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Spatiotemporal distribution and priority assessment of steroids in the estuarine environment: Implications for environmental risk management

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ABSTRACT

Steroids, known for their endocrine-disrupting capabilities, have become a subject of considerable concern in the scientific community. This research offers a thorough evaluation of steroid contaminants within the Jiulong River Estuary (JRE), examining their spatiotemporal distribution, multimedia distribution, and mass inventory. Seven steroids were detected in water samples, while ten steroids were identified in sediments, with concentrations ranging from 0.2 to 51 ng/L in water and no-detectable (ND) to 12 ng g^{-1} in sediments. In both water and sediments, natural steroids were the most prevalent throughout both the dry and wet seasons. The distribution of these compounds within the aquatic-sediment system was governed by their hydrophobicity and a suite of environmental factors, such as temperature, salinity, pH, chlorophyll-a, and total organic carbon content. Mass inventory analysis revealed that over 90 % of the total steroid mass inventory was stored in the sediments, underscoring their pivotal role as a repository for these substances within the JRE. Furthermore, this research represents the first comprehensive screening to identify priority contaminants in this region. Utilizing a multimetric evaluation approach, progesterone and testosterone were identified as high-priority pollutants during the dry season, with progesterone alone ranking as a high-priority pollutant in the wet season. This study provides crucial insights for the management of steroid-related pollution and the assessment of environmental risks in estuarine ecosystems.

1. Introduction

Steroids, ubiquitously detected in aquatic systems as emerging contaminants (Runnalls et al., 2010), have attracted considerable concern due to their potential carcinogenicity (IARC, 2020) and proven ecological risks. Even at low concentrations, as minimal as nanograms per liter (ng/L), steroids can induce intersex characteristics in fish and disrupt aquatic populations (Runnalls et al., 2015; Dang and Kienzler, 2019; Kidd et al., 2007; Azizi-Lalabadi and Pirsaheb, 2021). Endogenous steroids and their metabolites are naturally excreted by humans, livestock, and aquatic species through urine and feces. Additionally, synthetic steroids, which are commonly used in both human and veterinary medicine, as well as for promoting growth, further contribute to environmental pollution (Chang et al., 2009; Fent, 2015). Through sewage treatment plants and runoff from agricultural activities, these compounds make their way into the environment (Zhong et al., 2021; Adeel et al., 2017). Additionally, global steroid emissions have been rising at an approximate rate of 10 % annually (Zhang et al., 2021). As a result, a wide variety of different steroids are present at very low concentrations in surface waters across the world (Ojoghoro et al., 2021), highlighting a growing concern about their ecological impact.

Estuaries, as intricate and dynamic interfaces between terrestrial and marine ecosystems, serve crucial roles in facilitating interactions between land and sea and in channeling terrestrial compounds towards the

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ocean. However, they also face significant anthropogenic stressors (Zhen et al., 2021; Wang et al., 2022; Bianchi et al., 2018). Annually, an estimated 2.5 × 10^{10} tones of terrestrial-derived compounds are conveyed to the ocean, with approximately 90 % of this flux passing through estuaries (Wu et al., 2017a). Within the diverse mixture of contaminants found in estuarine environments, steroids have emerged as prevalent pollutants (Korkmaz et al., 2022; Omar et al., 2019; Meador et al., 2016), posing a significant threat to both fisheries and human health.

The Jiulong River (JR), Fujian Province's second-largest river, drains approximately 12 % of the region's land area (Guo et al., 2011). It traverses through the cities of Longyan, Zhangzhou, and Xiamen, providing essential water resources to these areas (Wu et al., 2019). The river eventually flows into the JRE, a shallow estuary in a subtropical region that connects Xiamen Bay to the Taiwan Strait. The merging of several tributaries, combined with the monsoon-driven coastal currents in the Taiwan Strait, makes the dynamics at the interface within the JRE highly complex (Guo et al., 2011). The Jiulong River Watershed (JRW) exerts a substantial impact on the water quality of the JRE and the Xiamen coastline, significantly contributing to the pollutant load within the estuary (Chen et al., 2014; Guo et al., 2011). Over recent decades, increasing human activities—such as population growth, urban and industrial wastewater discharge, expansion of agricultural fertilizer use, and livestock farming have intensified environmental pressures on the JRE (Zhang et al., 2011; Chen et al., 2015; Cao et al., 2014; Li et al., 2017). Especially noteworthy is the profound impact of the swift economic growth in the Xiamen Special Economic Zone, which has led to considerable anthropogenic perturbations in the estuarine ecosystems and adjacent regions (Yan et al., 2012; Wu et al., 2016). The synergistic impacts of these variables have markedly compromised the environmental integrity of the JRE. A wide range of research has emphasized the detection of different contaminants in the JRE, including perfluoroalkyl substances (PFAS) (Cai et al., 2018), pesticides (Lin et al., 2013), antibiotics (Zheng et al., 2011), and polybrominated diphenyl ethers (Wu et al., 2017b). Nevertheless, the prevalence of steroid pollution, particularly its metabolites, within this region remains largely underexplored. Given that agricultural runoff and household waste are primary sources of steroid contamination, it is essential to evaluate the

