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MICROBIAL QUALITY OF WATER, SEDIMENT, FISH AND SHELLFISH IN SOME BRAZILIAN COASTAL REGIONS

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Resumo

Os estudos sobre a microbiota de ambientes costeiros brasileiros foram basicamente focalizados nos aspectos sanitários envolvendo indicadores de poluição e estão revisados no presente capítulo. As populações de coliformes fecais encontradas nos ambientes estudados foram constituídas predominantemente da espécie *Escherichia coli*, e outras bactérias da família *Enterobacteriaceae*, provavelmente de origem fecal. Os indexes de coliformes fecais, encontrados em águas costeiras, apresentaram correlação positiva com outros parâmetros indicadores de poluição e com a ocorrência de alguns microrganismos patogênicos. Outros parâmetros microbianos, tais como as contagens de estreptococos fecais, leveduras, bactérias heterotróficas, *Pseudomonas aeruginosa* e *Staphylococcus aureus* podem ser utilizados como indicadores de poluição. As águas de praias brasileiras, localizadas em regiões urbanas, frequentemente apresentaram níveis de contaminação fecal superiores aqueles aceitáveis para uso em atividades de recreação e pesca. Os animais e sedimentos associados à ambientes aquáticos contaminados podem apresentar níveis mais elevados de organismos indicadores e patogênicos em relação as populações detectadas na água. Medidas de saneamento básico visando a melhoria da qualidade das águas nas regiões costeiras brasileiras são necessárias para proteção de recursos naturais importantes para as atividades econômicas de turismo e pesca.

Palavras-chave: bactéria, leveduras, organismos indicadores, poluição aquática, coliformes.

Abstract

Studies of microorganisms in Brazilian coastal regions have focused largely on pollution indicators and pathogens and are briefly reviewed here. Fecal coliform counts taken in Brazilian tropical waters have been confirmed to be mostly *Escherichia coli* and other *Enterobacteriaceae* of probable fecal origin, and to correlate well with other pollution parameters including frequency of occurrence of some pathogens. Other microbial counts such as fecal streptococci, yeasts, heterotrophic bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus* can also be useful as pollution indicators. Water of Brazilian urban beaches is frequently contaminated to levels above those acceptable for use as bathing or fish harvest areas. Where water is contaminated according to microbial indicators, the associated sediments and animals can have even more elevated levels of pathogens and indicator organisms. Improvement in the sanitary quality of Brazilian coastal waters is needed to protect resources of economic importance to the tourism and fishing industries.

Key-words: bacteria, yeasts, indicator organisms, aquatic pollution, coliforms.

Introduction

Human activities frequently result in damage to the environment, and coastal ecosystems are not an exception to this rule. During the last few decades, global awareness regarding the pollution of the oceans has increased. According to the United Nation Convention on the Oceans, marine pollution is the direct or indirect introduction by humans of materials or energy in the marine and estuarine environments when it causes or can induce deleterious effects to living resources and life in the marine environment, risks to human health, losses to marine activities such as fishing and other legitimate uses (Anonymous, 1998). Brazil is a maritime nation with 7408 km of coast, and with 22% of the population residing in coastal counties. Most of the wastewater and sewage generated by coastal cities in Brazil do not receive adequate treatment. Also, agricultural and industrial wastes, together with run-off from heavy rains, contribute as sources of aquatic pollution. The natural discharge of rivers in estuarine coastal zones can bring water polluted with sewage and industrial effluents, and contribute to dispersal of pollutants from the land. Another relevant source of marine pollution is the discharge of oil and wastes from boats with marine transportation activities, occasionally resulting in accidents like the oil spills causing considerable, and often long term, damage to the marine biota. Concern with chemical water pollution risks have overshadowed the equally significant risks associated with microbial pollutants. The main sources of pathogenic microbes are domestic and animal wastes. In addition, the increase of organic matter concentration in the water results in greater populations of the endemic microbiota which can include significant human pathogens.

Coastal communities rely on recreation activities and fishing as relevant economic resources. The use of contaminated recreation waters can result in transmission of various human diseases. Elderly people, pregnant women, children and imumuno-compromised patients are at higher risk than others from contact with pathogenic microbes present in polluted water. Also, microbial communities associated with sea food caught from contaminated beaches and estuaries can contain pathogenic species that are responsible for substantial outbreaks of food poisoning. The main microorganisms associated with waterborne diseases are listed in Table 1.

Data on waterborne disease outbreaks in Brazil is scant, but in the United States of America, the Centers for Disease Control (CDC) estimate that each year up to 900,000 cases of illness and possibly 900 deaths occur as a result of waterborne microbial infections (ASM, 1998). Pathogenic microbes found in water include many types of bacteria, fungi, protozoa and virus-causing diseases ranging from diarrhea to heart and respiratory diseases. In this chapter we review work regarding microbial quality of water, beach sediments, and marine animals along the Brazilian coast.

