Proceedings of the 9th World Congress on New Technologies (NewTech'23)
Brunel University, London, United Kingdom - August 09-11, 2023
Paper No. ICEPR147
DOI: 10.11159/icepr23.147

Non-Target Screening Of Organic Micropollutants In Durgam Cheruvu Lake, India

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Abstract – The main objective of this study is to evaluate the water quality of Durgam Cheruvu Lake, Hyderabad, India by comprehensively analysing organic micropollutants. Samples were collected from three different sites in the lake and nontargeted screening was performed using liquid chromatography-quadrupole time-of-flight (LC-QTOF). A total of 183 compounds were detected in all samples. This includes pharmaceuticals, herbicides, fungicides, pesticides, hormones, steroids, UV filters, plasticizers, cyanotoxins, and metabolites. In all samples, pharmaceuticals accounted for approximately 50%, herbicides 8%, and metabolites 9%. The high abundance values were observed for 17 α β – Dihydroequilin, Avobenzone, Sibutramine, Butachlor, Napropamide, and Estriol at all the sampling locations. Estriol and 17αβ – Dihydroequilin are classified as the largest endocrine disruptors among many micropollutants. Eutrophication-related cyanotoxins including Microcystin-LR and Anatoxin-A have been identified in the lake. Additionally, the urine metabolites of Clarithromycin, Flunitrazepam, and other transformed metabolites of cocaine-d3 and Amitriptyline were discovered. Overall, veterinary medications, narcotic pharmaceuticals, pain killers, anti-psychotic, anti-depressant, and anti-obesity drugs were found to be the most prevalent components in the lake samples, indicating the discharge of domestic and industrial wastewater into the lake.

***Keywords*:** Cocaine; Cyanotoxins; LC-QTOF; Metabolites; Micropollutants; PCA, Pharmaceutical

1. Introduction

Lakes are important components of the Earth's environment as they supply valuable water as well as offer natural habitats for plants and animals, regulating hydrological cycles, and influencing microclimate. In addition to being used for the aforementioned purposes, the lakes are also used for drinking water, the water supply system, fishing, and ecotourism. Several lakes have reportedly vanished around the nation as a consequence of illegal dumping, waste discharge, and evaporation of their catchment areas that have been reclaimed for urbanization. The majority of Indian lakes are prone to nutrient overload, which causes eutrophication. The combined effects of overpopulation, urbanization, and industrialization have a detrimental effect on surface water bodies. Ecosystems throughout the world have changed drastically as a result of the activities of humans. Organic micropollutants, which happen to be everywhere and may have unexpected ecological effects at low exposure concentrations (such as ng/L or g/L levels), are one of the well-known anthropogenic stressors on aquatic ecosystems [1], [2]. Pharmaceuticals and personal care products, plasticizers, food additives, wood preservatives, laundry detergents, disinfectants, flame retardants, pesticides, natural and synthetic hormones, and a few disinfection by-products are a few examples of categories that may contain micropollutants [3].

According to the literature, 57% of India's rising pollutants include pesticides, 17% are pharmaceuticals, 15% are surfactants, 7% are personal care items, and 5% are phthalates [4]. Pharmaceutical compounds found in water bodies have been associated with risk to aquatic life [5]. Multiple drug residues are prevalent in the aquatic environment where microorganisms exist, and this exposure can cause mutations and the formation of new resistant strains. Hyderabad is a hub for pharmaceutical companies. Many studies reported the presence of micropollutants in waterbodies of Hyderabad mainly Hussain Sagar, Musi river, kazipally lake, etc., [6], [7]. The aim of this study is to identify the organic micropollutants present in Durgam cheruvu lake of Hyderabad, India.

2. Materials and methods

2.1. Site selection and sample collection

Durgam Cheruvu is a freshwater lake which is located in Hyderabad, Rangareddy district of Telangana, India. The lake is 34 hectares or 83 acres in area. The lake was a drinking water source for the Golconda Fort during the Qutub Shahi dynasty. The lake's size and water quality decreased due to local pollution, urbanization, and domestic sewage discharge from nearby residential complexes. The Hyderabad Metropolitan Water Supply and Sewage Board, with permission from the Telangana government, built a separate sewage treatment plant near Durgam Cheruvu in 2022. Recently, the Lake in Hyderabad underwent a significant alteration to become a popular destination for tourists. Samples from the lake were collected in March 2023 using a boat at a depth of 30 cm. Sampling locations named A, B, and C are marked on the map using Google Earth shown in Figure 1. Collected samples were passed through a 0.22 µm filter and filtered samples were stored at 4 °C until analysis.

