

# Microbiological quality of cultivation water used for oysters marketed in Cananeia-SP, Brazil

## Qualidade microbiológica da água da cultura de ostras comercializadas em Cananeia-SP, Brasil

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### Abstract

Bivalves represent the environment conditions where oysters are farmed, thus is vital that these organisms are not contaminated because they can represent a serious risk to the public health. This study aimed to assess the microbiological quality of water for the cultivation of oysters of Cooperostra (cooperative of producer) in Cananeia-SP, Brazil. The total and fecal coliforms were analyzed. The results showed that water quality were not satisfactory, according to Brazilian legislation, except for some months. The seasonal interference was analysed and a high positive correlation was verified, with low coliforms concentration in winter. The pluviometric index showed a high positive correlation to the microorganisms analysed.

**Keywords:** Coliforms. Water. Cananeia. Microbiology. Ostreidae.

### Resumo

Bivalves representam as condições do ambiente em que são cultivadas ostras, sendo portanto vital que esses organismos não estejam contaminados, pois podem representar um risco grave para a saúde pública. Este estudo teve como objetivo avaliar a qualidade microbiológica da água para o cultivo de ostras da Cooperostra (cooperativa de produtor), em Cananeia-SP, Brasil. Os coliformes totais e fecais foram analisados. Os resultados mostraram que a qualidade da água não foi satisfatória, de acordo com a legislação brasileira, exceto por alguns meses. A interferência sazonal foi analisada e uma alta correlação positiva foi verificada, estando a concentração de coliformes baixa no inverno. O índice pluviométrico apresentou correlação alta e positiva para os microrganismos analisados.

**Palavras-chave:** Coliformes. Água. Cananeia. Microbiologia. Ostras.

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## INTRODUCTION

The coliform group was defined by *Standard Methods for the Examination of Water and Wastewater* as all aerobic and facultative anaerobic, gram-negatives, non-spore and rod-shaped, which ferment lactose with gas formation in 48h at 35 °C. This group includes organisms that differ in biochemical, serological and habitat. These organisms can be classified as *Escherichia*, *Aerobacter*, *Citrobacter*, *Klebsiela* and other genus that almost never appear in feces as *Serratia*.

The Cananeia Estuary waters fits in CONAMA resolution number 20 that defines brackish water in class 7 for: a) primary contact recreation; b) aquatic communities protection; c) natural or intensive (aquaculture) cultive of species to human consumption.

The estuary-lagoon complex of Cananeia, Iguape and Ilha Comprida, southern state of São Paulo (Brazil), represents one important coastal regions of Brazil, considered by IUCN (International Union for Conservation Nature) as the third biggest estuary of the world, in primary productivity and a nursery of Atlantic species. The region includes a Biosphere Reserve of Atlantic Forest and comprises several protected areas: Federal APA Cananeia, Iguape and Peruíbe, APA State of Ilha Comprida, Ecological Station of Chauás, Island State Park of Ilha do Cardoso and Mandira Extractive Reserve.

This study aimed to assess the microbiological quality of cultive water of oysters, analysing the presence of total and thermotolerant coliforms, since 1999 to 2004, in estuarine water of Cananeia.

## METHODS

Samples of estuarine waters of Cubatão Sea were collected, monthly, in front of Cooperostra (Cooperative of oysters producers) from Cananeia, during six years (January 2005 – December 2011), in flood and ebb tide phases.

Water samples were collected at a depth of 15 to 30 cm with the bottle turned against flow direction according to The Guidelines of Bier<sup>1</sup>, Harrigan and McCamce<sup>2</sup>, Guimarães<sup>3</sup>, Taylor<sup>4</sup> and standards of The Society of Environmental Technology and Sanitation<sup>5</sup>.

The samples were analyzed in three main steps: 1) the total number of bacteria; 2) the most probable number (MPN) of coliforms; and 3) the thermotolerant coliforms determination in each sample collected.

### The total number of bacteria

Samples integral and diluted (1/10, 1/100 and 1/1000) were seeded in simple Agar medium and incubated at 37 °C for 24-48 hours. The colony count was done with stereoscopic magnifiers and determined the number of bacteria per volume sown.

### The most probable number of coliforms (NMP)

Diluted samples, as described for CETESB<sup>5</sup>, were seeded in simple lactose broth. Tubes with gas production were considered positive and was used to confirmatory test. This analysis was performed on lactose-broth bile brilliant green, 2%, where also gas production was observed. The MPN of coliform per hundred milliliters was determined by the Taylor Table<sup>4</sup>.

### The thermotolerant coliforms determination (100ml)

Positive tubes were seeded in EC broth, specific to *Escherichia coli*, used as pathogenic enteric bacteria indicator, and were incubated at 44.5 °C, during 18 to 22 hours where it was found again, the gas production. The MPN of coliforms per 100ml was determined by Taylor Table<sup>4</sup>.

