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Human health risk attributed to consumption of seafood and recreation swimming in Negombo Lagoon, Sri Lanka: An assessment on lagoon water and inhabitant oysters (*Crassostrea cucullata* Born, 1778)

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A R T I C L E I N F O

Original content: Assessment of Ecological Health on Negombo Lagoon Datasheet (Original data)

Keywords:
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A B S T R A C T

The Negombo Lagoon is a coastal lagoon influenced by local communities that introduce waste into its ecosystem. This study examined seven sewage entry points, out of which five sites were chosen for oyster sampling based on availability. Physicochemical and microbiological parameters of water (measured in triplicate at each site, $n = 84$) and oyster samples (total length, TL > 6 cm, $n = 30$) were assessed. Variation in regional coliform contamination was analyzed employing a one-way analysis of variance (ANOVA). Results indicated that the northern part of the lagoon exceeded recommended coliform thresholds for swimming (total coliform concentration (TCC) < 126 most probable number (MPN)) and seafood consumption (TCC < 100 MPN/g), indicating the presence of *Escherichia coli*. Water quality indices affirmed fecal pollution, except in the southern part of the lagoon. Furthermore, the study found high oyster consumption (76.7 %), elucidating that oysters from the northern part of Negombo Lagoon pose health risks.

1. Introduction

Coastal lagoons, characterized by their shallow depths of less than five meters and are often aligned parallel to coastlines, are partially or completely separated from the sea by natural barriers (Kjerfve, 1994; Newton et al., 2018). These lagoons serve as ecologically significant habitats supporting a diverse range of species, including finfish, crabs, and oysters. Lagoon ecosystems, which comprise approximately 13 % of coastlines globally, play a pivotal role in sustaining biodiversity and the livelihoods of coastal communities by serving vital sources of dietary protein and economic opportunities (Newton et al., 2018; Derolez et al., 2023; Rodrigues-Filho et al., 2023).

Nonetheless, the invaluable economic and ecological functions of coastal lagoons are susceptible to anthropogenic disruptions (Derolez et al., 2020; Madarasinghe et al., 2020a, 2020b; Lacoste et al., 2023), especially the introduction of fecal contaminants originating from human and livestock waste. Anthropogenic activities, such as seepage from sewer systems, direct discharge of untreated sewage, and mismanagement of livestock waste can introduce a spectrum of pathogens and pollutants that critically compromise the overall quality of lagoon water. The microbial oxidation of organic matter initiated by these contaminants can lead to a cascade of effects, resulting in the depletion of dissolved oxygen and the development of anoxic or hypoxic conditions that adversely impact the survival and well-being of fish

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species (Selig et al., 2018; Richardson and Soloviev, 2021). Among those, water pathogens make a high impact on economically important seafood species such as oysters, mackerel, tilapia, and catfish (Pernet et al., 2012; Lafferty et al., 2015; Olugbojo and Ayoola, 2015).

The proliferation of exogenous bacteria, brought forth by the influx of human and livestock-associated pollutants, increases the concentration of coliform bacteria within the ecosystem. This poses a risk for the contamination of aquatic species, particularly sedentary organisms that are more prone to prolonged exposure (Kataržytė et al., 2018). The introduction of sewage into coastal lagoons also contributes positively to the availability of nutrients for primary productivity (Howarth et al., 2021). This creates a positive feedback loop for the plankton population within the ecosystem. As oysters are filter feeders, they actively filter plankton from the water for food. Hence, oysters mostly colonize such areas of the lagoon due to food availability (Bhattacharyya et al., 2010), often settling on hard substrates such as mangrove roots or rocky reefs. Studies have shown that food availability is an inducer of spawning in seafood species such as oysters (Nowland et al., 2021). This indicates that sewage discharge areas with high nutrient and plankton abundance are likely to have a high oyster population, which would be a hotspot for oyster harvesting by fishers, even though those areas are prone to harbor fecal pathogens.

Hence, systematic assessment of fecal contamination in coastal ecosystems becomes essential to protect the ecological integrity of these habitats and public health. Efforts to maintain the integrity of water bodies reserved for recreational activities and fisheries have been proven to work based on frequent monitoring of coliform concentrations. This, in turn, led to the establishment of standardized threshold values aimed at mitigating human health risks associated with fecal contamination (Loubersac et al., 2007) (Table 1).

Considering the continuous discharge of sewage into most coastal lagoons in developing countries such as in Sri Lanka, the widespread reduction in microbiological water quality becomes an immediate concern. This compromise threatens the well-being of aquatic organisms and endangers human consumers reliant on seafood as a primary dietary source. Specifically, the presence of fecal bacteria, including the commonly used indicator *Escherichia coli*, in sewage discharges from lagoon catchment areas emphasizes the potential for waterborne contamination (Islam et al., 2018; Kataržytė et al., 2018; Lenart-Boroń et al., 2017). Hence, fecal coliforms, a subset of gram-negative bacteria capable of fermenting lactose into gas and acid, serve as vital indicators in microbial assessments of lagoon water quality and seafood safety (Li et al., 2021). The adoption of fecal coliforms, including the aforementioned *E. coli*, enhances coliform-based analyses and forms the basis of regulatory frameworks in various countries to ensure the safety of recreational waters and fishery resources alike (Li et al., 2021). The holistic contamination assessment framework, which makes use of samples from both the biotic (i.e., oysters) and abiotic (i.e., physicochemical and microbiological parameters), helps provide insight into their mutual effects and offers a unique perspective on water quality and fecal contamination assessment.

Table 1
The standard threshold values for assessing the water quality of aquatic ecosystems set out by the Canadian Council of Ministers of the Environment (CCME) (Bilgin, 2018; Solaiman et al., 2020).

Parameters	CCME standard
pH	6.5–8.5
TDS (mg/L)	<500
Nitrate (mg/L)	<48.2
Temperature (°C)	–
Dissolved oxygen (mg/L)	>5
Orthophosphate (mg/L)	<0.05
Electrical Conductivity (uS/cm)	<2500
BOD_5 (mg/L)	<3
E. coli (CFU/100 mL)	<126

TDS – Total Dissolved Solids, BOD_5 – Biological Oxygen Demand, CFU – Colony Forming Units.

The consequences of fecal contamination extend to seafood resources, with sedentary species such as oysters being particularly susceptible. Filter-feeding behaviours of oysters expose them to heightened contamination risks, as they tend to accumulate substantial concentrations of fecal contaminants present in the lagoon waters (Wang et al., 2018; Ray et al., 2021). This contamination risk assumes critical significance within coastal communities heavily reliant on oysters as a dietary protein source (Jahan and Strezov, 2019; Hong et al., 2020).