Fig. 1: Sampling sites of the lake.

2.2. Non-target screening of micropollutants

The filtered samples were transferred to 2 mL amber LC vials and injected into the instrument with an auto-sampler for the identification of micropollutants. Agilent 6545 Liquid chromatography-Quadrapole time of flight (LC-QTOF) was used for screening of the samples. The system was equipped with a 1260 Infinity II pump. A 10 µL aliquot was injected into the instrument for screening. Gradient flow was used for the separation and identification of compounds of interest. In this study, an aqueous solution of 5 mM ammonium acetate and 0.1% acetic acid (mobile phase A), and 0.1% acetic acid in MeOH (mobile phase B) were used. All chemicals and solvents used in this study were MS grade.

Mobile phase A was started at 95% for 0.5 minutes and ramped linearly to 100% B over the next 10.5 minutes. 100% B was kept on hold for 2 minutes, then ramp to 95% A over the next 5 minutes. Then 95% A was held for the next 2 minutes. The total run time for each sample was 20 minutes. After each sample, a 1 minute post-run was maintained to remove any interference from the previous sample before injecting the next sample. A Zorbax Eclipse plus C18 (Narrow Bore RR) column (2.1*100 mm, 3.5-micron diameter) was used and a mobile phase flow rate of 0.22 mL/min was maintained throughout the study. The maximum column pressure limit was 450 and the steady column temperature was 40±0.4°C.

Mass spectrometry was performed using a dual Agilent Jet Stream Electrospray Ionization (AJS-ESI) source operating in both positive and negative modes. For AJS-ESI, the mass range was set from m/z 100 to 1200 with an acquisition rate of 3 spectra/s. Gas temperature of 150 ºC; drying gas (N_2) flow rate of 10 L/min; nebulizer gas pressure of 35 psi; sheath gas temperature of 375 ºC; sheath gas flow of 11 L/min; 3500 V capillary voltage and 125 V fragmentor voltage were maintained. During the measurement, a solution containing reference compounds with m/z 121.0509, 922.0098, (positive ionization mode), and m/z 112.9856, 966.0007 (negative ionization mode) was injected for mass drift correction.

The spectra files were processed using Agilent MassHunter Qualitative Workflows B.08.00 for target/suspect screening. Data files were screened with Agilent MassHunter Personal Compound Database and Library (PCDL)-water library for identification of compounds, with their respective abundance and Q-Score.

2.3. Statistical analysis

The principal component analysis was performed for the compounds whose abundance value is greater than 30000 using PAST 4.13 Statistical software.

3. Results and discussion

3.1. Screening of the pollutants

A total of about 183 compounds have been identified in the three sampling sites of the lake. These compounds were categorized as pharmaceuticals, metabolites, herbicides, fungicides, insecticides, pesticides, hormones and steroids, personal care products (primarily UV filters), plasticizers, cyanotoxins, and miscellaneous. The distribution of the compounds at different sites is presented in a pie chart shown in Figure 2. Overall, 119, 126, and 129 compounds were detected at Sites A, B, and C, respectively. In all the sample sites, 9% of the detected compounds are metabolites, 8% are herbicides, and 50% are pharmaceuticals. The two cyanotoxins Microcystin-LR and Anatoxin-a, which are generated by cyanobacteria during eutrophication, were also identified at all sample locations, indicating the presence of high nutrient concentrations in the lake.