Table 1. Principal pathogenic and opportunistically pathogenic waterborne microorganisms and some diseases they can cause.

Microorganism	Disease
Bacteria	
Aeromonas hydrophila	Diarrhea
Campylobacter sp.	Diarrhea
Cyanobacteria (blue-green algae toxins)	Diarrhea & Hepatic cancer
Escherichia coli (pathogenic serotypes)	Diarrhea
Flavobacterium meningocepticum	Meningitis
Helicobacter pylori	Gastritis
Legionella pneumoniae	Pneumonia
Leptospira sp.	Leptospirosis
Pseudomonas aeruginosa	Eye & Ear infections
Salmonella spp.	Diarrhea
Shigella spp.	Diarrhea
Vibrio cholerae	Cholera
Vibrio parahaemolyticus	Gastroenteritis
Vibrio vulnificus	Skin & Tissue infections
Staphylococus aureus	Skin infections
Yersinia enterocolitica	Gastroenteritis
Fungi	
Candida spp.	Skin & Tissue Infections
Trichosporum cutaneum	Skin & Tissue infections
Protozoa	
Acanthamoeba	Eye infection
Cryptosporidium spp.	Diarrhea
Giardia lamblia	Diarrhea
Entamoeba histolitica	Diarrhea
Pfiesteria (dinoflagellate toxins)	Neurological disease
Virus	
Adenoviruses	Gastroenteritis
Caliciviruses	Diarrhea
Coxsackieviruses	Encefalitis & Meningitis
Echoviruses	Meningittis
Hepatitisviruses	Hepatitis
Polioviruses	Poliomielitis
Rotaviruses	Gastroenteritis

Microbial Indicators of Water Quality

The presence of disease-causing microbes in water means it is of inadequate sanitary quality. It is important to assess the risk imposed by the microbial pollution by using monitoring programs and research to evaluate the meaning of the data obtained for different types of aquatic environments and water uses. It is not practical to detect and enumerate most water-born pathogens in routine monitoring programs. The traditional approach in monitoring microbial water quality is to employ groups of organisms of fecal origin as pollution indicators (*APHA, 1995*, Hagler & Mendonça-Hagler, 1988). An ideal indicator should fulfill the following characteristics: be present and in higher population when the pathogen is present, be associated with the source of pathogens, have a better survival in the environment and be more resistant to disinfection processes, be easy to detect by simple and fast methods, have populations proportional to the level of pollutants, be well distributed in the sample and not multiply in the environment. Unfortunately, no known indicator of sanitary quality fulfills all these criteria. The methodology used to assess the microbiological water quality is described in the reference work "Standard Methods for the Examination of Water and Wastewater" of the American Public Health Association. The most traditional microbial indicator of sanitary water quality is the coliform group which consists of bacteria belonging to the family *Enterobacteriaceae,* defined as all facultatively anaerobic, Gram-negative, non-spore forming rod-shaped bacteria that ferment lactose with gas and acid formation within 48 h at 35°C. The group includes a number of species belonging to the genera *Escherichia, Klebsiella, Citrobacter* and *Enterobacter*. When the coliform test is performed at 35 ± 0.5 °C the parameter is reported as total coliforms (TC), but at an incubation temperature of 44.5 ± 0.2 °C it is referred to as the fecal coliform (FC) test. More than 95% of the bacteria isolated from feces grow at this elevated temperature which is more selective for organisms of fecal origin. Seawater is toxic to coliform bacteria, but Oliveira (1990) found that counts were stable for 24hr in refrigerated samples of tropical marine and estuarine waters. The complete total coliform test is the traditional standard for drinking water whereas presumptive fecal coliforms are frequently used to monitor environmental water quality. The standard procedures to enumerate coliforms use the multiple-tube or most probable number (MPN) method or the membrane filter (MF) method. New methods have been proposed based on the detection of the enzyme b-galactosidase using chromogenic substrates. Coliform bacteria fulfill many of the criteria for an ideal fecal pollution indicator. However, they have some disadvantages regarding resistant pathogens such as protozoan cysts and viruses. Therefore, coliform indexes generally reflect recent contamination with feces of warm- blooded animals.