Coliforms in water bodies often signal the presence of pathogenic bacteria like *Salmonella* spp., *Shigella* spp., and *E. coli*, which are prevalent in the intestines of humans and other warm-blooded animals (Leight et al., 2018; Tambi et al., 2023). These microorganisms could pose a human health risk when ingested through seafood consumption or recreational swimming in contaminated lagoon sites (Guggenheim et al., 2020; Tambi et al., 2023). Therefore, coastal lagoons with anthropogenic impacts, especially in developing countries, require thorough investigations into fecal contamination patterns. This becomes especially important considering the potential transmission of gastroenteritis and other health issues through the consumption of contaminated seafood, particularly oysters (Obodai et al., 2010; Bell et al., 2021; Tambi et al., 2023). Therefore, the implementation of robust sanitation measures, encompassing proper sewer systems, effective farm waste management practices, and treatment facilities, emerges as a critical step to curbing the risk of seafood contamination, particularly in regions with inadequate sanitation infrastructure.

The west coast of Sri Lanka, including the Negombo Lagoon, hosts numerous coastal lagoons exposed to substantial anthropogenic pressures. Negombo, situated within the second most industrious city in Sri Lanka, contends with substantial anthropogenic pressures flowing from surrounding human activities (Cooray et al., 2021). According to the 2012 census, the Negombo Lagoon area has a population of about 5.8 million people, placing significant pressure on land use in that area (Athukorala et al., 2021). The Negombo Lagoon attracts tourists from all over the world for purposes such as boating and bird watching, consequently contributing to the booming hospitality sector. The residents are mostly engaged in fish farming, while other sectors in the area encompass hospitals, airports, and manufacturing industries (Jayewardenepura, 2021). Given its proximity to residential settlements, the Negombo Lagoon becomes vulnerable to fecal contamination, compelling a thorough investigation into its seafood resources and the associated health risks. This study focuses on sanitary health management, aiming to establish baseline information regarding the utilization of the studied sites of the Negombo Lagoon for recreational swimming and seafood consumption. The present study endeavours to achieve three main objectives: (1) assess the water quality index (WQI) across different sampling sites; (2) assess the distribution patterns of *Crassostrea cucullata* within various sectors of the lagoon; and (3) evaluate the health implications associated with the consumption of *Crassostrea cucullata* originating from the Negombo lagoon. In this study, we hypothesized that sewage contamination within the Negombo lagoon is uniform across different discharge points. Since Negombo is highly industrious, it implies a pervasive pollution scenario across the entire lagoon, emphasizing the potential health risks associated with its utilization. The investigation seeks to explore and validate this hypothesis, contributing to a comprehensive understanding of sewage pollution in the lagoon and its implications for both environmental health and seafood consumption.

2. Materials and methods

2.1. Study area overview

The study was centered within the Negombo Lagoon, a coastal lagoon situated on the west coast of Sri Lanka. The lagoon experiences a humid climatic regime (Cooray et al., 2021). The lagoon has a shallow depth of <5 m with dimensions spanning 12.5 km in length and 3.6 km in width (Mendis et al., 2020) (Fig. 1). This geographic location is notable for its status as one of Sri Lanka's most industrially developed and urbanized cities, thus subjected to a substantial influx of anthropogenic waste originating from the local population.

2.2. Sample collection procedure

Before commencing this study, ethical approval was obtained from the Ethical Review Committee of the Allied Health Science, University of Ruhuna, Sri Lanka (*Reference: 2021.08.17, 25th April 2022*). For this study, a preliminary survey ($n = 60$) was conducted using a purposive sampling technique to identify sewage discharge points into the lagoon. Additionally, to gather insights into local practices, interviews were conducted to ascertain the extent of lagoon oyster exploitation. Participants residing in the vicinity of the lagoon and its environs were specifically selected for this purpose.

Subsequently, seven conduits with the potential to introduce fecal contaminants into the lagoon were chosen for further investigation. The sources of these potential contaminants include sewage canals, avian activities, sewer systems, and piggery farms.

The water sampling, carried out from April 4th to May 6th, 2022 (Table 2), extended over four weeks to align with the southwest pre-monsoon season. This timing was selected for its climatic stability, ensuring reliable baseline measurements unaffected by excessive runoff during this period (Ganguly et al., 2015). Aseptic techniques were used to collect samples ($n = 84$) weekly into screw cups to ensure sample integrity for subsequent laboratory analysis. After collection, the samples were immediately placed in ice-cooling containers (2–6 degrees Celsius) to preserve the microbial integrity during the transportation phase.

Simultaneously, during the fourth week of sampling, the collection protocol extended to handpicking six mature oysters (with a total length, TL > 6 cm) within a 2-m radius of each designated sampling point, namely the northern, eastern, and western sides of the lagoon. Two of

Table 2
The study timeline for field survey and sample collection from the Negombo lagoon.

Date	Activities
14th March–1st April 2022	Conducting Interviews and analysing survey data
4th April 2022	Collection of water samples and measurement of physicochemical parameters
14th April 2022	Collection of water samples and measurement of physicochemical parameters
25th April 2022	Collection of water samples and measurement of physicochemical parameters
6th May 2022	Collection of water samples, measurement of physicochemical parameters and handpicking of oysters

