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Bacteriological Quality of the Mangrove Oyster (*Crassostrea rhizophorae*) in the Swamp of Mestizo - Cordoba (Colombia)

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Abstract

Objectives: To determine the bacteriological quality of the mangrove oyster and its aquatic environment, such as a risk factor in public health, through the consumption of these products from the natural environment. **Materials and Methods:** Data were collected about 60 samples of oysters and the surrounding water, between June and November 2004. Using the indirect method of counting multiple fermentation tubes, we estimated the concentration of bacteria indicators of total and fecal coliform contamination. The indicators of the bacteriological quality of the oysters presented a minimum of ≤ 3 NMP.100 mL^{-1} and a maximum of 46 × 104 MPN.100 mL^{-1} of total coliforms, fecal coliforms showed a seasonal variation in concentrations between ≤3 NMP.100 mL^{-1} and 2 × 103 MPN.100 mL^{-1}. **Findings:** The analysis of water samples recorded a range of concentration of coliforms from ≤ 3 NMP.100 mL^{-1} to 3.9 × 105 NMP.100 mL^{-1} and for fecal coliforms it ranged from ≤ 3 NMP.100 mL^{-1} to 7 × 103 MPN.100 mL^{-1}. This study suggests that there exists a directly proportional relationship between the bacteriological quality of the mangrove oyster *Crassostrea rhizophorae* and the water quality of the environment, by its status filtering, which contributes a factor of risk to public health, the consumption of this food, especially if the consumption is done in a direct way. **Applications/recommendations:** the potentiality of the Bay of Mestizo, Colombia, for the development of a sustainable aquaculture of the mangrove oyster (*Crassostrea rhizophorae*), requires baseline studies on various environmental and social aspects which will lay the basis for the socio-economic development of this region.

Keywords: Molluscs, total coliforms, fecal coliforms, sustainable aquaculture, public health.

1. Introduction

Estuaries are semi-aquatic systems where fresh water is mixed with the marine waters[1,2]. The pollution of these ecosystems for the discharge of wastewater of domestic origin and urban represents one of the main causes of alteration in the environmental quality health care. The bivalve molluscs represent some of the food of animal origin from the highest consumption in the world. Have a high protein content, representing 50% of the dry matter, also has a relatively high carbohydrate content (28% of the dry matter) and a low lipid content (11% of the dry matter)[3,4]. Within these molluscs is the oyster *Crassostrea rhizophorae*, its habitat are the estuaries where they are permanently fixed to hard surfaces and to the roots of the red mangrove (*R. mangle*) forming clusters. It displayed throughout the year, but with higher intensity from June until mid-December[5,6].

The oyster *Crassostrea rhizophorae* is an invertebrate filtrador [7], able to filter water at a rate of 2 to 5 liters/hour, assimilating the biotic and abiotic contaminants present in the environment[8,9]. Within the biotic contaminants are bacteria that are widely distributed in the aquatic environment which have recognized the family Vibrionacea: *Vibrio cholerae*, *V. parahaemolyticus* y, *V. vulnificos*, among

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other potentially pathogenic to man. On the other hand if the systems are fecal pollution filtradoras, by its terms, can be found in the interior of these bivalves' microorganisms such as *Salmonella sp*., *Shigella sp*., enteric viruses, total coliforms (TC) and fecal coliforms (FC). For all these implications the determination of fecal coliform bacteria of fecal origin such as *E. coli* provides reliable information on the hygiene-sanitary conditions of the water and of the oyster, since this species represents the 90% of fecal coliforms and is a microorganism indicator of faecal contamination to the molluscs have their origin solely of the digestive tract of the homeotherms organisms[10, 11, 12, 13, 14].

The ingestion of bivalve has been associated to food related to infectious disease[8, 15]; however marketing has been carried out on the beaches with greater tourism potential of the country, offering them to the consumer in a crude way, directly in the shell. Under this allegation, the study was conducted bacteriological monitoring of the oyster *Crassostrea rhizophorae* and body of water where it is developed in the swamp of Mestizo, in order to evaluate the sanitary conditions of the bivalve mostly marketed on the beaches and tourist attractions of the department of Cordoba.

