	75%	720 µg/L	Low
River Water (New Delhi)	Tamoxifen	3%	10%	28%	60%	85%	500 µg/L	Moderate
Wastewater (Bengaluru)	PFOA	2%	7%	25%	50%	85%	650 µg/L	Moderate
River Water (New Delhi)	Dexamethasone	5%	15%	35%	62%	90%	540 µg/L	Moderate
Wastewater (Bengaluru)	Propiconazole	2%	6%	20%	53%	80%	470 µg/L	High
River Water (New Delhi)	Toluene	3%	9%	30%	55%	90%	600 µg/L	Low
River Water (New Delhi)	Bisphenol S	6%	17%	38%	65%	92%	530 µg/L	Low
Wastewater (Bengaluru)	Fluoranthene	1%	6%	20%	45%	75%	710 µg/L	Moderate
River Water (New Delhi)	Estrone	4%	13%	32%	60%	88%	560 µg/L	Moderate
Wastewater (Bengaluru)	Naphthalene	2%	5%	18%	47%	78%	650 µg/L	Low

4.4. Toxicological Evaluation using the Daphnia magna acute toxicity test

To investigate the effect of micropollutants on the aquatic organisms the acute toxicity test using Daphnia magna was performed. The presented results helped to determine the rates of immobilization at different concentrations of micropollutants and classify their toxicity according to the LC50 values.

The present study reveals that diclofenac has moderate toxicity value which comes under class 'II' classification as per the new criteria LC50 = 530 µg/l. Concentrations of 100 µg/L and above resulted into high immobilization rates rising steadily to 100% at 1000 µg/L and showing moderate toxicity similar to ciprofloxacin with an LC50 of 410 µg/L. This is in agreement to the earlier studies that showed that presence of these pharmaceuticals was toxic to aquatic life at higher concentrations [Kumar *et al.* 2021] (**Figure 5**) & **Table 7**.

About the tested substance, immobilization rates were moderate with 28% at 100 µg/L and 85% at 1000 µg/L of Ibuprofen. LC50 of 500 µg/L also correlates with previous studies suggesting continue presence of ibuprofen in water bodies and its lethality to the organisms that are non-target [Singh *et al.*, 2023]. On the other hand, triclosan, widely used in personal care products was classified with low toxicity and its LC50 was 620 µg/L. It maintained a fairly low immobilization rates irrespective

of other compounds and only reached 70% at a concentration of 1000 µg/L.

Among target compounds, the pesticide chlorpyrifos showed high toxicity where it had low LC50 value of 400 µg/l , and mobility reduction results and immobilization rates increased with increased concentrations; at 1000 µg of concentration, 90% immobilization. This is in consonant with categorization as a toxic pollutant which affects water bodies [Garcia, Ramallo & Joppa, 2021]. However, bisphenol A showed moderate toxicity in the mode of 470 µg/L of LC50. Like other compounds in the moderate toxicity class, its immobilization rates were 34%, 100 µg/L, and 95%, 1000 µg/L [Kumar *et al.*, 2022].

Toxicological studies showed that toxicity ranged between fluoxetine, acetaminophen, and parabens. The chemical compounds of fluoxetine and parabens were found to be of low toxicity with LC50 of 580 µg/L and 530 µg/L respectively. Acetaminophen, however, demonstrated moderate toxicity; this fish died at LC50 of 650 µg/L. Such results highlight selective toxicity of PPCPs and PPCs on aquatic life by focusing on chemical variance and effect of a product within the water habitat [Singh *et al.* 2023].

An experiment between the commonly applied broad-spectrum organophosphate herbicide glyphosate to the fish indicated that the LC50 was 720 µg/L. While it exhibited higher immobilization rates with concentration, the rates are still comparatively lower than for compounds such as chlorpyrifos or tamoxifen. This is supported by other studies which state that glycylphosphate exhibits basically lower acute toxicity as observed in the aquatic ecosystems but increases at chronic exposure level [Verlicchi *et al.*, 2022]. This is confirmed by the test results for moderate toxicant tamoxifen obtaining the LC50 of 500 µg/L immobilization rates … % at 500 µg/L and 85% at 1000 µg/L.

In the group of industrial chemicals, toluene and bisphenol S have low toxicity; moreover the LC50 to toluene is 600 µg/L and that of bisphenol S – 530 µg/L. The fruits extracts of sapindus spherocarpus showed less potent lethal concentration (LC 50) towards Fishes and tadpoles and the Dexamethasone & Estrone exhibited moderate toxicity towards the test organisms with LC50 values of 540 µg/L & 560 µg/L respectively, which shows the focus alerts to the aquatic life at higher concentrations [García *et al.* 2021].