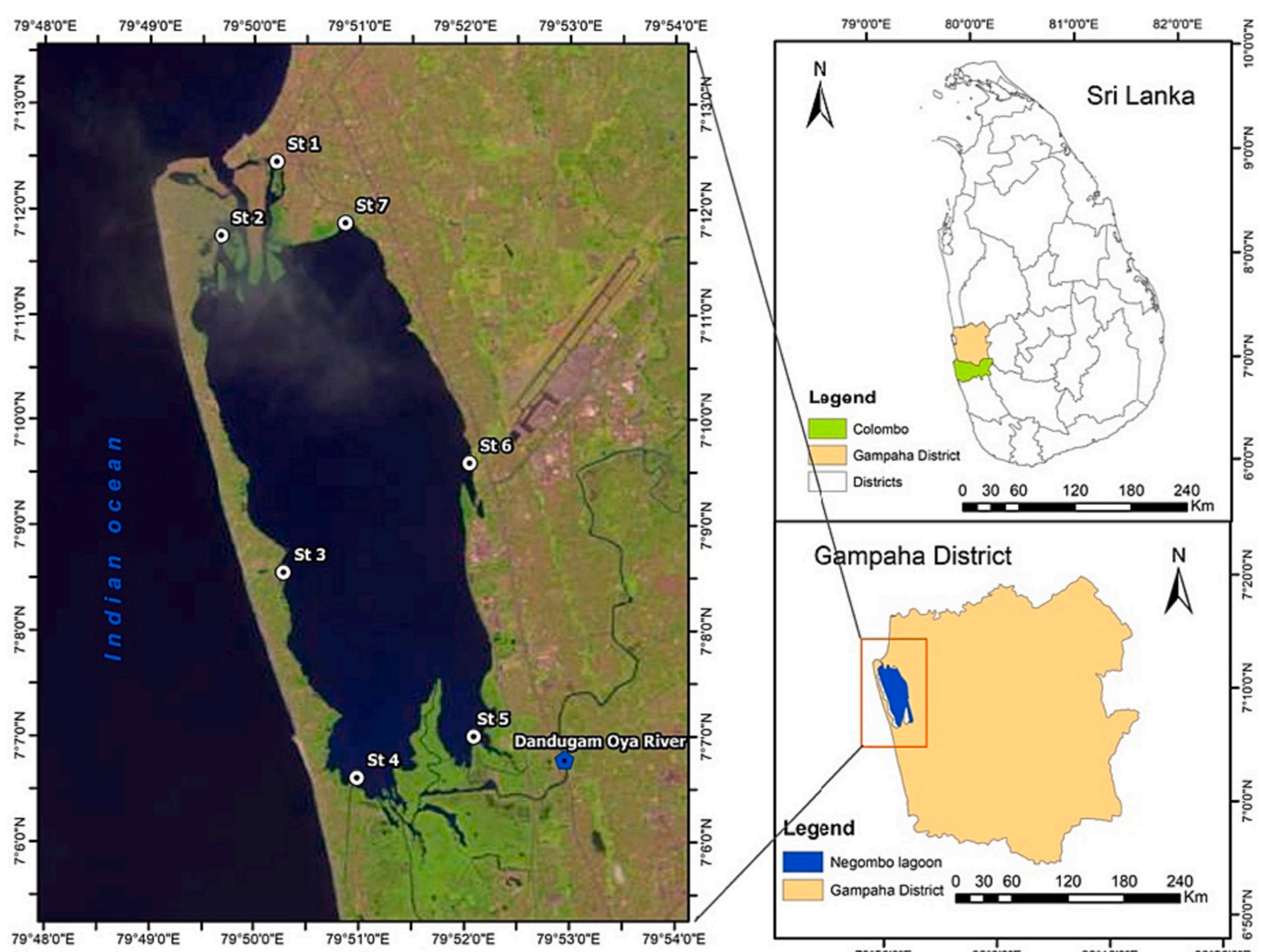

Fig. 1. The map of Negombo lagoon (left) along the west coast of Sri Lanka. ***St 1, St 2, St 3, St 4, St 5, St 6,*** and ***St 7*** are the sampling locations used for the study.

the sampling points in the southern part of the lagoon were excluded from the oyster study phase due to their absence in those areas. These oysters ($n = 30$) were carefully placed within sterile plastic bags and preserved within an ice cooler maintained at temperatures ranging between 2 and 6 degrees Celsius. At the laboratory, each of the oysters was shucked using a shucking knife. This process involved delicately opening the oyster valves to make their meat accessible for extraction and subsequent processing for microbiological investigation.

Furthermore, in-situ physicochemical parameters ($n = 84$) were assessed within each study site, with triplicate samples obtained at random intervals.

2.3. Species identification

The shucked oyster shells, previously used for microbiological studies, were employed for identification. A thorough examination of the shell's morphological features was conducted with the assistance of a marine biologist. These attributes were to identify the oyster's genus and species level with the help of identification keys outlined in the manual authored by Bussarawit et al. (2010).

2.4. Physicochemical parameter measurement

Physicochemical parameters, such as temperature, dissolved oxygen (DO), total dissolved solids (TDS), pH, electrical conductivity (EC), and salinity, were measured in-situ using a multiparametric probe (HI 98194). Water samples were collected into screw cups and transported to the laboratory to determine nitrate and phosphate concentrations through spectrophotometry (Jenway 6405, Cole-Parmer). At the laboratory, 1 mL of lagoon water was poured into the specimen vial and was used as a blank. Another 1 mL of the lagoon water sample was poured into the second specimen vial, and a reagent pack (nitrate or phosphate) (Hach) was added to it. The vial was gently inverted to allow mixing and was then placed in the spectrophotometric meter to record its wavelength as the concentration of nutrients present in the water.

In determining the biological oxygen demand (BOD_5) of the lagoon water, 250 mL of BOD_5 bottles were used. At each sampling point, two of the 250 mL BOD_5 bottles were submerged below the water surface to collect lagoon water (n = 84). The stoppers were used to close the bottles while still submerged to prevent atmospheric oxygen from contaminating the water sample. The concentration of dissolved oxygen in one of the BOD_5 bottles was fixed in situ by pipetting 1 mL of manganous sulfate which reacts with dissolved oxygen to form a brown manganic oxide precipitate. Afterwards, 1 mL of sulfuric acid was added to dissolve the brown manganic oxide into manganese ions. Potassium iodide (KI) was then added to the sample to react with manganese ion to yield Iodine which is stoichiometrically equivalent to dissolved oxygen. Both fixed and unfixed BOD_5 bottles were transported to the laboratory for Winkler titration. The unfixed BOD_5 bottle was incubated for five days after which the concentration of oxygen was fixed before measurement using the abovementioned chemical reagents. 200 mL of the sample was then titrated against sodium thiosulphate, which reacted with iodine in the samples until a pale yellow colouration was formed. Two drops of starch were added to the sample to form a blue color which was further titrated until it produced a clear color. The titer value was then recorded from the pipette. The final BOD_5 was computed using the equation below.

$$BOD_5 = (DO)_i - (DO)_f \tag{1}$$

where i is the initial dissolved oxygen level, and f is the final dissolved oxygen level.

2.5. Preparation of culture media

For the estimation of total coliforms, lauryl tryptose broth (LTB) was used. Eosin Methylene Blue (EMB) Agar served as the medium for detecting *E. coli*, with tryptone water employed for validation purposes. The media were prepared as follows:

Lauryl Tryptose Broth (Oxoid, United Kingdom): A conical flask was filled with 600 mL of distilled water. Single-strength LTB powder (21.36 g) was added and dissolved through heating. Subsequently, 10 mL of the LTB solution was poured into test tubes with inverted Durham tubes. The tubes were then autoclaved at 121 °C for 15 min. A double-strength LTB variant (42.72 g) was similarly prepared (Table 3).

