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Analysis of microbiological contaminants in mussel *Perna perna* (Linnaeus, 1758), before and after depuration, from mariculture of the lowland coast, Rio de Janeiro, Brazil

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Abstract

Mussels can be used in the assessment of contamination of marine environments because of their filtering particles. Since they are used as a food source, this study aimed to evaluate the efficiency of the (experimental) depuration process for the microbiological quality of mussels obtained from two regions of the Baixadas Litorâneas in Rio de Janeiro. Samples of cultivation water and mussels were collected seasonally from March 2019 to February 2020, in a total of 240 animals from both regions to perform the Most Probable Number of coliforms at 35 °C, at 45 °C (E. coli), *Enterococcus* spp., research *Salmonella* spp., count of heterotrophic aerobic mesophilic and psychrotrophic bacteria in control samples and debugged. A low count of *E. coli* was observed for the cultivation water, but in mussels the counts were high in the winter period in Armação de Búzios, exceeding the limits established in accordance with the current legislation. However, the depuration technique adopted after collection provided a reduction in bacteriological counts, proving to be an effective process. Therefore, if there is a bacterial reduction with the adoption of the depuration process, there is a need for mitigation measures adopted by mariculturers and city halls regarding the safety of marine waters and molluscs.

Keywords: coliforms; contamination; depuration; microorganism; bivalve mollusc.

Practical Application: The present study focused on the need for a depuration unit of low operational cost, to assess its functionality in the routine activities of producers, to reduce possible bacterial contamination in bivalve molluscs.

1 Introduction

The quality of bivalve molluscs is related to the sanitary conditions of the marine environment in which they live. The sources of contamination in these environments are a result of the inadequate sewage runoff in the habitat of these molluscs (Souza et al., 2012; Giangaspero et al., 2014).

Some changes in the ecosystems of the Brazilian coast are caused by environmental changes such as disorderly occupation of coastal slopes, port activities, real estate speculation, exploration of energy resources and related activities, ship construction and repair, among others that result in the discharge of domestic and industrial effluents. Therefore, such activities can affect aquaculture and marine fisheries (Soares et al., 2015).

For an efficient sanitary control in the production chain of bivalve molluscs, constant microbiological monitoring of cultivated molluscs and waters is necessary, particularly because they are filter feeders: in addition to feeding on phytoplankton and suspended material, they also absorb contaminants (trace metals, pesticides, biotoxins) and microorganisms (enterobacteria such as *Salmonella spp.* and *Escherichia coli*) widely related to food outbreaks and diseases (Austin, 2010; Sande et al., 2010).

Members of the United States Centers for Disease Control and Prevention (2013) have reported that fish and shellfish are involved in 5% of individual cases and in 10% of all foodborne disease outbreaks (FDO), and most outbreaks were caused by the consumption of bivalve molluscs (Olgunoğlu, 2012).

Given the need for constant improvement of sanitary control actions to guarantee the supply of safe food to the consumer, there are laws in Brazil that provide for microbiological standards for ready-to-eat foods and lists of microbiological standards (Brasil, 2019), a National Program for Hygiene and Sanitary Control of Bivalve Molluscs (PNCMB), which establishes the minimum requirements necessary to guarantee the safety and quality of bivalve molluscs for human consumption (Brasil, 2012) and the standardization of thermotolerant coliforms in saline water of cultivation of bivalve molluscs intended for human consumption. This standardization is the geometric mean of the density of thermotolerant coliforms, of a minimum of 15 samples from the same source, not exceeding 43 per 100 milliliters, and 90% percentile should not exceed 88 thermotolerant coliforms in 100 milliliters (Brasil, 2005).

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Depuration is a controlled technological process in which bivalve molluscs are held in clean seawater treated with agents such as chlorine, ozone or ultraviolet light for a few hours in order to reduce the amount of microorganisms in their tissues through the natural filtration process (Rong et al., 2014).

Pursuant to federal legislation, a depuration plant is the establishment intended for the reception, purification, packaging, labeling, storage and shipping of bivalve molluscs (Brasil, 2020).

Different types of depuration systems are used, as follows: the open system with constant flow of water; the closed system, a closed recirculated sea-water system or the "Batch-process" system where water is replaced at regular intervals (Corrêa et al., 2006). The closed recirculation system is the most used because it uses less water. Therefore, this study aimed to evaluate the microbiological quality of the cultivation water and mussels pursuant to the limits of the current legislation, before and after the purification process (experimental module).