The fecal streptococcus (FS) or enterococcus group is another widely used fecal indicator. They comprise Gram-positive bacteria that are usually more resistant to sea water and water treatment procedures than coliforms. The species included in this group give a positive reaction with Lancefield's group D antisera and have been isolated from feces of warm blooded animals. Fecal streptococcus counts have been supported by some studies as an alternative for fecal coliform counts in evaluating bathing quality of recreational waters (American Public Health Association, 1990). The extent of fecal pollution present in recreational surface waters measured by fecal streptococci have been shown to have a positive correlation with swimming-associated gastroenteritis in studies done by EPA in the United States and also, recently, by CETESB in São Paulo, Brazil. (M. I. Sato, personal communication). The EPA (1986) guidelines for primary-contact recreation in fresh water is 33 enterococci /100 ml and for marine waters it is 35/100 ml, based on the geometric means of at least five samples per 30 day period during the swimming season (*APHA, 1995*). These indexes are comparable with the level of 200 fecal coliforms per 100ml as a primary-contact recreational standard. The ratio of the fecal coliform to fecal streptococcus counts have been used to differentiate human fecal contamination from that of other warm blooded animals. This microbial parameter has the disadvantage of high levels of false positives detected by the standard methodology. Some biotypes of streptococci from non-fecal sources can grow in the media used to count fecal streptococci (Araujo *et al.*, 1990).

Other microbial counts can be valuable to complement coliform counts as indicators of less recent or non-fecal pollution.(*APHA, 1995*) Sulfate reducing anaerobic spore-forming bacteria, mainly *Clostridium perfringens,* have been used as an indicator of non-recent fecal pollution based on the better survival of this endospore forming bacterium than coliforms in the environment. Water polluted with a heavy load of organic matter can increase the population of opportunistic pathogens and this condition is reflected by high levels of heterotrophic bacteria and fungi including yeasts. Viruses such as bacteriophages have been proposed as indicators to represent this microbial group in aquatic environments. The use of virus standards is restricted because the methods to detect and enumerate them are time consuming and expensive for routine use. Microbial parameters based on pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, have sanitary significance in recreational waters, providing additional information to complement fecal coliforms in analysis of marine and chlorinated water. The detection of specific pathogens in the water is particularly important during a disease outbreak, for instance the enumeration of *Vibrio cholerae* during cholera epidemics.

Heterotrophic bacteria are normal inhabitants of marine and estuarine environments (Mendonça-Hagler & Hagler, 1991). They play an important role in the degradation and mineralization of organic matter and represent an important element in food chains in the environment. Their population size is closely dependent on organic matter concentration in the water. They can be present as free-living in the water mass or associated with different substrata. The numbers of heterotrophic bacteria usually increases in coastal waters as a result of run-off from land. In the water column, the heterotrophic population is higher at the thin surface layer (neuston), at the thermocline layer, and near the bottom (Reinheimer, 1969). Cultivation methods do not enumerate more than few percent of the population present in environmental samples due to lack of efficiency or selectivity of the culture media or the metabolic state of the cells. Gomes *et al.* (1997) found total bacteria determined by acridine orange direct counts on membrane filters to be 2 to 4 orders of magnitude higher than total heterophic bacteria as determined by plate counts in a coastal lagoon of Rio de Janeiro. Roszak *et al.* (1984), studying *Salmonella enteritidis* survival in water, used the term "viable but nonculturable" for bacteria that demonstrated metabolic function, but were not culturable by any available method. The low metabolic activity is assumed to be a strategy for survival of Gram-negative bacteria analogous to the dormant stages of Gram-positive endospores and protozoan cysts. *Vibrio* spp., *Campylobacter* spp. and other relevant waterborne human pathogens retain their viability under environmental stress by entering the nonculturable state (Rollins & Colwell, 1986). Bacterial cells that cannot be cultured are traditionally considered dead, but they continue to be viable for weeks, months and even years. Nonculturable *V. cholerae* cells detected by fluorescent microscopy were demonstrated to produce clinical symptoms of cholera in human volunteers triggering outgrowth of cells to the culturable state and confirming the potential pathogenicity of *V. cholerae* in the aquatic environment (Colwell & Huq, 1994).

Several molecular approaches have been applied to microbial ecology. A comprehensive review of these methodologies has recently been published by Rosado *et al.* (1999). The methods are mostly based on the polymerase chain reaction (PCR) applied to environmental DNA, cloning and sequencing of ribosomal RNA or other genes aiming to delineate a better picture of the microbial diversity in the environment. Molecular methods are also powerful tools to assess the potential risk posed by microbial water pollution; however, they are still too expensive and impractical for routine use. Another useful approach to detect microbes in the environment is the use of specific immunoassays. Rapid detection kits based on fluorescent antibodies are commercially available for field use to improve diagnostic capability.

Water quality of bathing beaches

Frequent reports have appeared in the popular press in recent years noting that a large portion of the urban bathing beaches from Ceará to Rio Grande do Sul are polluted to the point of not passing Brazilian bathing water standards. The microbial water quality of Brazilian beaches located in the vicinity of urban centers have been monitored by local environmental authorities such as CETESB in São Paulo and FEEMA in Rio de Janeiro states. The pollution standard used in the monitoring programs follows the legally binding guidelines published by "Conselho Nacional de Meio Ambiente" (CONAMA, 1988). Waters are classified accordingly to the type (fresh, brackish or marine) and the intended use. The recommended standard for satisfactory sanitary quality of water for primary contact recreational activities, including bathing, in brackish and marine waters is a maximum of 1000 fecal coliforms (FC) in 100ml of water of 80% of a minimum of 5 samples collected during a month. Waters with less than 250 FC/100ml are classified as excellent for bathing. When marine animals are to be consumed raw by humans, the standard is more rigid: 14 fecal FC/100ml and no more than 10% of samples exceeding 43 FC/100ml for fishing and aquaculture waters.