2. Materials and methods

The Swamp of Mestizo is located between the 9 or 25′ N, 75o 48′ W, in the Cispatá Bay, in the department of Córdoba. It is characterized by a coastal humid tropical climate, with temperatures above 27°C[16]. Samples of water and oysters were taken every 15 days for six months, from June to November of 2004, in three different stations called station one (E1), season two (E2), and three (E3) in the swamp of Mestizo. For the water samples, 300 cc of sterile bottles were used which were immersed in each of the stations to a depth of 30 cm approximately, to fill the 80% of the bottle sampled, the samples were stored at 4°C[5, 17,18]. For the samples of *Crassostrea rhizophorae* between 50 and 60 specimens of approximately 6 and 7 cm were collected, consecutively were stored in sterile plastic bags to 4°C[8], until the arrival at the Laboratory of Aquaculture Health of the University of Cordoba.

The biological samples were processed by scraping the outer surface with sterilized material, cleaning with rubbing alcohol to 70% and rinsed with distilled water and sterilized. The soft tissues of the oysters are aseptically removed and weighed (100 g); they were subsequently homogenated in a beaker with 100 ml of peptone water 0.1%[8]. For the enumeration of total coliforms and fecal coliforms in water samples, an in samples such as oysters, we applied the technique of multiple tubes expressed in the Most Probable Number in 100 mL (MPN/100 mL), with bright green bile broth. Presumptive tests were performed for total coliforms, registering a growth of bacteria by turbidity after 24 hours and the confirmatory tests, after 48 hours. For fecal coliforms, the presumptive tests were positive, when those tubes showed turbidity and gas production; the confirmatory test was performed by replication of the pipes with gas production incubated for 24 hours to 44.5 ± 0.5°C, to be analyzed by the biochemical tests[19,20].

3. Results and discussion

Both the water samples and the oyster (*Crassostrea rhizophorae*) samples exhibited for the growth of total and fecal coliforms. The plating of the tubes with gas production in the EMB agar (Eosin Methylene Blue Agar), exhibited colonies with metallic green brightness characteristic of the *Escherichia col*i and the biochemical tests confirmed the presence of *Escherichia coli* in the samples studied (Figure 1).

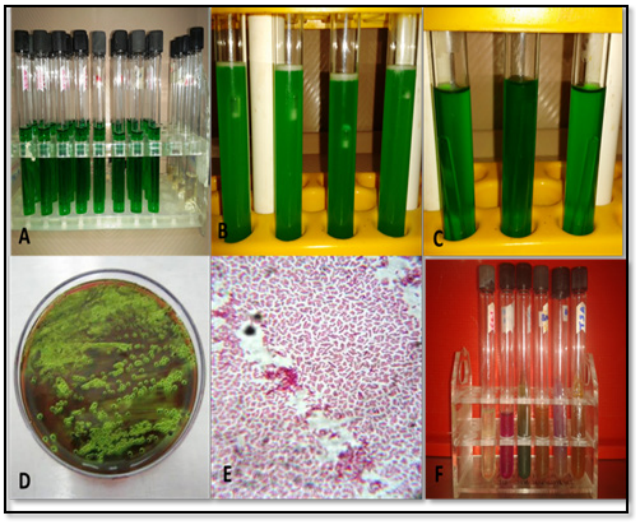

Figure 1. Colimetria test for samples of water and oysters (C. rhizophorae) of the Swamp of Mestizo. A: test of colimetrìa; B: Tubes with gas production; C: Tubes with production of turbidity; D: Plating in agar EMB; E: Gram Stain; F: Biochemical tests

The indicators of the bacteriological quality of the oysters taken in the swamp of Mestizo, presented a minimum of ≤ 3 NMP.100 mL^{-1} and a maximum value of

46×10^4 MPN.100 mL^{-1} of total coliforms, while the water introduced at least a value of ≤ 3 NMP.100 mL^{-1} and a maximum of 3.9×10^5 NMP.100 mL^{-1} of total coliforms, in relation to be statistically significant differences between samples with P<0.05 for total coliforms (Figure 2).