2 Materials and methods

2.1 Location of the study

The collections were carried out seasonally (autumn, winter, spring and summer) from March 2019 to February 2020. A total of 240 animals were obtained from the two regions of the Lowlands of Rio de Janeiro (Armação de Búzios and Arraial do Cabo) where there are commercial cultivations of mussels, *Perna perna* (*Linnaeus*, 1758).

The molluscs were randomly collected from only one producer at Praia Rasa in Armação de Búzios, in area 1 (22°`44' 20.1" S and 41° 56' 52.3" W) and from another producer at Praia do Forno in Arraial do Cabo, in area 2 (22° 58'2 83" S and 42° 0'25. 40" W) directly from the ropes used in the production and at different points (Figures 1 and 2).

In each selected region and at a certain season of the year, samples of seawater and mussels were collected (30 animals at each collection) for bacteriological analysis, and 25 liters of seawater were collected to supply the purifier used in the treatment of mussels. The collection of water samples was performed in duplicate using previously sterilized glass bottles (250 mL). To minimize external contamination, the protocol was followed, with water from approximately 0.5 m depth collected and the bottles dipped into the water preferably up-current.

The collected samples were kept refrigerated in isothermal boxes with filtered ice in polyethylene bags and sent to the Laboratory for Microbiological Control of Animal Origin Products (CMPOA), of the Department of Food Technology, Faculdade de Veterinária of Universidade Federal Fluminense (Niterói, RJ).

In the laboratory, the mussel samples were divided into three groups and evaluated for microbiological quality: control sample (CS) not subjected to depuration, samples obtained after the purification process (depuration) for 24 (D24) and 48 hours (D48).

In an air-conditioned room at an average temperature of 18 °C, the mussel samples were previously cleaned with the aid of a brush and under running water. The sediments and encrusting organisms were removed and allowed to air-dry in a plastic tray previously disinfected with 70% alcohol. For bacteriological analysis, six mussels were opened with the aid of a sterilized instrument beside a Bunsen burner to remove the shells and collected samples from the analytical unit, so that the amount required for analysis in 25 grams is obtained (International Organization for Standardization, 2003; Salfinger & Tortorello, 2015).

Bacteriological analyzes of the Most Probable Number (MPN) of Coliforms at 35 °C and Coliforms at 45 °C (*Escherichia coli*), were carried out with the multiple-tube fermentation technique