## RESULTS

The distribution of CF concentrations, from 2005 to 2011, was shown in Table 1. It was found that thermotolerant coliforms presented higher values than permitted by Law, in all studied years although the total coliforms indexes presented values according to CONAMA resolution to 2007, 2009 and 2010.

Throughout months it was observed high concentrations of CF, with the highest values (1600 NMP) in May and November 2009, August 2010, August and October 2011, which also showed the same maximum concentrations for coliforms. The lowest values occurred in December 2001, June 2002 and August, October and November 2010 (Table 1).

**Table 1.** Monthly averages of coliforms in NMP in 2005 to 2011

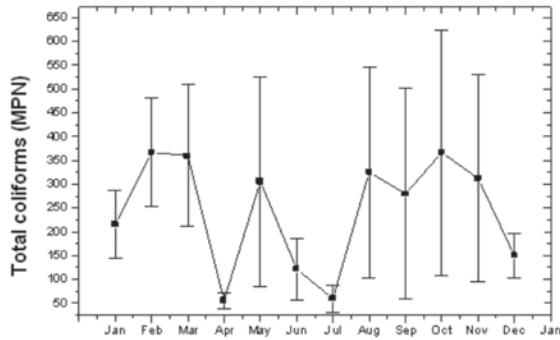
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	% of the samples above the threshold of CONAMA resolution	% of samples in accordance with the resolution CONAMA
<b>Year 2005</b>														
<b>Total Coli-forms</b>	324*	672*	72*	75*	143*	132*	182*	12	62	11	15	332*	66,66	33,33
<b>Thermo-tolerant Coliform</b>	92*	228*	49*	18	210*	110*	94,4*	11,3	63*	10,5	10	276*	66,66	33,33
<b>2006</b>														
<b>Total Coli-forms</b>	423*	102*	378*	10	12	20	12	10	180*	12	248*	14	33,33	66,66
<b>Thermo-tolerant Coliform</b>	236*	99*	218*	9	11	13	9	8	24*	13	279*	21*	58,33	41,66
<b>2008</b>														
<b>Total Coli-forms</b>	760*	592*	220*	190*	225*	80*	-	205*	31	72	7	18	63,63	36,36
<b>Thermo-tolerant Coliform</b>	551*	487,8*	83,5*	42,5*	176,8*	62,5*	-	153*	31*	62	6	4	72,72	27,27
<b>2009</b>														
<b>Total Coli-forms</b>	15	468*	210*	62	1200*	10	12	15	10	-	1600*	450*	45,45	54,54
<b>Thermo-tolerant Coliform</b>	19*	350*	150*	10	800*	8	7	6	4	-	1600*	300*	54,54	45,45
<b>2010</b>														
<b>Total Coli-forms</b>	16	26	23	13	20	80*	100*	17	1600*	8	16	46	25	75
<b>Thermo-tolerant Coliform</b>	8	26*	23*	6	16*	80*	40*	6	1600*	8	6	10	58,33	41,66
<b>2011</b>														
<b>Total Coli-forms</b>	80*	40	700*	12	35	800*	100*	1600*	17	1600*	250*	120*	66,66	33,33
<b>Thermotolerant Coliform</b>	18	30	700*	6	20	500*	70*	1600*	17	1600*	120*	100*	66,66	33,33

\* Values at odds with the limits of CONAMA resolution.

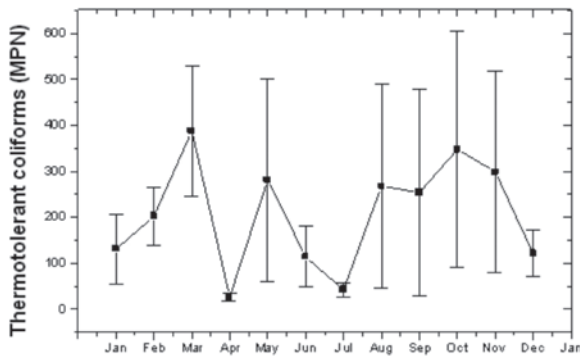
The monthly distribution of CF showed decrease in April and July (Figure 1 and 2). There were significant differences (ANOVA,  $p < 0.05$ ) for these months when compared to the others studied. The seasonal variation for total coliforms no showed statistical difference, although during winter there

was a clear decrease of the mean relative to other seasons (Figure 3). Thermotolerant coliforms averages for the summer season were statistically different in spring (Figure 4). Correlating the counts of FC with rainfall, there was high correlation for FC ( $R = 79$ ) and total ( $R = 86$ ) (Figure 5 and 6).

