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Bioaccumulation of 17β-estradiol in Freshwater Gastropod Sulcospira testudinaria under increasing Anthropogenic Pressure in the Brantas River, Indonesia

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ABSTRACT

The presence of endocrine-disrupting compounds in freshwater ecosystems has raised increasing concern due to their potential ecological impacts, particularly in rivers exposed to growing anthropogenic pressure. This study investigated the occurrence and bioaccumulation of 17β-estradiol (E2) in river water and in the freshwater gastropod *Sulcospira testudinaria* collected from ten sampling stations along the Brantas River, Indonesia. Water and tissue samples were obtained during the dry season to minimise hydrological variability and improve spatial comparability among stations. E2 was detected in river water at all stations at nanogram-per-litre levels, while concentrations in *S. testudinaria* tissues were substantially higher, reaching up to 470 ng g^{-1} wet weight, with elevated values generally observed in middle and downstream sections. Positive relationships were observed between E2 concentrations in water and gastropod tissues, indicating spatially dependent bioaccumulation along the river continuum. The results indicate that anthropogenic activities contribute to estrogenic contamination in the Brantas River system. The observed patterns, together with environmental conditions influenced by organic and nutrient loading, suggest enhanced persistence and bioavailability of E2 in impacted areas. *S. testudinaria* shows potential as a benthic indicator organism for reflecting estrogenic contamination in tropical freshwater environments, although its applicability should be interpreted within the context of site-specific environmental variability.

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INTRODUCTION

Freshwater ecosystems play a critical role in supporting biodiversity and providing essential ecosystem services; however, they are increasingly impacted by anthropogenic activities that deteriorate water quality (Khosrovyan, 2024). Rapid urbanisation, agricultural intensification, and industrial development have contributed to the continuous input of emerging contaminants into river systems worldwide. Among these, endocrine-disrupting chemicals (EDCs) are of particular concern due to their persistence in aquatic environments and their ability to elicit biological effects at very low concentrations, thereby interfering with endocrine regulation in

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aquatic organisms (Wang et al., 2023).

One of the most frequently detected EDCs is E2, a natural estrogen primarily derived from domestic wastewater, livestock operations, and pharmaceutical residues. Conventional wastewater treatment processes are often insufficient to fully remove E2, allowing its continuous release into surface waters (Grzegorzek et al., 2024). Even at nanogram-per-litre concentrations, E2 has been reported to induce endocrine disruption, reproductive impairment, and physiological alterations in aquatic organisms (Gonsioroski et al., 2020; Ma et al., 2022). Despite increasing attention, most previous studies have focused on its occurrence in water or its accumulation in fish, while benthic invertebrates remain comparatively underrepresented.

The environmental fate and bioavailability of E2 are strongly influenced by physicochemical parameters such as pH, temperature, dissolved oxygen, and organic matter content, which regulate its degradation, transport, and partitioning in aquatic systems (Georgin et al., 2024; Zhou et al., 2024). These interactions suggest that water-phase measurements alone may not adequately reflect organism-level exposure, particularly for benthic species that are in close contact with sediments where contaminants may accumulate.

Freshwater gastropods are widely recognised as suitable bioindicator organisms due to their limited mobility, direct exposure to both sediment and water, and capacity to accumulate contaminants. *Sulcospira testudinaria* has been previously applied as an indicator species for aquatic pollution owing to its sensitivity and bioaccumulative potential (Dashmahapatra et al., 2023; Ciślak et al., 2023). Previous studies have reported its use in monitoring heavy metals and microplastics (Hertika et al., 2023; Buwono et al., 2025); however, its application in assessing endocrine-disrupting hormones, particularly E2, remains largely unexplored, especially in tropical river systems.

The Brantas River, one of the largest river systems in Indonesia, is subjected to intensive anthropogenic pressures from domestic, agricultural, and industrial activities, resulting in continuous pollutant loading along its course (Lusiana et al., 2020; Irawanto et al., 2024; Anggraini, 2025). However, information regarding estrogenic contamination and its bioaccumulation in benthic organisms within this river system is still limited. Therefore, this study aims to (i) determine the concentration of E2 in surface water and in *S. testudinaria* tissues along the Brantas River, and (ii) evaluate its spatial distribution and bioaccumulation patterns. This research is expected to provide new insights into estrogenic contamination dynamics and the potential use of benthic gastropods as bioindicator organisms in tropical river ecosystems.