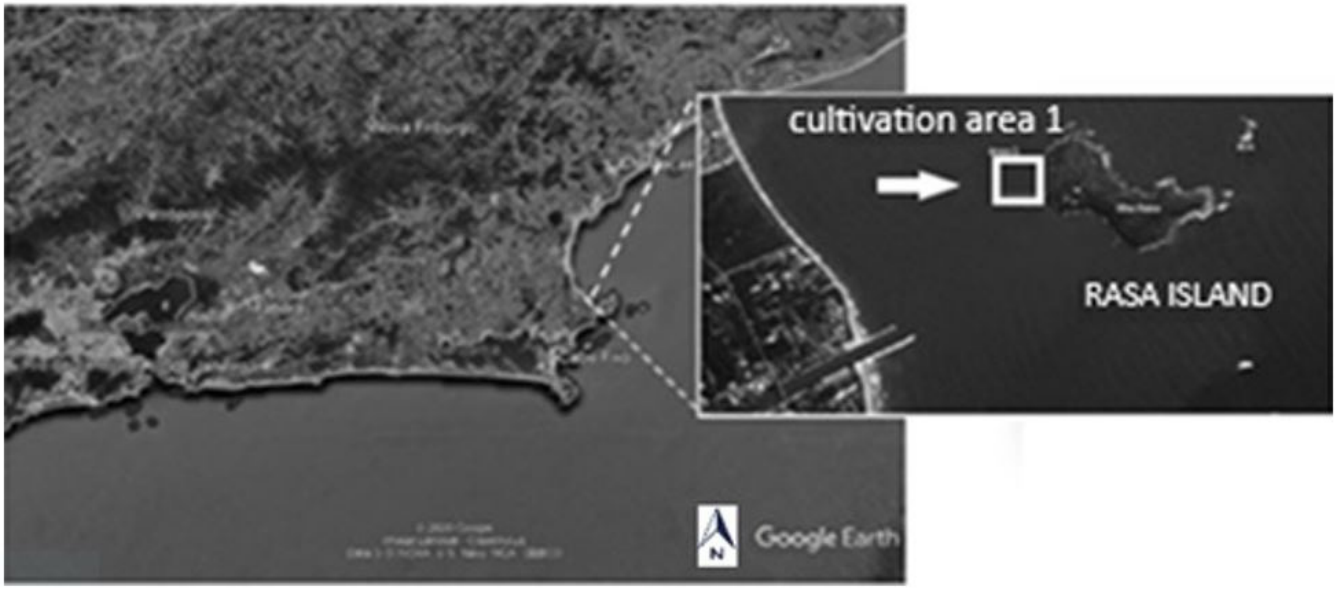

Figure 1. Location of the marine farm on Rasa beach near Rasa Island in Armação de Búzios (cultivation area 1). Source: Google Earth (2019).

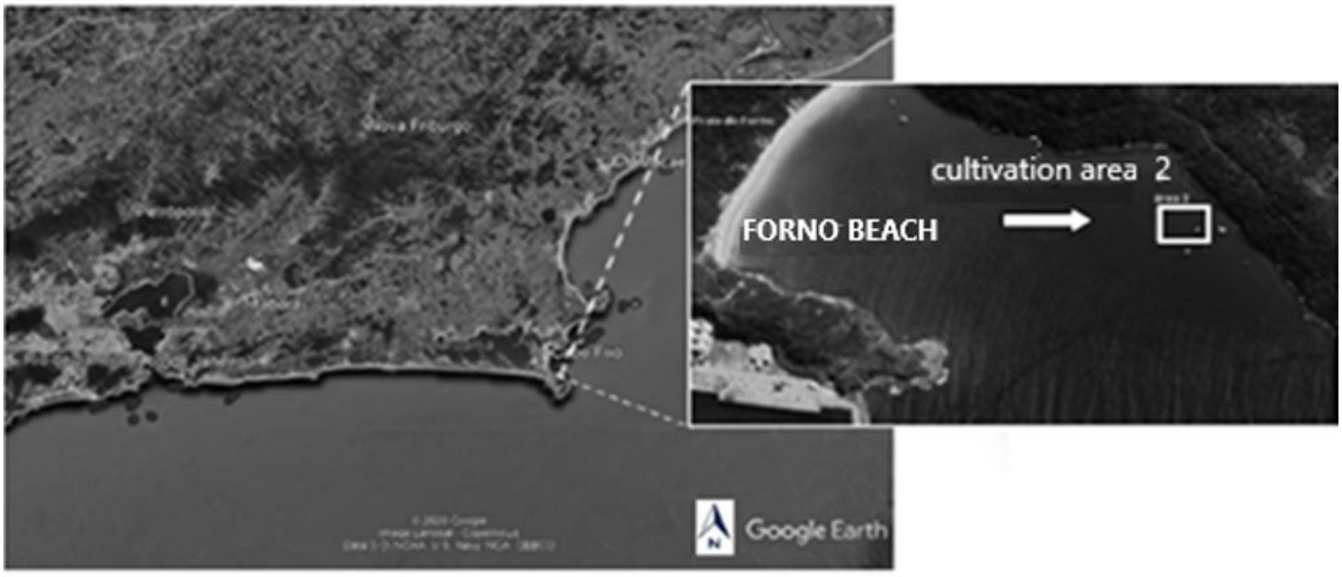

Figure 2. Location of the marine farm at Forno beach in the Marine Extractive Reserve of Arraial do Cabo (cultivation area 2). Source: Google Earth (2019).

