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Environmental risk of per- and polyfluoroalkyl substances (PFAS) in freshwater ecosystems: Integrating predictive and empirical toxicity models

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A R T I C L E I N F O

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A B S T R A C T

Per- and polyfluoroalkyl substances (PFAS) have garnered global attention due to their toxic nature, tendency to accumulate in organisms, and resilience to natural degradation processes in the environment. In this study, the distribution and risk assessment of ten (10) PFAS were determined in clean (Kuantan River) and moderately polluted (Kim Kim River) river. The toxicity data of aquatic organisms were obtained from the predictive Ecological Structure Activity Relationships (ECOSAR) and empirical Ecotoxicology Database (ECOTOX) databases to calculate the risk assessment. The highest PFAS concentration in the Kuantan River was in perfluorobutanoic acid (PFBA), with a mean value of 1.99 ± 0.44 ng/L. Meanwhile, the highest PFAS detected in the Kim Kim River was perfluoropentanoic acid (PFPeA), with a mean value of 35.80 ± 24.65 ng/L. Risk quotient (RQ) approaches were applied to assess the potential risk of PFAS in both databases. The RQ showed negligible risk towards all detected PFAS (RQ < 0.01) in both rivers. The output of this study produces extensive information on pollution risks, thereby providing valuable guidance to policymakers concerning regulatory choices, management of exposure, and reduction of PFAS in aquatic environments.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) represent a broad category of synthetic chemicals distinguished by the partial or complete fluorination of their carbon chains, which creates functional groups. The PFAS includes more than 5000 identified compounds that are extensively utilized in diverse applications, including food packaging, paper goods, non-stick cookware, textiles, carpets, electronics, medical equipment, industrial surfactants, and firefighting foams (AFFF) (Pontius, 2019). The widespread adoption of PFAS since the 1940s is attributed to their unique properties, such as resistance to heat and acids, water and oil repellency, and high surface activity (Li et al., 2022). These chemicals are frequently detected in riverine, estuarine, and marine environments, often associated with human activities and hydrological processes (Sabri et al., 2025; Ivantsova et al., 2024a,b). Their presence in food chains, food webs, and water systems has led to their identification in food and water sources, raising concerns about chemical risks due to dietary exposure (Razak et al., 2024). The global dispersion of PFAS has resulted in their presence in the environment and living organisms, leading to adverse effects such as immunotoxicity, neurotoxicity, genotoxicity, reproductive and developmental toxicity, and endocrine disruption (Logeshwaran et al., 2021; Razak et al., 2023; Wee and Aris, 2023a).

In light of growing environmental concerns and increased regulatory oversight, various measures have been introduced to limit the production and utilization of per- and polyfluoroalkyl substances (PFAS), especially those containing seven or more perfluorinated carbons in perfluorocarboxylates (PFCA) and six or more in perfluoroalkane sulfonates (PFSA) (Marchiandi et al., 2021). Specific compounds, such as perfluorooctanoic acid (PFOA), perfluorooctanesulfonate (PFOS), and perfluorohexanesulfonate (PFHxS), have been included in the International Stockholm Convention on Persistent Organic Pollutants. This

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global agreement prohibits the industrial production of PFOS and its derivatives, while PFOA is subject to restrictions and gradual phase-out efforts, with certain exceptions (UNEP, 2019). Despite these measures, PFAS analogues including shorter-chain variants and telomer-based compounds continue to be produced and used commercially (Abunada et al., 2020).

Manufacturers are increasingly reducing the production of long-chain PFAS, particularly PFOA and PFOS, and transitioning toward short-chain alternatives. As a result, short-chain PFAS, such as perfluoro-n-butanoic acid (PFBA) and perfluorobutanesulfonic acid (PFBS), are becoming more prevalent in environmental samples. For instance, PFBA and PFBS were the most abundant PFAS detected in water samples from Tokyo Bay, representing 52 % of the total PFAS identified. Similarly, these compounds were dominant in the Shuangtaizi Estuary, with concentrations of 45 ng/L and 4.7 ng/L, respectively (Chen et al., 2017). Additionally, other PFAS derivatives, including perfluorohexanoic acid (PFHxA), perfluorooctanesulfonamide (PFOSA), and perfluorinated phosphonic acid (PFPAs), are commonly found in drinking water samples across multiple regions, such as Europe, China, Malaysia, Thailand, Singapore, Vietnam, Jordan, Ethiopia, the USA, and Brazil (Ahrens et al., 2016; Gebbink et al., 2017; Guardian et al., 2020; Liu et al., 2021; Shigei et al., 2020; Wee et al., 2019)

Conducting thorough risk assessments of PFAS is vital for defining chemical concentration thresholds that minimize or eliminate adverse effects on environmental safety and human health. The European Commission has developed a technical guidance document outlining risk assessment procedures to determine the Predicted No-Effect Concentration (PNEC) through an assessment factor approach (European Commission Joint Research Centre, 2003). Most PFAS risk evaluations rely on the Risk Quotient (RQ) method, which employs a single-factor index to indicate the extent of PFAS-related environmental risks (Razak et al., 2021). However, the RQ method can be limited in accuracy due to its dependence on species-specific dose-response toxicity data, which may not fully represent risks at the population level. To address this limitation, this study incorporated predictive toxicity data from ECOSAR and empirical toxicity data from ECOTOX, utilizing species sensitivity distribution (SSD) to improve the accuracy of environmental risk assessments. Both databases compile aquatic toxicity data across multiple trophic levels, enhancing the reliability of risk evaluations.

In Malaysia, the Department of Environment (DOE) oversees water quality monitoring to assess the condition and detect variations in river water quality. Water samples are collected from designated monitoring stations for both field and laboratory analyses to evaluate physico-chemical and biological properties. The Water Quality Index (WQI), aligned with Malaysia's National Water Quality Standard (NWQS), is used to determine pollution levels and the suitability of water for various uses. The WQI incorporates parameters such as dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia nitrogen (NH_3N), suspended solids (SS), and pH. Based on the classification by Malaysia's Department of Environment (DOE), river water quality is categorized into three conditions: clean, moderately polluted, and polluted, which correspond to Class I-II, Class III, and Class IV-V respectively (Department of Environment, 2023). For this study, two rivers with differing pollution levels were selected as case studies: Kuantan River (Class II, clean) and Kim Kim River (Class III, moderately polluted) (Department of Environment, 2023). The research focused on the 10 most prevalent PFAS compounds, which are widely regarded as reliable markers of PFAS contamination due to their frequent detection in environmental samples, substantial contribution to exposure risks, and inclusion in regulatory frameworks such as those established by the United States Environmental Protection Agency (USEPA) and the European Union (EU) (UNEP, 2019; US EPA, 2024). The objectives of this study are threefold: (i) to investigate the presence and distribution of PFAS in the surface water of Kuantan River and Kim Kim River, (ii) to explore the relationships between physicochemical parameters and PFAS distribution, and (iii) to evaluate the ecological risks posed by PFAS in these rivers using toxicity data from the ECOSAR and ECOTOX databases. Assessing PFAS risks in these rivers is critical for developing effective mitigation strategies, supporting informed decision-making, and guiding policy development.

2. Materials and methods

2.1. Chemicals and reagents

The analytical standards PFAC-MXB and MPFAC-MXA, sourced from Wellington Laboratories (Ontario, Canada) with purity levels exceeding 98 %. PFAC-MXA comprises a composite standard solution of ten PFAS, including perfluoro-n-butanoic acid (PFBA), perfluorooctane sulfonate (PFOS), perfluoro-n-nonanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoro-n-hexanoic acid (PFHxA), perfluorohexanesulfonic acid (PFHxS), perfluoro-n-octanoic acid (PFOA), perfluoropentanoic acid (PFPeA), perfluoroheptanoic acid (PFHpA), and perfluorobutanesulfonic acid (PFBS). The MPFAC-MXA mixture, serving as an internal standard, contains isotopically labeled analogs of the same PFAS compounds. LC-MS grade methanol and ultrapure water were procured from Fisher Scientific (Massachusetts, USA), while analytical-grade ammonium acetate was supplied by Sigma-Aldrich (Missouri, USA). For sample preparation, weak anion exchange solid-phase extraction (SPE) cartridges (Oasis®Wax, 150 mg, 6 mL, 30 μm pore size) were acquired from Waters (Milford, Ireland).