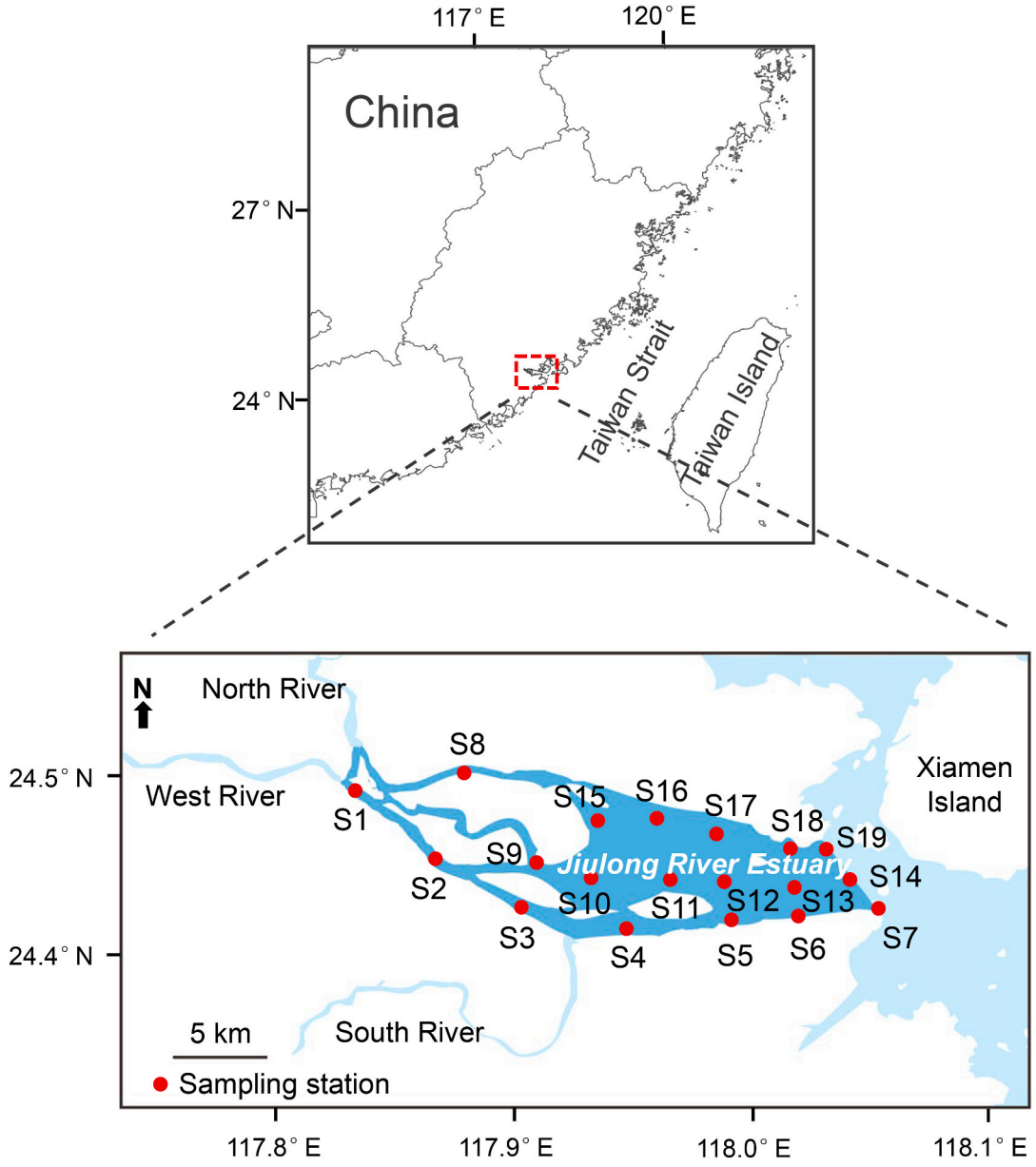

Fig. 1. Locations of the sampling sites in the JRE.

extent of steroid pollution in the JRE.

Therefore, the aim of this research was: (1) to clarify the spatial and temporal distribution of steroids within an estuarine water-sediment system, (2) to identify the factors shaping their distribution behaviors, (3) to quantify the pollution load via a mass inventory estimation, and (4) to prioritize pollutants employing a multi-criteria integrated assessment approach. The findings will contribute valuable scientific knowledge for the protection of estuarine ecosystems and pollutant management, providing a theoretical foundation for policy development and the optimization of pollution control strategies.

2. Materials and methods

2.1. Study area and sampling collection

The JRE, situated along the southeast coast of Fujian Province, China, is influenced by freshwater inputs from the JR, which has an average yearly runoff of 1.49×10^{10} m^3 (Chen et al., 2015). The JRE is primarily shaped by the confluence of three major streams, with two of the principal tributaries contributing significantly to its discharge. The North and West streams deliver average annual runoffs of 8.22×10^9 m^3 and 3.68×10^9 m^3, respectively (Fig. 1). Notably, the JRE is located in a region with high concentrations of sewage, livestock, and aquaculture activities, leading to substantial wastewater discharges, including steroids, directly into the estuary via river runoff (Zhang et al., 2011; Chen et al., 2015).

Samples of surface water and sediment were obtained from 19 locations (S1 − S19) within the JRE during both the dry season (November 2014) and the wet season (June 2015) (Fig. 1). A comprehensive overview of the sampling sites is provided in Table S1. At each site, 5 L of surface water was gathered using plastic containers, while sediment samples (from a 0–5 cm depth) were collected with a stainless-steel grab sampler. However, sediment samples could not be retrieved from several sites (S1, S3, S4, S7, S11, S15, S17) during the wet season. Following collection, the water samples were acidified to a pH of 3 with sulfuric acid (Yu et al., 2022) and preserved with a 5 % methanol solution (*v*/v) to prevent microbial activity. All samples were kept at −20 °C until they were analyzed further. Additional information about the sampling procedure is available in Text S1.

2.2. Sample extraction and instrumental analysis

Existing studies suggested that estrogens are likely to be very important steroids in aquatic environments (Ojoghoro et al., 2021), especially 17α-ethynylestradiol (EE2), which has been confirmed to have strong ecological toxicity (Runnalls et al., 2015; Caldwell et al., 2012). However, compared to estrogens, there was a significant knowledge gap in the environmental behavior and ecological risks of androgens, progestagens, and glucocorticoids, which severely limits the comprehensive ecological risk assessment of steroid pollution. Based on globally published data, this study selected three different types of steroids (androgens, progestagens, glucocorticoids) with high detection rates or concentrations in the environment as research targets, aiming to systematically analyze the spatiotemporal distribution patterns of characteristic pollutants in the JRE.

This research focused on analyzing a total of 22 steroids, including three androgens: 4-androstene-3,17-dione (AED), testosterone (T), and 17β-boldenone (17β-BOL); five glucocorticoids: cortisol (CRL), cortisone (CRN), 11-deoxycorticosterone (DOC), tetrahydrocortisol (3α,5β-THCRL), and prednisolone (PREL); and fourteen progestagens: progesterone (P), 17α,20α-dihydroxyprogesterone (17α,20α-DOHP), 17α,20β-dihydroxyprogesterone (17α,20β-DOHP), 17α-hydroxyprogesterone (17α-OHP), 17α-hydroxyprogesterone acetate (17α-OHPA), 11-ketoprogesterone (11-KP), 1-dehydroprogesterone (1-DHP), 16-dehydroprogesterone (16-DHP), medroxyprogesterone acetate (MPA), norvinisterone (NVT), dydrogesterone (DGT), norgestrel (NGT), cyproterone acetate (CPTA), and megestrol acetate (MGTA) (Table S2). A comprehensive list of the chemicals employed in this study is available in the Text S1.

The methods used for sample extraction were modified from previous research (Zhou et al., 2012; Liu et al., 2011). Initially, filtered water samples (5 L) were followed by processing with Oasis HLB solid-phase extraction (SPE) cartridges (6 mL, 500 mg). For sediment samples (2 g), ultrasonic-assisted extraction was performed using a mixture of acetonitrile and citric acid buffer, after which enrichment and cleanup were carried out with SAX-HLB cartridges. Steroid analysis was conducted using LC-MS/MS, employing an Agilent 1290 Infinity II liquid chromatograph in combination with an Agilent 6470 triple quadrupole mass spectrometer, operated in multiple-reaction monitoring (MRM) mode. Comprehensive details on the extraction and quantification methods are available in the Supplementary Information (SI).