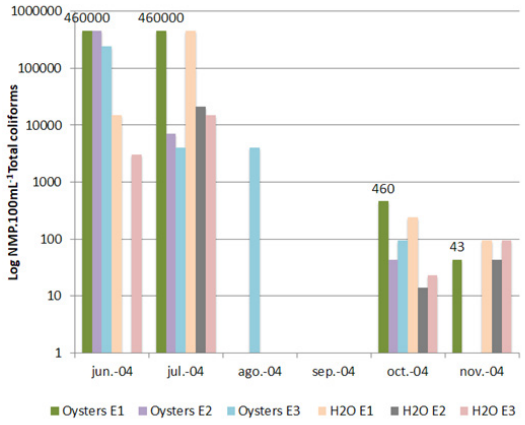

Figure 2. Values of Total Coliforms in samples of oysters and water from the swamp of Mestizos during the period from June to November of 2004

The indicators of the bacteriological quality of samples of oysters reported for fecal coliforms a minimum of ≤ 3 NMP.100 mL^{-1} and a maximum value of 2 × 103 MPN.100 mL^{-1}. The water samples were recorded for fecal coliforms at least a value of ≤ 3 NMP.100 mL^{-1} and a maximum value of 7×10^3 MPN.100 mL^{-1} and presented statistically significant differences between samples with P<0.05 for fecal coliforms (Figure 3).

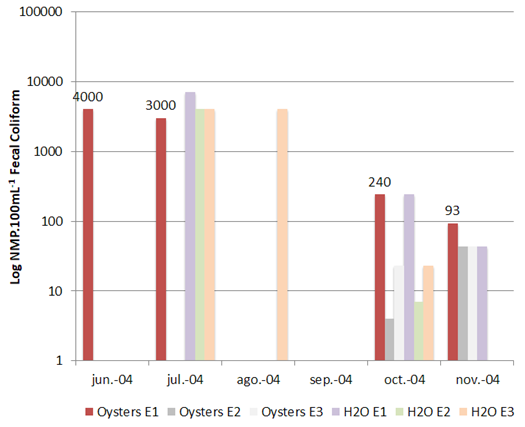

Figure 3. Values of fecal coliforms in the samples of oysters and water from the swamp of Mestizos during the period from June to November of 2004

The samples of *Crassostrea rhizophorae* in the E1 presented a total coliform concentration of 7.8 × 104 MPN.100 mL^{-1}, in the E2 is recorded concentrations of 2.5×10^3 MPN.100 mL^{-1} and the E3 2.1×10^3 MPN.100 mL^{-1} (Figure 4). There was no statistically significant difference found (P<0.05) between stations. The concentration of fecal coliforms in the E1 was 2.5×10^3 MPN.100 mL^{-1}, the E2 presented a value of 7.1×10^2 NMP.100 mL^{-1} and the E3 registered a concentration of 6.3×10^2 NMP.100 mL^{-1} (Figure 4), no statistically significant difference was found (P<0.05), between stations. With regard to the quality of the oysters in terms of total coliforms found that 42% (N = 15) had less than 3 NMP.100 mL^{-1}, the 75% (N = 27) were less than or equal to 1,000 NMP.100 mL^{-1}. For its part, the 50% (N = 18) were under the age of 3 NMP.100 mL^{-1} fecal coliforms and the 75% (N = 27) of the samples recorded concentrations less than or equal to 200 NMP.100 mL^{-1} (Figure 5).

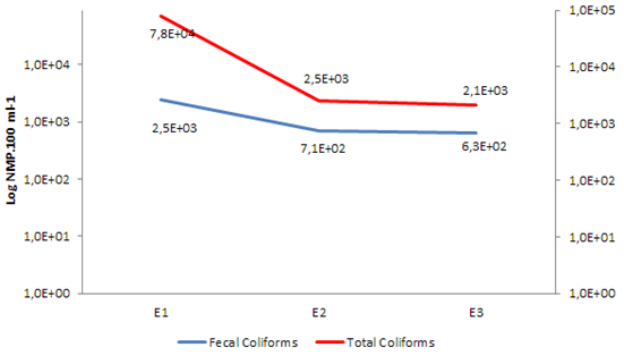

Figure 4. Average values of total and fecal coliforms in oysters of mangroves (C. rhizophorae) of the Swamp of Mestizo in the stations one (E1); two (E2) and three (E3)