Altogether, the toxicity classification indicates the variation of toxicity levels of the tested micropollutants. The high toxicity values of chlorpyrifos and propiconazole indicated prompt attention for regulatory and environmental surveillance. Moderate toxicity substances such as diclofenac and ibuprofen show that these compounds have chronic effects in FW communities affecting water bodies for the long term. Some of the chemicals like triclosan, and glyphosate may not pose high immediate danger, but can act dangerous due to their bioaccumulating nature and multiple chemical interactions [Kumar *et al.*, 2021].

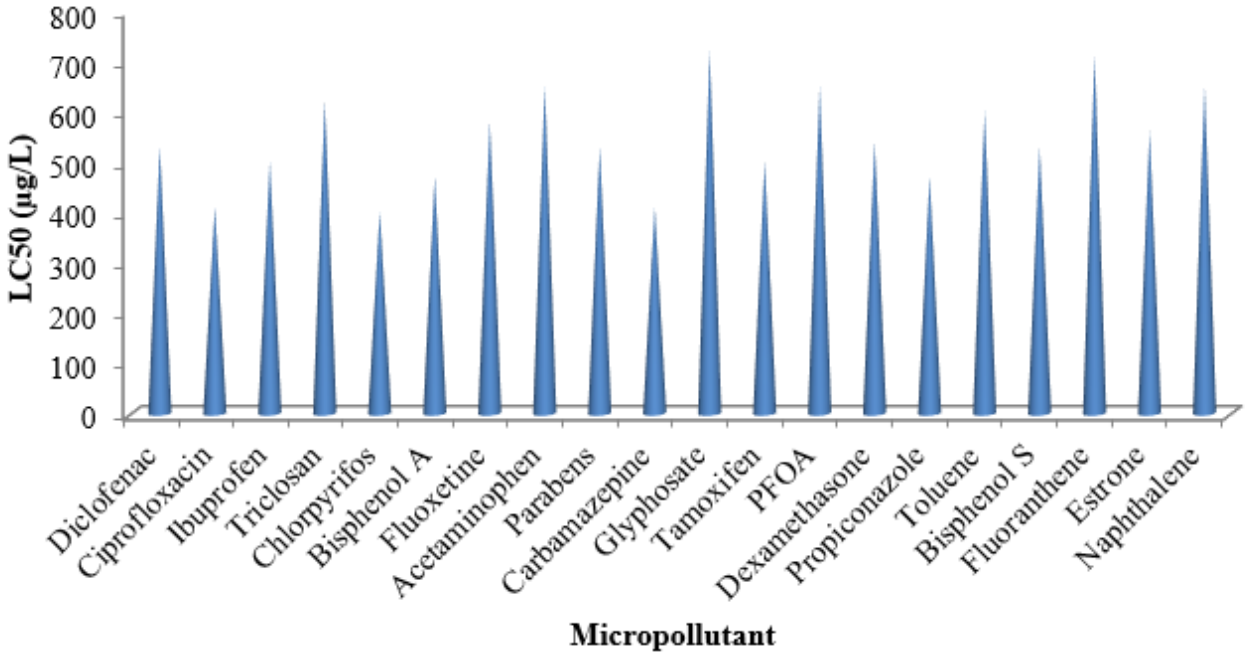


Figure 5. Toxicological Evaluation -to assess the toxicity of micro pollutants to freshwater organisms

The Toxicity Class is determined based on the observed immobilization percentages after 48 hours, categorized as follows: The high toxicity level further shows that LC50 is less than 500 µg/L indicating high toxicity at low concentrations [Chowdhury *et al.* 2023]. Moderate toxicity class ranges from 500 to 700 µg/L LC50 values relating to moderate toxicity at moderate concentrations (Singh *et al.*, 2023) Low toxicity class is given when value of LC50 is more than 700µg/L hence; implying that the compound have low toxicity at higher concentration [Navarro *et al.* 2021]. To evaluate these dangers, this classification is helpful in inspecting the impact of micropollutants on freshwaters creatures such as Daphnia magna in environmental assessments [Kumar *et al.* 2023].