(Feng et al., 2002), using Lauryl Sulfate broth (35-37 °C for 24/48 h) for the presumptive and other confirmed phases for total coliforms, using brilliant green lactose bile broth at 35-37 °C and *E. Coli* (EC) broth (44.5° ± 0.5 °C for 24/48 h). With the use of a platinum loop the EC broth tubes were inoculated in Eosin Methylene Blue Agar plates at 35-37 °C for 24 h, for observation of CFU that typically have a green metallic sheen. Confirmation was performed with biochemical tests Indole, Methyl Red, Voges Proskauer and Citrate (IMVic). The MPN was determined using Mac Crady's table, and contamination levels of coliforms expressed as MPN/100 g for molluscs (Salfinger & Tortorello, 2015), and table 9221, III, with levels of coliforms expressed as MPN/100 mL for the water sample (American Public Health Association, 1995). For the investigation of *Salmonella* spp. in mussels, 1% buffered peptone water was inoculated with the sample and incubated at 35 to 37 °C for 24 hours, in the pre-enrichment stage. For selective enrichment, Mossel and Rappaport Vassiliads broths were used. For selective plating, *Salmonella- Shigella* agar (selective differential medium), Hecktoen and Brilliant Green agar media were used. Typical colonies of *Salmonella* spp. were submitted to biochemical screening on triple sugar iron agar (TSI), lysine iron agar (LIA), in addition to biochemical tests in urea broth, phenylalanine agar, indole and Simons citrate agar. (Salfinger & Tortorello, 2015). In the count of Aerobic Mesophilic Heterotrophic bacteria (CAMHB) the pour plate method, Standard agar, was used for count and incubation at 35-37 °C for 24-48 h (Salfinger & Tortorello, 2015) and in the count of psychrotrophic bacteria (CBHAP) "spread plate", in Standard agar, was used for count and incubation of plates at 7 °C for seven to ten days, according to the methodologies recommended by Salfinger and Tortorello (2015).

To analyze the MPN of *Enterococcus* spp, an indicator of contamination with microorganisms, a fast miniaturized technique in multiple tubes (Eppendorf type) with chromogenic chromocult broth was used. The methodology was described by Merck (2000) modified by Franco and Leite (2005). For confirmation of the isolates obtained, the biochemical tests recommended in the methodologies were performed, and the study of morpho-tinctorial features was carried out with the Gram staining technique under oil immersion using a microscope.

To reduce possible bacterial contamination in bivalve molluscs, the depuration process was carried out in a low-cost miniaturized experimental system, with an ultraviolet light filter (36 watts), in a rectangular glass tank (66.8 L) 5 mm thick, 29 cm high, 59 cm long and 39 cm wide. A volume of 25 liters of seawater was used for 15 mussels, at an average temperature of 24 °C, in a closed recirculation system, with a SB1000C pump (110 v), with a maximum water flow of 1,000 L/h, in a cascade process to promote adequate ventilation. Samples for bacteriological analysis were removed after 24 and 48 hours of treatment.

The physicochemical parameters of water from cultures carried out (*in situ*) were also evaluated with the following instruments: for salinity measurement, a portable refractometer, model RTS – 101 ATC ("Instrutherm") was used; a Secchi disk was used for assessing turbidity; a box of universal application pH-indicator strips, model K36-014, brand Kasvi was used for measuring the pH of the water. Data on surface water temperature, rainfall and tidal levels were verified on the tide table site for each area where the bivalve molluscs were collected (Tábua das Marés, 2020).

2.2 Statistical analysis

The data obtained were expressed in Table 1 as the mean value and the respective standard deviation for temperature, salinity, pH and turbidity.

3 Results and discussion

Regarding the physicochemical parameters of the waters used in the culture of bivalve molluscs, in the four seasons of the

year (autumn, winter, spring and summer) (Table 1), the water temperature measured in Rasa beach ranged from 24.3 ± 2 .3 °C and at Forno beach, from 23.5 ± 1.7 °C, which are characteristic of subtropical regions. Salinity ranged between 35 ± 0.5 g/L at Praia Rasa and between 36 ± 1.0 g/L at Praia do Forno; pH was 7.0 (neutral) on both beaches, .Therefore, several studies report that seawater is a slightly alkaline solution, with a pH ranging on average between 7.5 to 8.4, and according to Conama Resolution No. 357 (Brasil, 2005), which regulates the cultivation of bivalve molluscs intended for human consumption, pH varies between 6.5 and 8.5. However, most pathogenic bacteria in the environment grow and multiply in acidic environments (with a pH ranging between 0 and 6.5). However, there are families of bacteria that only live in a neutral environment and others in an alkaline environment. Turbidity at Rasa beach ranged from 2.00 ± 0.5 m of visibility and at Forno beach it ranged from 5.40 ± 1.7 m of visibility, demonstrating that the region of Armação de Búzios had less transparency (amount of light reaching the water), greater amount of suspended matter, providing more food.

The results obtained with coliforms at 35 °C and *Enterococcus* spp. as hygiene sanitary indicators (as they do not have a standard in current legislation) were described in Table 2, and values greater than > 23 MPN/100 mL (coliforms at 35 °C) and up to 1.1 x 10^9 MPN/100 mL were observed (*Enterococcus* spp.).