In addition to routine monitoring of bathing beaches, some studies have been made along the coast to test the sanitary significance of microbial indicators by assessing the correlation between their levels and the presence of relevant pathogens. Botelho *et al.* (1980) studying estuarine and marine waters in Rio de Janeiro detected *Salmonella* and *P. aeruginosa* in primary contact recreational waters of Rio de Janeiro occurring when there were unacceptable levels of fecal coliforms. *P. aeruginosa* is considered a health risk to bathers due to its high incidence in water including those passing coliform based bathing standards, and the types of disease caused by this human opportunistic pathogen (Guimarães *et al.,* 1993). Hagler *et al.* (1986) studying microbial pollution indicators in Rio de Janeiro, evaluated the specificity of coliform tests for *E. coli* and other coliform bacteria, determining what level of coliform counts corresponded with the presence of some pathogens, and how microbial indicator counts correlate with other parameters in subtropical marine waters over a wide range of pollution levels. They found the fecal coliform group measured in the standard test to consist of 70% *Escherichia coli* and most of the others isolates were of the genera *Klebsiella*, *Enterobacter* or *Citrobacter*, confirming good specificity of the test for these waters. Fecal coliforms, fecal streptococci, heterotrophic bacteria and yeast counts showed correlation with above 99% confidence levels with most others microbial and chemical parameters studied. Waters with fecal coliforms levels above 1000/100ml (and about 480/100ml FS) had increased incidence of *Salmonella*, *P. aeruginosa* and pathogenic yeasts. Work done with beach waters in Santos (SP), using *Salmonella* as model pathogenic organism showed a highly positive correlation between the pathogen frequency in water samples and counts of enteric bacteria. *E. coli*, the main component of the coliform group, and *Salmonella* are closely related bacteria having similar behavior in the environment (Martins *et al.,* 1988). Low levels of fecal coliforms and *E. coli* can be found in apparently unpolluted tropical freshwaters such as mountain springs and bromeliad tanks (Hagler *et al.,* 1993). The Microbial Ecology and Taxonomy laboratory of Instituto de Microbiologia Prof. Paulo de Goes UFRJ recommends a fecal coliform based classification using geometric means of a minimum of 5 samples for recreational waters (Araujo *et al.,* 1991). In this classification waters with below 20 FC/100 ml (the minimum detected in a 5 tube MPN test with volumes of inoculum starting at 1 ml) are considered natural since low levels of fecal coliforms have been found to be normal even in unpolluted pristine tropical waters (Hagler *et al.,* 1986).

Counts between 20 and 100 FC/100 ml are of good bathing quality but not recommended for shellfish harvest. Most primary contact water standards are within the range of 100 to 1000 /100 ml falling into the suspect classification of marginal bathing quality. Bathing is not recommended in waters with FC counts above 1000/ 100 ml which are classified as contaminated, above 10,000 as very contaminated and above 100,000 as sewage (Table 2). These studies support the use of fecal coliforms and fecal streptococci as indicators of recent fecal pollution in subtropical marine waters. Yeasts and heterotrophic bacteria were suggested as complementary indicator parameters for non-fecal pollution.

Table 2. A quality classification of tropical surface waters based on geometric means of fecal coliform counts per 100 ml from a minimum of 5 samples as suggested in Araujo *et al.* (1991) for waters of Rio de Janeiro.

Classification (abreviation)	Color code	Fecal coliform count /100ml range
Natural (NAT)	blue	<20
Good bathing (BAN)	green	20 - $<$ 100
Suspect bathing (SUS)	yellow	100 - $<$ 1,000
Contaminated (CON)	red	1,000 - $<$ 10,000
Very contaminated (MCN)	brown	10,000 - $<$100,000
Sewage (ESG)	black	$\geq$ 100,000

Notes
1) NAT considered sufficient quality for shellfish harvest.
2) NAT and BAN considered good quality for bathing or other forms of primary contact recreation.
3) SUS is considered marginal quality for bathing and should be avoided by people with high susceptibility to infectious diseases.
4) CON, MCN, and ESG are not recommended for bathing and with increased contamination levels presumably representing increased risk of infection. Levels of fecal coliforms of above 10,000,000 per 100 ml have been recorded for some sites in Rio de Janeiro, where as uncontaminated marine beaches have typically less than 2 per 100ml.