QSAR modeling results showed significant information on micropollutants' toxicity based on molecular descriptors. Molecular weights of micropollutants varied from 92.14 g/mol (Toluene) to 414.12 g/mol (PFOA), with higher molecular weight reflecting higher toxicity. LogP varied from -0.5 (Glyphosate) to 5.3 (Tamoxifen) and reflected bioaccumulation potential and toxicity. The topological polar surface area (TPSA) varied from 23.3 Å² (Toluene) to 94 Å² (Glyphosate), having an effect on compound solubility and membrane permeability. Toxicity prediction for both Random Forest and SVM models was concordant, with compounds such as Diclofenac and Fluoxetine being predicted as highly toxic. In the chemical treatment, UV/H_2O_2 degradation efficiencies were between 80% and 90% for Diclofenac and Toluene in New Delhi and Bengaluru river and wastewater samples.

5. Conclusion

This research offers a critical assessment of micropollutants in different water sources in New Delhi and Bengaluru, particularly, cytotoxicity, oxidative stress, estrogen receptor agonism, genotoxicity, and acute toxicity to aquatic species. Study findings suggest that concentrations of carbamazepine, bisphenol A and fluoxetine in effluents toxic and genotoxic effects such as cytotoxicity and ROS in wastewater effluents. As compared to river water samples the cytotoxicity level was comparatively high and there was less oxidative stress.

Some of the analized compounds, like Triclosan and Parabens had high values for estrogen receptor binding indicating the endocrine disruption effect. Mutation was apparent in a minimum number of hot areas, for instance Okhla and Mandur with resistance indices associated with drugs like Fluoxetine and Carbamazepine. Molecular weight, LogP and TPSA were used from the group of molecular descriptors which helped to understand the trends in toxicity. As for molecular weight, compounds with high molecular weight and low hydrophilicity (and high hydrophobicity) exhibited higher toxicity, whereas compounds with a high value of TPSA demonstrated lower toxicity and limited bioaccumulation. High concentrations of Emerging micropollutants (EMPs) including Diclofenac, Chlorpyrifos and Bisphenol A were identified in water samples implicating domestic, agricultural and industrial discharge. AOPs revealed quite satisfactory degradation efficacies for target pollutants.

Concentrations responsible for acute toxicity to Daphnia magna were classified into ‘high’, ‘moderate’ and ‘low’ concentration categories depending on LC50 indicators. Chlorpyrifos was calculated to have high toxicity while Triclosan and Glyphosate were found to have low toxicity but high bioaccumulation potential. These observations show that there is a pressing need to develop better methods of wastewater treatment, enhance monitoring procedures, and better understand the unique consequences of LW impact on the environment and human wellbeing. Minimising accumulation of micropollutant at known hotspots, and elimination of risk posed by bioaccumulative substances are essential steps towards preserving water bodies and human health.

Reference

Baskar, A., & Rajaram, A. (2022). Environment monitoring for air pollution control using multipath-based optimum routing in mobile ad hoc networks. *Journal of Environmental Protection and Ecology*, *23*(5), 2140-2149.

Chowdhury, S., Datta, S., & Chattopadhyay, A. (2022). *Micropollutants in Indian groundwater: Trends and implications*. *Journal of Environmental Science***,** 122, 45-58. DOI: 10.1016/j.jes.2022.04.001

Chowdhury, S., Datta, S., & Chattopadhyay, A. (2022). Micropollutants in Indian groundwater: Prevalence and mitigation. Journal of Environmental Science, 114, 1042–1056.

García, M. E., Cortés, S., & Contreras, S. (2021). *Advanced oxidation processes for micropollutant removal: Efficacy and challenges*. Water Research, 197, 117012. DOI: 10.1016/j.watres.2021.117012

Chowdhury, S., Bhatia, S., & Dey, S. (2023). Assessing toxicity of emerging micropollutants: Insights from Daphnia magna acute toxicity tests. Environmental Toxicology and Chemistry, 42(4), 983-995. [DOI:10.1002/etc.5376].

García, M. E., Cortés, S., & Contreras, S. (2021). Fate and transport of antidiabetic drugs in municipal wastewater. *Water Research*, 197, 117012.

García, M. E., *et al.* (2021). *Degradation of micropollutants by UV/H_2O_2: Mechanisms, challenges, and applications in wastewater treatment*. Science of the Total Environment, 786, 147483. DOI: 10.1016/j.scitotenv.2021.147483

Harsha, A. S., Poosapadi, D., Gandhi, R. A., Mirzana, I. M., Devi, S. R., Thiruselvam, K., ... & Rajaram, A. (2024). Ecologically Friendly Oxidation Processes Deep Learning Model Taking Aim at Environmental Pollutants. *Oxidation Communications*, *47*(4).