Eosin Methylene Blue (Oxoid, United Kingdom): EMB powder (28.8 g) was dissolved in 800 mL of distilled water within a conical flask and boiled for complete dissolution. After cooling, the agar solution underwent autoclaving at 121 °C for 15 min. The resulting agar was poured into plates and stored at 2–8 °C, shielded from light.

Tryptone Water (Oxoid, United Kingdom): Tryptone powder (9 g) was dissolved in 600 mL of distilled water and boiled for a full dissolution. A portion (3 mL) of the tryptone solution was transferred to test tubes and autoclaved at 121 °C for 15 min.

2.6. Determination of coliform concentration in lagoon water samples (MPN/100 mL)

To assess coliform concentrations, three distinct volumes of water samples—namely, 10 mL, 1 mL, and 0.1 mL—were pipetted into separate sets of tubes, yielding a total of 15 tubes. Each tube contained Lauryl Tryptose Broth (LTB) along with a fermentation tube (refer to Table 3).

For the initiation of lactose fermentation and subsequent gas production, the tubes were incubated at 37 °C over a period of 24 to 48 h. After incubation, the tubes displaying positive gas production were counted and then matched against the most probable number (MPN) chart (Bartram and Ballance, 1996).

2.7. Detection of E. coli presence

Samples from tubes exhibiting positive gas production were acquired using an inoculating loop and subsequently streaked onto Eosin Methylene Blue (EMB) agar plates. The plates were incubated at 37 °C for a period of 24 h, after which colonies were observed for the emergence of a green metallic sheen which is indicative of *E. coli* presence within the sample.

For EMB plates that did not present a discernible green metallic sheen, a further biochemical examination known as the indole test was performed. This involved selecting a pristine colony from each plate and introducing it into tryptone water and incubating it at 37 °C for 24 h. After incubation, an addition of 5 drops of Kovac's reagent was performed to check for color change.

2.8. Enumeration of total coliforms in oyster samples

Each of the oysters was shucked, and the meat was ground using a sterile mortar and pestle. About 22.3 g of the ground meat was diluted in 200 mL of phosphate-buffered saline solution (PBS) to be used for the

Table 3
The volumes of lagoon water and medium used for the presumptive test for coliforms.

Dilution	Volume of inoculated sample (ml)/sample	Number of tubes	Volume of LTB in each test tube (ml)	Concentration of medium
10-fold	10 ml of lagoon water	5	10	Double-strength
10-fold	1 ml of lagoon water	5	10	Single-strength
10-fold	0.1 ml of lagoon water	5	10	Single-strength

LTB – Lauryl Tryptose Broth.

analysis. Three different volumes (i.e., 10 mL, 1 mL, 0.1 mL) of the oyster sample were pipetted into 5 tubes, each containing LTB with a fermentation tube (Table 3). The tubes were incubated at 37 °C for 24–48 h. After incubation, the tubes were checked for positive gas production, and the number of tubes that tested positive was counted, and their number was used to estimate the coliform abundance according to the guidelines of Bartram and Ballance (1996).

2.9. Assessment of the presence of E. coli in oyster samples

An inoculating loop was used to collect samples from the tubes that were positive for gas production and streaked onto EMB agar to check for the presence of *E. coli*. The plates were incubated at a temperature of 37 °C for 24 h and later observed for a green metallic sheen. An indole test was performed on EMB plates that did not show a clear green metallic sheen. This was done by taking a clean colony from each EMB plate and inoculating it into tryptone water, and then incubating it at 37 °C for 24 h. After incubation, 5 drops of Kovac's reagent were added to each test tube to check for indole positive or negative reactions.

2.10. Water quality index (WQI)

The water quality index at the sewage discharge locations was calculated in accordance with the Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI) (Bilgin, 2018). Temperature, nitrate, orthophosphate, pH, total dissolved solids, electrical conductivity, biological oxygen demand, and fecal coliforms were the variables used. The water quality index was calculated using the formula shown below:

$$\mathrm{CCME-WQI} = 100 - \left(\frac{\sqrt{F_1^2 \pm F_2^2 \pm F_3^2}}{1.732}\right) \tag{2}$$

where F_1 represents the number of variables/parameters whose value does not meet the standard value (failed variables) (Table 1), F_2 represents the number of individual tests/measurements that do not meet the standard values (failed test), F_3 represents the number of times by which an individual concentration is greater than the standard value. F_3 is then calculated from the normalized sum of the excursions (*nse*) from the standard values to yield a range between 0 and 100. The number of times by which an individual test is greater than the standard value is termed an "excursion". The following elaborates on how these values were obtained.

$$F_1 = \left(\frac{Number\ of\ failed\ parameters}{Total\ number\ of\ parameters}\right) \times 100$$

$$F_2 = \left(\frac{Number\ of\ failed\ individual\ tests}{Total\ number\ of\ individual\ tests}\right) \times 100$$

$$excursion_i = \left(\frac{Failed\ individual\ test\ value_i}{Standard\ value_i}\right) - 1$$

$$nse = \frac{\sum_{i=1}^{n} excursion_i}{Number\ of\ individual\ tests}$$

$$F_3 = \left(\frac{nse}{0.01nse \pm 0.01}\right)$$

The final water quality index value for each sampling site was compared to the standard water quality index chart (Table 4).

2.11. Data analysis

Data analysis was conducted using Microsoft Excel and R Statistical Software (v4.0.2; R Core Team, 2020). The hypothesis that sewage contamination was uniform across different lagoon point sources with no significant differences led to the choice of a one-way analysis of variance (ANOVA) as a suitable test for comparing responses across groups. Given the need to distinguish between various discharge sites, the Tukey HSD test was employed.

Prior to conducting the one-way ANOVA test, we assessed the parametric assumptions for the analysis. Subsequently, recognizing that the assumptions were not met, we applied a square root transformation to the response variable (Total Coliform Concentration, TCC). This transformation was done to align the data with the requisite parametric assumptions of homogeneity and homoscedasticity (Fox and Weisberg, 2019). The transformed data was used in constructing the one-way ANOVA model and for subsequent post-hoc analysis.