2.2. Study area

The Kuantan River is located in the northeastern area of Pahang, adjacent to the Terengganu-Pahang border. It is considered one of the future growth centres and trade, business, transportation and tourism hubs on the east coast of Peninsular Malaysia due to its strategic location. Land use changed dramatically, with major allocations for agriculture (40 %), transportation (2.25 %), housing (1.93 %), institutions and community facilities (1.32 %), industry (1.29 %), and other uses. Several tributaries receive and convey releases from agriculture, domestic, urban, and industrial activities into the main river before discharging into the South China Sea. The sampling points on the Kuantan River are shown in Fig. 1.

Kim Kim River is located in Pasir Gudang, Johor, and flows into Masai, Johor. Pasir Gudang, Johor is known as one of the largest and earliest ports in Malaysia. The Pasir Gudang area is defined as a natural area for port construction associated with the development of industrial areas and new urbanisation through existing infrastructure surrounded by the sea and abundant land for land reclamation. The Pasir Gudang City Council (MBPG) reported 801 mixed industrial factories (light, medium, and heavy industries) in urban areas. Industrial land use contributed the most (38 %), followed by housing (31 %), open and recreational areas (14 %), institutions and community facilities (10 %) and businesses (6 %) (MBPG, 2019). The presence of 2005 factories around densely populated residential areas in Pasir Gudang is a concern following an industrial disaster involving the accidental release of toxic and flammable chemicals into nearby residential areas (Academy of Sciences Malaysia, 2019). The selected sampling points along the Kim Kim River are shown in Fig. 2. The descriptions of each sampling point in the Kuantan River and Kim Kim River are presented in Tables S1 and S2, respectively.

2.3. Surface water sampling and analysis

Triplicate water specimens were gathered from designated sampling locations in May 2023. Collection was conducted at a depth of roughly 10 cm within the river channel, by employing polypropylene (PP) bottle (1 L). Immediately after collection, sample bottle was maintained in icebox at temperatures below 4 °C to ensure preservation (US EPA, 2019). Filtration was subsequently performed using 0.45 μm glass

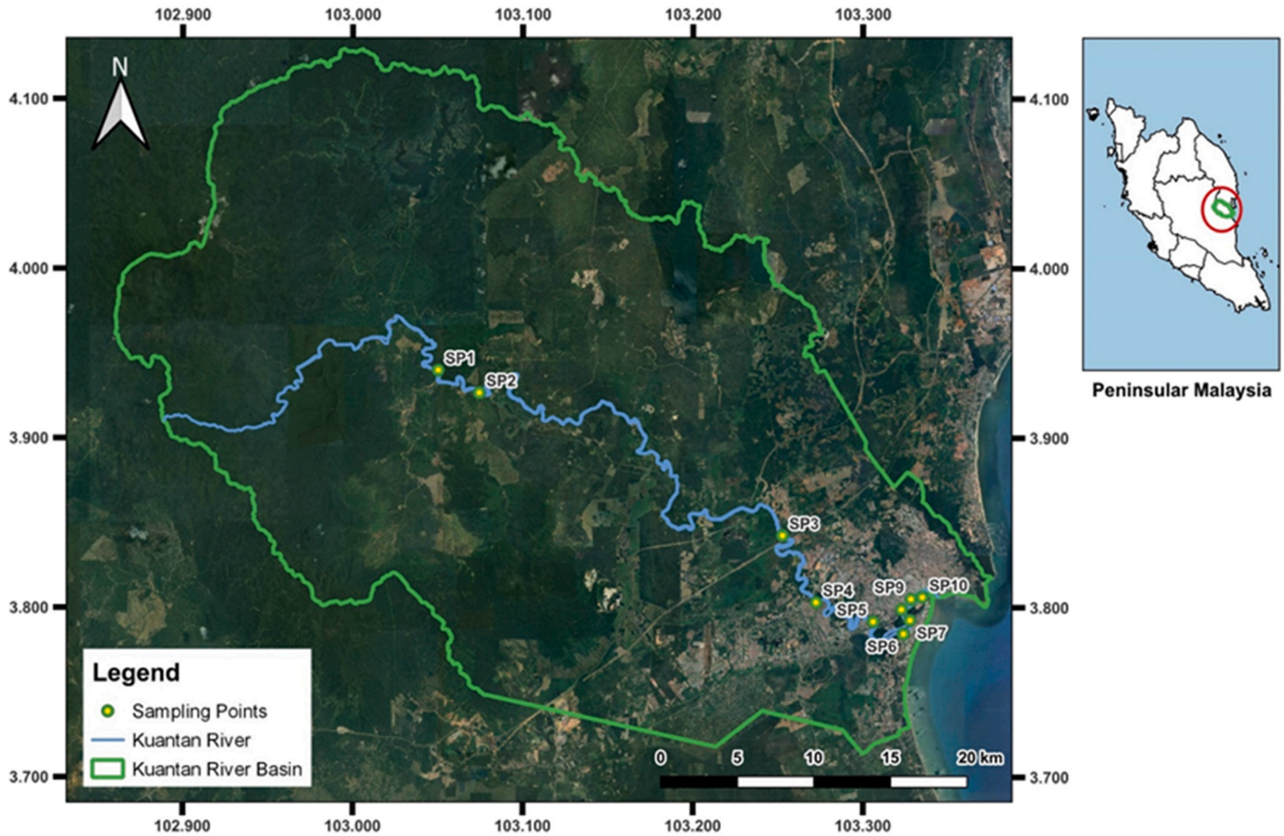

Fig. 1. The selected sampling points along the Kuantan River.

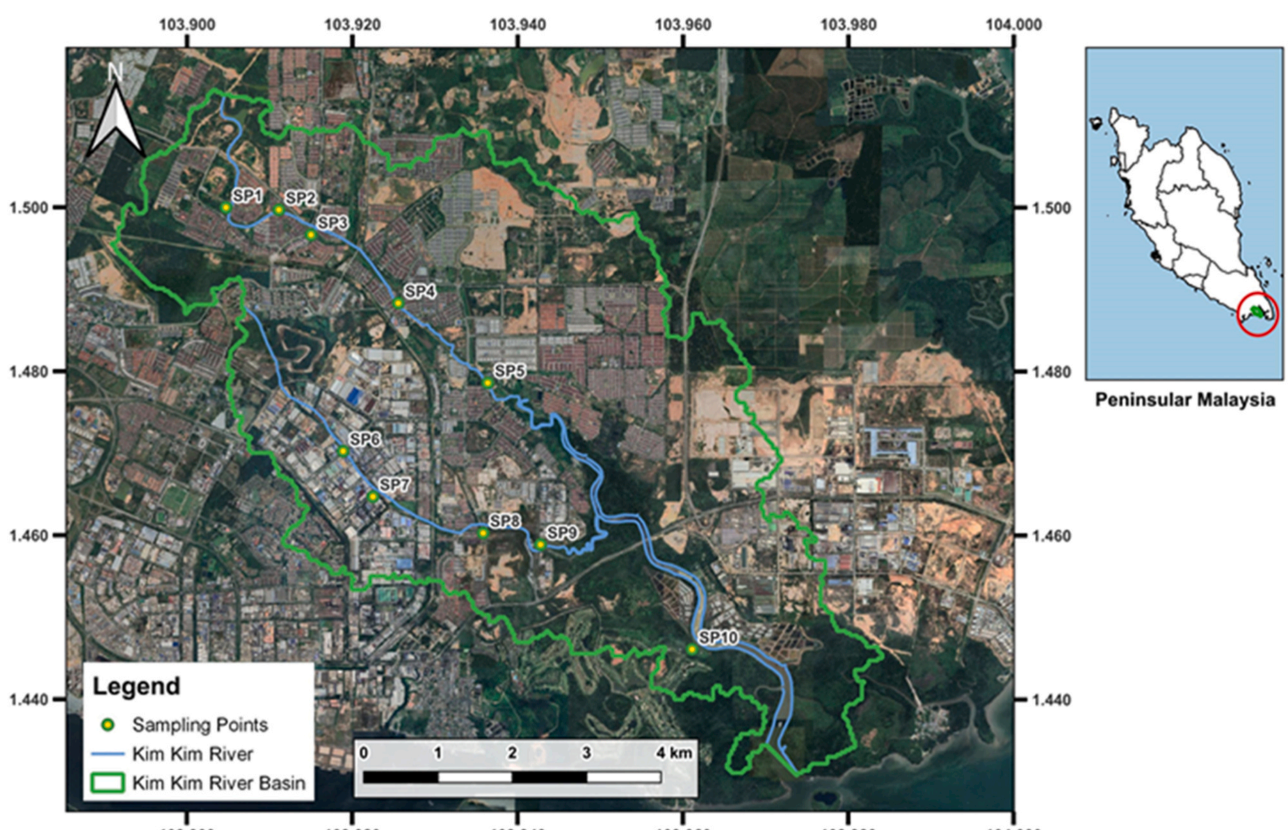

Fig. 2. The selected sampling points along the Kim Kim River.

microfiber filters (47 mm diameter, Whatman, UK), followed by the addition of ascorbic acid (100 mg per 1 L sample) to inhibit degradation. All protocols for sampling, preservation, and handling adhered to the guidelines outlined in the Standard Methods for the Examination of Water and Wastewater (APHA, 2017). Concurrently, in-situ physicochemical parameters (pH, temperature, conductivity, salinity, dissolved oxygen, total dissolved solids, and oxidation-reduction potential) were recorded using a YSI Pro Plus multiparameter instrument (Ohio, USA).

