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Assessment of microbial quality of oysters in Negombo Lagoon, Sri Lanka

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Abstract Oysters are known for their nutritional value as a protein source in the human diet. The present study assessed the microbial quality of the oysters collected from Negombo Lagoon. Oysters and associated water samples were collected from four locations in the lagoon on a monthly basis from October 2021 to August 2022. The salinity, temperature, and pH of each location were also measured in situ. The samples were tested for aerobic plate count (APC), total coliforms, faecal coliforms and *Escherichia coli* (*E. coli*) following Sri Lankan Standards (SLS). Statistical analysis was performed using MINITAB 17. The results indicated that the APC in oysters (from 0 to 1.14 x 10^2 CFU/g) resulted in<5x10^5 CFU/g, the maximum recommended bacterial count for good quality seafood, while the water showed APC from 1.6 to 1.8 x 10^3 CFU/mL. The total coliforms and faecal coliform counts in oysters were from 2.67x10^{-2} to 166×10^{-2} MPN/g and from 6.67 x 10^{-2} to 93.3 x 10^{-2} MPN/g, while these counts in water samples were from13.3 to 366.7 MPN/100mL and from 4.30 to 194 MPN/100 mL respectively. *E. coli* levels in oysters varied from 1.00 to 81.0 x 10^{-2} MPN/g, which were below the maximum recommended level (11 MPN/g) for good quality seafood. *E. coli* levels in water ranged from 1.33 to 93.3 MPN/100 mL. The findings further revealed that the correlations between *E.coli* in water and water quality parameters were statistically insignificant and weakly positive. But, the correlation between *E.coli* in oysters and respective water showed a strong positive correlation and it was statistically significant ($p<0.05$). Although the oysters collected are suitable for direct consumption as per European shellfish harvesting area classification criteria, the microbial counts in water were higher than those of the oysters. Therefore, depuration of oysters is recommended for safe human consumption while continuous monitoring is conducted.

Keywords: Aerobic plate count, total coliforms, faecal coliforms, *Escherichia coli*

INTRODUCTION

Oysters are considered to be a nutritious protein food item as they constitute a rich source of essential macro and micro-nutrients for providing a balanced diet. This food source is rich in protein, glycogen, essential vitamins and minerals such as zinc, iron, calcium, and selenium and contains approximately 5.6% high glycogen level, making it an excellent energy source, and this level is higher than the other mollusks (Jay, 2000). The chemical composition of oyster flesh varies over a wide range depending on the species, the place of origin, and the season (Jay, 2000).

The oyster consumption is influenced by several factors including local traditions, economic conditions, and environmental factors. Ayitey *et al.* (2024) stated that oysters are used as an export commodity, generating monetary value through their trade. There is a demand for oysters from hotels, as this delicacy is highly popular among foreign tourists. Oysters are often featured in various dishes either cooked (baked, boiled, steamed and fried) or in raw form for their unique taste and texture.

The economically important *Crassostrea madrasensis* and *Saccostrea cucullata* are the two available oyster species found in some coastal areas in Sri Lanka. Capture fishery of oysters for local consumption is therefore prevailed in Negombo, Chilaw, Kalpitiya, Mannar, Jaffna, Trincomalee and the Southern coastal belt of the country. Though bivalve farming is not commonly practised in Sri Lanka, there is a high potential for culturing these oysters in the above-mentioned areas in the country. Community-based bivalve farming, including, oysters has been started in many parts of the country through the government initiation.

However, the commercial oysters in the above-mentioned coastal areas receive untreated urban wastewater through freshwater streams. 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 (Ayitey *et al*., 2024). Being filter feeders, the oysters are capable of efficiently concentrating various contaminants together with microorganisms from their habitats (Kittigu, 2006). Salinity and temperature fluctuations due to continuous inflow of freshwater sources are another favourable condition for the growth and survival of different pathogenic microorganisms (Leda, 2015). Especially enteric bacteria such as coliforms, faecal coliforms and *Escherichi*a *coli* with human excreta are introduced into lagoons in which they can contaminate fauna inhabiting such environments (Mallin *et al.* 2000). There is a vast potential for health risks to consumers through the consumption of enteric bacteria contaminated oysters (SOFIA, 2006).