Descriptive analyses were carried out using Excel, and graphical representations were created using the ggplot package in RStudio (Wickham, 2016). This analytical approach ensured a robust exploration of the dataset and facilitated clear visualization of results.

3. Results

3.1. Physicochemical parameters of lagoon water

The assessment of various physicochemical parameters within the lagoon water revealed closely aligned average values across different sampling sites. Particularly, sites St 4 and St 5 exhibited relatively diminished average salinities (salinity <21 ppt) compared to the remaining sites, as outlined in Table 5. Of particular interest, St 5 demonstrated comparatively lower average coliform concentrations (TCC < 92 MPN) and biological oxygen demand ($BOD_5 < 3$ mg/L) when contrasted with the other sampling sites. These observations underscore the subtle differentiations in physicochemical attributes within the lagoon water matrix.

Table 4
The classification of the Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI) values in assessing the pollution level of the water (Bilgin, 2018).

Water quality condition	Range	Description
Excellent	95-100	The water quality is close to natural and is not under the threat of degradation
Good	80-94	The water quality is close to natural and is rarely under the threat of degradation
Fair	65-79	The water body is protected, however, sometimes can be subjected to degradation
Marginal	45-64	The water body is frequently under the threat of degradation
Poor	0-44	The water body is severely under the threat of degradation and not in the desired range for aquatic species

Table 5
Mean values (± standard deviation) of physicochemical and microbiological parameters at the different sampling sites (St) of the Negombo Lagoon.

Parameters	St 1	St 2	St 3	St 4	St 5	St 6	St 7
TCC	1129.43 ± 76.17	1826.17 ± 792.02	879.41 ± 456.45	411.67 ± 90.21	91.22 ± 83.61	791.93 ± 507.98	588.33 ± 267.52
Temp (°C)	31.19 ± 0.16	30.99 ± 0.18	31.72 ± 0.08	31.70 ± 0.41	32.15 ± 0.78	32.34 ± 1.06	32.74 ± 1.19
Salinity (ppt)	26.63 ± 4.86	27.41 ± 6.05	23.19 ± 6.09	20.98 ± 3.82	20.92 ± 5.16	25.96 ± 4.55	26.36 ± 5.99
DO (mg/l)	0.54 ± 0.50	0.56 ± 0.28	0.67 ± 0.45	0.82 ± 0.62	1.03 ± 0.86	0.75 ± 0.36	0.88 ± 0.30
TDS (ppt)	17.91 ± 0.55	21.39 ± 4.40	18.65 ± 3.82	18.20 ± 1.18	18.84 ± 1.63	20.42 ± 3.19	21.14 ± 4.78
pH	7.64 ± 0.14	8.04 ± 0.01	7.82 ± 0.09	7.68 ± 0.15	7.61 ± 0.06	7.77 ± 0.05	7.86 ± 0.18
BOD_5 (mg/l)	6.43 ± 1.52	7.21 ± 0.17	6.99 ± 1.31	6.27 ± 0.91	2.22 ± 0.32	7.213 ± 1.28	8.78 ± 1.83
NO_3^- (mg/l)	1.95 ± 1.11	1.91 ± 0.28	2.17 ± 0.32	1.84 ± 0.49	2.07 ± 0.57	2.25 ± 0.61	2.50 ± 0.37
PO_4^{3-}(mg/l)	0.21 ± 0.18	0.11 ± 0.06	0.12 ± 0.05	0.26 ± 0.18	0.13 ± 0.07	0.12 ± 0.08	0.15 ± 0.07
EC (uS/cm)	31.30 ± 7.35	42.79 ± 8.83	37.23 ± 9.25	38.00 ± 1.35	37.76 ± 3.06	40.84 ± 6.39	41.45 ± 8.46

TCC – Total Coliform Concentration, DO – Dissolved Oxygen, TDS – Total Dissolved Solids, BOD_5 – Biological Oxygen Demand, EC – Electrical Conductivity, Temp – Water Temperature, NO_3^- - Nitrate, PO_4^{3-}- Phosphate.

3.2. Spatial variation in total coliform concentrations

Significant variations in coliform concentrations were observed among the different sampling areas, as indicated by the outcomes of the one-way ANOVA ($F(6,21) = 11.24$; $p = 0.0000122$). The study site St 2 exhibited the highest coliform concentration, a trend identified through the Tukey HSD test. In contrast, site St 5 registered the lowest concentration of coliform contaminants attributed to anthropogenic sources. The remaining sites demonstrated intermediate levels of coliform contamination, signifying a moderate input of these contaminants (Fig. 2).

3.3. Assessment of *E. coli* contamination

E. coli contamination was assessed across various regions of the lagoon, yielding insightful findings. The northern segments of the lagoon, specifically sites St 1, St 2, and St 7, exhibited *E. coli* presence in both water and oyster samples. However, the eastern and western segments remained devoid of *E. coli* contamination, as depicted in Fig. 3. Particularly noteworthy was the heightened prevalence of *E. coli* at site St 7, evident within both lagoon water and oysters. *E. coli* detection was facilitated through the identification of a characteristic green metallic sheen (Fig. 3D), while samples devoid of *E. coli* predominantly displayed mucoid growth (Fig. 3C).

3.4. Environmental parameters and contamination levels

The quantified Total Coliform Concentrations (TCC) within oysters, present in all sampling points except points 5 and 4, surpassed the permissible threshold for seafood consumption (TCC < 100 MPN) across all sites. Except for St 6, where the mean TCC was found to be 55.83 ± 36.09 (mean ± standard deviation (SD)) (Table 6). The Water Quality Index (WQI) spanned a range of 45 to 64 across the sampling sites, with the exception of St 5, which demonstrated a WQI value of 73.76 (refer to Table 7). These assessments provide a comprehensive overview of *E. coli* contamination levels, TCC, and WQI values across the study sites.

3.5. Oyster consumption patterns

The consumption of oysters sourced from the Negombo Lagoon constitutes a prevalent practice, with 76.7 % of participants reporting its inclusion in their regular dietary habits. Oysters are important in the local food culture and are often part of their meals. However, it is noteworthy that a limited subset (6.7 %) of respondents perceived oysters as an export commodity, generating monetary value from their trade (Fig. 4).