Solid-phase extraction (SPE) was employed for analyte extraction, with Oasis WAX Plus Short cartridges serving as the stationary phase. Prior to extraction, cartridges were pre-conditioned sequentially with 5 mL methanol, 5 mL ultrapure water, and 5 mL methanol containing 0.1 % ammonia. Pretreated samples were passed through the cartridges at a controlled flow rate of one drop per minute. Cartridges were rinsed with 5 mL ultrapure water, vacuum-dried for 15 min, and eluted using a mixture of 5 mL methanol and 5 mL 0.1 % ammoniated methanol. The

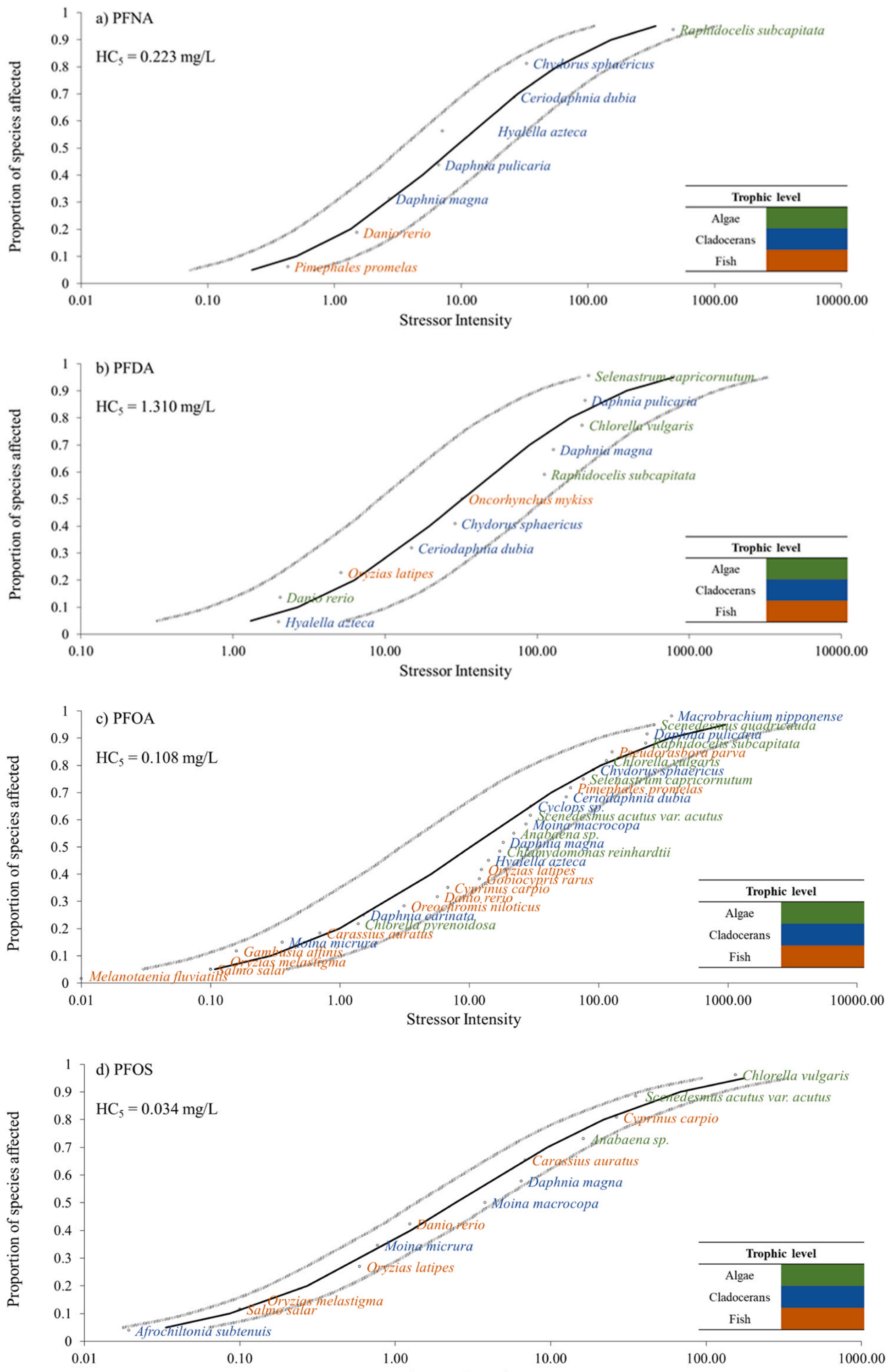

Fig. 3. Species Sensitivity Distribution (SSD) curves generated using the USEPA SSD Generator for (a) PFNA, (b) PFDA, (c) PFOA, (d) PFOS, (e) PFHpA, (f) PFHxA, (g) PFPeA, and (h) PFBA. SSD curves could not be generated for PFHxS and PFBS because toxicity data were unavailable for algae and cladocerans, respectively.