Enumeration of additional microbial groups such as heterotrophic bacteria and yeasts as eutrophication indicators offers useful data to complement the information on fecal indicators levels. In bathing waters the main sources of sanitary risk are pathogens. capable of causing infections upon contact with body surfaces, mucous membranes, ears, and eyes. These pathogens are not exclusively of fecal origin and indicators of fecal pollution do not necessary indicate their presence in the environment. In such cases, enumeration of significant pathogens like *P aeruginosa* and *S. aureus* can be used to evaluate bathing quality (*APHA, 1995*). They survive better in sea water but their sanitary significance is limited by inclusion of non-fecal strains, and they make up a lower portion of the fecal microbiota than do coliforms. Gram-positive bacteria are more resistant to marine and chlorinated waters than Gram-negatives, and *S. aureus* is an important pathogen causing various diseases including some skin and eye infections. The occurrence of *S. aureus* in waters with fecal coliform counts above 1000/ml was consistent with previous results with other pathogens; however, *S. aureus* was also present in waters that were considered good by current bathing standards (Araujo *et al.,* 1990). Correlation coefficients between *S. aureus* and fecal streptococci were similar to those with fecal coliforms. *S. aureus* is often from non-fecal pollution and probably originated by washing from the bathers themselves. In marine and chlorinated waters FS has an advantage over FC as indicator of *S. aureus* . The presence of *S. aureus* in waters passing bathing quality standards points out a limitation on the use of coliforms as the only indicator in monitoring water quality. The data suggest that waters with high density of bathers can also present a risk of contact with pathogens, thus recommending the use of non-fecal microbial indicators to complement coliforms in monitoring bathing waters.

Araujo *et al.* (1991) did a survey of water quality using levels of fecal coliforms as an indicator in the bathing beaches and shellfish harvest waters in 80 sites in the cities of Rio de Janeiro and Nitéroi. This metropolitan area has a population of about 9 million where most of the sewage does not receive adequate treatment. The authors reported that most ocean beaches of Rio de Janeiro had water with acceptable quality for bathing according to Brazilian standards. Exceptions included sites where polluted streams, canals or sewage effluents entered the beaches. These problems increased following rain storms resulting in large amounts of sewage entering the beaches. Beaches along the main channel near the entrance of Guanabara Bay were relatively clean, often passing bathing standards. Shoreline sites located inside the bay are more seriously polluted, especially the enclosed beaches in the northwest of the bay. Paranhos *et al.* (1995) observed an increase of fecal coliform counts in Guanabara Bay of 1.3 to 2.9% between 1980 and 1990. Sato *et al.* (1998a) found the main beaches in Santos inadequate for bathing especially during the weekend due to heavy use. Vieira *et al.* (1998) isolated *E. coli* in 76.8% of 108 water samples in Formosa, Meireles and Diários beaches in Fortaleza, but none were enteropathogenic when tested for sorotypes O25, O26, O91, O112, O119 ,O158 e O164. Vieira *et al.* (1999) found intermediate pollution levels at Barra do Ceará. Melo *et al.* (1997) isolated *Salmonella* from water samples collected on Kartódromo and Farol beaches in Fortaleza, Ceará, indicating inadequate sewage treatment of domestic effluents. Similar results were reported by Martins *et al.* (1988) from Santos and São Vicente beaches where 74,7% of water samples were positive for *Salmonella.* The presence of pathogenic bacteria showed a positive correlation with the coliform levels and often were found in samples that did not pass the bathing water standard. In some cases *Salmonella,* was not detected in water with high fecal contamination and inversely the pathogen was detected in samples with low level of coliforms present. Rodrigues *et al.* (1989) studied the incidence of *Salmonella* present in Rio de Janeiro ocean and bay beaches. It was found in water samples collected from Flamengo, Ilha do Governador, Ramos and Copacabana beaches. The serogroups found were *S. typhimurium*, *S. agona* e *S. oranienburg*.

Polluted waters can carry important pathogenic viruses whose presence are not well indicated by coliform indexes. Coliphages have been tested as a potential pollution indicator in a comparative study done in Canada in the Ontario River and in Brazil along the coast of São Paulo (Dutka *et al.,* 1987). Data from these studies showed positive correlations between fecal coliforms, fecal streptococci and *E. coli* levels, and counts of coliphages. The level of 20 coliphages /100 ml was proposed as a standard for river water. However, the presence of *Salmonella* and enterovirus could not be predicted well by coliphages in marine waters.