Therefore, assessing the suitability of oysters as a food source is important as oysters are high-nutrient seafood sources for the people who consume oysters frequently in such areas. Since there are limited studies on the microbial quality of oysters in Sri Lanka, the present study was planned to assess the microbial quality of *Crassostrea madrasensis* and associated water sources in selected culture locations in Negombo Lagoon.

MATERIALS AND METHODS

Study sites

The study sites of the present study in Negombo, Sri Lanka are illustrated in Figure 1. The sites were selected based on the oyster production areas in Negombo Lagoon. The site N1 is located in the northern area of the lagoon and receives various contaminants due to its location near the sea. The sites N2 and N4 are also in the northern area close to two land masses in the lagoon. The mixing of water in these sites is low when compared to the sites N1 and N3. The site N3 is located in the southern area of the lagoon, which receives contaminants through Dandugam oya and Hamilton Canal, the major freshwater flows.

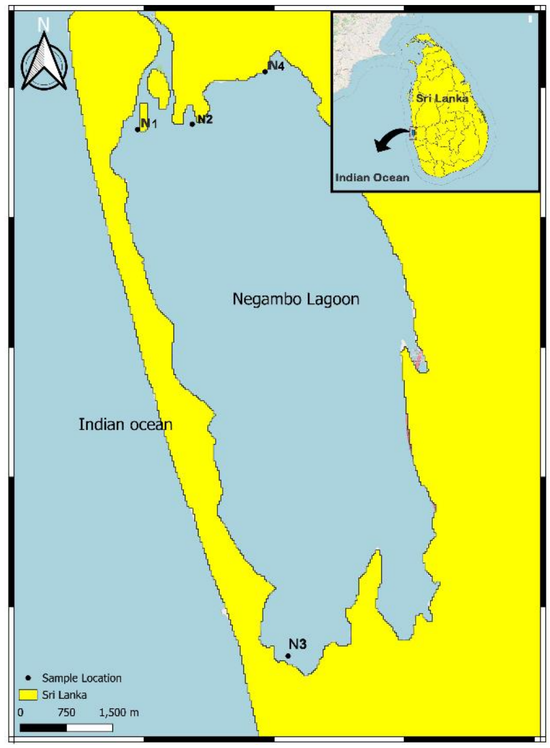

Fig 1. The study sampling sites of the Negombo Lagoon

Oyster and water samples

Approximately 5-10 oyster samples of *Crassostrea madrasensis* having a total length of 5 cm - 6 cm were collected from each location from Negombo Lagoon with the help of fishermen for ten months from October 2021 to August 2022. The samples were packed in cleaned polyethylene bags and stored in chill bins at 4^0C. The water samples were simultaneously collected with the bivalve samples from near the surface of all sampling sites (approximately 15 cm depth) into clean, sterile bottles. The water samples stored were transported in the same manner. All samples in chilled storage were transported to the Quality Control Laboratory of the Institute Post Harvest Technology at the

National Aquatic Resources Research and Development Agency (NARA) immediately to be analysed.

Physio-chemical parameters of water samples

Temperature, pH and salinity of the water samples were measured in situ using a portable multi-parameter (TSP WP 91 waterproof multi-parameter) of each sampling station at approximately 1m depth intervals from the surface to approximately 0.5m above the sediment surface. Depth was measured using a marked plumb line.

Microbial analysis of water and oyster samples

The collected oyster samples were cleaned to remove contaminants, such as dirt, on the surface. The flesh of the oysters was carefully removed from the shells to determine the microbial parameters. Oyster and water samples were tested for Aerobic Plate Count (APC) at 37ºC (SLS 516-1 Sec,1:2013 ISO 4833-1:2013) by the pour plate technique on plate count agar. Oyster samples for total coliforms (SLS 516-3-Sec,1:2013ISO 4831-2006), faecal coliforms and *E. coli* (SLS 516-12:2013 ISO 7251:2005), and water samples for total coliforms, faecal coliforms and *E.coli* were tested by MPN-technique (SLS 1461 Part 1/Sec 3: 2013).

Statistical analysis

A one-way ANOVA followed by Tukey's pairwise comparison test was performed to determine whether there are significant differences among selected microbial parameters of water samples and oyster samples across different sites within the lagoon. This analysis was conducted using Minitab 17 statistical software after confirming normality with the Anderson-Darling test. Additionally, a Pearson correlation was performed to identify any correlations between water quality parameters and *E. coli* count, as well as between *E. coli* in oysters and *E. coli* in the corresponding water samples.