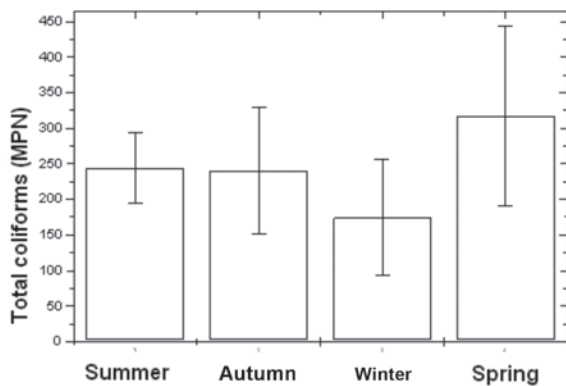
**Figure 1.** Monthly variation of total coliforms in the period from 2005 to 2011. The bars represent the respective standard deviations



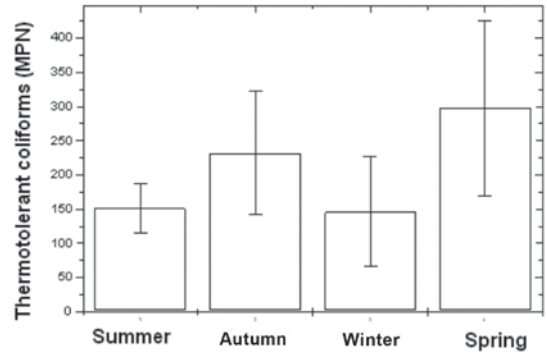
**Figure 2.** Monthly variation of thermotolerant coliforms in the period from 2005 to 2011. The bars represent the respective standard deviations



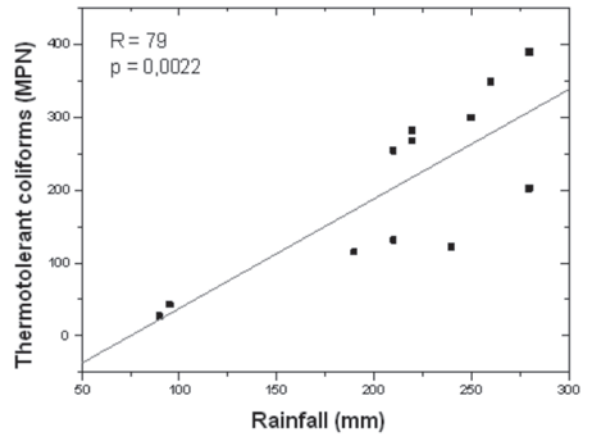
**Figure 3.** Seasonal variation of total coliforms during the period from 2005 to 2011. The bars represent the respective standard deviations



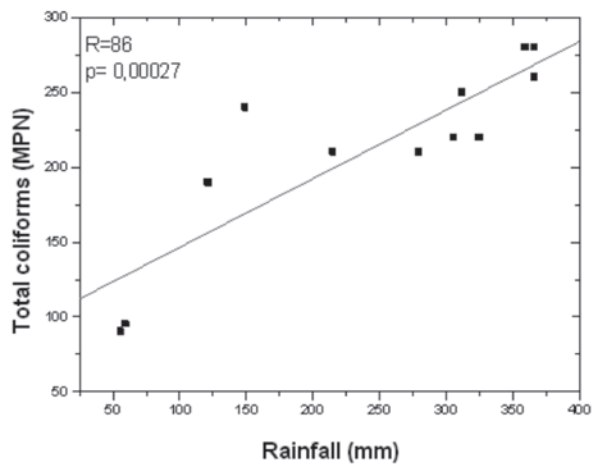
**Figure 4.** Seasonal variation of thermotolerant coliforms in the period from 2005 to 2011. The bars represent the respective standard deviations



**Figure 5.** Correlation between rainfall averages and the values for thermotolerant coliform



**Figure 6.** Correlation between rainfall averages and the values for total coliforms



## DISCUSSION

The CF indexes determination in Cananeia estuary Waters depends on this region is considered an important fishing area and is used to aquaculture.

The figures show the temporal variability in CF concentration in the study area. The values fluctuation has been mainly determined by variations and dynamics of tides. However, the pattern tends to become more complex with rainfall effect during the period from January to April.

The high correlation between thermotolerant coliforms, total coliforms and rainfall averages agrees with Galvão<sup>6</sup>, which states that the rains interfere in rates of microbiological quality of the water because it has the ability to drag sewage and solid waste to waterways that flow into the sea.

Cananeia is characterized by high rainfall, with annual average of 2670 mm<sup>7</sup>. The rains in the region are related to the seasons, with rainy summers and dry winters, with the average monthly maximum is 266.9 mm in summer and in winter of 95.3 mm<sup>7</sup>.