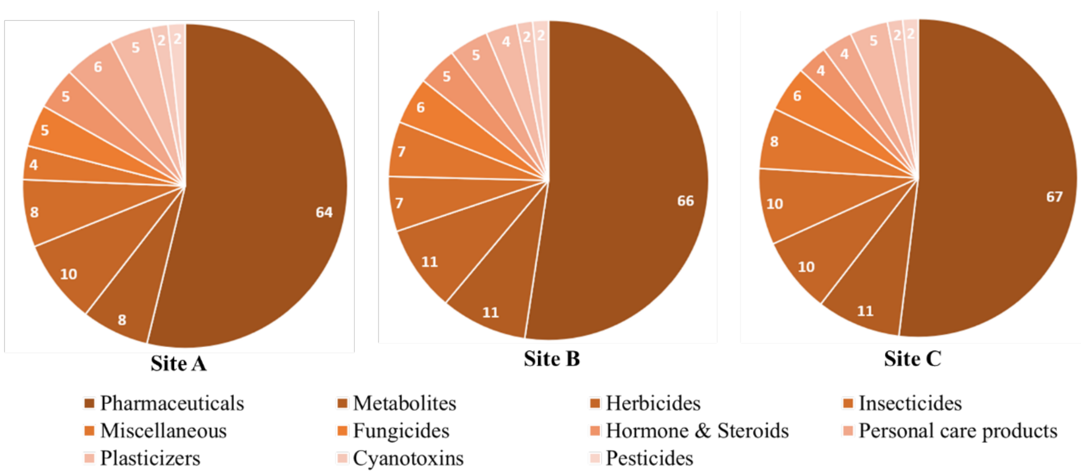

Fig. 2: Micropollutants distribution in the selected sites of the lake.

The micropollutants whose area of abundance (area under the peak) was greater than 10000, have been given in Table 1. All compounds were not detected in all the sampling sites. Nefazodone was detected only at sites B and C. This drug is used to treat severe depressive disorders, aggressive behavior, anxiety, and panic disorders. Similarly, Sulfaquinoxaline, a veterinary drug given to cattle and sheep; Clarithromycin-N-oxides, a urinary metabolite of antibiotics; and Temazepam, commonly used to treat severe insomnia were detected only at sites B and C. 17 α β – Dihydroequilin (estrogen sex hormone), Avobenzone (ultraviolet A (UVA) blocker), Sibutramine an anti-obesity drug were found in high abundance at the sampling locations and are widely distributed in the lake region. The mass scan spectra of Sibutramine in all the sites are given in

Figure 3. Mepivacaine drug and DDM / Dichlorophen a veterinary fungicide are detected at Site A only. The different distribution of pollutants at each location can be caused by changes in waste flow into the lake. The lake had been surrounded by pharma companies and pesticide dealers. The domestic wastewater discharge from the surrounding areas of Madhapur and Jubilee Hills might be one of the reasons for the presence of pollutants in the lake.

Table 1: Micropollutants identified at different sampling sites of Durgam cheruvu lake.

Compound Name	**Mass**	**Formula**	**Site A**	**Site B**	**Site C**
Butachlor (Machete)	311.1667	$C_{17}H_{26}ClNO_2$	223946	175011	23657
Napropamide	271.155	$C_{17}H_{21}NO_2$	12538	10639	12217
Estriol	288.1726	$C_{18}H_{24}O_3$	211027	20301	287810
17 α β - Dihydroequilin	270.1609	$C_{18}H_{22}O_2$	**203846**	**143541**	**213595**
Avobenzone (BM-DBM)	310.1553	$C_{20}H_{22}O_3$	**161399**	**215063**	**230327**
Sibutramine	279.1742	$C_{17}H_{26}ClN$	**199311**	**200701**	**167158**
Nefazodone	469.2219	$C_{25}H_{32}ClN_5O_2$	N.D.	70732	79963
Narasin	764.5077	$C_{43}H_{72}O_{11}$	84433	78173	73389
Erythromycin	733.4584	$C_{37}H_{67}NO_{13}$	61223	59400	64178
Dextilidine (Tilidine)	273.1715	$C_{17}H_{23}NO_2$	62705	55333	59747
Clozapine	326.1295	$C_{18}H_{19}ClN_4$	48454	42943	36943
4-Hydroxyantipyrine	204.0889	$C_{11}H_{12}N_2O_2$	45983	37335	31383
Temazepam	300.0687	$C_{16}H_{13}ClN_2O_2$	N.D.	N.D.	22903
Sulfaquinoxaline	300.0687	$C1_4H_{12}N_4O_2S$	N.D.	N.D.	22229
Morphine-d3	288.1571	$C_{17}H_{16}D_3NO_3$	16835	16940	21685
Apramycin	539.2818	$C_{21}H_{41}N_5O_{11}$	12898	16099	18596
Tolycaine	278.1655	$C_{15}H_{22}N_2O_3$	17480	17819	14755
Procainamide	235.1686	$C_{13}H_{21}N_3O$	14207	11384	11883
Zilpaterol	261.1464	$C_{14}H_{19}N_3O_2$	12176	11128	10909
Tramadol-N-oxide	279.1848	$C_{16}H_{25}NO_3$	14245	13350	9812
Abacavir	286.1556	$C_{14}H_{18}N_6O$	12677	9144	9376
Erythromycin A dihydrate-C13	735.4705	$C_{35}H_{67}NO_{13}$	20741	5177	4630
Mepivacaine	246.1727	$C_{15}H_{22}N_2O$	13092	N.D.	N.D.
Bis(2-ethylhexyl) phthalate (DEHP)	390.2761	$C_{24}H_{38}O_4$	14031	138550	20800
DDM / Dichlorophen	268.0066	$C_{13}H_{10}Cl_2O_2$	18162	N.D.	N.D.
Clarithromycin-N-oxide	763.4676	$C_{38}H_{69}NO_{14}$	N.D.	N.D.	21220
NMP / N-Methylpyrrolidone	99.0681	C_5H_9NO	2771	23503	30033