RESULTS AND DISCUSSION

Water quality parameters

Variations of pH, salinity and temperature in water collected from different sites of Negombo Lagoon throughout the study period are given in Figure 2, Figure 3 and Figure 4 respectively.

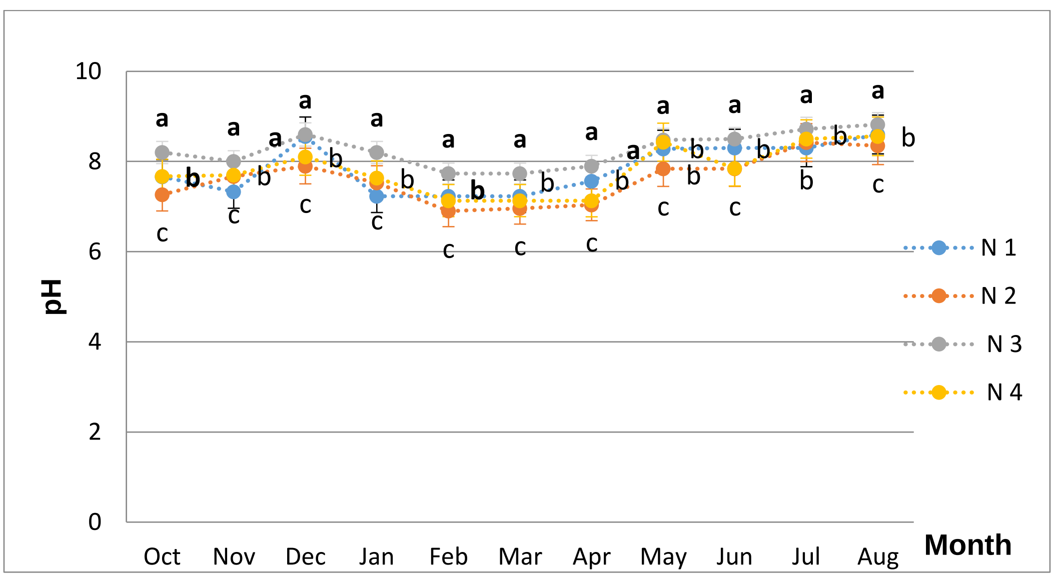

Fig 2. pH variation of water in four sites of the Negombo lagoon. Data shown in the graph are mean±SEM. The mean values indicated in the graph having different superscripts were significantly different. ($p \leq 0.05$; One-way ANOVA followed by Tukey's pairwise test)].

The pH values varied from 7.04 to 8.82, showing a slightly alkaline condition. The highest pH values were recorded at sites N3 in the month of August, while the lowest pH values was recorded during February, and it could be attributed to the low rainfall patterns.

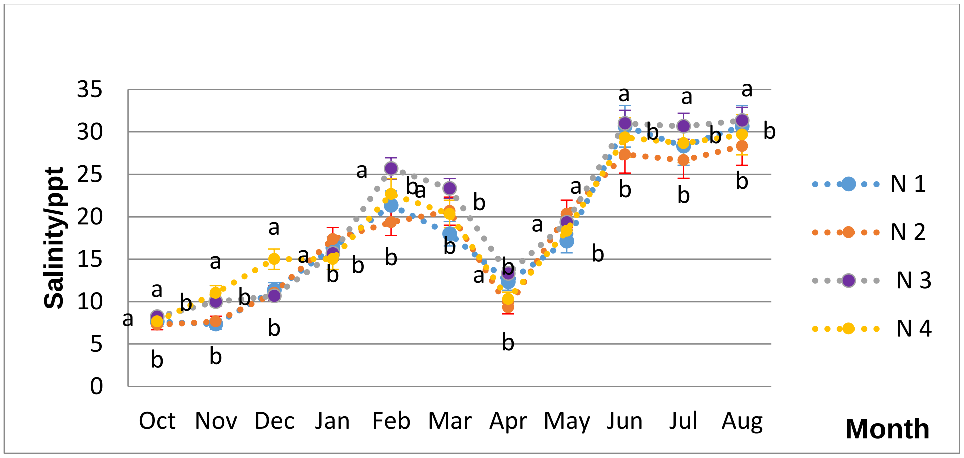

Fig 3. Salinity variation of water in four sites of Negombo Lagoon. Data shown in the graph are mean±SEM. The mean values indicated in the graph having different superscripts were significantly different. ($p \leq 0.05$; One-way ANOVA followed by Tukey's pairwise test)].