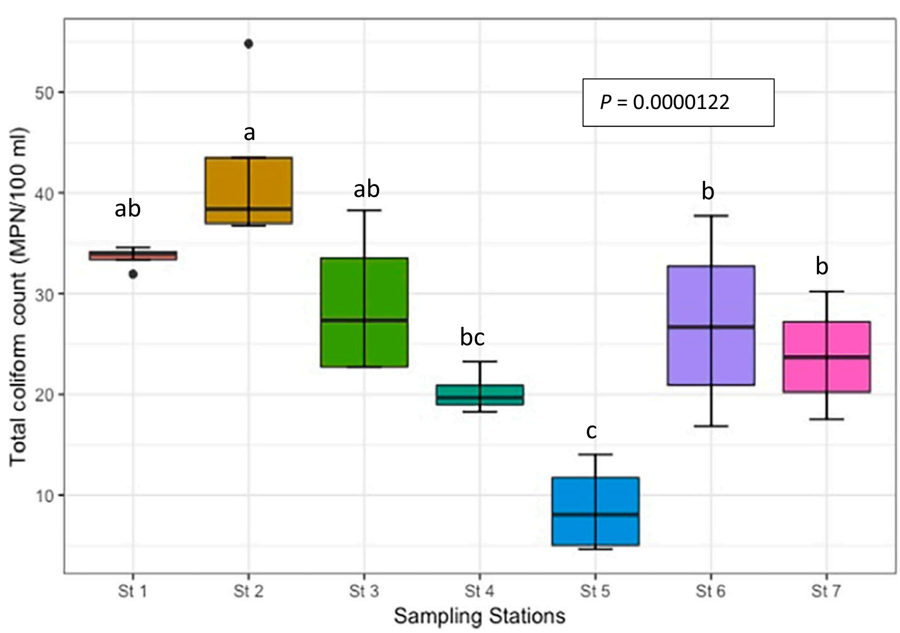

Fig. 2. A one-way analysis of variance (ANOVA) comparing total coliforms among the different sewage discharge points. The boxplots with the same letters on top of them represent no significant difference. The dark points are outliers. The horizontal lines in the boxplot represent the median. The lower and upper hinges correspond to the first and third quartiles, and the whiskers represent the minimum and maximum values for each group data.

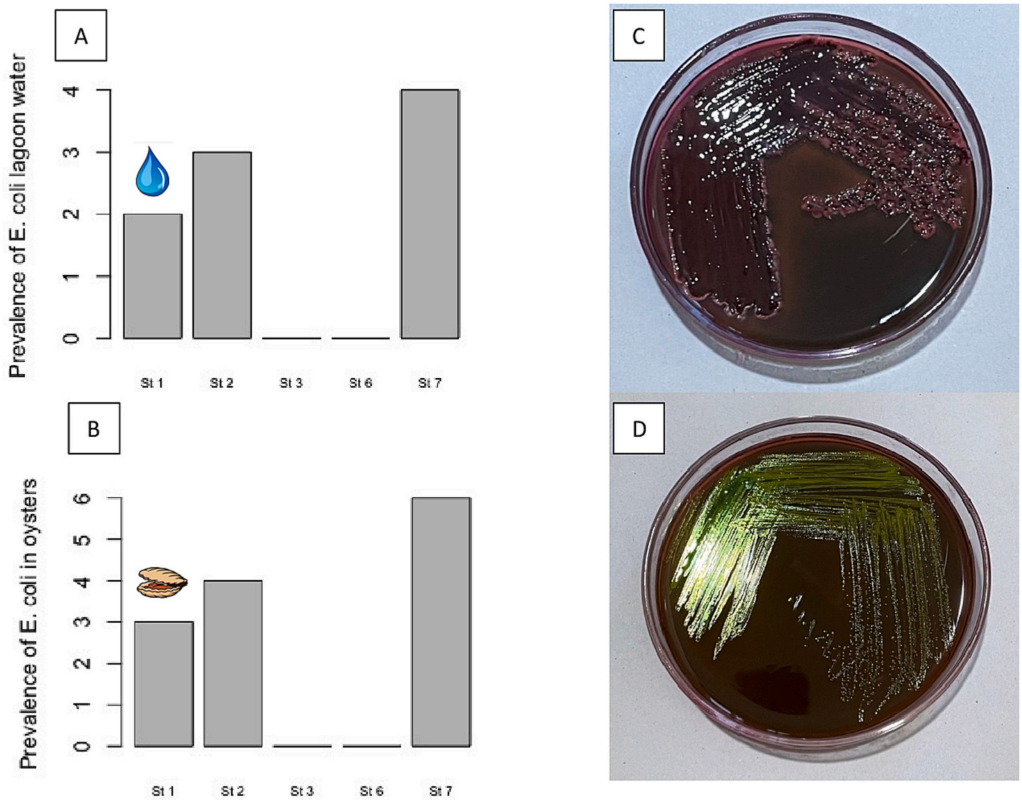

Fig. 3. A bar graph showing the prevalence of *E. coli* in (A) lagoon water and (B) oyster samples collected from the sewage discharge points. The eosin methylene blue (EMB) agar mostly showed (C) mucoid growth for *E. coli* contamination-free samples and (D) green metallic sheen growth for samples contaminated by *E. coli*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 6
Comparison of the total coliform concentration (TCC, mean ± standard deviation) in oysters to the standard allowable threshold value in frozen or fresh seafood and the status of *E. coli* contamination.

Stations	TCC	Standard limit of TCC in seafood (MPN/g)
St 1	**144.83 ± 59.90**	100
St 2	**115.67 ± 64.29**	100
St 3	**102.67 ± 50.92**	100
St 6	55.83 ± 36.09	100
St 7	**279.83 ± 149.40**	100

Note: TCC values in bold represent those that exceeded the standard threshold limit, MPN – Most Probable Number.

Table 7
The water quality index (WQI) computed using physicochemical and microbiological parameters obtained from each sewage discharge point.

Sampling station	WQI	Water quality condition
St 1	47.91	Marginal
St 2	45.23	Marginal
St 3	49.63	Marginal
St 4	51.50	Marginal
St 5	73.76	Fair
St 6	52.07	Marginal
St 7	50.89	Marginal

4. Discussion

4.1. Distribution of Crassostrea cucullata in the Negombo Lagoon

Oysters of the species *Crassostrea cucullata* were predominantly observed near the lagoon's mouth. This tendency can be attributed to the salinity levels in this vicinity, which are generally higher due to the influx of seawater from the open sea during tidal surges within the lagoon (Brookes et al., 2022; Muduli and Pattnaik, 2020). A Habitat Suitability Index (HIS) study conducted by Chowdhury (2019) highlighted the thriving nature of oysters in high-salinity environments. This aligns with the presence of oysters in the northern section of the Negombo Lagoon, which exhibited the conducive water conditions necessary for the flourishing of *C. cucullata*. In contrast, the southern part of the lagoon experiences freshwater input from the Dandugam Oya River, leading to water dilution and altering the chemical makeup of the local ecosystem, which affects the survival and development of oyster larvae (Cheng et al., 2016; Lawlor and Arellano, 2020).