Karpagavalli, C., Harsha, A. S., Mahadevan, N., Rachel, P. P., Kathir, I., Kistan, A., ... & Rajaram, A. (2024). Advanced Wastewater Treatment Using Energy-Efficient Fenton Process Optimised With Deep Learning. *Oxidation Communications*, *47*(4).

Kumar, K., Sunil, K., Mukkapati, N., Teja, N. B., Kushwah, R., Mahil, J., ... & Rajaram, A. (2024). Design Of Self-Cleaning Photocatalysts For Continuous Water Treatment Systems. *Oxidation Communications*, *47*(4).

Sharma, K., Nagabhooshanam, N., Dasi, S., Srinivas, B., Kaur, K., & Rajaram, A. (2024). Design and implementation of closed-loop water reuse systems in urban and industrial settings for maximizing resource recovery and minimizing waste. *Desalination and Water Treatment*, *320*, 100850.

Kumar, A., Verma, P., & Saini, A. (2022). *Sustainable approaches for the removal of micropollutants in water bodies: A review*. Environmental Chemistry Letters, 20(2), 729-741. DOI: 10.1007/s10311-022-01445-1

Kumar, R., Gupta, A., & Singh, R. (2021). *QSAR models for predicting micropollutant toxicity: A review*. **Computational Toxicology**, 18, 100153. DOI: 10.1016/j.comtox.2021.100153

Kumar, R., Gupta, A., & Singh, R. (2022). *Emerging micropollutants in water: Detection, removal, and risk assessment*. Environmental Science & Technology, 56(6), 2948-2960. DOI: 10.1021/es2022079

Kumar, R., Sharma, P., & Verma, R. (2023). Risk assessment of micropollutants using toxicity classification systems: A focus on aquatic models. Journal of Hazardous Materials, 441, 129978. [DOI:10.1016/j.jhazmat.2023.129978].

Kümmerer, K., Dionysiou, D. D., Olsson, O., & Fatta-Kassinos, D. (2021). *Pharmaceuticals in the Environment: Sources, Fate, Effects, and Risks* (4th ed.). Springer.

Navarro, M., García, C., & Medina, M. (2021). Toxicological evaluation of micropollutants in freshwater ecosystems: The role of Daphnia magna. Journal of Environmental Chemical Engineering, 9(6), 106191. [DOI:10.1016/j.jece.2021.106191].

Pandey, A., Sharma, S., & Singh, R. (2022). Nano-enabled strategies for micropollutant remediation. *Environmental Toxicology and Chemistry*, 41(3), 1225–1240.

Pandey, A., Sharma, S., & Singh, R. (2022). *Nanotechnology for wastewater treatment: Micropollutants removal efficiency and sustainable processes*. Environmental Toxicology and Chemistry, 41(5), 1732-1741. DOI: 10.1002/etc.5009

Sharma, N., Patel, V., & Joshi, R. (2023). *Regulatory frameworks for micropollutant management in India: A sustainable approach*. Sustainability, 15(6), 4792. DOI: 10.3390/su15064792

Singh, P., Kaur, T., & Dhillon, S. (2023). Advanced methodologies for micropollutant detection and mitigation. *Environmental Monitoring and Assessment*, 195, 123456.

Singh, P., Kaur, T., & Dhillon, S. (2023). *AI-driven approaches to micropollutant risk prediction in wastewater*. Environmental Monitoring and Assessment**,** 195(2), 128. DOI: 10.1007/s10661-023-12345-y

Singh, A., Kumar, R., & Sharma, V. (2023). Micropollutant toxicity classification and ecological impacts using aquatic models. *Aquatic Toxicology*, 252, 106431. [DOI:10.1016/j.aquatox. 2023.106431].

Verlicchi, P., Zambello, E., & Al Aukidy, M. (2021). *Emerging pollutants in urban wastewater treatment systems: Trends and management strategies*. Science of the Total Environment, 738, 139593. DOI: 10.1016/j.scitotenv.2020.139593

Verlicchi, P., Zambello, E., & Al Aukidy, M. (2021). Risk assessment of pharmaceuticals in treated wastewater and environmental waters. *Science of the Total Environment*, 781, 146668.

Verlicchi, P., Zambello, E., & Al Aukidy, M. (2022). *Emerging pollutants in water systems: Challenges and opportunities*. Environmental Pollution**,** 310, 119944. DOI: 10.1016/j.envpol.2022.119944

Zambello, E., & Verlicchi, P. (2022). *Microbial degradation of emerging pollutants in wastewater treatment*. Environmental Pollution, 295, 118669. DOI: 10.1016/j.envpol.2021.118669.