However, the results found by Soares et al. (2015) for coliforms at 45 °C, in the same cultivation of marine molluscs at Praia Rasa (Armação de Búzios), were as follows: in February (summer) a concentration of > 2419.6 MPN/100 mL was recorded; 1413.6 MPN/100 mL in March (summer/fall), 128.3 MPN/100 mL in October (spring) and 93.4 MPN/100 mL in December (spring/summer) 2014. These data showed an increase in the density of coliforms at 45 °C because of the increase in population during the high season, which provides greater discharge of effluents, contrasting with the results obtained in the winter and spring periods. Compared to the present study, carried out in the same place of mollusc cultivation, in Armação de Búzios (RJ), the results of coliform densities at 45 °C in spring were 16.1 MPN/100 mL, but with a pathogenic strain classified as EIEC.

In winter, at Forno beach (Arraial do Cabo) 9.2 MPN/100 mL were recorded, a pathogenic strain classified in serology as EIEC and 2.2 MPN/100 mL in the spring, with a pathogenic strain identified in the serology as EPEC class. These results were below the allowable limit of 43 MPN/100 mL (Brasil, 2005).

Therefore, this study corroborates the finding of Kolm and Nowicki (2011) who recorded in Gamboa do Maciel, Paraná, Brazil, densities of coliforms at 35 °C and coliforms at 45 °C in the dry period (winter).

Table 1. Physicochemical parameters of water from cultivation in coastal lowlands municipalities (Armação de Búzios and Arraial do Cabo), in the state of Rio de Janeiro.

		Seasons of the year				
Beaches	Parameters	October/19	Winter/19	Spring/19	Summer/20	Mean/SD
Rasa	Turbidity (m)	1.30	2.04	2.10	2.55	2.00 ± 0.5
	pH	7	7	7	7	7
	Salinity (mg/L)	34	35	35	35	35 ± 0.5
	Temperature (°C)	26	21	24	26	24.3 ± 2.3
	Tidal coefficient	63 (mid)	58* (mid)	85 (high)	90 (high)	
Forno	Turbidity (m)	3.70	4.60	7.75	5.55	5.40 ± 1.7
	pH	7	7	7	7	7
	Salinity (mg/L)	37	36	37	35	36 ± 1.0
	Temperature (°C)	23	22	23	26	23.5 ± 1.7
	Tidal coefficient	64 (mid)	83* (high)	39 (low)	40 (low)	

SD: Standard Deviation. *Collection made after the undertow.

Table 2. Results of bacteriological analyzes of seawater samples collected on the beaches of Armação de Búzios and Arraial do Cabo in the state of Rio de Janeiro

		Seasons of the year			
Beaches	Analyzes	October/19	Winter/19	Spring/19	Summer/20
Rasa	*E. coli* (MPN/100 mL)	< 1.1	< 1.1	16.1*	< 1.1
	Coliforms at 35 °C (MPN/100 mL)	< 1.1	< 1.1	23	> 23
	Enterococcus spp. (MPN/100 mL)	< 3	< 3	9 x 10^2	23
Forno	*E. coli* (MPN/100 mL)	< 1.1	9.2**	2.2*	< 1.1
	Coliforms at 35 °C (MPN/100 mL)	< 1.1	16.1	5.1	< 1.1
	Enterococcus spp. (MPN/100 mL)	< 3	2.3 x 10^7	1.1 x 10^9	< 3

*Pathogenic strain (EIEC). **Pathogenic strain (EPEC).

Silva et al. (2008) used *Enterococcus* as an indicator in the rainy season (summer) and obtained high values in all beaches, up to 2.4 x 10^4 MPN/100 mL. However, in the dry season (winter), values were lower than those in the rainy season, but also high, up to 1.6 x 10^3 MPN/100 mL. Nevertheless, values of 2.3 x 10^7 MPN/100 mL (winter) and 1.1 x 10^9 MPN/100 mL (spring) were observed in this study, as described in Table 2, proving that they are more resistant to seawater.