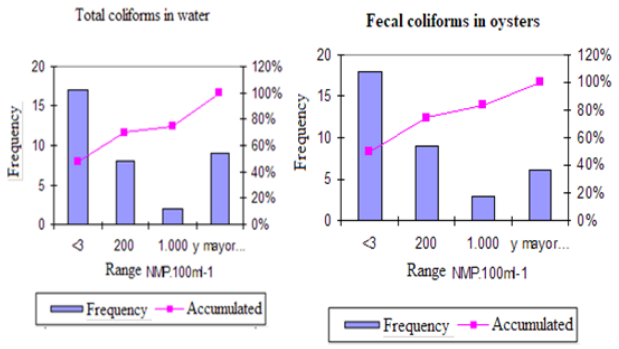

Figure 5. Frequency distribution of the total and fecal coliforms in oysters from the morass of Mestizos, during the period from June to November 2004

The mean values of the water samples in the sampling sites from the train one (E1) presented a value of 7.7 × 104 MPN.100 mL^{-1} of total coliforms, season two (E2) reported 4.1 × 104 MPN.100 mL^{-1} and the three (E3), 2.1 × 104 MPN.100 mL^{-1} (Figure 6). There was no statistically significant difference was found (P<0.05) between stations. With regard to the determination of fecal coliform bacteria was determined average values in the train one (E1), 9.5 × 10^2 NMP.100 mL^{-1}, 3.4 × 102 NMP.100 mL^{-1} in the season two (E2) and in the season three (E3) were estimated 6.8 × 102 NMP.100 mL^{-1} (Figure 6). There was no statistically significant difference (P<0.05) between these stations. Of the 36 water samples taken during the period June−November 2004, in The Swamp Mestizo, it was found that 47% (N = 17) of the cases presented lower total coliform concentrations of 3 NMP.100 mL^{-1}, the 69% (N = 25) less than or equal to 200 NMP.100 mL^{-1} and 75% (n = 27) of less than 1000 NMP.100 mL^{-1}. The 61% (N = 22) of the water samples showed less than 3 NMP.100 mL^{-1} fecal coliforms and the 83% (N = 30) less than or equal to 200 NMP.100 mL^{-1} (Figure 7).

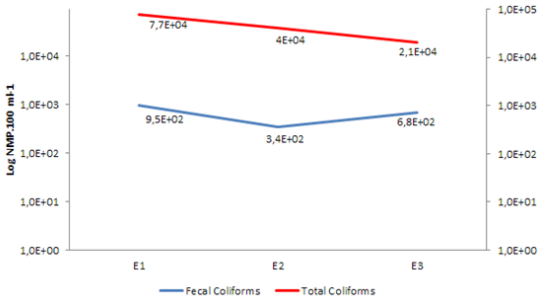

Figure 6. Average values of total and fecal coliforms in water of the Swamp of Mestizo in the stations one (E1); two (E2) and three (E3)

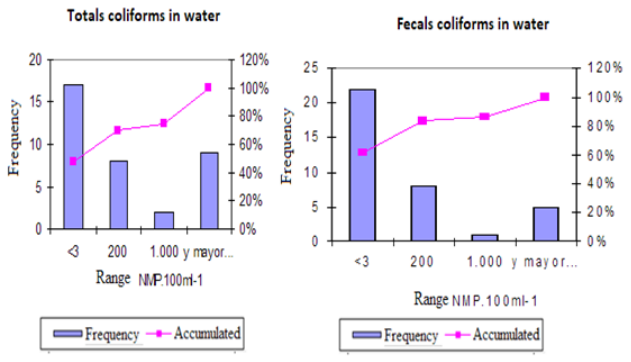

Figure 7. Frequency distribution of the total and fecal coliforms in water of the Swamp of Mestizos, during the period from June to November 2004

We evaluated the correlation of the bacteriological quality of the water and the bacteriological quality of the oyster and found a high correlation between the total coliforms present in the water and those found in mangrove oysters (0.94; p 0,005); in the same way we found a high correlation between fecal coliforms present in the water and the of the mangrove oyster (0.81; p 0,005). The presence coliform group is closely related to the growth of the population at the global level, and in the same way with the increase of the domestic, agricultural and industrial, that due to the constant dumping in natural bodies of water are the source of deterioration of the environment with significant implications at the ecological, socio-economic and health[20].