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

The steroid recovery rates for water samples ranged from 63 % to 149 %, whereas for sediments, the recovery values fluctuated between 68 % and 155 % (Table S3). For each steroid analyzed, the detection limit (LOD) and quantitation limit (LOQ) for each steroid analyzed were calculated based on the signal-to-noise ratio and the corresponding value for the target compounds in the extraction process fell within the following ranges: for water samples, 0.029–1.4 $ng{\cdot}L^{-1}$ and 0.10–4.6 $ng{\cdot}L^{-1}$, and for sediments, 0.033–1.4 $ng{\cdot}g^{-1}$ and 0.11–4.8 $ng{\cdot}g^{-1}$ for sediments (Table S3). To evaluate background contamination and ensure the proper performance of the instrument, several blanks including field blanks, procedural blanks, solvent blanks and standard solutions were included in the analysis. The calibration curves for all the target analytes, spanning concentrations from 1.0 to 100 $\mu g{\cdot}L^{-1}$, demonstrated excellent linearity with correlation coefficients exceeding 0.995. The relative standard deviations for standard solutions, both intra-day and inter-day, varied between 1.4 % and 12 % and 6.4 % and 22 %, respectively.

2.4. Priority pollutant screening

In recent years, pinpointing priority pollutants has emerged as a critical environmental concern; however, a standardized protocol for their identification remains to be developed. Prior studies have focused on pinpointing priority contaminants in wastewater treatment plants (WWTPs) and surface waters, predominantly utilizing ecological risk assessments as a framework (Wang et al., 2023; Lu et al., 2023). However, such methodologies may fall short in capturing the intricacies inherent to actual environmental settings. Thus, it is imperative to encompass a holistic approach that accounts for the prevalence and ecological hazards of contaminants, alongside their propensity for persistence and bioaccumulation, especially within the context of estuarine ecosystems.

To address these concerns, a modified methodology was developed, which employs a multi-criteria scoring technique to prioritize contaminants (Gong et al., 2024; Wei et al., 2024; Zhang et al., 2024). This methodology integrates four pivotal criteria: occurrence (O), persistence (P), bioaccumulation (B), and ecotoxicity (E), with each criterion being accorded equal significance in the assessment. The criterion for occurrence encompasses the concentration levels and detection frequencies of the targeted pollutants within the JRE, with each factor being given equal consideration in the evaluation. Persistence is assessed based on the biodegradation half-life data available through the CompTox Chemicals Dashboard, whereas the bioaccumulation potential is evaluated using data from the EPI Suite 4.1 (Table S4). Ecotoxicity is determined by calculating the ecological risk of each compound (Table S4). The values assigned to each criterion are categorized into five classes using geometric progression, and a two-thirds cumulative rating method is applied to derive a conclusive score (Table S5). The total score for each compound is calculated by summing the scores across the four

criteria. Based on the total scores, the target contaminants are categorized into five priority classes (I – V), with Classes I and II representing high- and medium-priority pollutants, respectively. Further details on the identification process for priority contaminants can be found in Supporting Information (Text S2).

2.5. Data analysis

Details on how the distribution coefficients (K_d and K_{oc}) were calculated in Text S3. Further information on the mass inventories and deposition flux of steroids in the JR can be found in Text S4. The sampling map of the JRE was created using ArcGIS Pro v.2022. Mann-Whitney *U* test was carried out with IBM SPSS Statistics® 24, while Origin Pro v.2021 was used for generating figures and performing correlation analysis. A significance level of *p*-values <0.05 or < 0.01 was applied for all statistical tests.

3. Results and discussion

3.1. Occurrence of steroids in the JRE

3.1.1. Water

A total of seven steroids were identified in water samples collected over two seasons, with concentrations varying between 0.27 and 51 ng·L^{-1} (Fig. 2A and B, Table S6). The observed levels were found to exceed those previously reported in the Malaysia Estuary (Omar et al., 2019), the Sado River Estuary (Portugal) (Rocha et al., 2013), and Pearl River Estuary (Xu et al., 2024).

Among the identified steroids, natural steroids AED and P were found to have the highest prevalence, with detection rates reaching 89 %