Bacteriological analyzes of soft tissue of molluscs before and after the purification process (experimental module) were monitored by the MPN of coliforms at 45^{0}C (*E. coli*), as a parameter of the National Program for Hygiene and Sanitary Control of Bivalve Molluscs (PNCMB) defined as approved (concentration less than 230 MPN/100 g of edible part), approved under controlled condition (between 230 and 46,000 MPN/100 g) and suspended (above 46,000 MPN/100 g). Therefore, according to Table 3, in the winter season at Praia Rasa there was a higher density in the control sample (CS) of > 1.1 x 10^{18} MPN/g and in the purified sample (D48) of 2.4 x 10^{10} MPN/g, both confirming the results of the biochemical tests (IMVic) and the serology of the pathogenic strain identified in the enteroinvasive *E. coli* class (EIEC).

Despite the lack of a standard for the analysis of coliforms at 35 °C (Table 3), in the winter period, they showed the highest density in the control sample (CS) of > 1.1 x 10^{18} MPN/g and in the purified sample (D48) of 2.4 x 10^{10} MPN/g. However, in the results for the count of aerobic mesophilic heterotrophic bacteria, the highest density in the control sample (CS) was 3.1 x 10^{10} CFU/g (spring).

However, in the count of aerobic psychrotrophic heterotrophic bacteria, the highest density in the control sample (CS) was 1.8 x 10^6 CFU/g and in the purified sam*ple* (D48) 2.8 x 10^3 CFU/g (both in winter), and in the analysis of *Enterococcus* spp. the highest density was found in the control sample (CS) of 1.1 x 10^4 MPN/g (summer) and in the purified sample (D48) of > 1.1 x 10^6 MPN/g (winter).

The results obtained in the depuration (D24, D48) at Praia Rasa (Table 3), in spring, showed a significant reduction for CAMHB with the control sample (CS) of 3.1 x 10^{10} CFU/g and for sample (D24) of 8.4 x 10^4 CFU/g. However, in the summer, there was also a reduction for CAMHB with the control sample (CS) of 5.9 x 10^5 CFU/g, for sample (D24) 40 CFU/g, and for CAPHB with the control sample (CS), of 3.7 x 10^4 CFU/g for sample (D24) < 3 CFU/g.

However, during the winter, in the sample (D48) there was no such reduction in the microbial load for coliforms at 45^{0}C *(E. coli*) (2.4 x 10^{10} MPN/g), coliforms at 35 °C (2.4 x 10^{10} MPN/g), *Enterococcu*s spp. (>1.1 x 10^6 MPN /g) and aerobic mesophilic heterotrophic bacteria (4.2 x 10^{13} CFU/g), due to power outage in the laboratory. So, there was no proper ventilation and efficient ultraviolet light treatment in the depuration unit, deviated from the study objective.

However, at Forno beach (Table 4), in autumn, the control sample (CS) was 93 MPN/g, confirming the results of the biochemical tests (IMVic) and the pathogenic strain serology identified in the enteroinvasive class *E. coli* (EIEC). However, in winter, the control sample (CS) was 2.3 x 10^{10} MPN/g, contrasting with the results of biochemical tests (IMVic) and serology.

The results obtained in the depuration (D24, D48) showed a significant reduction for coliforms at 45 °C (*E. coli*), in winter, with the control sample (CS) of 2.3 x 10^{10} MPN/g, for sample (D24) < 3 MPN/g, and for CAMHB with the control sample (CS) of 1.8 x 10^{11} CFU/g for the sample (D48) 5.4 x 10^5 CFU/g, but such reduction was not observed for *Enterococcus* spp. (>1.1 x 10^9 MPN/g).

Therefore, in the spring, there was a significant reduction for CAMHB with the control sample (CS) of 4 x 10^7 CFU/g for sample (D24) 1.4 x 10^2 CFU/g and for CAPHB with the control sample (CS) of 5.3 x 10^6 CFU/g for sample (D24) 320 CFU/g.

Bacteriological analyzes for *E. coli* densities (CS; D24 and D48) detected in this study were within the limits established by the National Program for Hygiene and Sanitary Control of Bivalve Molluscs (PNCMB), in autumn, spring and summer. However, in winter, the levels of these bacteria were very high, exceeding the limits of 46,000 MPN/100g or 460 MPN/g. The processing activities were considered "suspended", according to the PNCMB, with criteria for authorized removal under the condition of bivalve molluscs.

Table 3. Results of microbiological analysis of MPN of E. coli, Coliforms at 35 °C and *Enterococcus* spp., Count of Aerobic Mesophilic and Psychrotrophic Heterotrophic Bacteria and search for Salmonella spp. in mussel samples, from Rasa beach, in the municipality of Armação de Búzios, State of Rio de Janeiro.

