IMPACT OF MICROPOLLUTANTS ON THE ENVIRONMENT AND DETECTION BY BIOMARKER-BASED APPROACH

Abstract

Pollutants in many forms have an influence on the environment, particularly micro pollutants generated by industry and human consumables, which have a crucial impact on the ecosystem's water and soil. Micro pollutants are a wide range of categories involving organic and inorganic pollutants causing harm to the environment even at very low concentrations. The most significant barrier that stands in the way of accurately determining the degree to which the water is safe for human consumption is the challenge posed by the difficulty of identifying micro pollutants. Recent advances in detecting micro pollutants have been made thanks to multiple distinctive methodologies, including biomarkers for a specific target in the detection of micro pollutants in an ecosystem. Biomarkers are the essential agents used to identify the presence of causative substances in a sample, and the amount of impact that it is capable of causing may be investigated in more detail. Both the degree of infection that was produced and the quantity of chemical that were entrapped may be determined. In this study, a comprehensive analysis of the various biomarkers used to detect micro pollutant populations in the environment has been carried out. Additionally, the process that is followed to detect these populations, as well as the results of the research has been investigated in great detail.

Keywords: Micro pollutants, Wastewater treatment plants, Pollution, Biomarkers, Detection, Ecosystem

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I. INTRODUCTION

The ever-increasing world population is accelerating the production of a wide range of chemical agents, leading to their unsafe disposal in the environment and posing a severe threat to all living beings. Micro pollutants, which comprise organic and inorganic substances, are particularly concerning as they can cause environmental harm even at low concentrations. While various treatments are available to eliminate hazardous pollutants, micro pollutants prove challenging to remove through these processes. These harmful substances take diverse forms, such as effluents from pharmaceutical industries, dyes from factories, and various chemical agents, with water being a major reservoir for these contaminants.

Even at minimal concentrations, micro pollutants can damage the ecosystem, affecting aquatic organisms and humans [1]. The toxicity of these micro pollutants is extensive and includes adverse effects such as male infertility [2], endocrine disruption, carcinogenicity, antibiotic-resistant bacteria [3], teratogenic, and mutagenic impacts [4]. Researchers worldwide are actively exploring advanced technologies to treat and eliminate micro pollutants from various surfaces. However, the initial stage of identifying these contaminants poses a significant obstacle. Detecting low-concentration levels of micro pollutants proves to be a challenging task when evaluating treatment procedures. Consequently, the precise and efficient detection of micropollutants has become a primary area of emphasis for numerous researchers.

Micro pollutants come in various types, including pharmaceutical and personal care products (PPCPs), Biocides, and Perfluoroalkyl and poly-fluoroalkyl substances (PFAs), originating from diverse sectors. PPCPs play a prominent role in sewage water and pose challenges for wastewater treatment processes. Notable micropollutants produced from PPCPs include Tramadol, Lofepramine, Fluoxetine, Mebeverine, and Diclofenac [6]. Different analytical protocols and sensitive detectors are utilized to identify food and water micro pollutants. Chromatographic techniques such as HPLC, HPTLC, and GC are combined with spectroscopic detection methods like NMR, Mass spectrometry, and PCR. Sensing devices detect microbes and their metabolites, especially in low molecular weight micro pollutants. Molecular detection techniques involve using quantum dots, nanoparticles with modified proteins and DNA, and nanodiamonds [8]. Additional methods utilized for micro pollutant detection in wastewater include fluorescence quenching studies, Ultraviolet and visible spectrophotometer methods, fluorescent probes, and flame ionization detection. These approaches collectively contribute to precisely and comprehensively detecting micro pollutants in various environmental samples. There are different kinds of biomarkers used for a wide range of studies, such as diagnostic biomarkers, Predictive biomarkers, and monitoring biomarkers, as shown in Figure 1.
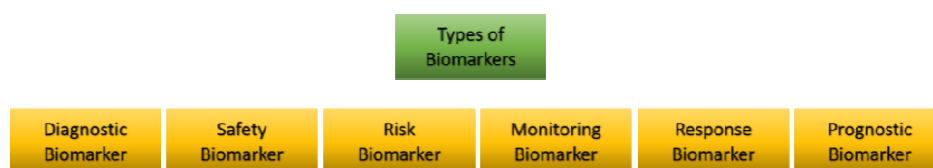

Figure 1: Various types of biomarkers are based on their detection and assessment methods

Diagnostic markers serve the purpose of confirming the presence of a disease or medical condition. On the other hand, safety biomarkers are employed to predict the toxicity of drugs and their related adverse effects and analyze agents responsible for environmental pollution. Risk biomarkers aid in assessing an individual's susceptibility to developing certain diseases or medical conditions. Monitoring biomarkers is crucial in understanding the disease's progression, spread, and response to treatment. Response biomarkers, part of pharmacodynamic studies, detect the specific responses from drug consumption or clinical interventions. Prognostic biomarkers are instrumental in screening for disease recurrence and predicting the progression of a diagnosed patient's medical condition. All these diverse types of biomarkers are valuable in detecting various micro pollutants in an extensive environment.