The salinity of lagoon water ranged from 7.27 ppt to 31.33 ppt. The maximum values were recorded from N3 and N1 sites in August. The lowest values were recorded from the N2 site in October. The salinity of Negombo Lagoon is strongly related to the monsoon rains (Rajapaksha, 1997). The salinity reached its maximum in April and gradually decreased with the southwest monsoon from May to August.

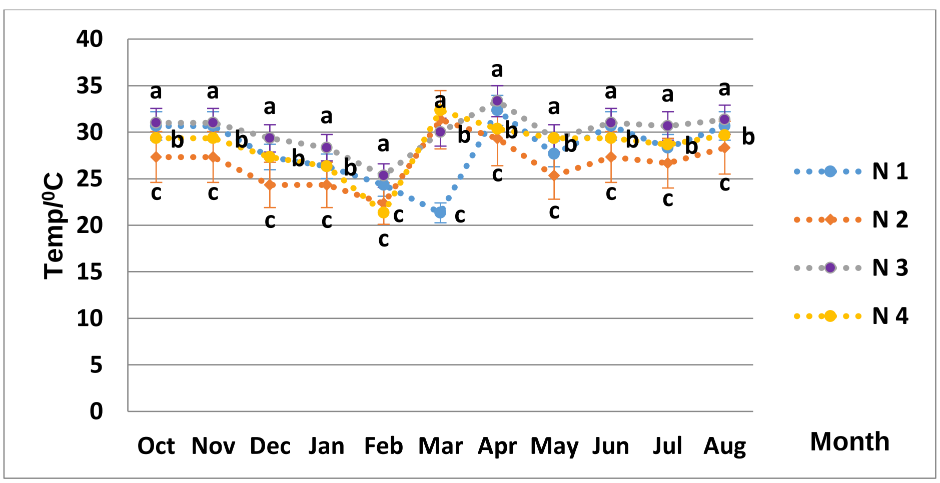

Fig 4. Temperature variation of water samples in the four sites of Negombo lagoon. Data shown in the graph are mean±SEM. The mean values indicated in the graph having different superscripts were significantly different. ($p \leq 0.05$; One-way ANOVA followed by Tukey's pairwise test)].

The lagoon water temperature ranged between 21.33^0C and 33.33^0C, and the highest lagoon water temperature was observed in April at site N3, and a gradual temperature reduction was observed with the onset of southwest monsoons. The lowest temperature was recorded in March at site N1.

Microbial analysis of oyster and water samples

Aerobic plate counts (APC)

The APC in oyster and water samples collected are given in Figure 5. Aerobic plate counts on fish and fishery products generally do not relate to food safety hazards but sometimes can be helpful in indicating quality, shelf life, and post-heat-processing contamination. Fresh fish and fishery products often have an APC of 10^4 to 10^5CFU/g (ICMSF, 1986). A previously recorded APC value for the same species collected from Kalpitiya, Kandakuliya, Anaiwasal, Janasavipura, and Gagngewadiya areas in Puttalam district range from 10^3 to 10^7 CFU/g, with 88% of the oysters having less than 5 x 10^5 CFU/g (Ariyawansa *et al.*, 2017).