*N.D. means not detected

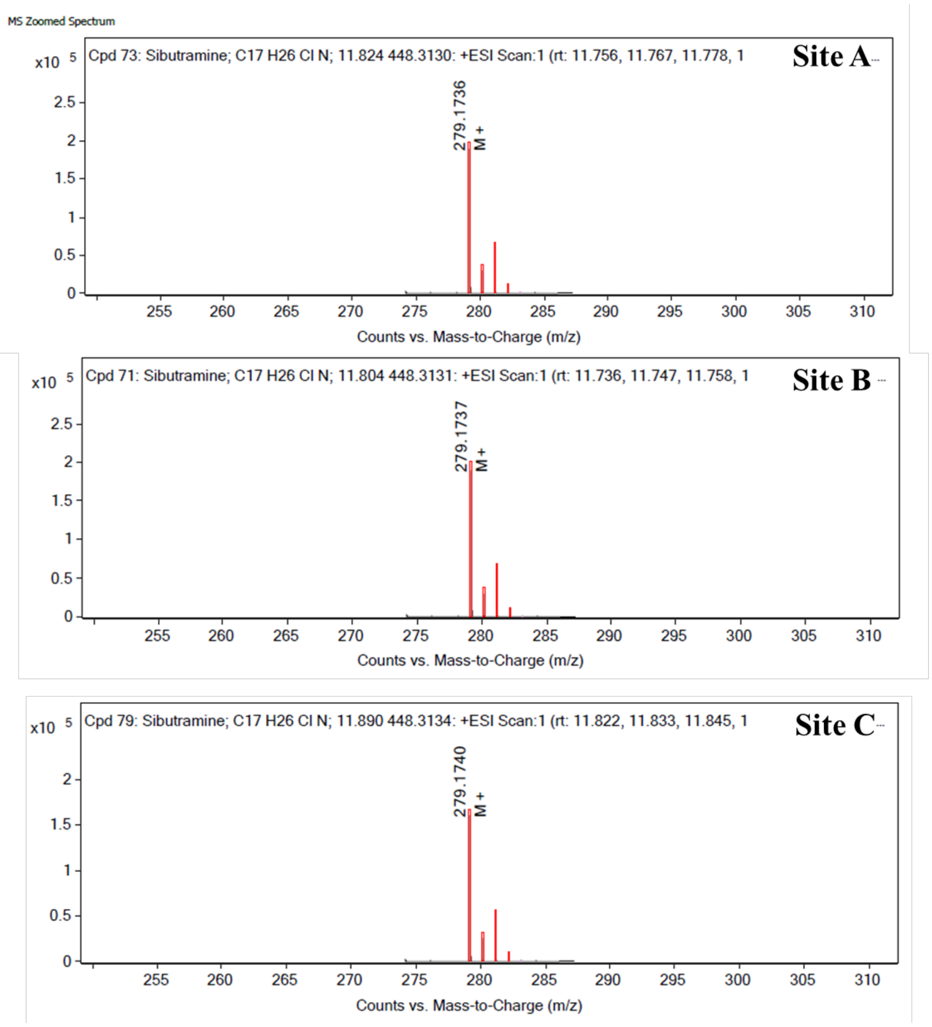

Fig. 3: MS Spectrum of Sibutramine in all sampling sites of the lake.