Detecting biomarkers in sewer waste poses a considerable challenge due to the varying spatiotemporal conditions inside the sewer system. While some studies have shown minor results concerning temperature changes caused by suspended solids in the sewer, a more significant impact is observed when biomarkers are transformed by changes in the pH of the sewer environment [9].

II. METALS AND CHEMICALS AS BIOMARKERS

A human trial comparing groundwater-consuming and tap water-consuming populations revealed higher levels of heavy metals like arsenic and lead in groundwater than in tap water. The hazardous levels exceeded the normal range of 0.01 to 16.34, with an average acceptable level of 1.20 ± 2.50. Biomarkers, such as hair, fingernails, and urine, were used to assess heavy metal exposure, with urine being suggested as a biomarker for daily analysis and fingernails for long-term evaluation [10]. The marine ecosystem is affected by hazardous chemicals like polycyclic aromatic hydrocarbons (PAHs), non-degrading chemicals, and heavy metals such as polychlorobiphenyl. Biomarkers indicating responses to glutathione S-transferase and catalase activity in native mussels can be used to detect their toxic levels. For instance, these biomarkers' activity helped detect heavy metals like zinc, lead, and cadmium in *Mytilus galloprovincialis* from the coastal region [11].

III. PHARMACEUTICAL MICROPOLLUTANTS AND THEIR BIOMARKERS

The study involved a control group that examined the genetic expression patterns of pharmaceutical products in zebrafish. Two specific pharmaceutical micropollutants, norfluoxetine and venlafaxine, were introduced as pollutants in separate and mixed conditions. The gene expression patterns were analyzed on 32 genes to assess the effects of these micro pollutants on gene regulation. These expression patterns were then utilized as a method to detect pharmaceutical micro pollutants [17]. In farming practices, pesticides like organophosphates and carbamates are commonly used to control insects on plants. However, these pesticides pose severe toxicity to the environment. Zebrafish were exposed to water containing pesticide micro pollutants, and the study focused on their effects on acetylcholinesterase signals in the model organisms. The inhibition of acetylcholinesterase was considered a biomarker for the presence of pesticides, as it led to behavioural and developmental impairments in the zebrafish [19]. For detecting xenobiotic contaminants in in-vivo fish models, ethoxy resorufin-O-demethylase was used as an efficient biomarker to assess CYP1A catalytic activity in the fish. Additionally, antioxidant enzymes such as

glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase were studied as defence mechanisms against generating oxyradicals [20].

IV. MICROPOLLUTANTS DETECTION USING REPORTER GENE ASSAY

To detect micro pollutants in highly polluted aquatic samples, researchers can perform the CALUX in vitro bioassay. This assay allows them to study various physiological processes of water extracts, including immune activity, metabolism, response to oxidative stress, and cytotoxicity. The in vitro reporter gene assay has proven to be a valuable tool in understanding the toxicity levels of the environment and the hazards caused by the accumulation of micro pollutants [21].'

In the Australian reverie environment, a hazard rating system was proposed for marine endemic systems based on the effects observed in the reporter gene assay in vitro. This system categorizes micro pollutant levels as low (1ng/L E2 eq.), medium (1-10ng/L E2 eq.), or high (10ng/L E2 eq.). However, it is important to note that these values are determined based on laboratory exposure alone and do not account for other external factors. To conduct the study, water samples were collected during pre and post-monsoon periods, considering the rainfall received from June to September. Environmental conditions such as temperature, pH, turbidity, and dissolved oxygen (DO) levels are noted during sample collection. Solid-phase extraction (SPE) is used to extract the samples. CALUX cells are then seeded in 96 well plates, and the water sample extracts treated with DMSO are added. The control solvent has a mean standard deviation of ±3 for LOD (Limits of Detection) and ±10 for LOQ (Limits of Quantification). The final quantification data analysis indicates that extracts from the Allahabad sites during the pre-monsoon period exhibited upstream estrogenic activity when treated with Biomarkers [22].

V. PLANAR ENVIRONMENTAL CONTAMINANTS BIOMARKERS

PCBs, highly carcinogenic chemicals commonly found in coastal debris, can harm aquatic organisms. To examine the environmental impact of these compounds on Dab fish (*Limandalimanda*) in the coastal region of the Dutch, scientists utilized the gene Cytochrome p450 1A (CYP1A) as a biomarker. A total of 25 Dab fish samples were collected from four sites in the North Sea, including one coastal area. The protein induction level of CYP1A was measured using an enzyme-linked assay (ELISA), and the activity of another enzyme, EROD (7-ethoxyresorufin O-deethylase), was also analyzed. The data obtained from the CYP1A and EROD assays performed on the Dab fish revealed that the level of PCB was higher in the coastal area compared to the other three sites. However, the values showed considerable variations due to background levels. In summary, the CYP1A biomarker proved useful in distinguishing the coastal population of Dab fish from other groups in the sea. These biomarkers indicate environmental conditions that may influence fish migration during spawning [23].