MATERIALS & METHODS

Study Area and Sampling Design

This study was conducted in the Brantas River watershed, East Java, Indonesia, from June to August 2025. Sampling was carried out biweekly at ten stations representing upstream, middle, and downstream sections of the river. The stations were selected to capture spatial variability in anthropogenic pressure, land use, and water quality conditions. Geographic coordinates of each station were recorded using a Global Positioning System (GPS) and are presented in Table 1, while the spatial distribution of sampling sites is shown in Figure 1. These stations formed the spatial framework for assessing E2 concentrations and associated environmental parameters along the river continuum.

Water Quality Measurement Methods

Water samples were collected at approximately 30 cm below the surface using pre-cleaned polyethylene bottles. Samples were stored in ice-cooled containers at 4 °C and transported to the laboratory for analysis. To evaluate environmental relevance, measured water quality

Table 1. Location and Conditions of Sample Collection Sites

Point	Coordinate	Regional Name	Site Description
ST. 1	-7.754103, 112.526556	Brantas Village, Bumiaji, Batu	Upstream area with the main spring source of the Brantas River
ST. 2	-7.924853, 112.639662	Polowijen Village, Blimbing, Malang	Densely populated area with light industries receiving domestic wastewater discharges
ST. 3	-7.445773, 112.257702	Jatigedong, Ploso, Jombang	Area dominated by agricultural activities and food seasoning industries
ST. 4	-7.794250, 112.005750	Semampir, Kediri	Highly populated area with cigarette manufacturing industries
ST. 5	-7.775368, 112.019220	Putih Village, Kediri	Agricultural area serving as a transition zone between industrial and rural regions
ST. 6	-8.115940, 112.052969	Buntaran Village, Rejotangan, Tulungagung	Residential and agricultural area receiving runoff from upstream sections
ST. 7	-8.094269, 111.978788	Pulosari Village, Ngunut, Tulungagung	Area with intensive agricultural activities
ST. 8	-8.143674, 112.161880	Kanigoro, Blitar	Area dominated by residential settlements and small-scale industries
ST. 9	-7.347581, 112.682405	Warugunung Village, Karangpilang, Surabaya	Downstream area with a high concentration of large-scale industries and multisectoral wastewater discharges
ST. 10	-7.446525, 112.462144	Lengkong Village, Mojokerto	Textile industrial area

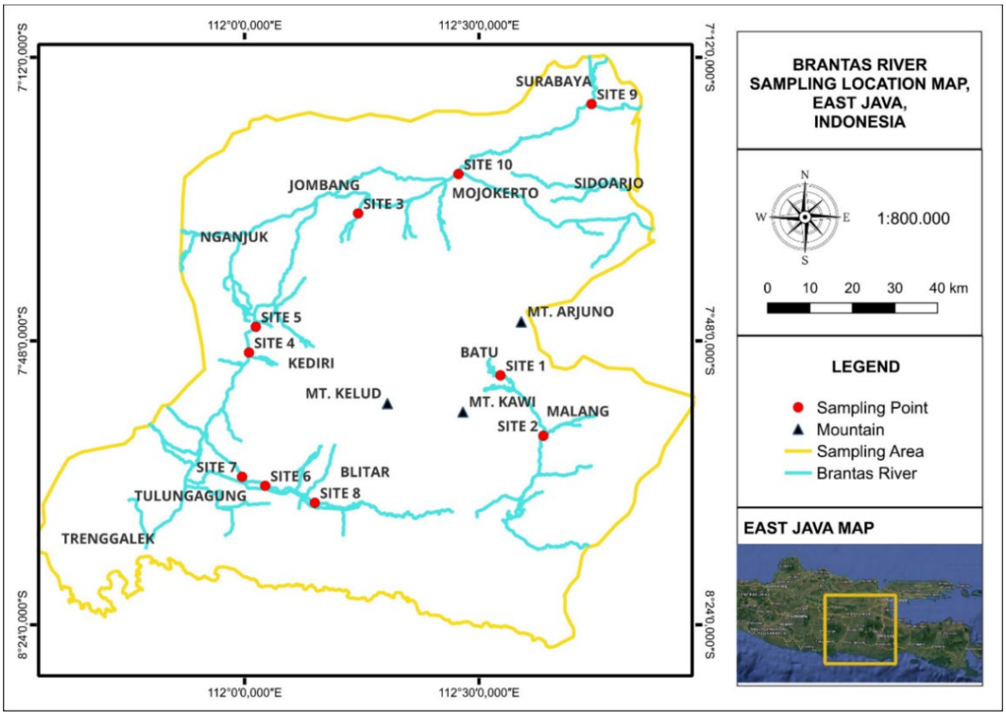

Fig. 1. Research map

parameters were compared with Indonesian Government Regulation No. 22 of 2021 (Class II water quality standards).