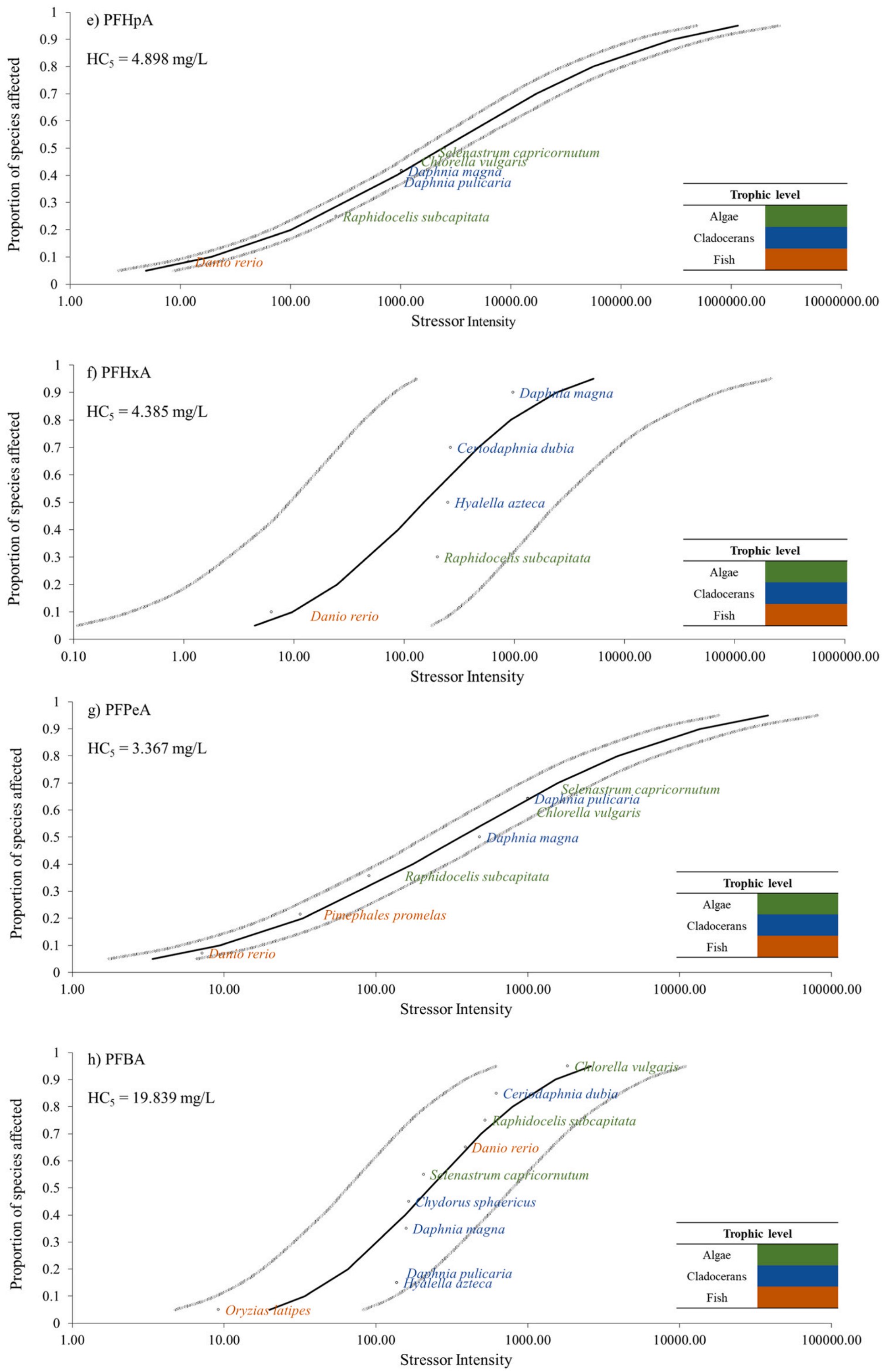

Fig. 3. (*continued*).

eluate was concentrated via centrifugal evaporation and reconstituted in 250 µL methanol prior to instrumental analysis. Quantification of target PFAS was performed using a Shimadzu LC-MS-8030 system (Kyoto, Japan) equipped with electrospray ionization (ESI) in negative mode. Separation was achieved using a Gemini C6-Phenyl column (150 × 2.00 mm, 5 µm; Phenomenex, USA) with a mobile phase comprising 10 mM ammonium acetate in ultrapure water (A) and 100 % methanol (B). Chromatographic conditions included a flow rate of 0.35 mL/min and a 10-minute runtime. Multiple reaction monitoring (MRM) transitions specific to each analyte are detailed in Supplementary Table S3.

2.4. Risk quotient (RQ)

The RQ methodology was employed to evaluate the ecological risks associated with PFAS compounds, including PFBA, PFOS, PFNA, PFDA, PFHxA, PFHxS, PFOA, PFPeA, PFHpA, and PFBS. As outlined in Eqs. (1)–(3), the RQ values were derived to categorize risks into four tiers:

negligible ($RQ < 0.01$), low ($0.01 \leq RQ < 0.1$), medium ($0.1 \leq RQ < 1$), and high ($RQ \geq 1$) (Razak et al., 2021).

$$\text{Risk quotient, } RQ = \frac{MEC}{PNEC} \quad (1)$$

$$\text{Predicted no effect concentration, } PNEC_{ECOSAR} = \frac{ChV}{AF} \quad (2)$$

$$\text{Predicted no effect concentration, } PNEC_{ECOTOX} = \frac{HC_5}{AF} \quad (3)$$

In these equations, the mean environmental concentration (MEC) of PFAS was compared against the predicted no-effect concentration (PNEC), which reflects thresholds for adverse ecological impacts. Toxicity data for PFAS were specifically curated from freshwater organisms across three trophic levels (algae, crustaceans, and fish) within the ECOSAR and ECOTOX databases to ensure methodological consistency. For $PNEC_{ECOSAR}$, the lowest chronic toxicity value (ChV) identified among the trophic groups was divided by an assessment factor (AF) of 10. This conservative AF was selected to account for uncertainties inherent in chronic toxicity predictions (Sadutto et al., 2021). In contrast, $PNEC_{ECOTOX}$ was computed using empirical toxicity endpoints (EC_{50}, LC_{50}, NOEC) extracted from the ECOTOX database (US EPA, 2020a), with detailed values provided in Supplementary Table S4. The species sensitivity distribution (SSD) curves were constructed via the USEPA SSD Generator (US EPA, 2020b), incorporating toxicity data from ECOTOX to represent multiple trophic levels (Fig. 3). From these curves, the hazardous concentration for 5 % of species (HC_5) was extrapolated. $PNEC_{ECOTOX}$ was then determined by applying an AF of 5 to the HC_5 value, a method justified by the incorporation of multispecies empirical data and recent methodological validations (Razak et al., 2023; Zainuddin et al., 2023).

2.5. Quality assurance and quality control

To ensure data integrity, quality assurance and control protocols were applied to verify the accuracy, consistency, and reliability of the results. Prior to use, laboratory apparatus and glassware underwent thorough cleaning by immersion in Decon 90, a non-ionic detergent, followed by sequential rinsing with tap water, deionised water, and methanol. Subsequent drying was carried out in an oven at 65 °C for 24 h. Procedural blanks were systematically evaluated during method optimization, validation, and real-sample analysis to identify and mitigate potential background contamination from laboratory sources. Each analytical method was validated to confirm its suitability for PFAS quantification, with parameters such as linearity, precision, recovery, limit of detection (LOD), and limit of quantification (LOQ) being rigorously assessed.

Calibration curves were constructed using six concentration levels (5–500 ng/L) of the target compounds to evaluate linearity. Method precision was determined through five replicate analyses ($n = 5$) at 100 ng/L, with relative standard deviation (RSD) serving as the metric. Recovery efficiency was examined by spiking river water samples from a control site (SP1, Kuantan River) at 100 ng/L. Iterative adjustments were made to achieve recoveries within 80–120 % for most analytes. LOD and LOQ values were derived from signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively, using spiked samples at 100 ng/L. Matrix effects were minimized by incorporating isotopically labelled internal standards, which mirrored the ionization characteristics of the analytes (Cabello et al., 2016). All samples for method development, optimisation, and validation were analysed three times, and the average concentration was calculated as the final analysis result.

2.6. Statistical analysis

The distribution of per- and polyfluoroalkyl substances (PFAS) in river water was statistically evaluated through IBM SPSS software (version 22.0). Descriptive statistical methods were applied to assess parameters including mean values, variability (standard deviation and coefficient of variation), detection ranges, and proportional distributions of PFAS. The detection percentage for individual compounds was derived by calculating the ratio of samples with measurable concentrations to the total number of samples analyzed (Wee et al., 2020). Similarly, the distribution percentage was computed by dividing the concentration of a specific PFAS compound by the cumulative concentration of all detected compounds. To investigate associations between PFAS levels and physicochemical characteristics of river water, a bivariate correlation analysis was performed, with statistical significance rigorously evaluated during this process. All analytical procedures were structured to ensure methodological consistency and alignment with established computational frameworks.

3. Results and discussion

3.1. Occurrences and distribution of PFAS in Kim Kim and Kuantan River

This study investigated the occurrence of 10 per- and polyfluoroalkyl substances (PFAS) in surface water samples from the Kuantan and Kim Kim Rivers, comprising PFBA, PFOS, PFNA, PFDA, PFHxA, PFHxS, PFOA, PFPeA, PFHpA, and PFBS. Spatial distributions of PFAS across sampling locations are detailed in Supplementary Data (Table S5). In the Kuantan River, mean PFAS concentrations (ng/L) followed a descending order: PFBA (1.99; range: <LOD–2.53) > PFOA (1.67; range: <LOD–2.06). PFOS, PFNA, PFDA, PFHxA, PFHxS, PFPeA, PFHpA, and PFBS remained undetected at all sites (Fig. 4). Contamination variability, assessed via coefficients of variation (CV: 19.69–22.26 %) suggested moderate heterogeneity in PFAS levels along the Kuantan River (Table S6). Conversely, the Kim Kim River exhibited higher PFAS concentrations, with PFPeA (35.80; range: <LOD–63.87) as the dominant compound, followed by PFOS (10.11), PFBA (9.39), PFOA (5.54), PFHxA (3.73), PFHxS (3.52), and PFHpA (1.84). Meanwhile, PFNA, PFDA, and PFBS were not detected. Elevated CV values (27.52–92.38 %) indicated pronounced spatial variability in PFAS contamination within this river.

Anthropogenic activities, such as industrial discharges, domestic waste inputs, land-use changes, and soil erosion in both river basins, are likely contributors to PFAS contamination through point and nonpoint sources (Wee et al., 2023a; Wee et al., 2023b). Additionally, inadequate wastewater treatment infrastructure may exacerbate PFAS persistence in aquatic systems. Conventional water treatment facilities lack specialized processes to effectively eliminate PFAS, leading to their gradual accumulation in surface waters. These factors collectively underscore the challenges in mitigating PFAS pollution in rapidly developing regions.