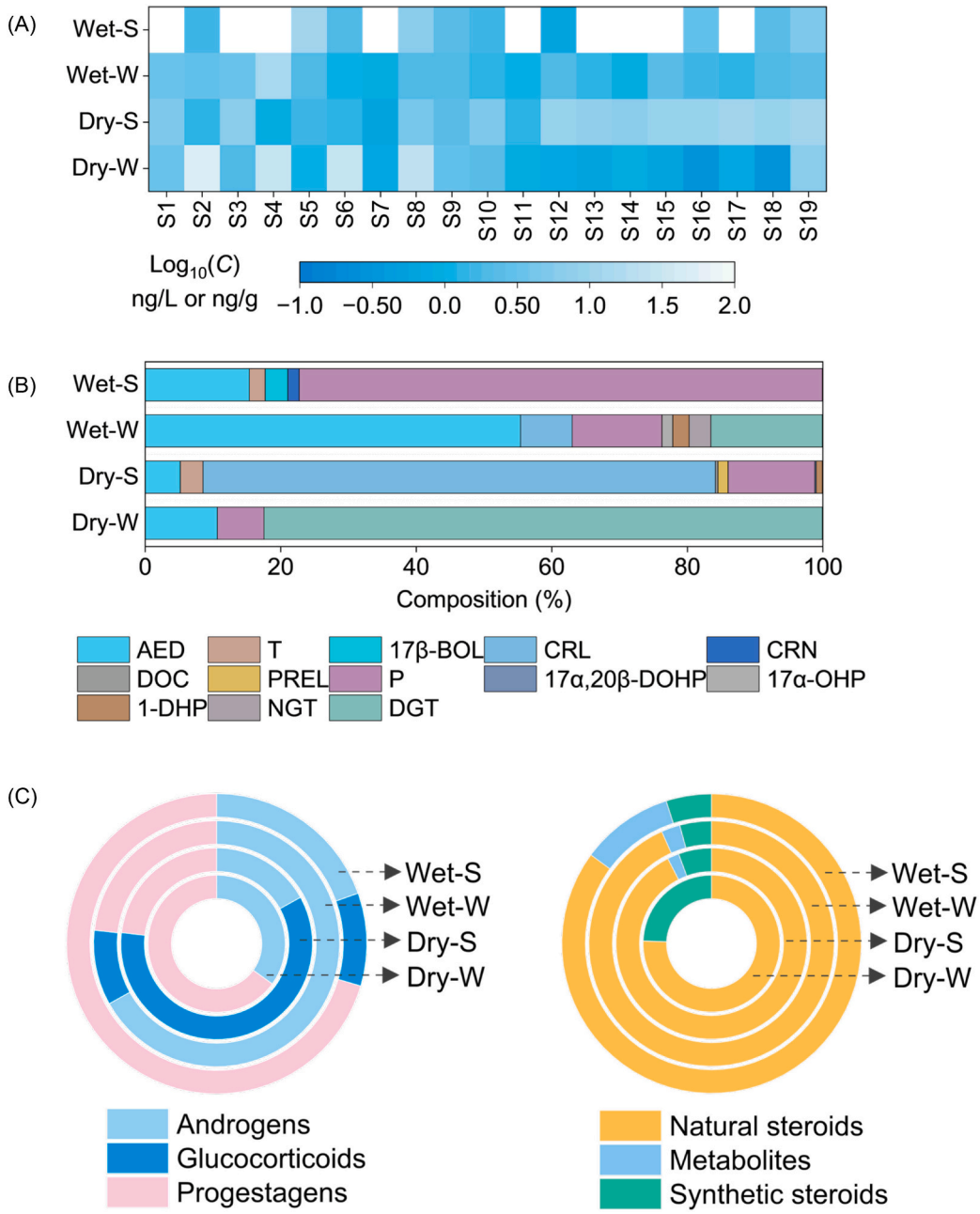

Fig. 2. Total concentrations (A), composition profiles (B), and percentages (C) of steroids in the surface water (Dry-W and Wet-W) and sediments (Dry-S and Wet-S) in dry and wet seasons.

and 100 % in the dry and wet seasons, respectively (Table S6). Concentrations of AED and P peaked at 3.2 ng·L^{-1} and 1.4 ng·L^{-1}, respectively (Table S6). The higher concentration of AED in water relative to P is likely due to its lower Log K_{oc} value (2.8), enhancing its mobility in water, in contrast to a higher Log K_{oc} (3.5) of P, which favors binding to sediments (Table S2). During the dry season, synthetic steroid DGT predominated in water, with concentrations peaking at 48 ng·L^{-1} (Fig. 2B). Its ubiquity in the dry season may be attributed to DGT's extensive application in contraception and for treating menstrual irregularities and endometriosis in the human and veterinary sectors within the JRW (Rižner et al., 2011). Other steroids such as CRL, 17α-OHP, 1-DHP, and NGT were only sporadically detected during the wet season. In terms of steroid classification, progestogens and androgens were predominant in both seasons, constituting 65 % and 67 % of the total steroid levels, respectively, surpassing glucocorticoids (Fig. 2C). Natural steroids constituted the majority of detected steroids, representing 76 % and 96 % of the total steroid levels in the dry and wet seasons, respectively (Fig. 2C). The higher occurrence of natural steroids in water may be attributed to the high human population density and extensive farming activities in the JRW.

3.1.2. Sediments

Overall, ten steroids were identified in sediments, with total concentrations varying from non-detectable (ND) to 12 ng·g^{-1} (Fig. 2A and B, Table S7). These values were similar to those observed in the Pearl River Estuary (Xu et al., 2024), but exceeded the levels found in estuarine sediments in Portuguese Estuaries (Amorim et al., 2024), Markman Canal and Swartkops River Estuary (Ohoro et al., 2021), and mariculture sediments in Malaysia (Ismail et al., 2020). However, they were lower than concentrations reported in the sediments of the Golden Horn Estuary (Korkmaz et al., 2022), the fishing ports in China (Liu et al., 2022) and river in Canada (Yarahmadi et al., 2018).

Paralleling the aqueous findings, natural steroids AED and P were widely identified in sediments. In the dry season, the detection rate for these compounds was 100 %, whereas in the wet season, detection rates varied from 58 % to 75 % (Table S7). The mean concentration of P (8.6 ng·g^{-1}) in sediments exceeded that of AED (1.6 ng·g^{-1}), contrasting with their aqueous concentrations where AED predominated. This disparity may stem from the higher Log *K*oc value of P, enhancing its affinity for sediment sorption relative to AED (Table S2). Interestingly, steroid metabolites like CRN were only detected in sediments during the wet season, whereas DOC and 17α,20β-DOHP were detected solely in the dry season (Table S7). This suggests that steroid metabolite profiles are highly influenced by seasonal variations. In terms of steroid classification, glucocorticoids and progestagens were dominant in the sediments, constituting 60 % and 70 % of the total steroids in the dry and wet seasons, respectively (Fig. 2C). In both seasons, natural steroids constituted the majority of detected compounds, accounting for 85 % to 92 % of the total steroid concentrations (Fig. 2C), consistent with their aquatic prevalence.

3.1.3. Potential threats posed by steroids to aquatic organisms

The concentrations of steroids and their metabolites detected in the JRE water samples were notably low (0.27–51 ng/L), yet these trace levels merit significant concern. Aquatic organisms, especially fish, demonstrate remarkable sensitivity to steroids in the water (Ojoghoro et al., 2021). While individual steroid concentrations might fall below thresholds for observable ecological impacts, the mixture of multiple steroids can induce adverse biological responses, a synergistic phenomenon termed the "a lot from a little" (Thrupp et al., 2018). Of particular note was the detection of 17α-OHP (ND − 0.28 ng/L), a hydroxylated progesterone metabolite. Unlike mammalian systems where parent compounds typically exhibit greater bioactivity, fish physiology shows heightened responsiveness to such hydroxylated metabolites, which function as biologically active progestagens (Scott et al., 1982). These combined findings suggest that multiple steroids and their metabolites, even at environmental concentrations of ng/L, require significant attention.

3.2. Spatiotemporal variations of steroids in the JRE

3.2.1. Water

No statistical variations in steroid concentrations were observed between two seasons (Mann-Whitney *U* test, $p > 0.05$) (Fig. 2, Table S6). This aligns with previous studies in the Pearl River estuary (Xu et al., 2024) and Jiaozhou Bay (Lu et al., 2021). In the dry season, three steroids (AED, P, and DGT) were detected in water, while seven steroids (AED, CRL, P, 17α-OHP, 1-DHP, NGT, and DGT) were detected in the wet season. The heightened riverine discharge during the wet season is hypothesized to facilitate the transport of a greater volume of steroids from the JRW, subsequently enriching steroid levels in the JRE. Furthermore, the increased anthropogenic activities, notably shipping and sewage discharge, is likely responsible for the elevated steroid concentrations observed in the wet season compared to the dry season (Wu et al., 2019). The maximum concentration of DGT in water was notably higher in the dry season (48 ng·L^{-1}) compared to the wet season (7.8 ng·L^{-1}), likely due to the dilution effects of precipitation and seawater influx during the wet season.