Yeasts are predominantly unicellular fungi that include several pathogens and have some potential as pollution indicators (Hagler *et al.,* 1995). They are typically present in clean open ocean waters but at populations of only a few per liter and with *Debaryomyces hansenii* and basidiomycetous species of the anamorphic genera *Cryptococcus* and *Rhodotorula* typically among the prevalent groups. Yeast populations are higher in coastal areas ranging to over a thousand per liter in polluted waters (Hagler & Ahearn, 1987). Hagler & Hagler-Mendonça (1981) studied polluted estuarine waters along Ilha do Fundão in Rio de Janeiro, and found 67 species mostly of the genera *Candida, Rhodotorula, Hanseniaspora, Debaryomyces and Trichosporon.* Oliveira (1990) found large populations of *Saccharomyces* and other fermentative yeasts in the North Paraiba River estuary near sugar refineries and distilleries. Intertidal sediments from polluted urban sites had similar yeasts to those in polluted water, but with *Candida krusei* and other *Pichia membranifaciens* clade species being more prevalent and the colorless algae *Prototheca* also frequently present (Hagler *et al.,* 1982). The species *Kluyveromyces aestuarii* appears to be associated with the mangrove ecosystem, especially with detritus feeding invertebrates and sediments (Araujo *et al.,* 1995, Soares *et al.,* 1998)). *C. krusei and Candida tropicalis* are yeasts frequently associated with aquatic pollution and may be good indicator organisms (Cook & Matsura, 1963; Hagler & Ahearn, 1987). *Rhodotorula mucilaginosa* (syn. *R. rubra*) was the prevalent basidiomicetous yeast found, but is also typical of clean waters although at lower populations. Incubation at 40°C can select for the opportunistic pathogenic yeasts more typical of polluted waters and can serve as counts of presumptive pathogenic yeasts to be used as pollution indicators (Hagler *et al.,* 1986).

Microorganisms associated with sand of coastal beaches

Sandy beaches are transition regions between the land and the sea, and suffer influences from both. Kolm & Correa (1994) determined variations of the heterotrophic bacterial populations at Pontal do Sul, Paraná in relation to abiotic factors. The results showed three different zones with decreasing populations: between the sand dunes and the level of high tide, at the high tide level and between the high tide and low tide. The higher population at the high tide zone is related with the organic matter contents and the deposit of materials from the infralitoral areas. In contrast, the continuous flush of waves and currents contributes to decreasing the population of bacteria and the washing of the sand grains in the infralitoral zone. Kolm *et al.* (1997) analised sand, muddy-sand and sediment in Antonina e

Paranaguá bays, Paraná from seven stations with a salinity gradient. The sediments collected from the entrance of Paranaguá bay had the lowest level of heterotrophic bacteria and muddy sediments had similar levels of bacteria to sandy ones. There was a higher proportion of halophilic bacteria detected at the entrance of Maciel Bay. The results indicated no correlation between the size of the sand grain and the numbers of bacteria. There was a positive correlation with the current velocities and sedimentation of organic matter. The high salinity can limit the population of non-halophilic and non-halotolerant bacteria in the sediment. The bacterial population in the sediment was influenced by heavy rainfall, although this was not the case for the water column.

The presence of pathogenic microorganisms in sand has been a sanitary concern. Sato *et al.* (1998b) noted that quality control of bathing areas is normally focused on microbial water quality. However, in recent years there has been an increasing preoccupation with beach sand contamination especially as a result of inadequate disposal of garbage, presence of animal wastes, or pollution brought by the tides, all factors that can carry pathogenic microbes and parasites to this environment. Beach sand was sampled during the spring of 1997 and 1998 by Sato *et al.* (1998b) to study the sanitary conditions of beaches of the coast of São Paulo. The higher level of contamination of beach sand was observed in the summer, especially with respect to fecal coliforms and fecal streptococci. Spring sampling showed only one beach was above the Portuguese standard of 10^5 fecal coliforms /100g, whereas this limit was exceeded during the summer at 4 ocean beaches in the north and 6 in the south of the state. These authors observed that there was no improvement or a significant reduction in microbial quality of beach sand based on indicator bacteria and the *Candida albicans* yeast count data reported by Sanchez *et al.* (1986). However, no eggs, cysts or larvae of parasites were observed by Sato *et al.* (1998) indicating an improvement relative to the previous study of Sanchez and colleagues in which nearly all the beaches were contaminated by eggs and helminth larvae. It was concluded that the higher levels of fecal contamination observed for the sand during summer demonstrated the necessity to inform the population about the diseases transmitted by contaminated sand and on the preventive measures that can be adopted. Also criteria should be established to monitor the level of risk posed by the use of these beaches since there is no microbial standard for sand in Brazil.

Heterotrophic bacteria in coastal waters

The distribution of heterotrophic bacteria was determined in the surface waters of Paranaguá e Antonina Bays in the state of Paraná (Kolm & Absher, 1995). Seven collection sites were sampled from the entrance of Paranaguá Bay to Antonina Bay during a year and bacterial counts made using ZoBell's medium 2216. Temperature, dissolved oxygen, pH and rain levels were also recorded. The results showed a gradient increasing from the inner to the outer parts of the bays.