While adult oysters can withstand varying salinity gradients ranging from 5 to 40 ppt, they exhibit greater proliferation under conditions of elevated salinity (Chowdhury et al., 2019; Naik and Gowda, 2013). As outlined by Griffiths et al. (2021), oysters possess a degree of resilience against short-term exposure to reduced salinity. However, prolonged exposure leads to stress, rendering them more susceptible to diseases and triggering instances of mass oyster mortality, as demonstrated by Campbell et al. (2022).

4.2. Fecal contamination of lagoon water and oysters by total coliform bacteria and E. coli

4.2.1. Total coliform concentrations as indicators of fecal contamination

Total coliform concentrations were employed as an indicative measure to evaluate the extent of fecal contamination within the lagoon water. A similar approach was adopted by Leite et al. (2018) in their study of fecal pollution in a coral reef ecosystem off the coast of Porto Seguro, Brazil. This underscores the usefulness of coliforms as indicators for assessing fecal contamination levels within lagoon ecosystems. The discernible spatial variations in coliform concentrations across the

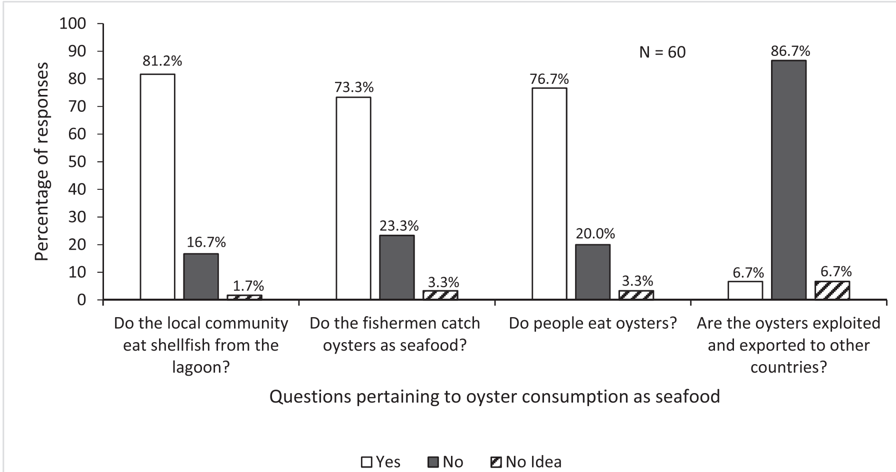

Fig. 4. Responses from the respondents regarding the use of the Negombo lagoon as a source of oyster exploitation and consumption. N represents the sample size.

sampled regions in this study highlighted certain sections of the lagoon bearing a higher load of fecal contaminants than others (Fig. 2). For instance, the pronounced elevation of coliform concentrations at site St 2, located in the northern sector of the Negombo Lagoon, can be attributed to its dense human settlements (Personal observation). Mohapatra et al. (2022) conducted a similar investigation on spatial variations in fecal coliform load in India's Chilika lagoon, revealing higher averages of coliform load in areas proximate to fishing villages. The prevalent practice of disposing of human and animal waste in coastal lagoons in communities around such ecosystems worsens the problem. This consequently accounts for the high concentrations of coliforms recorded at this site. In contrast, the relatively lower coliform concentration at site St 5 could be attributed to its status as a protected zone, sheltered from anthropogenic stressors and less inhabited, unlike other sections of the Negombo Lagoon (Athukorala et al., 2021; Wear et al., 2021). The remaining sites exhibited moderate fecal contamination levels, likely sourced from shrimp farms, industries, and sewer systems within less densely populated communities. These differences in contamination levels are indicative of varying waste production and contamination levels across the studied sites.

The discharge of sewage effluents at various sampling points, particularly in the northern sector of the lagoon, resulted in poor water quality parameters, including DO, BOD_5, and coliform concentrations (Morsy et al., 2020). Numerous studies have demonstrated that microbial decomposition activities progressively degrade water quality by consuming dissolved oxygen to break down organic material, leading to anoxic or hypoxic conditions unsuitable for fish respiration (Bhat and Qayoom, 2021; Singh et al., 2021). This could potentially result in fish migration or mortality. The discharge of fecal coliforms, such as *E. coli*, into lagoon water and oysters poses a health risk to both recreational swimmers and seafood consumers (Pakingking et al., 2022; King and Leonard, 2023). Recreational swimmers may ingest pathogenic bacteria by coming into contact with lagoon water. Seafood consumers who consume oysters, either raw or partially cooked, are at risk of infection by these pathogens. Several studies have shown that depuration is an effective method for removing contaminants before processing seafood for consumption (Martinez-Albores et al., 2020). Many aquaculture farmers have adopted this method to ensure the safety of their products.

4.2.2. Oyster contamination and health implications

Oysters harvested from the lagoon sampling sites exhibited coliform concentrations surpassing recommended threshold values (<100 MPN/g) for safe consumption, except for site St 6 (See Table 6). This was because sites St 1, St 2, St 3, and St 7 received inputs from human and animal waste, including sewer systems, piggery farms, and aquaculture activities, which resulted in elevated coliform concentrations (Wear et al., 2021). Oysters, being sessile filter-feeders, are susceptible to coliform contamination from the lagoon (Nin Gan and Xu, 2022). The tendency of oysters from sites exposed to fecal waste to exhibit high coliform contamination makes them unsuitable for consumption. However, site St 6 registered coliform levels below 100 MPN/g, possibly due to effluents from industries that harbor lower fecal waste contamination.