The principal components analysis projections on PC1 and PC2 were presented in Figure 4. Results of the PCA showed a separation of the parameters related to herbicides, hormones, personal care products, pharmaceuticals, and plasticizers on the two independent axes. The first component (PC1), which accounts for 67.5% variance discriminates herbicides, hormones, and personal care products on the positive side, and pharmaceuticals and plasticizers together on the negative one. The second component (PC2), accounting for 24.4%, seems to discriminate selected sampling sites into two sets. Site B was plotted towards the positive direction of PC2, while the second set (Site A and C) was distributed on the negative side of PC2. Estriol and 17αβ – Dihydroequilin had high positive loadings (>0.90) for PC1, while they had negative loadings for PC2. These compounds are estrogens which are classified as the greatest endocrine disruptors among many micropollutants. The concentration of these hormones, which play a role in the development of feminine features in a variety of living creatures, has increased recently in aquatic environments [8]. Humans as well as animals regularly produce the natural estrogens estriol and 17-Dihydroequilin [9]. Butachlor (Machete), Avobenzone (BM-DBM), and Sibutramine had positive loadings for both PC1 and PC2. Butachlor is a common herbicide, used for stopping weeds from developing new plant tissues. Avobenzone is a UVA blocker and an active ingredient in sunscreens, more than 60 disinfection by-products of avobenzone were identified to be transformation products in chlorine and bromine disinfected waters [10]. Sibutramine is an anti-obesity drug. Bis(2-ethylhexyl) phthalate (DEHP) had high positive loadings for PC2 and negative loadings for PC1. DEHP is most commonly used as a plasticizer in various flexible polyvinyl chlorides. DEHP is present in multiple environmental matrices including water, wastewater, landfill leachate, sludge, soil, and sediments [11]. DEHP is mostly released into the soil compartment, with a possibility that some of it may later be released into the atmosphere or surface waters. The remaining pharmaceutical compounds Nefazodone, Narasin, Erythromycin, Dextilidine (Tilidine), Clozapine, 4 Hydroxyantipyrine, Bis(2-ethylhexyl) phthalate (DEHP), NMP / N-Methylpyrrolidone had both negative PC1 and PC2 loadings.

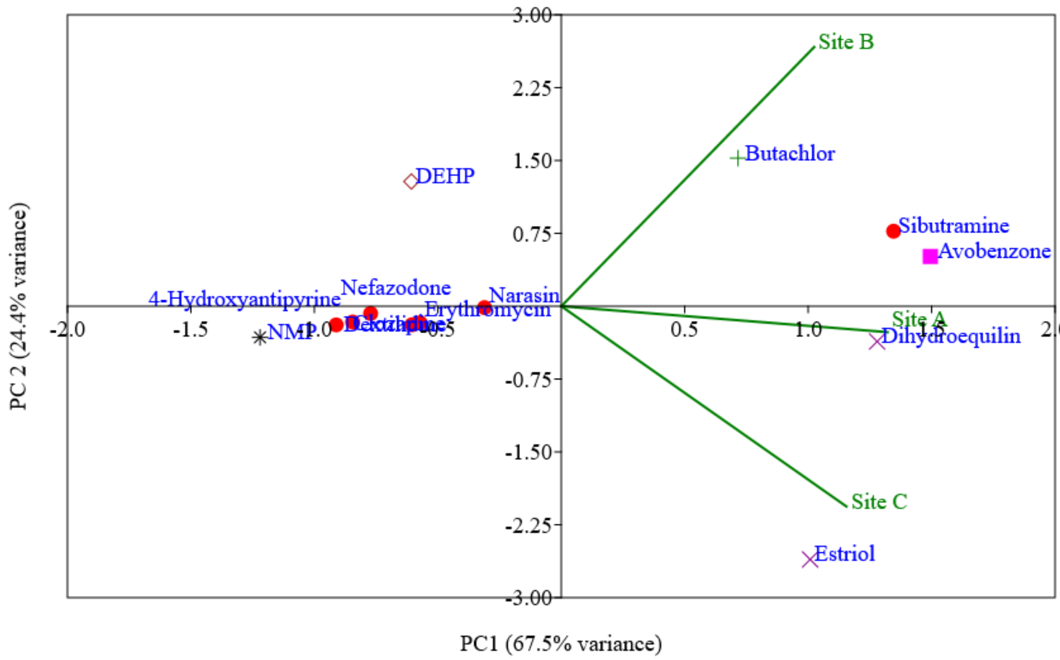

Fig. 4: Principal component analysis (PCA) projections on PC1 and PC2.