The present research highlights the possible impact of physicochemical properties of water bodies on the dispersion of PFAS in tropical river water. The physicochemical characteristics and their correlation coefficients for sampling sites in the Kuantan and Kim Kim Rivers are detailed in Tables S7–S9. In the Kuantan River, oxidation-reduction potential (ORP) demonstrated a notable positive association ($p < 0.05$) with PFOA. Elevated ORP values promote oxidative conditions, stabilizing minerals like Fe(III) and Mn(IV) oxides that serve as PFAS adsorption surfaces. Under reducing conditions, metal oxides such as Fe(III) and Mn(IV) that serve as key adsorption surfaces for PFAS can be reductively dissolved to Fe(II) and Mn(II), respectively, leading to the release of previously sorbed PFAS into the aqueous phase (Wang et al., 2022; Smith et al., 2021; Huang and Zhang, 2020). In the Kim Kim River, temperature exhibited a strong positive correlation ($p < 0.01$) with PFOS, likely due to enhanced PFAS solubility at higher temperatures (Abunada et al., 2020). Conductivity, total dissolved solids (TDS), and salinity displayed significant positive relationships ($p < 0.05$) with shorter-chain PFAS (PFBA, PFPeA) and PFOS. These parameters

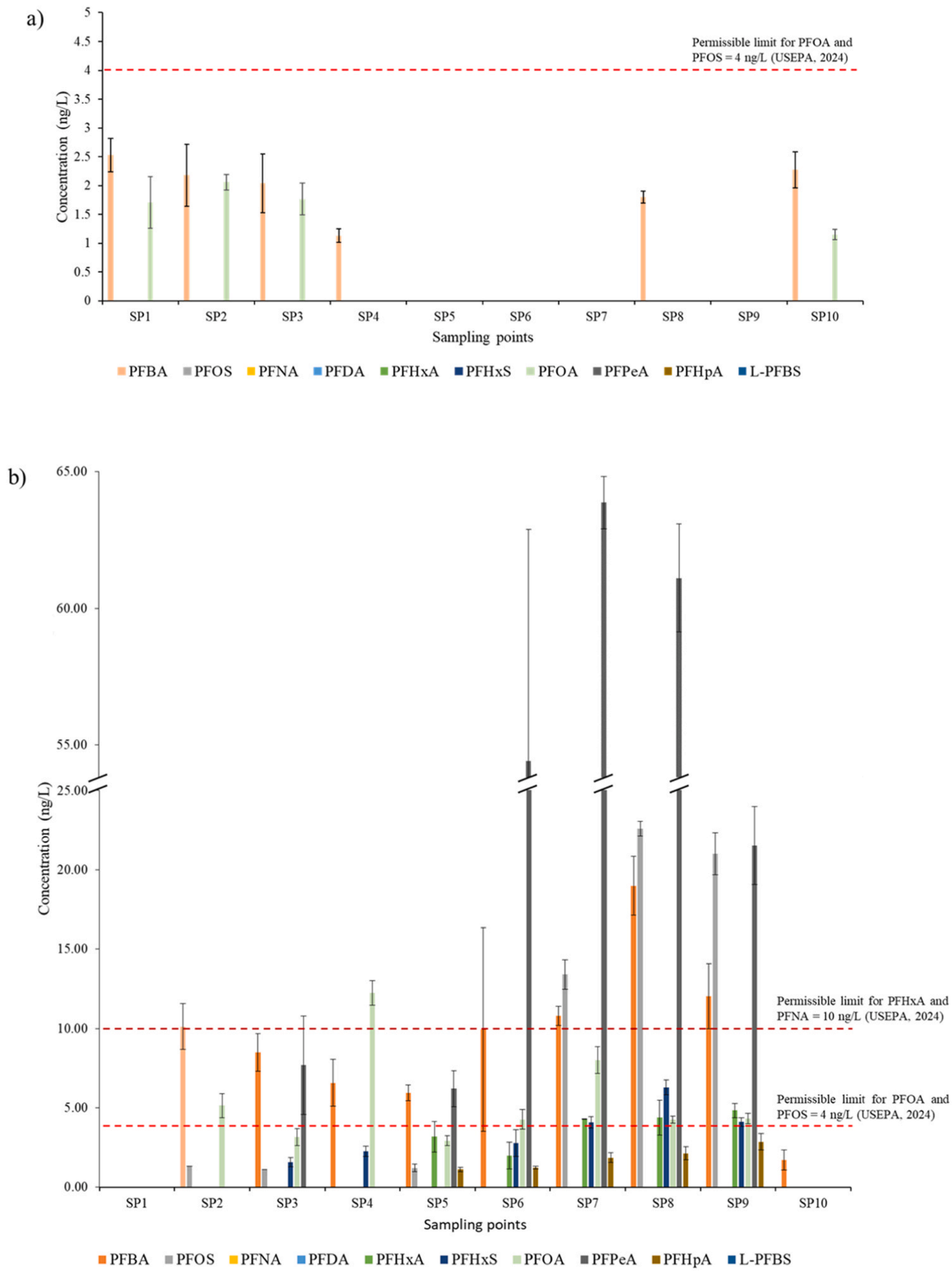

Fig. 4. Distribution of PFAS in surface water of the a) Kuantan River and b) Kim Kim River.

collectively increase ionic strength, promoting PFAS partitioning to sediments and organic particulates, thereby reducing aqueous concentrations. Hydrophilic PFAS variants (e.g., PFBA, PFBS, PFHxA, PFHxS) diverged from this pattern, as their polar functional groups may enhance solubility in high-ionic environments (Wang et al., 2022). Dissolved oxygen (DO) showed inverse correlations with PFOS ($p < 0.01$) and PFHxS ($p < 0.05$). While PFAS stability persists across redox states, DO fluctuations may indirectly modulate PFAS mobility by altering co-pollutant degradation pathways or mineral binding site accessibility (Abunada et al., 2020). These findings underscore the role of water chemistry in governing PFAS transport and retention in tropical aquatic ecosystems.

The levels of PFAS identified in this study were evaluated against data from prior research on regional and global rivers, as well as the permissible limits set by the USEPA, as outlined in Table 1. Among the 5000 PFAS-related compounds, PFOA and PFOS have been identified as the most prevalent in groundwater and surface water (Razak et al., 2023; Wee and Aris, 2023b). Nonetheless, other PFAS variants have also been detected in river ecosystems across the globe. For example, Sharma et al. (2016) noted that PFBS and PFHxA were the most abundant PFAS in the Ganges River (India), with mean concentrations of 5.55 ng/L and 2.32 ng/L, respectively. Similarly, Si et al. (2021) found that PFHpA was the dominant PFAS in the Liuxi River (China), with a mean concentration of 647.00 ng/L, significantly higher than PFOA, which had a mean concentration of 27.60 ng/L. PFAS derivatives have also been identified in various rivers, including the Tagus River (Spain), Shaying River (China), Vindeln River (Sweden), Las Vegas Wash (USA), Truckee River (USA), Pra River (Ghana), Ka Kum River (Ghana), Youngsan River (South Korea), Nakdong River (South Korea), Chao Phraya River (Thailand), and Laguna Lake (Philippines) (Navarro et al., 2020; Nguyen et al., 2017; Bai and Son, 2021; Essumang et al., 2017; Si et al., 2021; Zhang et al., 2023; Sharma et al., 2016; Hong et al., 2013; Lam et al.,

Table 1
Comparison of PFAS in average and range (min-max) in surface water data reported from regional and worldwide rivers and the permissible limit of PFAS based on USEPA.