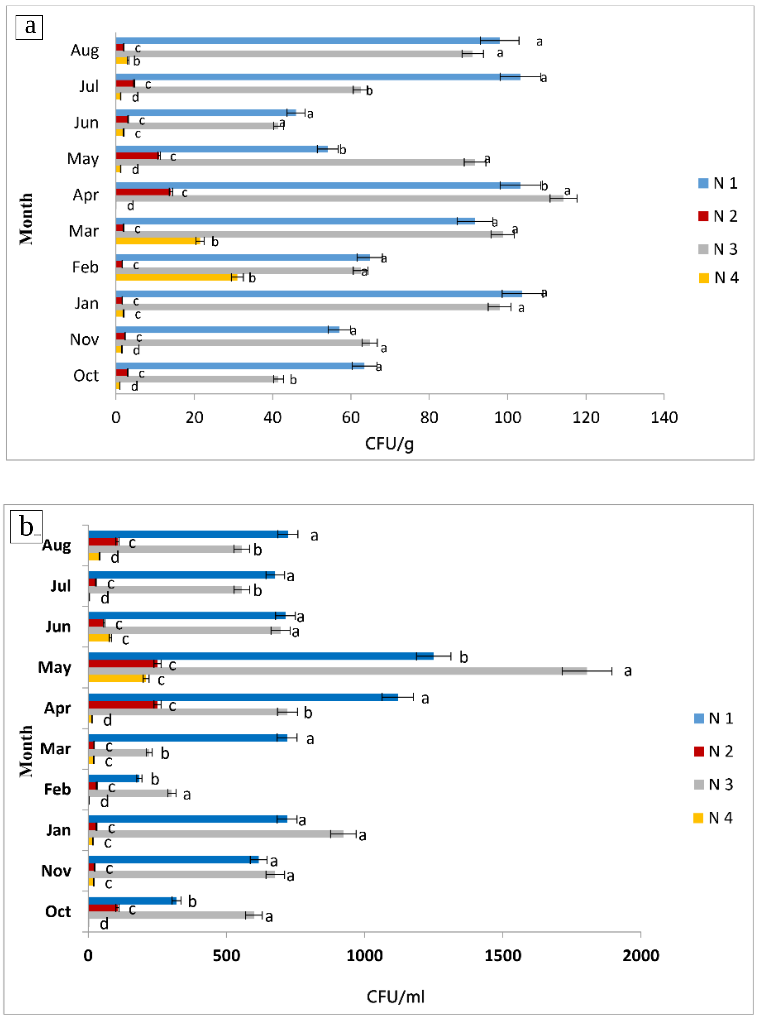

Fig 5. Aerobic plate count in oyster (a) and water (b) samples. Data shown in the graph are mean±SEM. The mean values indicated in the graph having different superscripts were significantly different. ($p \leq 0.05$; One-way ANOVA followed by Tukey's pairwise test).

APC of the oyster samples collected from four sites ranged between 0 and 1.14×10^2 CFU/g. Among these sites, the highest APC counts were recorded in site N3, followed by site N1 (Figure 5a) for oyster samples. This could be attributed to the location of the fish market (Lellama) near the lagoon. The increase in the APC value of site N1 may be due to the discharges of effluents from the fish market. The highest APC values were recorded in April. Nevertheless, all samples were below the 5 $\times 10^5$ CFU/g acceptable limit of European regulation standards.

The quality and safety of oyster is dependent upon the quality of the growing water areas (Peralta and Andalecio 2011). The APCs at 37^{0}C in water samples in four sites of Negombo lagoon ranged between 1.60 CFU/mL and 1.80x10^3 CFU/mL. The highest APC counts were recorded from site N3, followed by site N1 in May. A significant increase in the APC in the month of May may be associated with average rainfall amounts. Rainfall runoff is another possible source of contamination and could include sewage discharges from municipal treatment plants and septic tanks, stormwater overflows, and runoff from lands. It can be evidenced that non-point source pollution carried by surface runoff significantly affects bacterial levels in water resources. According to meteorological data, November, May, and July (meteorology department data 2021/2022) were high-rainfall months.

Total coliform count

The total coliform in oyster and water samples collected is given in Figure 6. The total coliform in all oyster samples collected from all sites of Negombo lagoon during the experiment period ranged between 2.67×10^{-2} and 166×10^{-2} MPN/g (Figure 6a). According to European standards, the standard acceptable limit for the total coliform count in live oysters should be less than or equal to 2.3MPN/g. These values in samples collected from site N1 and site N3 were observed to be comparatively higher than those of the other two sites. The Negombo fish market and lots of dried fish processing areas, situated near the experimental site N1 in the lagoon and the locating of restaurants near site N3, could be the possible sources for coliforms in these areas. The highest total coliform values were recorded in May in site N3.