VI. BIOMARKERS FOR IDENTIFYING SEWER CROSS CONTAINMENT

Fecal contamination in storm sewers can be accessed through various biomarkers, including the 16S rRNA Bacteroide Human-specific biomarker, Mitochondrial DNA genetic markers, and chemical markers. The investigation involves studying wastewater

micropollutants (WWMPs) like ACE, CAF, THEO, and CBZ about these biomarkers, using their threshold reference values as a basis. The concentration values of these micropollutants in cross-contaminated samples were also examined. The process begins with isolating Bactericidal DNA and then amplifying it using human-specific HF183 marker and human-specific mitochondrial marker (HMT) primers through PCR. Different combinations of studies were conducted while keeping the WWMP common in nearly all the samples. Notably, the concentration of E. coli was found to be particularly sensitive to the HF183 human-specific marker and CAF, falling below or above the threshold of approximately 235 CFU or MPN 100mL-1 [24].

VII. EFFECTIVENESS OF BIOMARKERS IN MODEL ORGANISMS

1. **Gammarus Fossarum :** *Gammarus fossarum*, a freshwater amphipod, was used as a test organism to explore the relationship between pollutants and biomarkers. Two groups of amphipods were studied: one group was exposed to conventionally treated wastewater, and the other was exposed to river water. After 22 days, the survival rates were analyzed using a two-way ANOVA test for biomarker data. The amphipods exposed to wastewater exhibited significantly higher values than those in the river water samples. Among the five biomarkers studied, glycogen, lipid content, and phenoloxidase biomarkers showed the most significant p-values [25]. However, it should be noted that the biomarker response in amphipods depends on various factors, including the characteristics of the pollutant mixture, duration of exposure, pollutant concentrations, and the metabolic activity of the amphipods [26]. In a similar study, the sublethal effects of the disrupting agent Bisphenol A (BPA) were assessed in Gammarus pulex. The evaluation involved various biomarkers, including SOD and CAT activities. The study demonstrated that biomarkers like GSH and TBARS were significantly enhanced, indicating metabolic and biochemical responses in G. pulex to assess environmental BPA pollution [27].

2. **Caenorhabditis Elegans:** *C. elegans*, a model organism, was utilized as a biomarker to assess micropollutants in wastewater treatment analysis. The study examined larval growth and cytochrome P-450 expression in treated wastewater samples. Specifically, the expression of CYP-35A3 served as a biomarker for the presence of PAHs and polychlorobiphenyls [18].

3. **Zebra Mussels:** PAHs, originating from coal, crude oil, and gasoline production industries, tend to associate with sediments and cause significant contamination as they do not readily dissolve in water. Wastewater treatment plants (WWTPs) often assess pollutant levels using zebra mussels and bioindicators to evaluate water quality. Several genetic approaches have been employed on these organisms, including the comet assay, micronucleus test (MN test), condition index (CI), gonado-somatic index (GSI), diffusive gradients in a thin film (DGT), and analysis of digestive enzymes such as amylase and cellulase. Among these, the MN test and Comet assay have emerged as the most sensitive tools for monitoring geno toxicity in freshwater environments [28][29].

4. **Cyprinus Carpio:** Fish, the most abundant aquatic organisms, are highly sensitive to contaminants. Malachite green, a colorant used in the textile industry for dyeing clothes, is considered a micropollutant in water. In Cyprinus carpio, various biomarkers are employed to detect the presence of malachite green dye. Hemoglobin levels are evaluated

using cyanmethemoglobin, blood glucose is estimated with O-Toluidine, protein levels with Lowry's method, blood cholesterol with the Zak method, and total amino acids with Moore and Stein method. Lipid content in the muscle is determined using the Folch method. At the same time, antioxidant activity in the gills, liver, and kidney areanalyzed through catalase activity (Luck method) and lipid peroxide estimation (Ohkawa method). Glutathione s-transferase is estimated using the Habig method, glucocorticoid receptor activity with the David and Richard method, ascorbic acid using Roe and Kuether method, and glutathione using the Moron method [30]. In addition to malachite green, the study analyzed organochlorine pesticides and polychlorinated biphenyls in Cyprinus carpio tissues. Over four weeks, the exposure of fish to different aquatic sites in the region allowed the determination of micropollutant contamination in marine systems. Metals that were absorbed and distributed into fish tissues were observed. To assess contamination levels, acetylcholinesterase activity, condition factor, hepatosomatic index, and osmolality in the fish were suggested as useful biomarkers [15]. Furthermore, behavioural and genotoxic alterations were observed in common carp due to exposure to organophosphate pesticides such as dimethoate and chlorpyrifos. These alterations included delayed opercular movements, retarded swimming activity, and lowered swimming velocity, all of which can serve as biomarkers for detecting the presence of these pesticides [31].