Location	Mean and range concentration (ng/L)										References
	PFBA	PFOS	PFNA	PFDA	PFHxA	PFHxS	PFOA	PFPeA	PFHpA	PFBS	
Tagus River, Spain	3.00 (<0.02–5.30)	5.90 (<0.01–34.00)	0.70 (<0.02–2.10)	0.80 (<0.01–2.60)	2.00 (<0.02–13.00)	4.40 (<0.03–12.00)	3.60 (<0.01–11.00)	1.80 (<0.10–4.70)	1.30 (<0.05–2.90)	1.70 (<0.06–5.30)	(Navarro et al., 2020)
Vindeln River, Ume River and Göta River, Sweden	2.10 (0.47–3.70)	3.50 (0.04–6.90)	2.90 (0.09–5.80)	2.20 (0.02–4.40)	2.40 (0.51–4.20)	9.00 (0.05–18.00)	2.20 (0.21–4.20)	NA	1.00 (0.36–1.70)	9.50 (0.03–19.00)	(Nguyen et al., 2017)
Las Vegas Wash, USA	2.60 (ND–21.80)	ND	ND	ND	80.70 (1.50–187.00)	11.20 (ND–31.80)	27.30 (ND–65.50)	52.30 (2.30–170.00)	11.60 (ND–32.50)	17.70 (ND–44.70)	(Bai and Son, 2021)
Truckee River, USA	ND	2.2 (ND–17.4)	ND	ND	18.00 (3.20–59.60)	6.4 (ND–33.90)	6.90 (1.60–19.2)	5.8 (ND–21.80)	1.70 (NA–10.40)	5.2 (2.30–11.40)	(Bai and Son, 2021)
Ka Kum River, Ghana	NA	113 (77.20–163)	NA	0.01 (<LOD–0.04)	0.01 (<LOD–0.02)	NA	167.40 (1.78–301.00)	NA	<LOD	NA	(Essumang et al., 2017)
Pra River, Ghana	NA	193.00 (95.4–277.00)	NA	0.03 (<LOD–0.06)	0.03 (0.02–0.06)	NA	205.00 (86.20–321.00)	NA	<LOD	NA	(Essumang et al., 2017)
Liuxi River, China	NA	NA	59.20 (31.60–170.00)	NA	0.50 (<LOD–3.61)	NA	27.60 (10.90–74.00)	NA	647.00 (212–1450)	NA	(Si et al., 2021)
Shaying River, China	0.28 (<LOD–1.34)	0.44 (<LOD–2.95)	0.02 (<LOD–0.018)	0.50 (0.14–4.21)	1.10 (0.15–4.05)	0.16 (<LOD–0.82)	2.16 (0.77–4.01)	1.41 (<LOD–6.58	0.64 (0.43–0.86)	1.24 (0.37–8.19)	(Zhang et al., 2023)
Ganges River, India	1.00 (<LOD–1.00)	0.51 (<LOD–1.73)	0.08 (<LOD–0.19)	0.07 (<LOD–0.19)	2.32 (0.37–4.73)	0.12 (<LOD–0.30)	0.40 (0.08–1.18)	NA	1.31 (0.34–3.27)	5.55 (<LOD–10.19)	(Sharma et al., 2016)
Youngsan River, South Korea	2.60 (1.10–4.00)	20.00 (0.38–68.00)	1.70 (0.62–3.20)	NA	3.60 (1.30 –6.90)	7.40 (1.00–16.00)	6.20 (2.60–10.00)	3.4 (0.85–5.70)	4.50 (1.60–8.30)	4.50 (2.10–8.60)	(Hong et al., 2013)
Nakdong River, South Korea	3.10 (1.40–5.20)	26.00 (5.30–66.00)	6.70 (2.50–12.00)	NA	7.60 (3.10–17.00)	9.10 (0.83–17.00)	16.00 (8.90–28.00)	4.40 (2.00–8.40)	11.00 (4.70–21.00)	7.40 (3.70–12.00)	(Hong et al., 2013)
Sai Gon River, Vietnam	NA	0.41 (0.03–1.62)	0.30 (0.06–1.04)	0.13 (0.03–0.67)	1.19 (0.52–1.34)	0.47 (0.07–1.69)	2.28 (0.92–6.39)	NA	1.22 (0.45–3.10)	0.31 (0.30–1.35)	(Lam et al., 2017)
Chao Phraya River, Thailand	2.32 (ND–6.52)	0.47 (0.08–1.30)	0.64 (0.07–2.73)	NA	1.18 ND–2.86)	0.44 (ND–0.73)	4.18 (2.19–10.70)	3.87 (ND–30.26)	19.49 (1.80–50.48)	0.91 (ND–2.73)	(Guardian et al., 2020)
Laguna Lake, Philippines	2.40 (0.52–5.15)	0.74 (0.11–2.85)	0.64 (0.12–1.54)	NA	0.90 (<LOD–2.08)	0.38 (ND–0.90)	4.31 (2.60–8.43)	0.92 (ND–1.57)	3.74 (0.68–6.19)	0.61 (<LOD–1.30)	(Guardian et al., 2020)
Selangor River, Malaysia	2.12 (1.33–5.67)	0.42 (<LOD–6.24)	0.44 (0.20–1.05)	0.22 (<LOD–1.82)	2.12 (0.09–2.73)	0.03 (<LOD–0.48)	14.22 (0.31–126.95)	NA	NA	NA	(Ramli, 2019)
Langat River, Malaysia	7.83 (0.12–104.46)	0.75 (<LOD–8.79)	0.97 (<LOD–15.45)	0.10 (<LOD–0.61)	1.27 (0.08–2.73)	0.10 (<LOD–1.14)	2.85 (0.42–35.52)	NA	NA	NA	(Ramli, 2019)
Kuantan River, Malaysia	1.99 (<LOD–2.53)	<LOD	<LOD	<LOD	<LOD	<LOD	1.67 (<LOD–2.06)	<LOD	<LOD	<LOD	Current study
Kim Kim River, Malaysia	9.39 (<LOD–18.99)	10.11 (<LOD–18.99)	<LOD	<LOD	3.73 (<LOD–4.83)	3.52 (<LOD–6.28)	5.54 (<LOD–12.24)	35.80 (<LOD–63.87)	1.84 (<LOD–2.86)	<LOD	Current study
Permissible limit (ng/L)	NA	4.00	10.00	NA	NA	10.00	4.00	NA	NA	NA	(US EPA, 2024)

<LOD: Below limit of detection.
NA: Not available.
ND: Not detected.

2017; Guardian et al., 2020; Ramli, 2019). In Malaysia, limited studies have been conducted on PFAS monitoring and regulation. Ramli (2019) investigated PFAS concentrations in the Selangor and Langat Rivers, both classified as moderately polluted (Class III) under Malaysia's National Water Quality Standard (NWQS). PFBA, PFOS, PFNA, PFDA, PFHxA, PFHxS, and PFOA were detected in these rivers. PFOA was the most abundant PFAS in the Selangor River, with a mean concentration of 14.22 ng/L, while PFBA was the most prevalent in the Langat River, with a mean concentration of 7.83 ng/L.

The increasing global presence of per- and polyfluoroalkyl substances (PFAS) and their derivatives in the environment has raised significant health concerns due to their documented and potential harmful effects. Consequently, environmental policies have been implemented to phase out these chemicals at both national and international levels. For instance, the Stockholm Convention identifies PFOS and PFOA as emerging persistent organic pollutants (POPs) and advocates for restrictions on their usage (Singh et al., 2023). However, in many low- to middle-income countries, such as Malaysia, where exposure risks are elevated and regulatory frameworks are insufficient, there is a lack of established PFAS regulations to mitigate this problem (Tahziz et al., 2021). Recently, in April 2024, the US Environmental Protection Agency (USEPA) finalized the National Primary Drinking Water Regulation (NPDWR) for six PFAS compounds. This regulation sets legally binding limits, known as Maximum Contaminant Levels (MCLs), for these substances in drinking water, including PFOA, PFOS, PFHxS, PFNA, and HFPO-DA (GenX). The MCLs established are as follows: PFOS at 4 ng/L, PFOA at 4 ng/L, PFHxS at 10 ng/L, PFNA at 10 ng/L, GenX at 10 ng/L, and a hazard index of 1 for mixtures containing two or more of PFHxS, PFNA, GenX, and PFBS (US EPA, 2024). According to these new standards, the levels of PFOA and PFOS detected in the Kim Kim River surpass the allowable limits. As a result, urgent measures are necessary to identify and control pollution sources, ensuring the river's water quality complies with the required standards to safeguard aquatic ecosystems.

3.2. *Risk assessment*

The detection of PFAS in the surface waters of the Kuantan River and Kim Kim River has raised significant concerns about their potential adverse impacts on aquatic ecosystems. Numerous studies have utilized individual toxicity metrics, such as LC_{50}, EC_{50}, and no observed effect concentration (NOEC), to derive predicted no-effect concentration (PNEC) values and conduct risk assessments for chemical substances (Omar et al., 2019; Razak et al., 2023; Zhang et al., 2020). However, these approaches often result in large assessment factors (AFs), ranging from 10 to 1000, due to uncertainties in the measurements, which can compromise the precision of the calculated RQ values. To improve the reliability of the risk assessment, this study utilized toxicity data from the ECOTOX and ECOSAR databases. The ECOSAR database employs quantitative structure-activity relationship (QSAR) models to predict toxicity, while the ECOTOX database provides experimentally derived toxicity data. Both databases incorporate ecotoxicological data from three trophic levels to compute RQ values. In this research, algae, crustaceans, and fish were chosen as representative aquatic organisms due to their prevalence in Malaysian freshwater ecosystems (Yusoff, 2015).