Examination of *E. coli* contamination revealed oysters from sites St 1, St 2, and St 7 as contaminated. These sites, situated within the highly populated and urbanized region of the lagoon (Athukorala et al., 2021), are characterized by waste inputs from piggery farms, sewer systems, and community seepages, all potential contributors of *E. coli* contamination to aquatic environments (Wear et al., 2021). In contrast, sites St 3 and St 6, characterized by shrimp farms and industrial effluents, demonstrated an absence of *E. coli* contamination due to their lesser potential as fecal contamination sources in aquatic waters. These findings underscore the complex interplay between contamination sources, urbanization, and aquatic health within the lagoon ecosystem. In addition to utilizing traditional physicochemical and microbiological parameters for assessing sewage pollution in coastal lagoons, recent studies have embraced innovative approaches like stable isotope systems to monitor the bioaccumulation of heavy metals, including copper, lead, nickel, cadmium, and others, in oysters (Jeong et al., 2021; Wang et al., 2022). Stable isotopes serve as valuable environmental forensic tools, enabling researchers to infer the origins of anthropogenic contaminants (Araújo et al., 2021). This technique provides insights into the dynamics of pollution sources, contributing to a more comprehensive understanding of pollution in coastal lagoons. The integration of such advanced tools enhances our ability to address pollution challenges in coastal lagoons, paving the way for more informed and targeted environmental management practices.

4.3. Comprehensive assessment of water quality across sampling sites in Negombo Lagoon

The comprehensive evaluation of water quality across the various sampling sites revealed an overall water quality index ranging from 45 to 64, aligning with the criteria outlined by the Canadian Council of Ministers of the Environment (CCME). While most of the examined sites displayed a marginal water quality status, site St 5 exhibited a favourable water quality condition (see Table 7). This divergence can be attributed to the dominant influence of river discharge from the Dandugam Oya River at site St 5, which introduces comparatively less anthropogenic waste into the Negombo lagoon (Chandrasekara et al., 2013; Hsieh et al., 2021). Our findings align with Miyittah et al. (2020), who conducted a water quality assessment of the Tendo lagoon in Ghana, revealing a range between 45 and 65. The authors identified most of the sites as marginally polluted, attributing this to deviations in water quality parameters from their recommended values. Similarly, Seiler et al. (2020) associated the high water quality index observed in their study of the Patos Lagoon in Brazil with the discharge of freshwater. Freshwater inputs are considered less contaminated compared to sites directly impacted by sewage discharge. Although the physicochemical and microbiological parameters considered for the water quality index demonstrated fewer instances of non-compliance at site St 5, the overall index showed that the site receives lower waste inputs compared to the other study sites, thereby contributing to its improved water quality condition.

Across the studied sites, the parameters that most commonly failed to meet quality standards were coliform counts, dissolved oxygen levels, biological oxygen demand, and phosphate concentrations (see Table 5). Gomes De Quevedo and da Silva Paganini (2016) identified domestic household cleaning detergents as the primary sources of phosphate (PO_4^{3-}) input into water bodies. Given that many sampling sites receive effluents from local communities, elevated phosphate (PO_4^{3-}) concentrations in these areas can be attributed to cleaning detergents from domestic households. Further, the influx of external microbes into coastal ecosystems intensifies the decomposition process, leading to a reduction in dissolved oxygen levels within the lagoon ecosystem (Hamdhani et al., 2020; Hsieh et al., 2021; Ramos-Ramírez et al., 2021). This increased decomposition activity contributes to a heightened biological oxygen demand, further undermining water quality by creating unfavourable conditions for fish species dependent on adequate dissolved oxygen levels for survival. The cascade of events set in motion by sewage within aquatic systems results in compromised water quality, which adversely affects the ecosystem.

4.4. Potential health implications for seafood consumers

Findings from participant interviews revealed a significant reliance on oysters, *Crassostrea cucullata* (Born, 1778) for everyday meals within local communities. This observation aligns with the work of Edirisinghe et al. (2021), which identifies seafood, particularly oysters, as a primary protein source in Negombo City. The northern region of the Negombo Lagoon stands out as a hub of fishing activities due to its proximity to the open sea, rendering it a diverse resource for fisheries in contrast to its southern counterpart (Athukorala et al., 2021; Hsieh et al., 2021). However, oysters procured from the northern sampling sites exhibit contamination by *E. coli*, posing a health risk to the local inhabitants who regularly consume these oysters.

The consumption of contaminated oysters *C. cucullata* from the studied northern sites carries potential health risks spanning from mild to severe gastroenteritis, dehydration, and extra-intestinal ailments (Obodai et al., 2010). It is important to underscore that the contamination observed in these oysters raises concerns about the well-being of the local communities that depend on them for sustenance. Moreover, the interview responses showed that oysters harvested from the studied sites remain largely within the local consumption sphere and are not exported to foreign markets for commercial gain. Hence, the potential risk of exporting contaminated oysters to other countries is minimal.

5. Conclusion

The present study comprehensively evaluated ecological health risks associated with selected sites within the Negombo lagoon, along with the potential health risks linked to consuming *C. cucullata* oysters. The investigation revealed that the discharge of coliforms due to fecal contamination, including sewer system seepage, direct sewage discharge, and surface runoff, reached its peak at site St 2. In contrast, site St 5 exhibited the lowest coliform contamination, positioning it as the least impacted location among the studied sites. This underscored its suitability for both seafood consumption and recreational swimming, in contrast to the other sites which showed elevated coliform concentrations.

The overarching water quality index analysis indicated a widespread vulnerability to coliform contamination across the Negombo Lagoon's sampling sites. However, site St 5 emerged as an exception, showing a favourable water quality status. However, in the context of seafood consumption, caution is advised regarding *C. cucullata* oysters obtained from sites St 1, St 2, and St 7. These sites exhibited coliform concentrations surpassing recommended thresholds (<100 MPN/g) and tested positive for *E. coli* contamination. Thus, caution should be taken when consuming *C. cucullata* oysters from these specific sampling sites within the Negombo lagoon or when engaging in recreational swimming activities. Further studies can be conducted on this lagoon to assess sewage pollution and its health implications during both the monsoon and non-monsoon seasons, aiming to understand the impact of stormwater and surface runoffs on the dynamics of fecal contamination and water quality in the lagoon.

CRediT authorship contribution statement

Samuel Ayitey: Conceptualization, Methodology, Formal analysis, Data curation, Validation, Investigation, Writing – original draft, Writing – review & editing. **T.W.G.F. Mafaziya Nijamdeen:** Writing – review & editing, Supervision. **Harshini Peiris:** Writing – review & editing, Supervision, Resources. **Sunanda Kodikara Arachchilage:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Validation, Resources. **Isabelle George:** Writing – review & editing, Methodology, Validation, Supervision. **Farid Dahdouh-Guebas:** Writing – review & editing, Methodology, Supervision, Funding acquisition, Validation. **K.H.M. Ashoka Deepananda:** Writing – review & editing, Methodology, Supervision, Resources, Validation, Project administration.

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.

Data availability

Assessment of Ecological Health on Negombo Lagoon Datasheet (Original data) (Figshare)

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