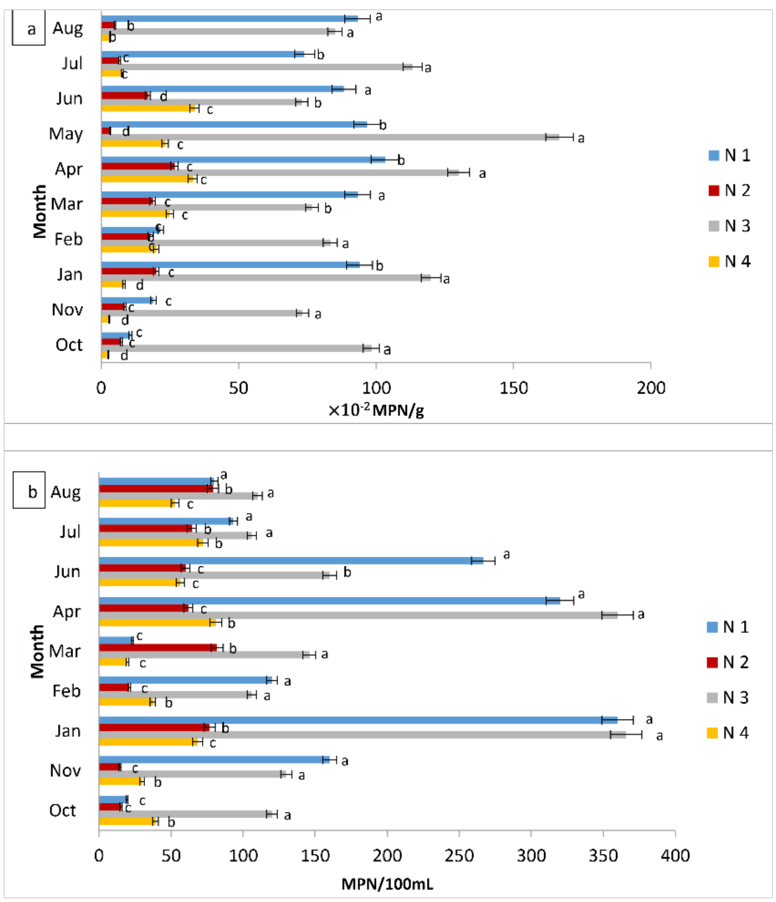

Fig 6. Total coliform count in oyster (a) and water (b) samples. Data shown in the graph are mean±SEM. The mean values indicated in the graph with different superscripts were significantly different. ($p \leq 0.05$; One-way ANOVA followed by Tukey's pairwise test)].

Nevertheless, some water samples exceeded the acceptable limit (≤70MPN/100 mL) of European regulation for total coliform. Among all four sites, higher values of total coliform count were recorded from N1 and N3 (Figure 6b) in January.

Faecal coliform count

The faecal coliforms in oysters and water samples collected are given in Figure 7. The faecal coliform in oysters varied from 6.67× 10^{-2} to 93.3 × 10^{-2} MPN /g. The highest values for faecal coliform content in oyster samples were recorded in April from site N3.