In Colombia in the year 2004 was marketed a total 55.71 tonnes of mollusc of which the oyster is one of the most traded molluscs with a total of 20 tonnes. Its high consumption increases the risk of enteric diseases if they are in contaminated sites. In Colombia, through the Ministry of Health and Social Protection by means of the resolution 000122 of 2012 established for microbiological requirements that must be met for the fishery products, in particular, the fish, molluscs and crustaceans for human consumption; fresh and frozen molluscs established a maximum allowable rate of good quality of *E. coli* 10 cfu/ g and a maximum allowable index to identify the acceptable level of good quality of *E. coli* 400 cfu/g. In the present study, according to the resolution of the Ministry, the 50% of the samples of oysters are of good quality and 75% are of acceptable quality. The International Commission on Microbiological Specifications for Foods (ICMSF) sets the maximum allowable limit 106 cfu/g. In the present study the 100% of the samples of oysters analyzed did not pass the set limit mentioned above, however, the possibility of transmission of disease by the consumption of oyster already exists that *E. coli* besides being considered indicator of fecal contamination, causes a diversity of diseases capable of producing haemorrhagic colitis and haemolytic uraemic syndrome[5].

Previous study resulted in the contamination of the mangrove oyster *Crassostrea rhizophorae* of the Cienaga from Santa Marta with pollution levels exceeding 11 × 10^8 NMP.100 ml and the present study corroborates the high levels of coliform contamination that can acquire the oysters to be found in ecosystems exposed to the introduction of organic matter[21, 22]. In the course of the present study we found variations in the concentration of coliforms (≤ 3 up to 4.6 × 10^5 MPN/100 ml) and fecal coliforms

(≤ 3 up to 7.0×10^3 MPN/100 ml) in the water samples. The highest incidence was observed during June, July, October and November while August and September are the months of lowest presence. When the *E. coli* are present in a high percentage, there has been a heavy contamination and/or represented by human or animal waste. If the number of *E. coli* found in small proportions means that pollution is of the same type but less recent or less important. If coliforms are detected but not E. coli, it indicates that pollution is recent but of fecal origin or of faecal origin but distant, so that intestinal coliforms have not survived[5].

The Colombian legislation has been established by chapter IV of Decree 1594 of 1978, the criteria for the control of water pollution, article 41 sets out the criteria of quality eligible for the destination of the resource for recreational purposes of primary contact (bathroom, swimming pools). Studies of the samples of water from the swamp of Mestizo showed that 75% of the samples are accepted for total coliforms and 83% of the samples for fecal coliforms not surpassing the limits permissible for primary contact. Similar studies were carried out in the beaches of Cordoba which showed permissible levels for the fate of these waters in activities of primary contact[20]. The bacteriological quality of the oysters and water stations proved to be statistically different, being the one with the lowest quality, possibly caused by the environmental characteristics of the site, such as precipitation, sea currents and the anthropic factors that bring the residual material in the natural sources.

There is a directly proportional relationship between the coliform bacteria present in the water and the oysters for their habit and physiology of power; however, there is a lag of concentrations within and outside of the oyster with relation to the quality of the surrounding water over time, due to the retention capacity of microorganisms oysters have in its filtering system.

4. Conclusions

The bacteriological quality of the water and oysters depends on environmental factors such as precipitation and the marine currents, as well as the factors that bring the residual material in the natural sources. There is a directly proportional relationship between the coliform bacteria present in the water and the oysters for their habit and physiology of power; however, there is a lag of concentrations within and outside of the oyster with relation to the quality of the surrounding water over time, due to the retention capacity of microorganisms that oysters have in its system. The quality of the oyster (*Crassostrea rhizophorae*) harvested in the swamp of half-bloods will be directly related to the quality of the surrounding water in the last few days before the harvest. The bacteriological quality of the water and oysters in the stations proved to be statistically different. Station 1 exhibited for the lowest quality with a poor rating in 75% of the samples. The oysters analyzed in the station 2 and 3 presented an acceptable level in 80% of the samples.

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