Sampling Sulcospira testudinaria

Specimens of the freshwater gastropod *Sulcospira testudinaria* were collected manually from river margins and shallow littoral zones (0–50 cm depth) at each sampling station. At each station, three replicate samples consisting of five adult individuals per replicate (total 15 individuals per station) were collected to ensure statistical representation. Only adult individuals with shell lengths of 2.5–3.5 cm were selected to minimise variability associated with ontogenetic differences. Individuals were carefully detached from submerged substrates (rocks and stones) using gloved hands and plastic forceps to avoid tissue damage. Collected specimens were rinsed with site water to remove adhering debris and placed in clean, labelled polyethylene bags. Samples were transported in an ice box at approximately 4 °C to the laboratory. Upon arrival, specimens were stored at −10 °C until analysis. Prior to analysis, individuals were thawed at room temperature. Soft tissues (whole-body excluding shell) were dissected, pooled per station to form composite samples, and homogenised for subsequent E2 analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

Concentrations of E2 in water and gastropod tissues were determined using a competitive ELISA kit due to its high sensitivity and specificity. Water samples were filtered through 0.45 µm membrane filters prior to analysis. Tissue samples were homogenised in phosphate buffer solution (pH 7.4) and centrifuged, and the supernatant was used for analysis. Aliquots (50 µL) of standards, controls, and samples were added to antibody-coated microplates, followed by 50 µL of enzyme-labelled E2 (HRP conjugate). After incubation for 1 hour at room temperature, plates were washed to remove unbound reagents. TMB substrate was added and incubated for 15 minutes, and absorbance was measured at 450 nm using a microplate reader. Calibration curves ranged from 0.1 to 1000 ng L^{-1} for water samples and 0.1 to 1000 ng g^{-1} wet weight for tissue samples. The method has been validated in previous studies with good agreement to chromatographic techniques (Uraipong et al., 2018).

Data Analysis

Descriptive statistics (mean, standard deviation, minimum, and maximum) were used to summarise E2 concentrations in water and *S. testudinaria* tissues. Spatial distribution was visualised using tables and bar charts. The relationship between water and tissue concentrations was evaluated using Pearson correlation analysis. Scatter plots with linear regression and coefficient of determination (R^2) were used to describe relationships between variables. Statistical significance was set at $p < 0.05$. All analyses were performed using Microsoft Excel 2021.

RESULTS & DISCUSSION

Water Quality

The physicochemical water quality of the Brantas River was evaluated to provide an environmental context for the occurrence and distribution of estrogenic compounds in the aquatic system. Key parameters, including temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD_5), and ammonia (NH_3), were analysed to assess spatial variations across the sampling stations. The results are presented in Figures 2, which illustrate the distribution

Table 2. Methods or instruments for water quality measurement

Parameter	Method / Instrument
Temperature (°C)	Lutron PDO-520
pH	pH meter
Dissolved Oxygen (DO) (mg/L)	Lutron PDO-520
Biological Oxygen Demand (BOD_5) (mg/L)	5-day incubation method
Ammonia (NH_3) (mg/L)	Spectrophotometric method

patterns of each parameter at each station. These parameters are essential for understanding environmental conditions that may influence the persistence, transport, and bioavailability of E2 in the river system.

The physicochemical characteristics of the Brantas River were assessed to provide environmental context for E2 distribution (Figure 2). Temperature and pH remained relatively stable across all stations and complied with Indonesian Government Regulation No. 22 of 2021 (Class II), indicating limited spatial variability in these parameters. In contrast, dissolved oxygen (DO), biological oxygen demand (BOD_5), and ammonia (NH_3) showed clear spatial variation. DO met the Class II standard (≥ 4 mg L^{-1}) at ST.1–ST.8 but decreased at ST.9–ST.10, indicating localized oxygen depletion in downstream areas. BOD_5 exceeded the standard (≤ 3 mg L^{-1}) from