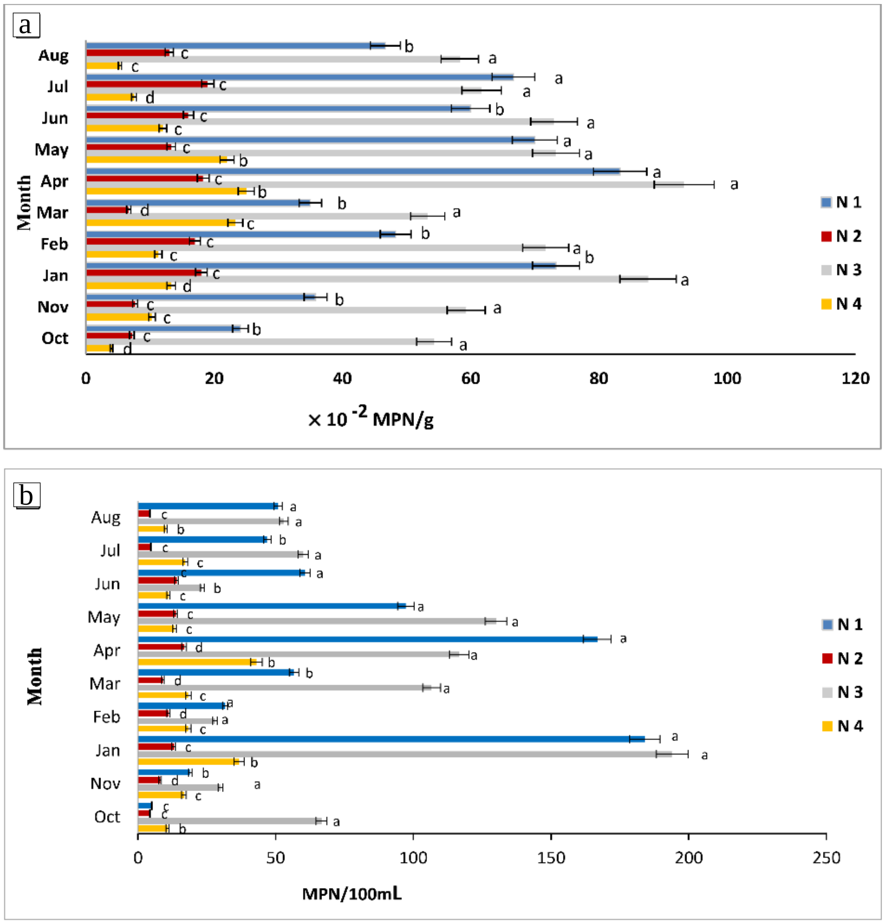

Fig 7. Faecal coliform count in oyster (a) and water (b) samples. Data shown in the graph are mean±SEM. The mean values indicated in the graph having different superscripts were significantly different. ($p \leq 0.05$; One-way ANOVA followed by Tukey's pairwise test)].

All water samples collected during the experimental period were positive for faecal coliform bacteria and values varied from 4.30 to 1.94x10^2 MPN/100mL. The highest value in samples was recorded in January in site N3 (Figure 7b). Considering temporal variations, higher faecal coliform values were recorded from January and April. Most samples collected in sites N1 and N3 were generally higher than the standard limit of 14 MPN faecal coliform / 100 mL (NSSP/ISSC/HHS/PHS/FDA, 2003). In addition, faecal coliform counts in water samples of four sites showed significant differences ($p<0.05$).

Escherichia coli (*E. coli*)

E. coli is a good indicator of faecal contamination, accounting for 90% of the faecal coliforms (Pereira *et al.*, 2006). *E. coli* in oysters and water samples collected are given in Figure 8. Almost all oyster samples collected from the selected sites of Negombo Lagoon during the experiment period were positive for *E.coli*, and it ranged from 1.00 x 10^{-2} to 81.0 × 10^{-2} MPN/g, which were below the maximum recommended level (11 MPN/g) for good quality seafood (Figure 8a). The highest *E. coli* value in oyster samples was recorded in January from site N1. Significant differences in the collected *E.coli* values of oyster samples were observed among four sites and months ($p<0.5$).