Season of the year	Hours	MPN/g of *E. coli*	MPN/g of Colif. at 35 °C	MPN/g of *Enterococcus*	UFC/g of CAMHB	UFC/g of Psychrotrophic bacteria	*Salmonella* 25 g
Autumn	0	< 3	< 3	< 3	7 x10^3	< 3	Absent
	48	< 3	< 3	< 3	4.9 x10^2	< 3	
Winter	0	>1.1x10^{18}*	>1.1 x10^{18}	3	3.9 x10^6	1.8 x10^6	Absent
	48	2.4x10^{10}*	2.4 x10^{10}	>1.1 x10^6	4.2 x10^{13}	2.8 x10^3	
Spring	0	< 3	2.1 x10^2	1.2 x10^3	3.1 x10^{10}	5.2 x10^2	Absent
	24	< 3	< 3	4.2 x10^2	8.4 x10^4	2.6 x10^2	
Summer	0	< 3	1.1 x10^4	1.1 x10^4	5.9 x10^5	3.7 x10^4	Absent
	24	< 3	1.1 x10^2	2.8 x10^2	40	< 3	

Control sample - CS (0 hour), Purified Sample (D24/ D48 hours). UFC - colony forming unit. *Pathogenic strain (EIEC).

Table 4. Results of microbiological analysis of MPN of E. coli, coliforms at 35 °C and *Enterococcus* spp., Count of Aerobic Mesophilic and Psychrotrophic Heterotrophic Bacteria and search for Salmonella spp. in samples of mussels, from Forno beach in the municipality of Arraial do Cabo, State of Rio de Janeiro.

Season of the year	Hours	MPN/g of *E. coli*	MPN/g of Colif. at 35°C	MPN/g of *Enterococcus*	UFC/g of CAMBH	UFC/g of Psychrotrophic bacteria	*Salmonella* 25 g
Autumn	0	93*	93	< 3	7 x10	< 3	Absent
	48	< 3	< 3	< 3	3 x10	< 3	
Winter	0	$2.3x10^{10}$**	$1.1 x10^{12}$	$>1.1 x10^{9}$	$1.8 x10^{11}$	$1.0 x10^{6}$	Absent
	48	< 3	$4.6 x10^{3}$	$>1.1 x10^{9}$	$5.4 x10^{5}$	$7.6 x10^{5}$	
Spring	0	< 3	93	90	$4 x10^{7}$	$5.3 x10^{6}$	Absent
	24	< 3	< 3	6	$1.4 x10^{2}$	320	
Summer	0	< 3	$4.6 x10^{3}$	< 3	$3.6 x10^{5}$	$3.4 x10^{9}$	Absent
	24	< 3	$2.4 x10^{3}$	< 3	$2.8 x10^{4}$	$1.0 x10^{7}$	

Control sample - CS (0 hour), Purified Sample (D24/ D48 hours). UFC - colony forming unit. *Pathogenic strain (EIEC). **There was no biochemical confirmation for E. coli growth.

In addition to the fact that the results obtained exceeded the limits recommended by the current legislation, the occurrence and presence of *E. coli* strains of the EIEC class (enteroinvasive *E. coli*), EPEC (classic enteropathogenic *E. coli*) in samples of water used in the cultivation of molluscs and EIEC (enteroinvasive *E. coli*) in mussel soft tissue deserve mention. Therefore, the use of a national and international system for the safety of products of animal origin is necessary (Franco et al., 2008).

Farias et al. (2010a) reported the occurrence of enteroinvasive *E. coli* (EIEC) and classical enteropathogenic *E. coli* (EPEC) in sururu (*Mytella guayanensis*) from the Vaza Barris river estuary (Sergipe, Brazil). The results for coliforms at 35 °C ranged between 1,700 and 54,000 MPN/g and for coliforms at 45 °C ranged between 200 and 22,000 MPN/g, and both the mussel and river water were positive for EPEC and negative for EIEC. However, the presence of *E. coli* in food represents a risk to consumers, as some species are proven to be pathogenic, and responsible for serious illnesses and diarrhea, such as uremic syndrome and hemorrhagic colitis (Nascimento et al., 2007).

Farias et al. (2010b) reported a probable relationship between the levels of coliforms at 45 °C and *Salmonella* spp. in the meat of bivalve molluscs. Microbiological analysis of bivalve molluscs in the Ceará River estuary, in Fortaleza, Ceará, Brazil detected *Salmonella* spp. in these molluscs at a level greater than that of coliforms at 45 °C.