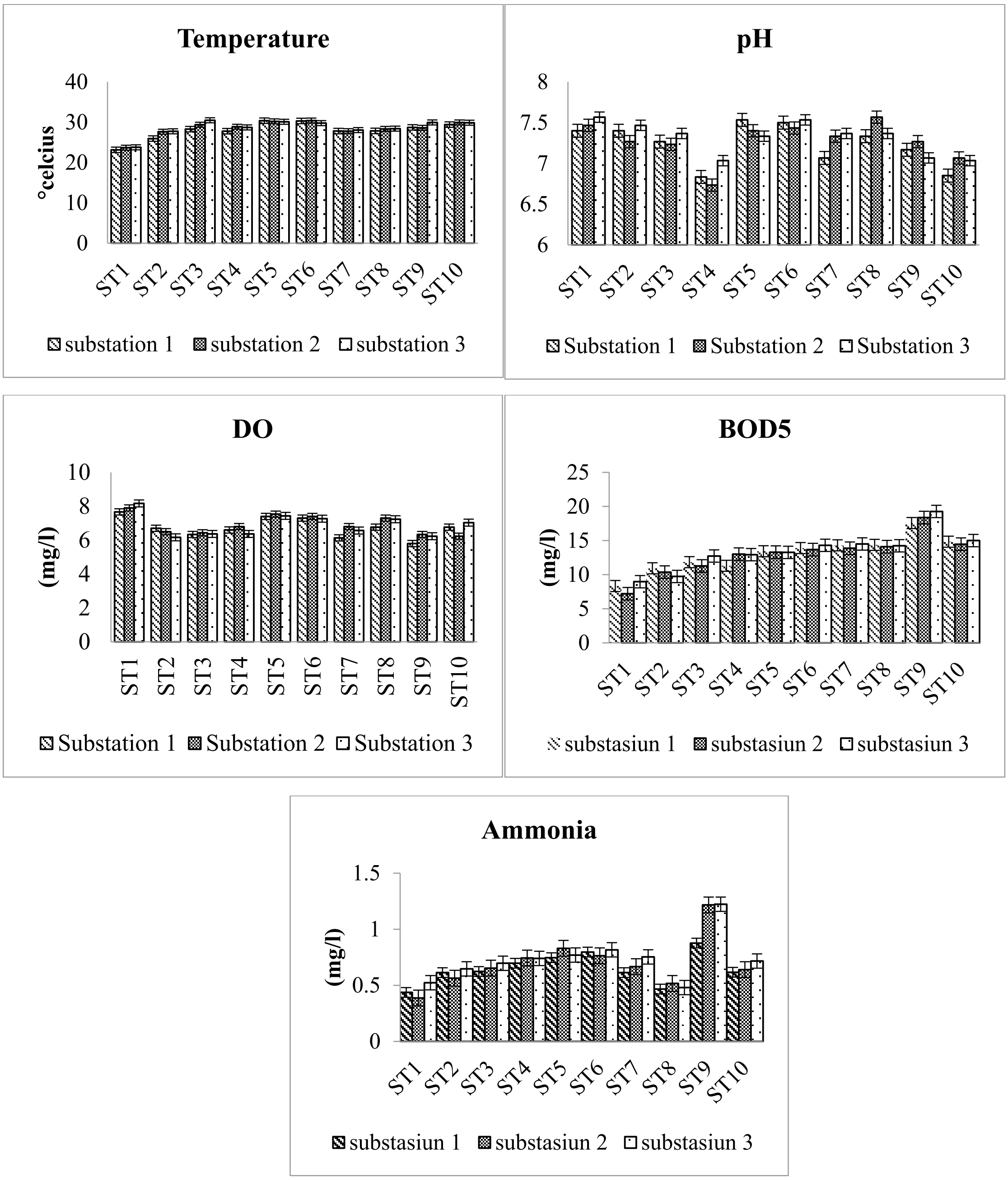

Fig. 2. Results of water quality analysis

ST.5 onward, while NH_3 exceeded the permissible limit (≤0.2 mg L^{-1}) at ST.4–ST.10.
The co-occurrence of elevated BOD_5 and NH_3 with reduced DO reflects increased organic and nutrient loading, particularly in middle and downstream sections (Yuan et al., 2024). Such conditions may reduce oxygen availability and slow the degradation of organic micropollutants, thereby enhancing their persistence in aquatic environments. In addition, NH_3 enrichment is commonly associated with wastewater inputs, which are also primary sources of estrogenic compounds, suggesting overlapping pollution sources along the river continuum (Georgin et al., 2024; Grzegorzek et al., 2024).