Spatial gradients in steroid concentrations within the estuary exhibited a decline from the riverine confluence towards the marine environment (Fig. S1). For instance, total steroid concentrations at sampling sites S1 and S7 in the dry season were 3.4 ng·L^{-1} and 0.68 ng·L^{-1}, respectively, while in the wet season, the concentrations were 3.3 ng·L^{-1} and 1.0 ng·L^{-1}. Similar trends were observed in previous studies (Sun et al., 2016; Yan et al., 2013). High steroid concentrations proximal to the river mouth likely indicate significant anthropogenic contributions to the area, encompassing discharges from sewage treatment plants in the downstream region of the western JR and the presence of untreated wastewater (Lv et al., 2014). The decline in steroid levels towards the open sea can be attributed to dilution by seawater. In the dry season, higher concentrations were observed at sampling sites S2 (57 ng·L^{-1}), S4 (26 ng·L^{-1}), and S8 (25 ng·L^{-1}), which located at the outlets where the North Stream, West Stream, and South Stream, respectively, enter the estuary. These regions experience substantial pollution from dense human populations, livestock farming, and aquaculture, which are likely significant contributors to the elevated steroid concentrations observed (Xu et al., 2023). Notably, site S4, which is near an aquaculture area and the outlet of the South Stream (Li et al., 2017), recorded the highest steroid concentration during the wet season (13 ng·L^{-1}). This is mainly attributed to substantial surface runoff, which carries a high volume of pollutants, including steroids, into the estuarine environment (Wang et al., 2010; Zhang et al., 2017). Site S14, located near the estuarine outlet, recorded the lowest concentration (0.91 ng·L^{-1}) in the wet season, influenced by the dilution effects of rainfall and river runoff (Liu et al., 2022).

3.2.2. Sediments

Based on steroid concentration levels, no notable seasonal variations were detected in the sediments when comparing the dry and wet seasons. (Mann-Whitney *U* test, $p > 0.05$) (Fig. 2a, Table S7), suggesting relatively stable steroid levels in the sediments. A similar seasonal trend has been reported in the Pearl River Estuary (Xu et al., 2024). The greater occurrence rates of natural steroids (such as AED, T, CRL, and P) were significantly reported in the dry season (74 %–100 %) (Fig. S2, Table S7). The diminished detection frequency of steroids in the wet season can be ascribed to heightened precipitation and the intensified hydrodynamic forces resulting from the interplay of riverine and marine waters in the estuary, potentially facilitating sediment resuspension (Wu et al., 2017b).

Spatially, steroid concentrations in the sediments of the lower estuary zone (sites S11-S19) were higher than those in the upper zone (sites S1-S4, S8-S10) during the dry season. Specifically, the northern bank of

the estuary (sites S15-S19) exhibited higher steroid concentrations compared to the southern bank (Fig. S1). The observed fluctuations in steroid concentrations could be attributed to a multitude of factors, such as localized pollution sources, hydrological regimes, and the influence of coastal effluents. Notably, the maximum concentration of steroids during the dry season was recorded at site S19 (12 ng·g^{-1}), located at the outlet of the JRE (Fig. S1, Table S7). This high concentration is presumably influenced by coastal effluents, enhanced sedimentation rates, and diminished sediment mobility, due to the proximity to adjacent islands (Lao et al., 2022). Conversely, the lowest concentration was observed at site S7, near the open ocean (0.59 ng·g^{-1}). During the wet season, steroid concentrations at individual stations exhibited variable patterns of increase and decrease (Fig. S1, Table S7). Specifically, the total steroid concentrations gradually declined from sites S8 and S16 to S18, suggesting that the upstream areas along the northern bank contribute significantly to the steroid load (Fig. S1). Relatively high total steroid concentration (11 ng·g^{-1}) was observed at Site S5, likely due to sewage discharge from the South Stream, a tributary that receives substantial wastewater inputs. Similarly, the total steroid concentration was 6.0 ng·g^{-1} at site S8, situated downstream of the North Stream outlet. High steroid concentration was also observed at the JRE outlet (S19, 5.5 ng·g^{-1}), reinforcing the idea that coastal discharges play a crucial role in the distribution of these contaminants. Interestingly, steroid concentrations were below detectable levels at sites S13 and S14, suggesting minimal contamination at these locations. This highlights the complex spatial distribution of steroids within the estuary and suggests that local hydrological and anthropogenic factors influence the sedimentary load of these compounds.

3.3. Partitioning behavior and influencing factors of steroids in the JRE

To better understand the distribution of steroids within the water-sediment system of the JRE, Log K_d values for the target steroids were determined over two seasons. The Log K_d values varied between 1.7 and 3.9 in the dry season and between 2.4 and 4.3 during the wet season (Fig. S2). A strong positive correlation was found between Log K_d and Log K_{ow} for the detected steroids (AED and P) during the wet season ($R = 0.88$, $p < 0.05$) (Fig. S2), indicating that hydrophobicity is a key factor in determining how steroids partition between water and sediments in this season. In contrast, the relationship between Log K_d and Log K_{ow} was weak ($R = 0.39$, $p > 0.05$) (Fig. S2), implying that steroid partitioning in the dry season is also influenced by factors other than hydrophobicity.

Besides hydrophobicity, various environmental variables influenced steroid distribution between water and sediments across both seasons (Fig. 3A). Temperature showed a significant positive relationship with AED, P, and overall steroid concentrations in water throughout the seasons (Fig. 3A). This could be attributed to its effect on enzyme activities such as arylsulfatase and β-glucuronidase, which aid in the deconjugation process of steroid conjugates present in water (Liu et al., 2015). Moreover, in the dry season, a negative correlation was observed between pH and both AED and total steroid concentrations in water (Fig. 3A), possibly because the fact that fluctuations in pH can affect steroid sorption by modifying the physical and chemical properties of organic matter (Neale et al., 2009). Salinity showed a negative correlation with AED and the total steroid concentration in water across both seasons (Fig. 3A), suggesting that steroid distribution may be influenced by salting-out and dilution, which are driven by factors such as rainfall, river inflows, and the discharge of domestic wastewater from land-based sources (Liu et al., 2022). Furthermore, chlorophyll-a (Chl-a) exhibited a