The $PNEC_{ECOSAR}$ values were determined by dividing the lowest NOEC value from the three trophic levels obtained from the ECOSAR database by an assessment factor of 10. Based on QSAR predictions, the most hazardous PFAS compounds were ranked in the following order: PFDA > PFNA > PFOA > PFHpA > PFHxA > PFOS > PFHxS > PFPeA > PFBA > PFBS. The corresponding $PNEC_{ECOSAR}$ values were as follows: (1.21×10^4 ng/L), PFNA (4.05×10^4 ng/L), PFOA (1.34×10^5 ng/L), PFHpA (4.15×10^5 ng/L), PFHxA (1.13×10^6 ng/L), PFOS (1.43×10^6 ng/L), PFHxS (2.50×10^6 ng/L), PFPeA (3.00×10^6 ng/L), PFBA (7.68×10^6 ng/L) and PFBS (1.87×10^7 ng/L) (Table 2). These findings align with the understanding that PFAS toxicity is influenced by the length of the alkyl chain, with shorter-chain compounds generally exhibiting lower toxicity (Logeshwaran et al., 2021). Longer-chain PFAS tend to be more toxic due to their greater environmental persistence and higher potential for bioaccumulation in organisms (Chambers et al., 2021). The alkyl chain length, determined by the number of carbon atoms, was ordered as follows: PFDA (10 carbons) > PFNA (9 carbons) > PFOS and PFOA (8 carbons) > PFHpA (7 carbons) > PFHxA and PFHxS (6 carbons) > PFPeA (5 carbons) > PFBA and PFBS (4 carbons).

The $PNEC_{ECOTOX}$ values were calculated by dividing HC_5 values

Table 2
Calculation of risk assessment of PFAS in Kuantan River and Kim Kim River based on the ECOSAR database.

Compound	Organisms (NOEC value)			Lowest NOEC value (ng/L)	$PNEC_{ECOSAR}$ (ng/L)	Kuantan River		Kim Kim River	
	Fish (ng/L)	Daphnid (ng/L)	Green Algae (ng/L)			RQ_{mean}	RQ_{max}	RQ_{mean}	RQ_{max}
PFBA	1.31×10^8	7.68×10^7	1.61×10^8	7.68×10^7	7.68×10^6	2.59×10^{-7}	3.29×10^{-7}	1.22×10^{-6}	2.47×10^{-6}
PFOS	3.03×10^8	3.13×10^8	1.43×10^7	1.43×10^7	1.43×10^6	NA	NA	7.07×10^{-6}	1.58×10^{-5}
PFNA	4.05×10^5	5.30×10^5	3.35×10^6	4.05×10^5	4.05×10^4	NA	NA	NA	NA
PFDA	1.21×10^5	1.86×10^5	1.47×10^6	1.21×10^5	1.21×10^4	NA	NA	NA	NA
PFHxA	1.40×10^7	1.13×10^7	3.68×10^7	1.13×10^7	1.13×10^6	NA	NA	3.31×10^{-6}	4.28×10^{-6}
PFHxS	3.34×10^7	2.50×10^7	7.32×10^7	2.50×10^7	2.50×10^6	NA	NA	1.41×10^{-6}	2.51×10^{-6}
PFOA	1.34×10^6	1.49×10^6	7.58×10^6	1.34×10^6	1.34×10^5	1.25×10^{-5}	1.54×10^{-5}	4.14×10^{-5}	9.13×10^{-5}
PFPeA	4.36×10^7	3.00×10^7	7.84×10^7	3.00×10^7	3.00×10^6	NA	NA	1.19×10^{-5}	2.13×10^{-5}
PFHpA	4.37×10^6	4.15×10^6	1.69×10^7	4.15×10^6	4.15×10^5	NA	NA	4.44×10^{-6}	6.89×10^{-6}
PFBS	3.45×10^8	1.87×10^8	3.52×10^8	1.87×10^8	1.87×10^7	NA	NA	NA	NA

$PNEC_{ECOSAR}$: Predicted no effects concentration based on ECOSAR database.
RQ_{mean}: Risk quotient based on the mean concentration.
RQ_{max}: Risk quotient based on the maximum concentration.
NA: not available.

derived from SSD curves by an assessment factor of 5. Analysis of the ECOTOX database revealed HC_5 values ranked in descending order: PFOS > PFOA > PFNA > PFDA > PFPeA > PFHxA > PFHpA > PFBA. Corresponding $PNEC_{ECOTOX}$ values were PFOS (n = 736) = 6.70 × 10^3 ng/L, PFOA (n = 1091) = 2.16 × 10^4 ng/L, PFNA (n = 247) = 4.47 × 10^4 ng/L, PFDA (n = 136) = 2.62 × 10^5 ng/L, PFPeA (n = 40) = 6.73 × 10^5 ng/L, PFHxA (n = 147) = 8.77 × 10^5 ng/L, PFHpA (n = 56) = 9.80 × 10^5 ng/L and PFBA (n = 163) = 3.97 × 10^6 ng/L (Table 3). However, HC_5 estimation for PFHxS and PFBS was hindered by insufficient toxicity data in algae and cladocerans, respectively. This gap underscores how limited ecotoxicological information may compromise risk assessments, hindering policy development and mitigation strategies for PFAS contamination.

In this study, RQ_{mean} values were derived from the average PFAS concentrations, while RQ_{max} values were determined from the highest detected PFAS levels in the studied rivers. Risk evaluations utilizing $PNEC_{ECOSAR}$ and $PNEC_{ECOTOX}$ criteria indicated both RQ_{mean} and RQ_{max} values remained below 0.01, suggesting minimal ecological risks associated with the analyzed PFAS compounds. These findings suggest minimal immediate ecological threats from these substances in the aquatic environments of the Kuantan and Kim Kim Rivers. However, despite the current low-risk estimates, their persistent and bioaccumulative nature, particularly in higher trophic organisms, remains a crucial concern. (Gosset et al., 2017; Razak et al., 2021). PFBA emerged as the dominant short-chain PFAS variant in both water systems, with existing studies confirming its documented toxicity to aquatic species (Ivantsova et al., 2024a,b; Ma et al., 2022).

The environmental co-occurrence of multiple PFAS derivatives presents potential cumulative ecological impacts, as demonstrated in this study. Such mixtures may result in synergistic interactions that amplify ecological risks, underscoring the need for proactive regulatory measures (Wee and Aris, 2023a). Moreover, future monitoring initiatives should include samples from biota and sediment to enhance the comprehensiveness of PFAS risk evaluations. Conducting a thorough exposure assessment across various matrices will offer deeper insight into the presence of PFAS in riverine ecosystems. Overall, the findings of this study emphasise the widespread presence and variability of PFAS concentrations in riverine ecosystems, underscoring the need for ongoing monitoring and regulation to reduce potential environmental and health risks. Adequate protection of aquatic organisms in freshwater environments is achieved by employing the lowest PNEC value when determining the RQ (Razak et al., 2021). This conservative approach is considered essential because it provides a broader safety margin by accounting for variations in species sensitivity and mitigating potential ecological harm. The significance of using the lowest PNEC is further emphasised by its role in addressing uncertainties in toxicological data, ensuring that even the most vulnerable species are safeguarded against harmful contaminants. The summary of the lowest PNEC and RQ values for both the Kuantan River and the Kim Kim River is provided in Table 4.

The ongoing global phase-out of legacy PFAS, including PFOA and PFOS, has precipitated the introduction and widespread use of alternative, often shorter-chain fluorochemicals. The environmental fate, behavior, and toxicological properties of these novel PFAS are often only poorly understood (Wee et al., 2023b). While emerging evidence suggests that shorter-chain PFAS may exhibit reduced bioaccumulation potential compared to their longer-chain counterparts, they often demonstrate enhanced mobility in aquatic systems and persistent environmental half-lives, raising considerable concerns regarding chronic, low-level exposure (Li and MacDonald Gibson, 2022).