17β-estradiol (E2) in water

The concentration of E2 in river water varied among sampling stations, indicating differences in contaminant inputs and environmental processes. Spatial patterns showed that E2 was detected at all stations, with varying concentrations reflecting localised sources and environmental conditions (Schuh et al., 2011). Stations exhibiting elevated organic and nutrient loads tended to show higher E2 concentrations, suggesting that water quality conditions may influence its persistence and distribution. These findings support the role of environmental factors in regulating the occurrence of estrogenic compounds in freshwater systems. The spatial distribution of E2 concentrations in water is presented in Figure 3.
E2 was detected at all sampling stations along the Brantas River, indicating widespread occurrence of estrogenic compounds (Figure 3). Concentrations exhibited spatial variation, with lower levels at upstream stations (ST.1–ST.2) and gradual increases toward downstream areas. The downstream station ST.9 showed relatively higher concentrations compared to upstream sites, reflecting cumulative inputs from domestic, agricultural, and industrial activities along the river system. The observed gradual increase rather than abrupt changes suggests diffuse and continuous estrogenic inputs typical of mixed land-use watersheds (Fairbairn et al., 2016). The spatial pattern of E2 is consistent with water quality conditions, where elevated BOD_5 and NH_3 in middle and downstream sections may contribute indirectly to its persistence by reducing degradation efficiency under oxygen-limited conditions. These findings indicate that environmental stressors and contaminant sources are co-occurring within the same spatial zones, particularly in downstream regions. Overall, the longitudinal distribution of E2 demonstrates cumulative enrichment toward downstream stations, consistent with previous studies in urbanized river systems impacted by incomplete wastewater treatment (Dai et al., 2016; Rodríguez-Hernández et al., 2022).

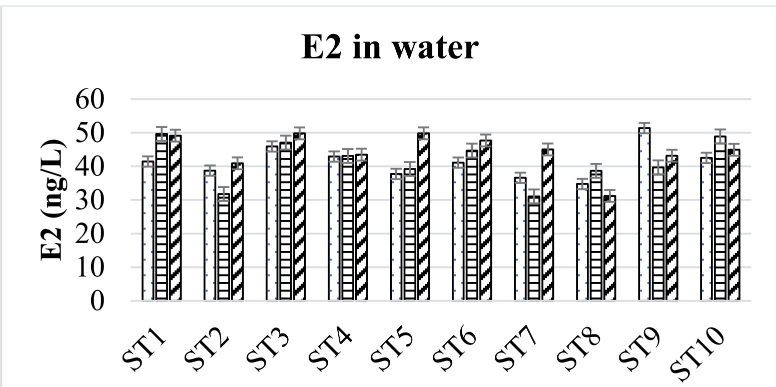

Fig. 3. Concentration E2 in water

E2 concentrations in Sulcospira testudinaria

The concentration of E2 in *S. testudinaria* tissues showed spatial variation among stations, indicating differences in exposure and accumulation patterns. As a benthic organism with continuous contact with sediments and overlying water, *S. testudinaria* is particularly susceptible to contaminant uptake. Higher tissue concentrations observed at certain stations suggest that local environmental conditions, including water quality and contaminant availability, may influence bioaccumulation processes. These results highlight the potential of *S. testudinaria* as a representative organism for assessing estrogenic contamination in freshwater ecosystems. The spatial distribution of E2 concentrations in *S. testudinaria* tissues is presented in Figure 4.

The concentration of E2 in *S. testudinaria* showed clear spatial variation along the Brantas River (Figure 4). Lower tissue concentrations were observed at upstream stations, with values around ~150 ng g^{-1} wet weight, while higher concentrations were recorded in downstream stations. The highest accumulation was observed at ST.9, reaching 470 ng g^{-1} wet weight, indicating strong downstream enrichment. Intermediate values were observed at middle-stream stations, reflecting gradual increases in exposure along the river continuum.

This pattern indicates progressive bioaccumulation of E2 in gastropod tissues in response to increasing environmental contamination. As a benthic organism, *S. testudinaria* is continuously exposed to both water and sediment compartments, which act as reservoirs for organic contaminants, including steroid hormones. The elevated concentrations in downstream stations suggest prolonged exposure to contaminated environments rather than short-term episodic pollution events. Sediment-associated organic matter and reduced oxygen conditions in downstream areas may further enhance the persistence and bioavailability of E2, increasing uptake by benthic organisms (Gibson et al., 2015). Overall, the spatial distribution of E2 in *S. testudinaria* closely follows environmental contamination gradients, supporting its potential as a bioindicator species for estrogenic pollution in freshwater ecosystems.