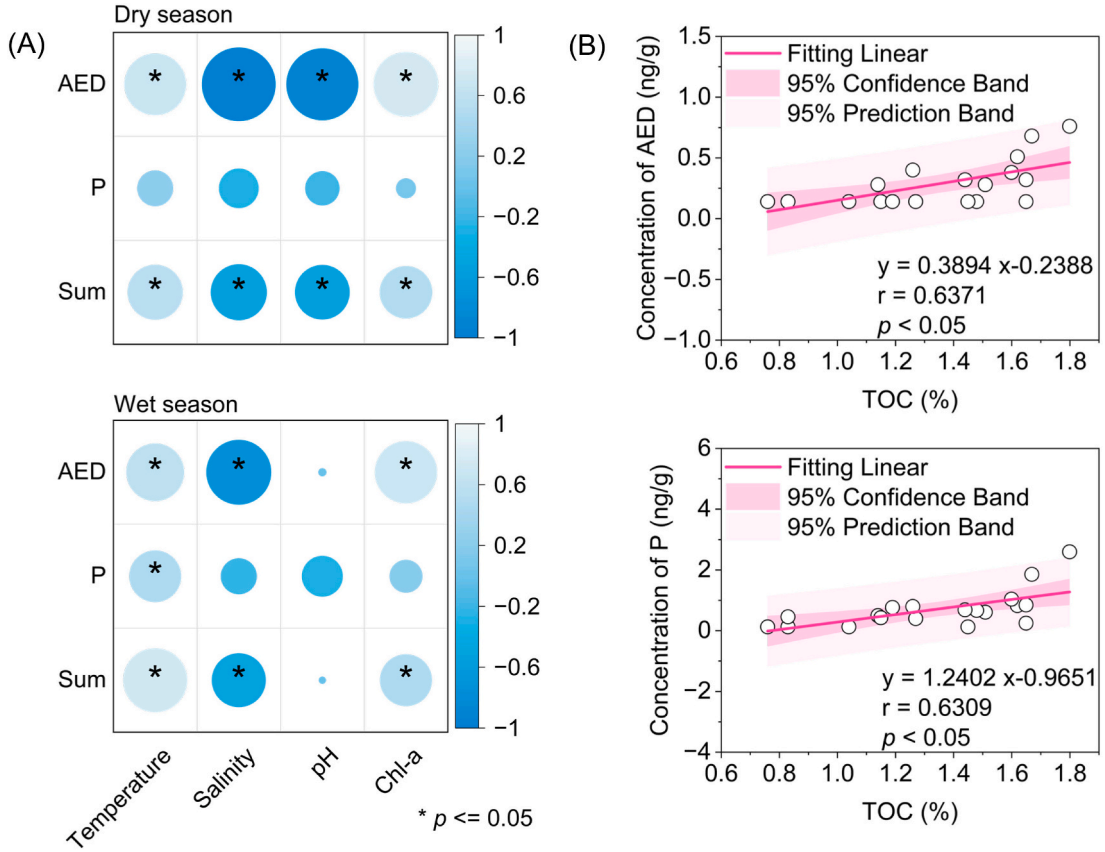

Fig. 3. (A) Correlations between detected steroids and environmental parameters for water in the JRE. Sum, total concentrations of steroids; (B) Correlations between detected steroids and TOC in sediments of the JRE during the dry season.

positive correlation with AED and the total steroid concentration in water during both seasons (Fig. 3A). This relationship can be attributed to the role of Chl-a as an indicator of phytoplankton biomass, which might be influence the bioavailability and distribution of pollutants including steroids in aquatic environments (Falkowski, 1994; Davies et al., 2018; Boyer et al., 2009). Higher Chl-a concentrations might reflect increased biological activity, which could affect the cycling and transformation of steroids in the water column.

In sediment samples, a significant positive relationship was observed between total organic carbon (TOC) and most of the steroids identified, such as AED and P, during the dry season ($p < 0.05$) (Fig. 3B). This suggests that higher TOC concentrations enhance the capacity of sediments to adsorb steroids (Huang et al., 2020; Li et al., 2019). Similar trends have been documented in other research on sediments collected from fishing ports and estuaries (Liu et al., 2022; Omar et al., 2018). However, in the wet season, no significant correlation was found between TOC and the detected steroids (AED, P, and the total steroid concentration) in the sediments (Fig. S3, $p > 0.05$), indicating that other factors could have a more prominent influence on the adsorption of steroids to sediments in this period.

3.4. Mass inventory and deposition flux

In the study, the mass inventories of steroids in the water of the JRE ranged from 0.36 to 0.76 ng cm^{-2} (androgens), 0 to 0.14 ng cm^{-2} (glucocorticoids), and 0.34 to 2.8 ng cm^{-2} (progestogens), respectively, over two seasons. Notably, the greater steroid mass inventory in the water was found during the dry season (Table S8). Glucocorticoids and progestagens collectively constituted over 75 % of the total mass inventories across both seasons (Table S8). Concordantly, the higher sedimentary steroid mass inventory was reported in the dry season, with natural steroids being the predominant contributors (Table S9). The estimated sedimentary steroid mass inventory was 0.11–0.32 mg m^{-2}, which is notably one to two orders of magnitude lower than the concentrations observed in fishing ports (2.1–16 mg m^{-2}) (Liu et al., 2022) and the Pearl River estuary (17–20 mg m^{-2}) (Xu et al., 2024). The sedimentary steroid mass inventory accounting for 91 % and 90 % of the overall mass inventory over two seasons, respectively (Fig. 4, Table S10). These results underscore the important role of sediments as a major reservoir for steroids in the JRE.

As a key reservoir for steroids, the deposition flux of steroids in the sediments warrants further attention. Deposition flux is a widely used indicator of contaminant accumulation in marine sediments, as it is not influenced by dilution from contaminant input sources or land-based runoff (Fang et al., 2015; Wang et al., 2020). The deposition flux of steroids in the JRE ranged from 0.28 to 5.4 ng cm^{-2} yr^{-1} (average: 2.5 ng cm^{-2} yr^{-1}) in the dry season and from 0 to 5.1 ng cm^{-2} yr^{-1} (average: 1.4 ng cm^{-2} yr^{-1}) in the wet season (Fig. 4). Notably, the burial capacity of steroids exhibited considerable spatial variability (Fig. 5). In the dry season, the highest deposition flux (5.4 ng cm^{-2} yr^{-1}) was observed at the outlet of the JRE (S19), likely due to the elevated sedimentation rates. In contrast, the highest flux during the wet season (5.1 ng cm^{-2} yr^{-1}) was recorded at site S5, possibly influenced by high-intensity human activities in the surrounding area.