No significant temporal variation was found in the bacteria population except during February 1986, after an increase in temperature and rainfall. Data on bacteria collected near the Paranaguá harbor and the city did not show much variation and was considered as having low pollution levels. Kolm & Lesnau (1997) studied the variation of heterotrophic bacteria and abiotic parameters in the water column in two stations located in Paranaguá Bay and found no vertical gradient for bacteria in 9 of 12 monthly samples, but a positive correlation was found between bacterial counts and the seston values. The population of cultivable bacteria ranged from less than $1x10^2$ to a maximum of $5x10^4$ UFC/ml in the samples from the two sites. Mesquita & Fernandes (1996) determined the temporal variation of the plantonic community (bacteria, picophytoplankton and nanoheterotrophs) on the coastal region of Ubatuba, São Paulo, during 7 days in 1988, at low and high tide. They found bacterial populations in the range of 1,0 to 2,7 x 10^6 cells per ml . The average density of nanoheterotrophs were 2,3 x 10^3 cells per ml and picofitoplancton mainly representing cyanobacteria ranging 1,0 to 7,6 x 10^4 cells per ml. The authors suggested a predator-prey interaction between the populations of nanoheterotrophs and the cyanobacteria considering the variation pattern. Pagnocca *et al.* (1991) found average values ranging from 10^3 and 10^4/ml for cultivable heterotrophic bacteria in Sepetiba Bay, RJ sampling at the beach and 2 km from shore at Pedra de Guaratiba. Oliveira (1990) did an extensive work on the estuary of the Paraiba do Norte river and found the heterotrophic bacteria populations to be highly correlated with the levels of pollution measured by microbial indicators, the biochemical oxygen demand, and concentration of organic matter in the water.

Bacteria isolated from fish and shellfish

Several studies have been done concerning the sanitary quality of fishery products from the Brazilian coast. Similar to the situation in other countries, fish and shellfish stocks are decreasing over the years due to overfishing and lack of sanitary water quality. According to data from IBAMA, lobster production dropped from about 3,500 tons in 1979 to less than 2,000 in 1997. *Lutjanus purpureus* (the "pargo") is another resource for export lost to Northeast Brazilian industries. However, many others such as shrimp are still exploited.

A quality control plan for the fishing industry was initiated by an act of the Ministry of Agriculture (CIPOA/DNDA n° 082/92 of June 23, 1992) HACCP, should improve Brazilian fish products and market acceptance. Polluted oceans yield contaminated fish which have a reduced shelf life. Matté *et al.*(1994) studied pathogenic vibrios associated with the muscle *Perna perna* from the state of São Paulo during one year finding ranges of MPN/100ml for *Vibrio alginolyticus* ($<3x10^0$ $-2.4x10^4$), *V. parahaemolyticus* ($<3x10^0$ $-2.4x10^4$), *V. fluvialis* (<3 $x10^0$-$1.1x10^3$), *V. cholerae* non-O1 (<3 $x10^0$- 23) , *V. furnissii* ($<3x10^0$-$3.0x10^1$), *V. mimicus* ($<3x10^0$- $9x10^0$) and *V. vulnificus* ($<3x10^0$-$3x10^0$).

Virulence tests were positive for 34,1% of these samples. Fecal coliform counts /100g in these mussels ranged from 1.1×10^3 to 4.4×10^4 whereas in the harvest waters they ranged from 1.8×10^1 to 3.3×10^3. *Perna Perna* from Guanabara Bay were also found by Costa *et al.* (1991) to concentrate microbes including coliforms by about an order of magnitude or more from water of their habitat. These results showed the importance of the microbial quality control for these filter-feeding shellfish.

Pagnocca *et al.* (1989, 1991) studied the heterotrophic bacteria and yeasts associated with shrimp *Penaeus schmitti,* sediment and water of Sepetiba Bay, Rio de Janeiro. The collection sites at Pedra de Guaratiba, near the city of Rio de Janeiro were at the beach, about 2 km offshore, and a commercial fishing region in the southeast of Sepetiba Bay. Counts of heterotrophic bacteria at the more polluted beach site reached 3.1×10^4 UFC /ml while offshore samples were $6{,}5 \times 10^2$ UFC/ml. Heterotrophic bacterial counts of sediments at the beach were about 10 times those of the water. Intestines of shrimp from the commercial fishing sites were 7.6×10^9 for heterotrophic bacteria and $6. \times 10^7$ for presumptive *Vibrio* spp., compared with 7.2×10^9 and 9.5×10^7/g for shrimp from the more polluted site. The bacterial and yeast populations in the shrimp intestines reflected those of their environment and consequently the level of pollution at their location of capture.