Correlation between E2 concentrations in water and Sulcospira testudinaria

To evaluate the relationship between environmental exposure and biological accumulation, the correlation between E2 concentrations in river water and in *S. testudinaria* tissues was examined. This analysis provides insight into the extent to which waterborne E2 contributes

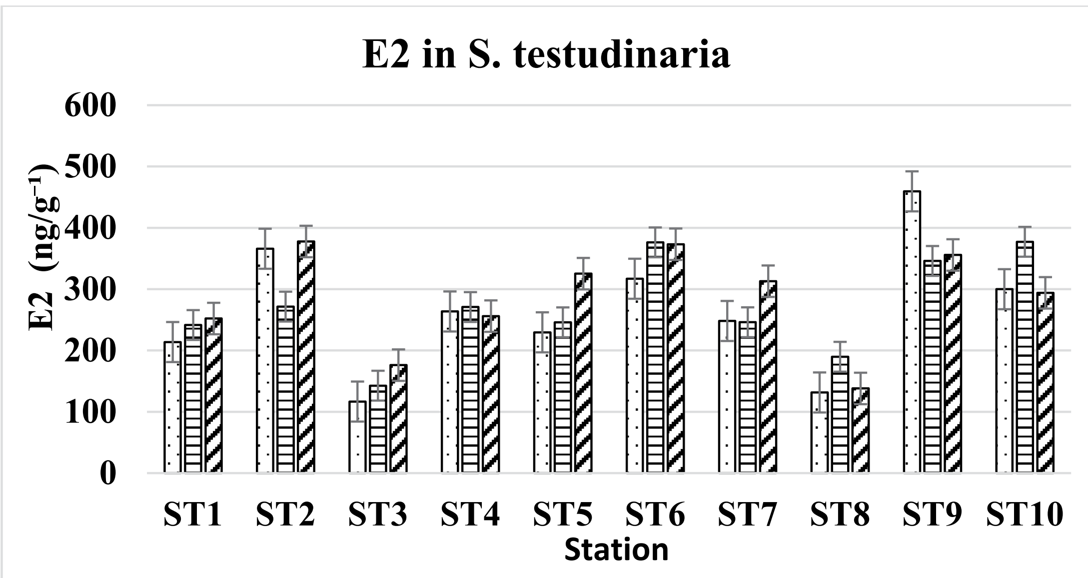

Fig. 4. Concentrations of E2 in S. testudinaria

to hormone accumulation in benthic organisms. Understanding this relationship is essential for assessing the suitability of *S. testudinaria* as a bioindicator of estrogenic contamination in riverine ecosystems. The correlation analysis also helps to clarify whether spatial variations in water contamination are reflected in tissue-level E2 burdens.

Pearson correlation analysis was performed to evaluate the relationship between E2 concentrations in water and *S. testudinaria* tissues at each sampling station (Table 2). In this analysis, n = 9 refers to the number of biological replicates per station used for statistical evaluation, consisting of composite tissue samples derived from pooled individuals.

The results showed positive correlations between water and tissue concentrations across all stations, with correlation coefficients ranging from moderate to strong (r = 0.508–0.857). This indicates that waterborne E2 contributes to its accumulation in benthic gastropods; however, the strength of this relationship varies spatially among sampling locations. Significant correlations ($p < 0.05$) were predominantly observed at downstream stations (ST.5–ST.9), which are characterized by higher anthropogenic pressure, including dense population areas and intensive industrial and domestic wastewater inputs. These conditions likely result in more continuous E2 inputs, leading to relatively stable environmental concentrations and enhanced bioavailability for benthic organisms. Similar patterns have been reported in river systems receiving sustained wastewater discharge, where benthic invertebrates exhibit stronger contaminant-body burden relationships under stable exposure conditions (Bergman et al., 2013; Sornalingam et al., 2021).