The rainy period extends from December to April, with monthly average of 200 mm, and the dry period from May to November, with lower values never below 80 mm<sup>7,8</sup>.

Natural flow in estuaries scales involve a phenomena of physical quantities transport, which occurs in the turbulent regime. These physical quantities can be mass of pollutants, concentration of microorganisms or thermally affected mass. Although ideally balanced, transport turbulent present high difficulties of quantification for natural environments. Despite efforts its detailed quantification is momentarily a question unanswered<sup>9,10</sup>. Natural processes, such as dispersion of pollutants in water, depend essentially on the hydrodynamic flow or current. However, the actual flow is dependent on factors external, as physiographic characteristics, meteorological, physical-chemical or anthropogenic, biological<sup>9,10,11,12,13,14,15</sup>.

Ability of dispersion, dilution or depuration in a body of running water is a particular feature, highly variable, hence the difficulty of generalizing study characteristics of these processes. Velz<sup>9</sup>

states that the character of water resources is dynamic and complex (emphasizing estuarine regions, with spatial and temporal variations), and the stable equilibrium in the hydrological environment is established through complex interactions between water flow channel and the vegetation cover, which are relatively time-varying (theme also seen in Cunha<sup>13</sup>).

Drainage systems nature is radically influenced by physiography (in a stable watershed, determining factors as climate, precipitation, temperature, wind speed, vapor pressure, solar radiation, are highly variable in time and space), which induces variations in flow and assimilation capacity of waste<sup>9,11,14,15,16</sup>. This influences channels characteristics, which have a major role on the dispersion, dilution or self-purifying water bodies, thus influencing the concentration of coliforms. For this reason we did not find a TC pattern of distribution over the months studied, although there is a trend of decreasing values during the winter and the months of April and July.

According to CONAMA Resolution No. 20, which defines brackish waters, in which the waters of this work can be inserted, the usage for primary contact recreation which should be obeyed to Article 26. For the use of natural creation or intensive species for human consumption and that will be eaten raw should not be exceeded an average concentration of 14 coliforms per 100 milliliters of no more than 10% of the samples exceeding 43 coliforms per 100 milliliters. For other uses shall not exceed a limit of 1,000 thermotolerant coliforms per 100 milliliters in 80% or more of at least 5 monthly samples collected in any month, in case there in the region, means available for the FC examination, the index threshold is up to 5000 coliforms per 100 milliliters of 80% or more of at least five monthly samples collected in any month<sup>17</sup>. In this study there were few months where this resolution could be accomplished.

Since Cananeia is a 11,000 inhabitants city, with 50% of sewage is treated it is difficult to explain the high levels of coliform. The effectiveness of treatment could be questioned or assume that these values are natural. Therefore, a study of CF

levels in various parts of the estuary, with the goal of ascertaining changes during low tides and high efficiency and especially the sewage treatment plant Cananeia is necessary. Furthermore, the microbiological determination in several areas where farming oysters is essential to avoid risk to the health of the consumer<sup>18,19</sup>.

If the treatment has not effectively and emphasizing the relationship issues of sanitation to human health, we can relate some waterborne diseases and exemplify the different types of relationships between the environment, diseases and sanitation. For example, hepatitis A and typhoid fever, as well as most of diarrheal diseases are acquired through consumption of water contaminated by manure, and are related, so with sewage, distribution and treatment of water supply. Due to the strong relationship with sanitation (water quality and destination of the waste) and the significant number of cases, according to WHO (2000), more than 10 million children die every year due to diarrhea.

The main diseases related to the ingestion of contaminated water and its causative agents<sup>16</sup> are: cholera (*Vibrio cholerae*), bacillary dysentery (*Shigella* sp.), typhoid fever (*Salmonella typhi*), infectious hepatitis (Hepatitis Virus Type

A), paratyphoid fever (*Salmonella paratyphi* A, B, C), gastroenteritis (other types of *Salmonella*, *Shigella*, *Proteus* sp.), infant diarrhea (enteropathogenic types of *Escherichia coli*), leptospirosis (*Leptospira* sp.). These bacteria have not been detected in the estuary, but its determination would be interesting to get an idea of the degree of contamination (if any), with a view to a policy of environmental and public health and especially for growing oysters.

## CONCLUSION

These results may suggest that a simple diagnosis of the environmental conditions of crop fields is insufficient to assess the water quality of shellfish cultivation, due to fluctuations in the levels of contamination throughout the year and by the fact of contaminants accumulation in marine biota, especially filtering organisms. Thus, a program of continuous monitoring of planted areas would be suitable not only for assessing the potential of these for mariculture and for ensuring the safe consumption of seafood, but also constitute an important tool for understanding the relationship between this contamination and environmental variables involved.

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