No correlation was found in this study between the levels of the two microorganisms, as the results of controls (CS) and depurated samples (D24, D48) for *Salmonella* spp. were absent in 25 grams, according to Normative Instruction No. 60, of December 23, 2019. As for the results of *Enterococcus* spp. (Praia do Forno), in winter (Table 4), a high density was observed in the control sample (CS), as well as in the purified sample (D48). This suggests that the microorganism's resistance is due to its high tolerance to adverse growth conditions, such as: ability to grow at temperatures between 10° and 45 °C, in the presence of 6.5% sodium chloride and at pH 9,.6 (Hartman et al., 1992; Brasil, 2001). This group is composed of gram-positive bacteria that are usually more resistant to seawater and water treatments than the coliform group (Mendonça-Hagler et al., 2001).

Corroborating high microbiological results for coliforms at 35 °C and 45 °C (*E. coli*), CAMHB, CAPHB and *Enterococcus* spp., in both Tables 3 and 4, one factor that can influence the presence of these microorganisms was the fact that the tidal coefficient was between medium to high (Table 1). The reason for this is that large coefficients imply large high tides and low tides, with the occurrence of large currents and movement of the seabed (Tábua das Marés, 2020), which impacts the safety of seawater and mussels.

According to Croci et al. (2002), the oyster depuration rate for *E. coli* was evaluated. In this experiment, oysters were collected after 5h, 24h and 44h of depuration and microbiological tests were conducted for counting these bacteria. The levels of *E. coli* quickly dropped from 1.1×10^5 MPN/g to 2.4×10^2 MPN/g at the end of the experiment.

In this study, depuration was carried out for 24h and 48h, and the MPN/g of *E. coli* in mariculture of Praia do Forno recorded was 93 MPN/g (CS) to < 3 MPN/g (D48) in the autumn season. As for the winter season, there was a reduction from 2.3×10^{10} MPN/g (CS) to < 3 MPN/g (D48). Therefore, it is not possible to assess the efficiency of the depuration unit (D48) during the winter, in the mariculture of Praia Rosa, due to a power outage in the laboratory, which made it difficult to ensure proper ventilation and efficient UV light treatment.

Given the results obtained in this study and other data available in the literature (Love et al., 2010; Rong et al., 2014), and in order to ensure the food safety of molluscs, the importance and efficiency of the depuration process in the elimination of contaminants is unquestionable. However, it is worth reflecting on the parameters required by legislation. Causative agents of food diseases, such as Vibrio species (*Vibrio parahaemolyticus*, *V. cholerae* and *V. vulnificus*) were not the focus of this study.

Other factors must be considered in the assessment of the efficiency of purification, such as temperature, salinity and the level of initial contamination, as well as the nature of that contamination. Furthermore, artificially contaminated molluscs have a faster depuration rate than those that have been naturally contaminated (Oliveira et al., 2011).

4 Conclusion

It is concluded that the cultivation water analyzed in both beaches had low levels of *E. coli,* according to the current legislation. However, in the bacteriological analyzes of meat of bivalve molluscs, the detected densities of *E. coli* were high in the winter period (Armação de Búzios), exceeding the limits established in by the National Program for Hygiene and Sanitary Control of Bivalve Molluscs. However, the presence of *E. coli* strains of the EIEC class (enteroinvasive *E. coli*) and EPEC (classical enteropathogenic *E. coli*) poses a risk to the safety of water and meat consumption. Hence further studies that address the limits established by the current legislation are needed, mainly due to the presence of pathogenic strains.

On the other hand, the present study focused on the need for a depuration unit of low operational cost, to assess its functionality in the routine activities of producers. The results obtained were effective after 24 and 48 h for coliforms at 45 °C *(E. coli*), coliforms at 35 °C, CAMHB and CAPHB. However, the depuration plant proved to be ineffective in the purification process for *Enterococcus* spp, which belong to a group of gram-positive bacteria generally more resistant to seawater and water treatments than the group of coliforms. Therefore, it is believed that the two investigated locations need: incentive to infrastructure and promotion of Malacoculture, a bivalve mollusc seed production unit, public policies, depuration units, the implementation of the National Program for the Monitoring of Bivalve Molluscs, as well as mitigation measures to be adopted by mariculturers and municipalities, in order to guarantee the safety of seawater and cultivated molluscs.

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