Epidemiological and ecotoxicological investigations are increasingly linking PFAS exposure to a spectrum of adverse health outcomes, including but not limited to endocrine disruption, immunotoxicity, metabolic dysfunction, and developmental effects (Cappelli et al., 2024). Given the inherent environmental persistence of PFAS, often spanning decades, it is imperative to establish robust, long-term monitoring programs and conduct longitudinal human cohort studies. These efforts, complemented by the application of effect-based assays, are critical to fully ascertain the ecological and human health risks posed by both legacy and novel PFAS compounds.

While this study offers a valuable snapshot of PFAS concentrations within the regional watershed, it is important to acknowledge that the single-moment, grab-sampling approach inherently limits the resolution of temporal variability influenced by seasonal cycles, hydrological events, and long-term trends (McCarthy et al., 2018). Episodic discharges, such as those stemming from industrial releases coinciding with maintenance activities, can lead to transient concentration spikes that current study's discrete measurements may not adequately capture (Muir and Miaz, 2021). Similarly, seasonal shifts in flow regimes and temperature can significantly alter PFAS partitioning between dissolved and particulate phases, thereby affecting their transport and bioavailability (Loken et al., 2025). Future research endeavors should integrate more sophisticated sampling strategies, such as repeated measurements, time-weighted passive samplers, or automated sampling devices (autosamplers), to accurately characterize these dynamic fluctuations and provide a more comprehensive exposure assessment (Fang et al., 2021).

Future research should prioritize understanding PFAS dynamics in riverine ecosystems by incorporating temporal and seasonal variability into water quality monitoring frameworks. Seasonal fluctuations in rainfall, temperature, and land use activities critically influence PFAS distribution, bioavailability, and ecological risks (Alam et al., 2024). Year-round sampling campaigns are essential to capture these dynamic trends, particularly given growing evidence of PFAS remobilization

Table 3
Calculation of risk assessment of PFAS in Kuantan River and Kim Kim River based on the ECOTOX database.

Compound	Number of toxicity study retrieved from ECOTOX database	HC_5 (ng/L)	$PNEC_{ECOTOX}$ (ng/L)	Kuantan River		Kim Kim River	
				RQ_{mean}	RQ_{max}	RQ_{mean}	RQ_{max}
PFBA	163	1.98×10^6	3.97×10^6	5.02×10^{-7}	6.38×10^{-7}	2.37×10^{-6}	4.79×10^{-6}
PFOS	736	3.35×10^4	6.70×10^3	NA	NA	1.51×10^{-3}	$3.37\ 10^{-3}$
PFNA	247	2.23×10^5	4.47×10^4	NA	NA	NA	NA
PFDA	136	1.31×10^6	2.62×10^5	NA	NA	NA	NA
PFHxA	147	4.39×10^6	8.77×10^5	NA	NA	4.26×10^{-6}	5.51×10^{-6}
PFHxS	18	NA	NA	NA	NA	N/A	N/A
PFOA	1091	1.08×10^5	2.16×10^4	7.73×10^{-5}	9.52×10^{-5}	2.56×10^{-4}	5.66×10^{-4}
PFPeA	40	3.37×10^6	6.73×10^5	NA	NA	5.32×10^{-5}	5.32×10^{-5}
PFHpA	56	4.90×10^6	9.80×10^5	NA	NA	1.88×10^{-6}	2.92×10^{-6}
PFBS	1031	NA	NA	NA	NA	NA	NA

HC_5: hazardous concentration for 5 % of species.
$PNEC_{ECOTOX}$: Predicted no effects concentration based on ECOTOX database.
RQ_{mean}: Risk quotient based on the mean concentration.
RQ_{max}: Risk quotient based on the maximum concentration.
NA: Not available.

Table 4
Summary of the lowest PNEC and RQ values for both the Kuantan River and the Kim Kim River.

Chemicals	Lowest PNEC. (ng/L)	Database	Kuantan River			Kim Kim River		
			RQmean	RQmax	Status	RQmean	RQmax	Status
PFBA	3,967,854.05	ECOTOX	5.02×10^{-7}	6.38×10^{-7}	Negligible Risk	2.37×10^{-6}	4.79×10^{-6}	Negligible Risk
PFOS	6702.15	ECOTOX	NA	NA	NA	1.51×10^{-3}	$3.37\ 10^{-3}$	Negligible Risk
PFNA	40,500.00	ECOSAR	NA	NA	NA	NA	NA	NA
PFDA	12,100.00	ECOSAR	NA	NA	NA	NA	NA	NA
PFHxA	877,050.16	ECOTOX	NA	NA	NA	4.26×10^{-6}	5.51×10^{-6}	Negligible Risk
PFHxS	2,500,000.00	ECOSAR	NA	NA	NA	1.41×10^{-6}	2.51×10^{-6}	Negligible Risk
PFOA	21,630.92	ECOTOX	7.73×10^{-5}	9.52×10^{-5}	Negligible Risk	2.56×10^{-4}	5.66×10^{-4}	Negligible Risk
PFPeA	673,343.51	ECOTOX	NA	NA	NA	5.32×10^{-5}	5.32×10^{-5}	Negligible Risk
PFHpA	415,000.00	ECOSAR	NA	NA	NA	4.44×10^{-6}	6.89×10^{-6}	Negligible Risk
PFBS	18,700,000.00	ECOSAR	NA	NA	NA	NA	NA	NA

PNEC: Predicted no effect concentration.
RQ_{mean}: Risk quotient based on the mean concentration.
RQ_{max}: Risk quotient based on the maximum concentration.
NA: Not available.

during sediment resuspension and stormwater influxes. Furthermore, integrating sediment sampling into study designs is equally vital, as benthic sediments act as long-term reservoirs for PFAS accumulation and secondary contamination. For instance, recent mesocosm experiments demonstrated that short-chain PFAS, such as PFBA and PFHxA, exhibit high vertical mobility in sediments (Liu and Li, 2024). Subsequently, coupling water column data with sediment-phase measurements enables researchers to conduct a more holistic assessment of PFAS partitioning and persistence across environmental matrices. Such multidimensional approaches elucidate interactions between hydrological cycles, sediment dynamics, and PFAS behaviour.

4. Conclusion

This study presents findings on the presence, distribution, and risk assessment of PFAS in the Kuantan River and Kim River in Malaysia. The findings of the present investigation indicate that only two types of PFAS were identified in the Kuantan River, which is classified as a clean river (Class II). In contrast, seven types of PFAS were detected in the Kim Kim River, which is considered a moderately polluted river (Class III) in Malaysia. The mean PFAS concentration in the Kuantan River was in the following order: PFBA (1.99 ng/ L, range: <LOD - 2.53 ng/ L) > PFOA (1.67 ng/ L, range: <LOD - 2.06 ng/ L). Moreover, mean PFAS concentration (ng/L) in Kim Kim River were in the following order: PFPeA (35.80 ng/ L, range: <LOD - 63.87 ng/ L) > PFOS (10.11 ng/ L, range: <LOD - 22.59 ng/ L) > PFBA (9.39 ng/ L, range: <LOD - 18.99 ng/ L) > PFOA (5.54 ng/ L, range: <LOD - 12.24 ng/ L) > PFHxA (3.73 ng/ L, range: <LOD - 4.83 ng/ L) > PFHxS (3.52 ng/ L, range: <LOD - 6.28 ng/ L) > PFHpA (1.84 ng/ L, range: <LOD - 2.86 ng/ L). Anthropogenic activities in industrial and residential areas were among the main sources of PFAS pollution in both rivers. Although the ecological risk assessment results are considered negligible ($RQ < 0.01$) for both rivers, they cannot be ignored, as ecological risk assessments might increase in the future due to biomagnification and bioaccumulation factors. Comprehensive framework for risk management and monitoring of PFAS in drinking water supply systems must be implemented to ensure the safety of water resources. It is essential to implement and maintain world-class standards that focus on controlling surface water pollution and enhancing PFAS treatment efficiency. This study aims to minimise the risks associated with PFAS exposure, both for the environment and for human health. The findings of this study are of great significance for future research into the management of risks associated with river water and offer essential guidance for decision-making processes aimed at conserving and maintaining river ecosystems.

CRediT authorship contribution statement

Ahmad Zaharin Aris: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Muhammad Raznisyafiq Razak:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Didi Erwandi Mohamad Haron:** Writing – review & editing, Validation. **Muhammad Qusyairi Jori Roslan:** Writing – review & editing, Investigation. **Nawaltul Akma binti Ahmad Sabri:** Writing – review & editing.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118739.

Data availability

Supplementary data associated with this article can be found in the online version

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