3.2. Transformed products

In all the samples five compounds and their transformed metabolites had been identified. The details of the compounds and the metabolites are shown in Figure 5. Clarithromycin N-oxide is a urinary metabolite of Clarithromycin, an antibiotic, was mainly detected at Site C. Cocaine d3 was detected in all samples along with its two major urinary metabolites, benzoylecgonine d3 and ecgonine methyl ester d3 (ecgonine methyl ester). Compared to the parent substance, benzoylecgonine is more toxic and remains in the body longer, up to 4 days, by which time it can be detected in urine. For this reason, benzoylecgonine is frequently used as a marker to check people's history of cocaine use and to estimate the amount of cocaine in water and wastewater [12]. The metabolites of flunitrazepam, N-Desmethylflunitrazepam (Flunitrazepam-M), and 7-Aminoflunitrazepam were mainly detected at Site C. Amitriptyline is used to treat depression by interfering with the reuptake of norepinephrine and serotonin in the central nervous system. The principal mechanism through which amitriptyline is metabolized in humans is by liver demethylation, which produces the physiologically active metabolite nortriptyline and (Z)-10-Hydroxyamitriptyline it was detected mainly in Site B [13].

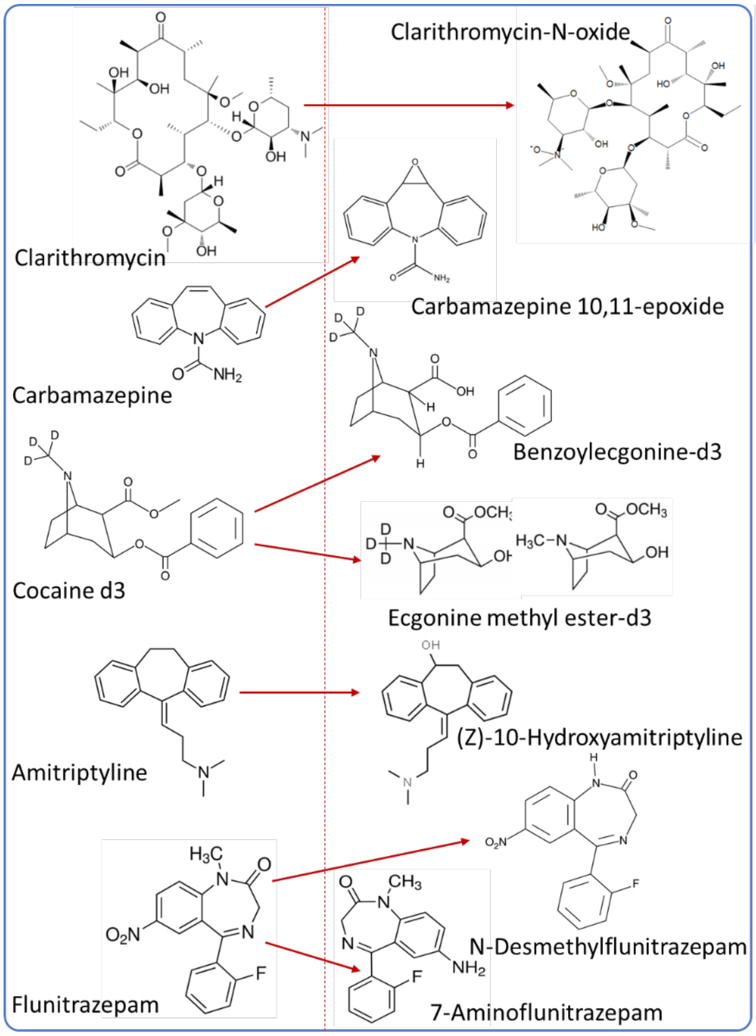

Fig. 5: Identified compounds and their transformed metabolites detected in the lake samples.

4. Conclusion

The non-target screening analysis of the organic micropollutants in Durgam Cheruvu Lake revealed the presence of several compounds, predominantly pharmaceuticals, including narcotic drugs, painkillers, anti-psychotic, anti-depressant, and anti-obesity medication. The presence of urinary metabolites is a sign that the discharge of domestic wastewater discharge into the lakes. The high abundance of the herbicide butachlor and pharmaceutical compounds suggests that nearby companies may be discharging their waste into the sewage system that flows into the lake. The presence of cyanotoxins indicates that the lake has an excessive nutrient concentration. The study's findings offer important guidance for programmes aiming to lower micropollutant emissions to aquatic habitats, such as through enhancing wastewater treatment technologies and emission controls. However, a continuous monitoring effort must be made because a lot of new pollutants are constantly entering the market. Furthermore, it is important to consider that aquatic animals encounter mixtures of compounds rather than simply single ones and that this may result in a greater potential for toxicity.

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