5. **Anguilla Anguilla** *:* Researchers studied the presence of micropollutants in the aquatic system by examining fish's gut microbiome and analyzing microbiota changes. They proposed using the microbiota as a biomarker to determine the presence or absence of a normal microbial population in the gut region of *Anguilla anguilla* [16]. In European eels, an increase in Ethoxyresorufin O-deethylase (EROD) activity was observed, indicating the presence of PAHs like naphthalene and beta-naphthoflavone. Additionally, there was a rise in the level of liver cytochrome P450 content, further suggesting the impact of micropollutants on the eels [32].

6. **Seaweed:** Seaweed serves as both an accumulator and biomarker for detecting exposure to organic chlorine pesticides and PAHs. It plays a crucial role in absorbing and retaining organic micropollutants, contributing to the maintenance of the ecosystem. Ulva fasciata, a type of seaweed, was found to contain 30.38% efficient carcinogenic fractions [13]. In coastal regions, biomarkers such as glutathione s-transferase and catalase were utilized to identify the toxicity levels of PAHs, polychlorobiphenyls, and heavy metals like copper, zinc, lead, and cadmium [14]. These biomarkers help assess the impact of micropollutants on the coastal environment and its inhabitants, including seaweed.

7. **Bivalve Molluscs***: Bivalve mollusks* were subjected to environmental pollution caused by microplastics (1 mg L^{-1}, size 0.1–0.5 mm) and the pharmaceutical drug ibuprofen (0.8 µg L^{-1}). The study was conducted over a period of 14 days, both with separate exposures and in a mixed environment. The research revealed a significant increase in the citrate synthase level as a biomarker in response to the mixed micropollutants, indicating the potential use of biomarkers for detecting microplastic pollution [12]. Another group of researchers conducted a systematic review to establish basic recommendations for micropollutant detection, particularly focusing on *Bivalve mollusks*. The results suggested a minimum sample size of fifty in a specific area, using 10% KOH for preparing the digestion solution, and a filter membrane with a pore size less than 5 µm. Following these

parameters in long-scale biomarker studies could lead to the identification of novel components to combat pollution effectively [33]. Moreover, the enzyme caspase-3 has been found to be responsive to Ibuprofen's diverse functionality under various conditions and environments. Changes in caspase-3 activity could be employed as a detection method to identify the levels of microplastics and the presence of ibuprofen in test samples [12].

VIII. CONCLUSION

The presence of hazardous micro pollutants poses a significant threat to the environment and can also impact human health. The contamination of natural habitats raises concerns about the need to reclaim and restore unpolluted ecosystems. However, detecting these micro pollutants has proven to be a challenging task, given their low concentrations. The advanced methods and biomarkers discussed in this study offer promising solutions for accurate and efficient micro pollutant detection. By monitoring the concentration and types of micro pollutants using biomarkers; we can gain valuable insights into the extent of contamination in different environments. The recent developments in biomarker-based detection are being actively employed in many countries to address the issue of micro pollutants Overall; this enhanced understanding of biomarkers and their application in detecting micropollutants will foster further research and raise awareness about the importance of identifying and mitigating these contaminants in our daily lives. By reducing the risk of contamination and preserving a pollution-free natural ecosystem, we can work towards a healthier and sustainable environment.

Table 1: Biomarkers for specific model organisms to detect the micro pollutants

ORGANISMS	BIOMARKER	TEST	DETECTION
Gammarus fossarum	Bisphenol A	Superoxide dismutase, Catalase	Bisphenol A pollution
Caenorhabditis elegans	CYP-35A3	Larval growth expression, Cytochrome p450 expression	Polycyclic aromatic hydrocarbons, Polychlorinated biphenyls
Zebra mussels	polycyclic aromatic hydrocarbons sediments	Micronucleus test, Comet assay	Cold crude oil, Gasoline
Cyprinus carpio	Acetylcholinesterase, condition factor,hepatosomatic index and osmolality	malachite green dye	Organophosphate pesticides of dimethoate and chlorpyrifos

Anguilla anguilla	Microbiota	Ethoxy resorufin-O-demethylase activity	Polycyclic aromatic hydrocarbons (naphthalene and beta-naphthoflavone)
Seaweed	Seaweed	glutathione s-transferase and catalase	Organic chlorine pesticides and Polycyclic aromatic hydrocarbons
Bivalvia	Citrate synthase	Liposomal functionality test	Ibuprofen, Microplastics

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