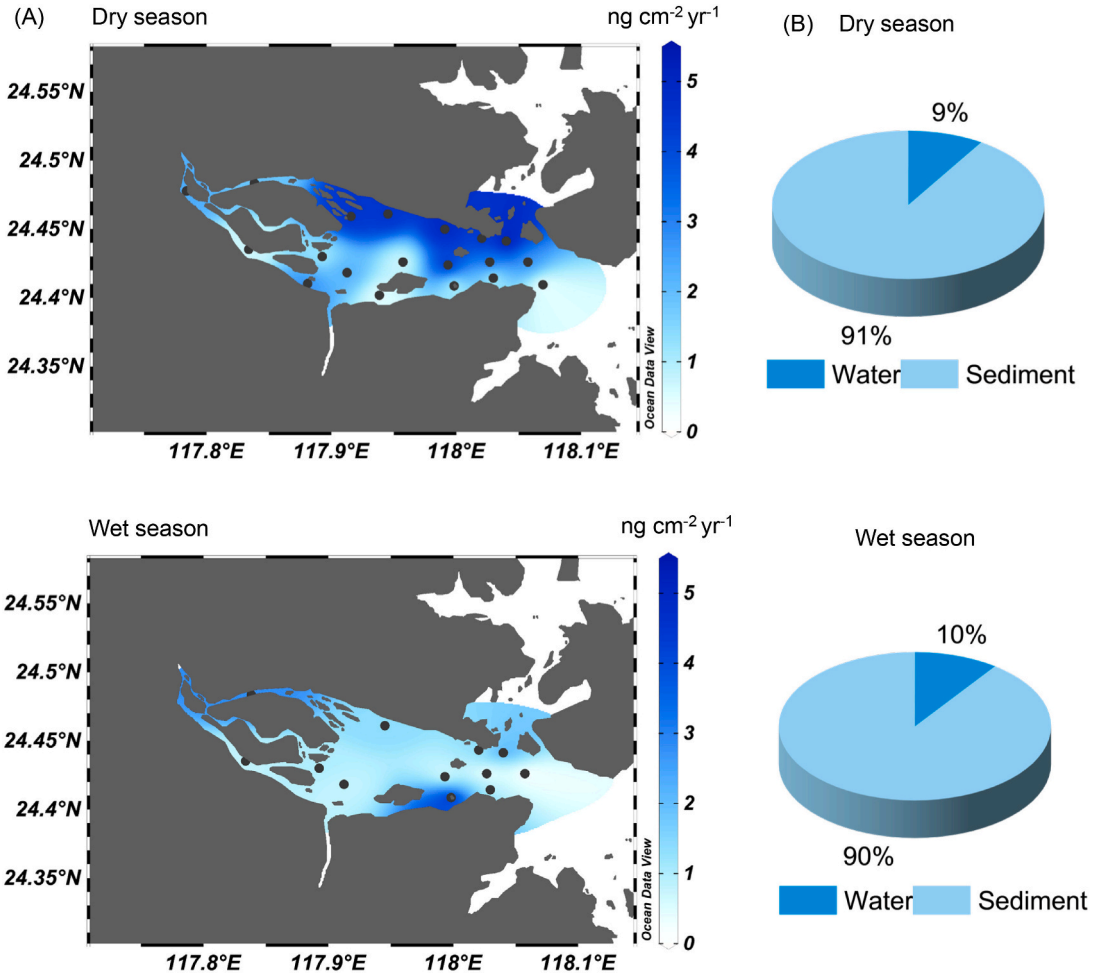

Fig. 4. Distribution pattern of steroids deposition fluxes (ng cm^{-2} yr^{-1}) in dry season and wet season (A) and the percentage of steroids in water and sediment during dry and wet season (B).

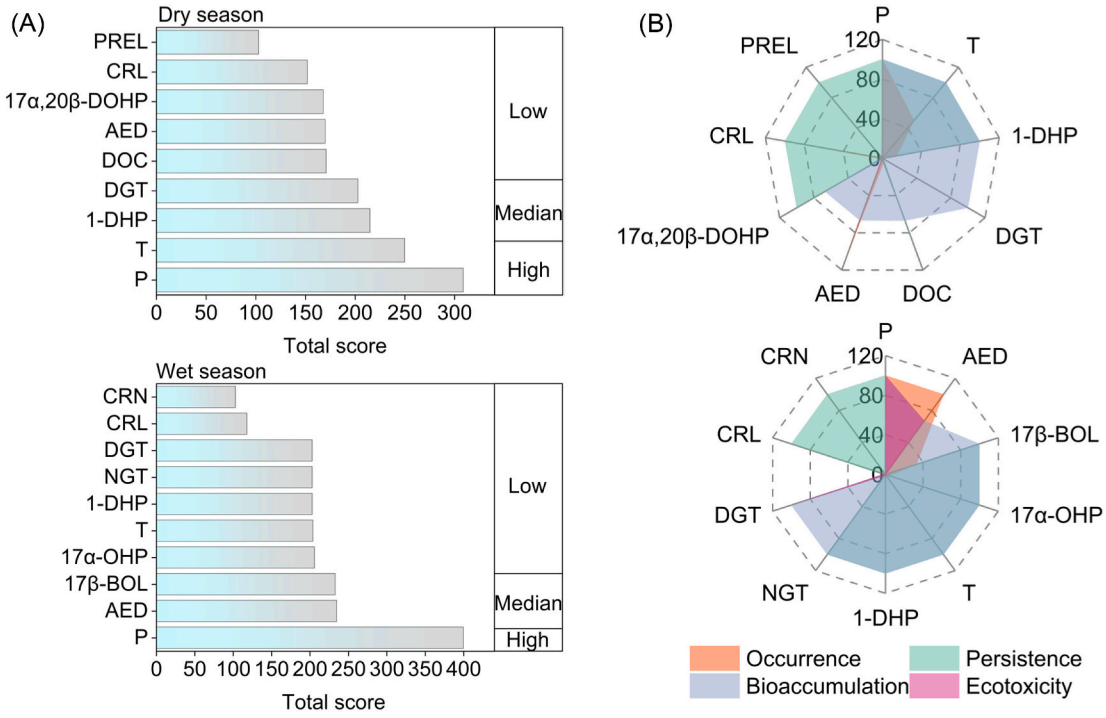

Fig. 5. The total scores for priority contaminants were determined using the multi-criteria scoring method (A). High, median, and low represent high-, medium-, and low-priority contaminants, respectively. The score for each criterion responsible for prioritization (B).

Based on our prior research, the riverine flux of steroids from the JR to the JRE was estimated at 94 kg per year (Xu et al., 2023). Steroids in the estuarine environment originate from both river discharge in the Jiulong River watershed and from aquaculture activities, undergoing various environmental processes, including degradation, water-sediments transformations, burial in deeper sediments, and transport to the open ocean (Xu et al., 2024). Despite the relatively low levels of steroid mass inventories and deposition fluxes in the JRE, the risk of steroid contamination remains significant and should not be overlooked.