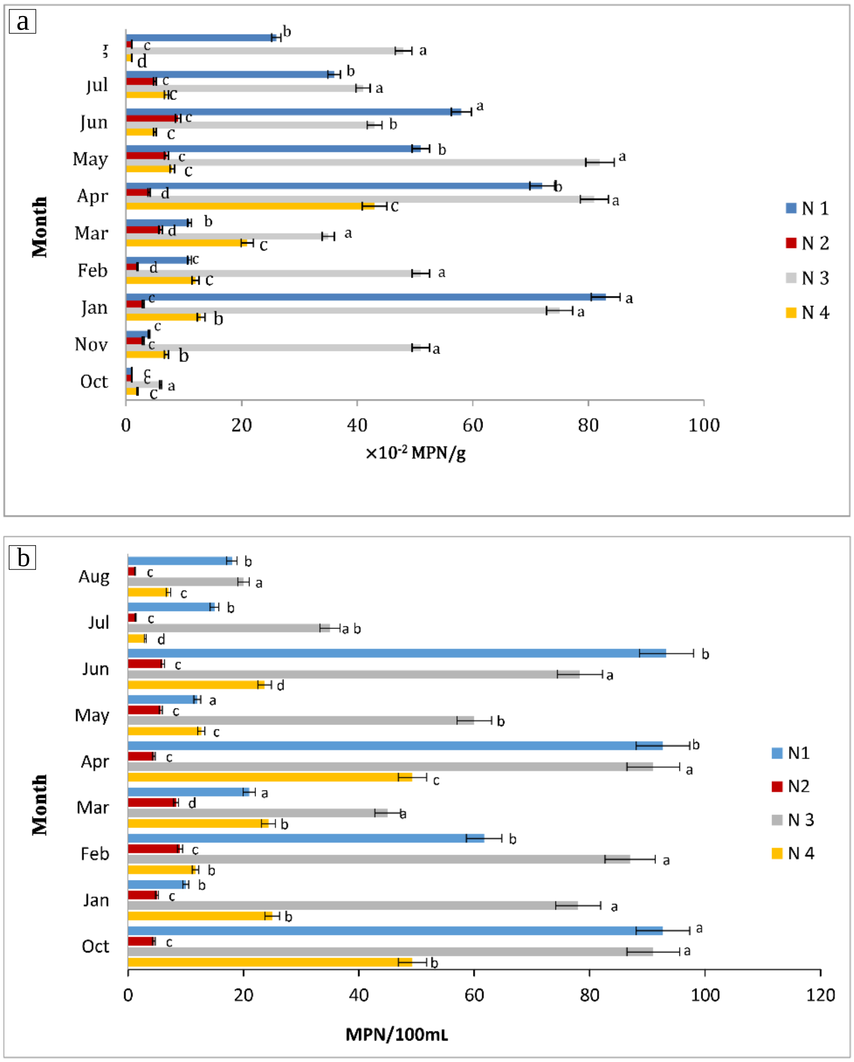

Fig 8. *E.coli* count in oyster (a) and water (b) samples. Data shown in the graph are mean±SEM. The mean values indicated in the graph with different superscripts were significantly different. ($p \leq 0.05$; One-way ANOVA followed by Tukey's pairwise test)].

All water samples were also positive for *E.coli*, ranging from 1.33 to 93.3 MPN/100 mL (Figure 8b). Significant differences in *E. coli* in samples collected were also observed among months ($p<0.05$). Higher *E. coli* values were recorded in the samples collected from sites N1 in April, June and October. In almost all events, sites N1 and N3 showed the highest microbial parameters. N3 receives effluents contaminated water mainly from the water flow of Hamilton Canal and Dandugam oya. Site N1 is directly connected to the sea. Therefore, these two sites receive a higher load of contaminants than the other two sites.

Correlation between water quality parameters and E.coli counts

E. coli is the primary bacterium in faecal coliforms and is considered the most precise indication of faecal contamination since it does not come from the environment but is only seen in high quantities in the faeces of humans and warm-blooded animals (Mets *et al.*, 2020). Therefore, the correlation between the water quality parameters and *E.coli* count may provide valuable insights into the faecal contamination of oysters. The correlation between the water quality parameters (pooled) and *E.coli* counts in water is shown in Table 1.

Table 1. Correlation between water quality parameters and *E.coli* count in water

Variable	Correlation (r)	P-value
salinity	0.242	0.194
pH	0.425	0.018
temperature	0.246	0.137

E. coli showed a weak positive correlation with all the water quality parameters. However, upon significance testing at a 95% confidence level, the p-value is higher than 0.05 (Table 1), indicating that the correlations between water quality parameters and *E.coli* counts are statistically insignificant. This may be due to the dilution of tested water quality parameters along with less polluted freshwater inflows.

Correlation between E.coli in oysters and E.coli in respective water

E.coli in oysters showed a strong positive correlation with *E.coli* in respective water (r=0.783). The correlation was statistically significant(p=0.00). This suggests a direct relationship where the oysters are likely accumulating *E. coli* from their surrounding environment highlighting the critical need for continuous water quality monitoring and management to ensure the safety of oysters harvested from these waters.

Conclusion and recommendation

As a whole, the oysters and water samples collected from sites N1 and N3 showed the highest microbial contamination due to the site-specific microbial contamination. Comparatively high microbial loads in oysters and water samples were observed in April, May and January. The correlations between *E.coli* in water and water quality parameters were statistically insignificant. Nevertheless, *E.coli* in oysters showed a strong positive correlation with *E.coli* in respective water and the correlation was statistically significant. Although the oysters collected from Negombo lagoon are safe for consumption, it is recommended to employ depuration, a critical step in ensuring the safety of bivalves to further reduce the microbial load of oysters prior to consumption.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this work.

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