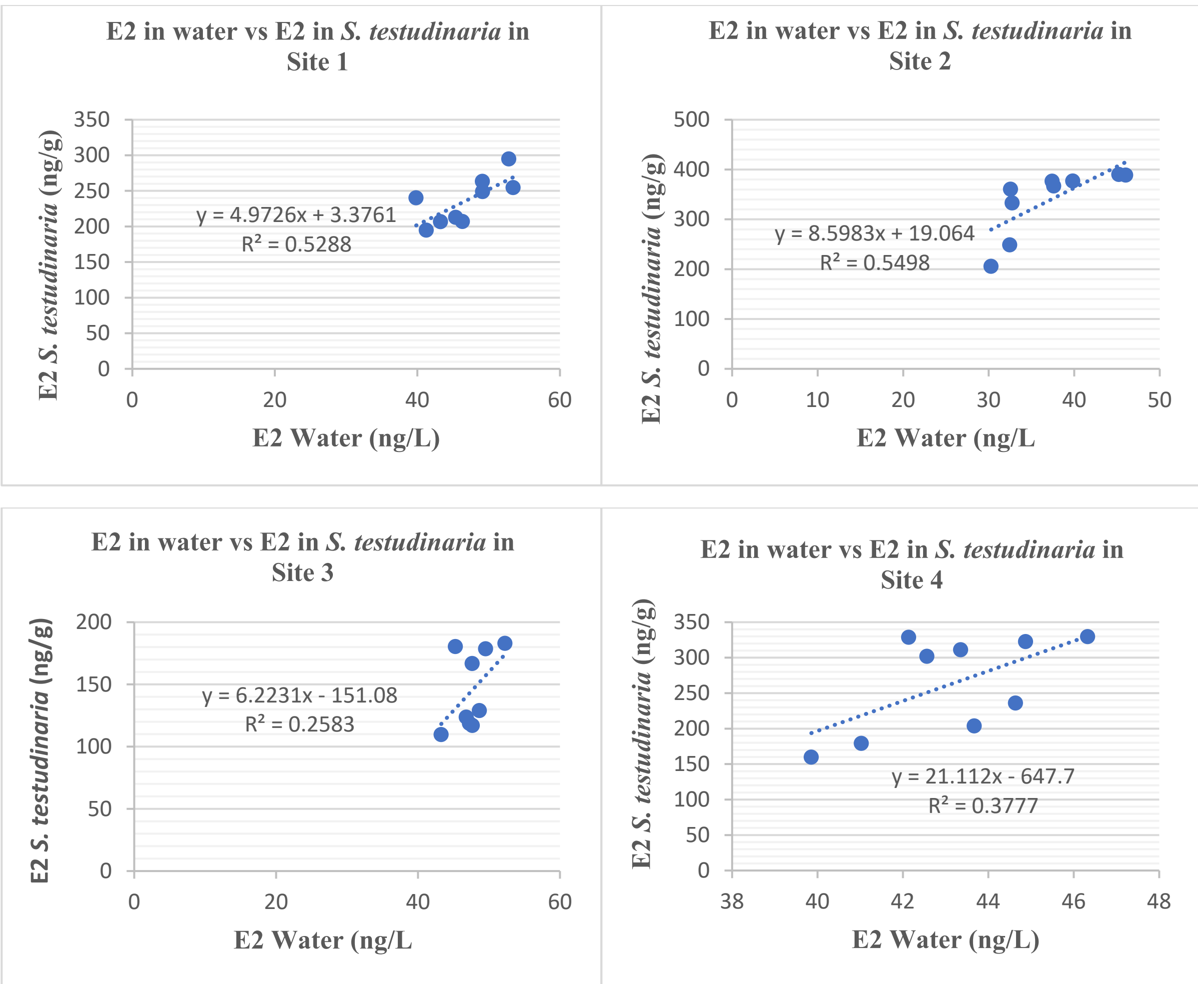

Fig. 5. Correlation E2 water vs E2 in S. testudinaria

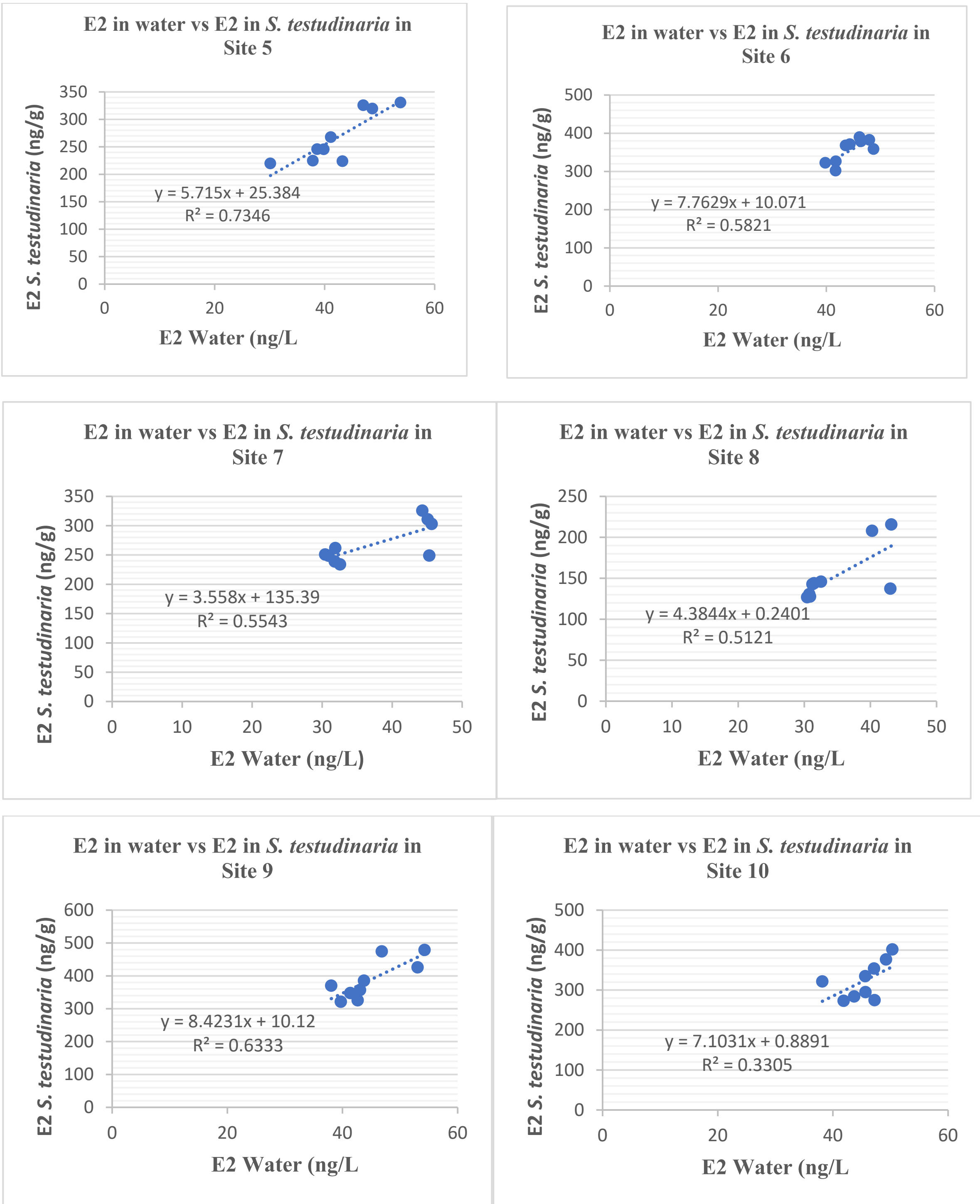

Fig. 5. Correlation E2 water vs E2 in S. testudinaria

In contrast, weaker or non-significant correlations observed at certain upstream and midstream stations (e.g., ST.3, ST.4, and ST.10) may be attributed to more variable exposure conditions, including dilution effects, intermittent pollutant inputs, and environmental partitioning processes. E2 can adsorb to suspended particles and sediments, which may decouple short-term water concentrations from tissue accumulation in benthic organisms (Combalbert & Hernandez-Raquet, 2010; Liu et al., 2019). Overall, the spatial variability in correlation strength highlights the influence of local environmental conditions on E2 exposure dynamics. Rather than weakening the bioindicator relevance of *S. testudinaria*, these findings emphasize the importance of considering site-specific environmental heterogeneity in interpreting estrogenic contamination in tropical river systems.

Analytical quantification was conducted using ELISA, which provides high sensitivity for screening estrogenic compounds in environmental samples. However, potential cross-reactivity with structurally similar compounds may influence compound-specific accuracy. Therefore, future studies are recommended to apply confirmatory techniques such as LC–MS/MS to improve analytical specificity and strengthen compound-level interpretation.

CONCLUSION

This study demonstrates the presence and bioaccumulation of E2 in the freshwater gastropod *S. testudinaria* along the Brantas River, Indonesia, reflecting increasing anthropogenic pressure within the river system. E2 was detected across all sampling stations, with spatial patterns indicating higher accumulation in downstream areas influenced by more intensive human activities. Positive correlations between E2 concentrations in water and gastropod tissues were observed across stations, with stronger and statistically significant relationships occurring primarily at middle and downstream sites. These results indicate that *S. testudinaria* responds to spatial variations in estrogenic contamination through bioaccumulation processes associated with environmental exposure gradients.

Overall, *S. testudinaria* shows potential as a useful benthic organism for reflecting estrogenic contamination in tropical freshwater systems due to its sedentary lifestyle and close association with sediment–water interfaces. However, the variability in correlation strength across stations highlights the importance of local environmental conditions, contaminant sources, and ecosystem dynamics in influencing bioaccumulation patterns. Therefore, its application as a bioindicator should be considered within a broader environmental and ecological context. This study provides baseline information for future assessments of estrogenic pollution and supports further exploration of benthic gastropods in biomonitoring frameworks for tropical river systems.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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