3.5. Identification of priority pollutants

A multi-criteria assessment approach was employed to rank steroids within the JRE. The target compounds were scored and subsequently categorized into five categories (I – V) using a geometric progression scale (Table S11). Compounds with scores ranging from 248 to 309 in the dry season, and from 305 to 400 in the wet season were designated as high-priority pollutants (Class I). Similarly, compounds scoring between 199 and 248 in the dry season, and between 232 and 305 in the wet season, were classified as medium-priority pollutants (Class II). Any remaining contaminants with scores below 199 (dry season) or 232 (wet season) were designated as low-priority pollutants (Class III – V). Notably, P was identified as a high-priority pollutant in both seasons, while T was considered high-priority only during the dry season (Fig. 5). The elevated scores for P were attributed to its widespread presence (with a 100 % detection rate), strong bioaccumulation potential (log BAF values of 2.7), its persistence (half-life of 98 days), and its high toxicity, as shown by the predicted no-effect concentration (PNEC) of 0.1 ng/L. Despite relatively low detection rates and concentrations of T in the JRE, its high scores could be explained by its significant bioaccumulation potential (log BAF value of 2.2) and notable persistence (half-life of 98 days). This suggests that evaluating contaminants based solely on their environmental concentrations may not always be appropriate. Moreover, considering factors like bioaccumulation potential and biodegradability could offer more reliable indicators of contamination levels, particularly for steroids. Surprisingly, AED, a widely detected contaminant, was classified as a low-priority pollutant (Class III) during the dry season and a medium-priority pollutant (Class II) in the wet season. This classification was due to AED's rapid degradation and minimal ecotoxicity, respectively. In contrast, synthetic steroids (such as 1-DHP, DGT, and 17β-BOL) were identified as a medium priority (Class II), emphasizing their significance for monitoring and management efforts within the JRE. As for compounds like 17α,20β-DOHP, CRL, PREL, and CRN, despite their low degradability in the JRE, their overall scores were low due to their infrequent detection, limited bioaccumulation, and low toxicity, making them less likely to pose considerable ecological threats to estuarine ecosystems. Therefore, to optimize environmental monitoring and regulation efforts, it is recommended to focus on compounds classified as high- and medium-priority.

Although certain parameters within this comprehensive multi-criteria assessment rely on predictive modeling, the approach reveals that substances with lower scores could substantially affect the outcomes, thereby underscoring the limitations inherent in relying solely on a single indicator—such as toxicity—to assess and characterize contaminants. Incorporating temporal and spatial variability, distribution patterns, physicochemical properties, and ecological risks of target pollutants, a holistic system for identifying and prioritizing pollutants surpasses the efficacy of relying on static priority lists. This framework can provide critical technical support to governments in monitoring steroid pollutants, facilitating more scientifically grounded and efficient pollutant management and environmental safeguarding.

3.6. Environmental implication and limitations

Incorporating prioritized assessment into environmental risk management is crucial for effectively addressing complex environmental contaminants like steroids in estuarine environments. A prioritized assessment allows for the identification and ranking of risks based on their occurrence, persistence, and potential impacts on human health and ecosystems. This approach enables environmental managers and policymakers to allocate resources more efficiently, targeting the most harmful pollutants first and devising specific management strategies for

each identified risk. For instance, in the case of steroids in the JRE, a prioritized assessment can help distinguish between high-priority areas with severe contamination and low-priority zones where risk levels are manageable. Factors such as the concentration of steroids, their toxicity, bioaccumulation potential, and their effects on aquatic life can all be considered in the prioritization process.

Our research still has several limitations. Due to the lack of experimentally derived aquatic ecotoxicity data for the majority of steroids, we had to rely on ECOSAR model predictions for risk assessment. Although ECOSAR provides comparable toxicity benchmarks for substances with limited data through structure-activity relationships, its predictions mainly focus on acute lethal endpoints (e.g., LC50) and functional group generalization, which may not accurately reflect the unique low-concentration chronic effects of steroids (such as endocrine disruption and reproductive suppression). For instance, an experimental study demonstrated that the fish reproduction NOEC (100 ng/L) for synthetic progestin levonorgestrel was four orders of magnitude lower than ECOSAR-predicted chronic values (Runnalls et al., 2013), suggesting ECOSAR may significantly underestimate ecological risks of certain steroids. Furthermore, ECOSAR does not incorporate receptor-mediated toxicity pathways, which represent the core mechanisms through which steroids induce ecological effects at ng/L concentrations. This omission may result in predictions failing to reflect the most critical toxic effects. To address these limitations, we propose two key directions for future research: First, prioritizing standardized chronic testing of steroids could provide more robust experimental evidence for ecological risk assessment. Second, enhancing predictive models by incorporating additional environmental factors and biological response variables would help improve model predictive capability and accuracy.

4. Conclusions

This research provides an in-depth examination of the spatiotemporal patterns and behavior of 22 steroids and their metabolites in the JRE, marking the first attempt to establish a prioritized list of pollutants for management in this region. Among the steroids examined, seven steroids were detected in water samples, and ten steroids in sediments, with natural steroids being more prevalent. The distribution patterns of these compounds varied significantly across different temporal and spatial scales. Higher concentrations of steroids in water were predominantly observed near the outflows of the three primary tributaries of the JR. In the dry season, elevated steroid levels were mainly found in sediments on the northern side of the JRE, whereas during the wet season, the southern side exhibited higher concentrations. The variation in steroid distribution can be attributed to both their hydrophobic characteristics and environmental factors such as temperature, salinity, pH, chlorophyll *a*, and total organic carbon content. Mass inventory analyses revealed that over 90 % of the steroid mass was retained in sediments during both dry and wet seasons, highlighting a significant accumulation of steroids in sedimentary environments. The highest deposition flux was recorded at 5.4 ng cm^{-2} yr^{-1}, which, though low, suggests a gradual buildup of these compounds. A multi-criteria assessment approach identified two steroids (P and T) as the highest-priority pollutants in the dry season, while steroid P was singled out as the primary concern in the wet season. Notably, AED, a commonly detected natural steroid, was classified as a medium-to-low priority pollutant. These findings provide crucial insights into steroid pollution dynamics and offer valuable information for effective environmental management and risk mitigation in estuarine ecosystems.

CRediT authorship contribution statement

Ru Xu: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Nian-Nian Wu:** Methodology, Formal analysis. **Shan Liu:** Writing – review & editing, Methodology, Investigation. **Hui Chen:** Methodology, Investigation. **Qin-Wei Hao:** Methodology, Investigation. **Yong-Xia Hu:** Methodology, Investigation. **Bing Hong:** Investigation. **Shen Yu:** Resources, Conceptualization. **Xiang-Rong Xu:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2025.117980.

Data availability

Data will be made available on request.

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