Silva & Hofer (1995) isolated 520 strains of *E. coli* from the fish manjuba (*Anchoa* ssp.), mullet (*Mugil* ssp.) and croaker (*Micropogon* ssp.) captured along the coast of the city of Rio de Janeiro. Of these strains, 40% were resistant to one or more antibiotic with a standard of mono and bi- resistance to tetracyclin (29.2%), streptomycin (21.7%) and ampicilin (16.9%). Only 7.3 % of the strains were colicinogenic. An association of colicin types with the more frequent resistant markers and expression of colicin type Ib was evident. It could not be concluded that colicin production was an important factor for competition between *E. coli* strains, since antibiotic resistance and colicin production are frequently coded by the same plasmid. It is possible that other factors such as resistance to heavy metals were inserted in these replicons and contribute to the survival of *E. coli* in habitats contaminated with these metals.

Rodrigues & Hofer (1986) studied the presence of *Vibrio* in oysters and water from Sepetiba Bay, RJ. Cultures of *Vibrio* (576) were isolated and 390 of these were identified as 7 species: non O1 *V. cholerae* (65%); *V. alginolyticus* (14%); *V. parahaemolyticus* (11.%); *V. fluvialis* (6.7%); *V. harveyi* (1.5%); *V. damsella* (1.3%) and *V. vulnificus* (0.5%). Pathogenic vibrios are significant contaminants associated with marine animals captured in polluted waters and a frequent cause of food poisoning. Since these organisms are part of the normal heterotrophic population of seawater their numbers are expected to increase with increased organic load in polluted seawater. The reported studies demonstrated the presence of pathogenic bacteria within these marine animals in concentrations superior to the adjacent waters.

Concluding remarks

Interpretation of the significance of microbial counts from aquatic environments is complex, depending on both qualitative and quantitative differences between polluted and clean waters. A minimum of 5 samples should be analyzed for each site to generate reliable data. To obtain statistically significant data, adequate experimental design is necessary to establish the collection sites, the number of, and time for collections, adequate water quality indicators, complementary microbial counts and relevant abiotic parameters. Seawater is toxic to coliform bacteria and similar enteric organisms and this toxicity increases with warmer temperatures (Farage, 1987). Temperature is an important factor to consider in interpreting microbial data obtained from tropical beaches. High temperature tests are more significant in their selection of warm-blooded animal-associated microbiota when applied in cooler temperate zones (Hagler *et al.,* 1986). Microbes that are endemic in tropical environments are adapted to high ambient temperatures. The significance of microbial indicators applied to different environmental conditions should be assessed to extrapolate their use. This can result in lower counts for the same amount of pollution in warm than in cool environments. However, bacterial cells dynamically adapt to shifts in environmental parameters by employing a variety of genotypic and phenotypic mechanisms. Metabolic regulation permits bacteria to adapt to nutrient starvation and other stress factors using strain-dependent survival strategies. Important factors to be considered in predicting the risk associated with water pollution are the infective dose of ingestion or contact with the pathogenic microbe and the difference in individual susceptibility to diseases.

Water quality at a geographic point can vary considerably due to currents, tides, polluted effluents and rainfalls. Abiotic factors such as sunlight, salinity and chemical pollutants can change microbial populations in the environment. As a result of the multiple influences, the coliforms and other microbe populations in the water are in a dynamic equilibrium. They can differ by several orders of magnitude when taken at intervals of a few hours or days or a few meters of distance. Because of these variations and the inherent lack of accuracy of microbial counts and the magnitude of differences in the microbial population, classification of recreational waters should be interpreted with caution, using a logarithmic scale with small differences have little significance (Araujo *et al.,* 1991).

Generally, the method of choice to monitor fecal pollution of bathing and shellfish harvest waters is fecal coliforms counts. Coliform-based standards have limitations, including the fact that they are not suitable to indicate non-fecal pollution, resulting from chemical contaminants that favor growth of autochtonous pathogens, or non-fecal origin human pathogens such as *P. aeruginosa* and *S. aureus* and dermatophytes washed from bathers. Microbial parameters to complement coliform indexes are needed to assess the sanitary quality of water. Fecal streptococci counts have been considered as an alternative to fecal coliforms for bathing beaches; however, data obtained in studies in marine and fresh waters sites of Rio de Janeiro demonstrated high correlation between these parameters. The level of coliforms being always superior and, therefore, a more sensitive indicator of fecal pollution. Similar results were found by Sato (CETESB, personal communication) in São Paulo. Fecal streptococci has a better survival in sewage and marine waters and can be appropriate for non-recent pollution. Nevertheless, when low levels are detected the interpretation of the results is dubious because a large proportion of species included in this group have non-fecal origin (Araújo *et al.,* 1990). Microbial water quality data should not be interpreted only on a pass-or-fail standard. An intermediary or suspect category for bathing water that marginally pass the fecal coliform standard is recommended for counts ranging between 100 and 1000 /100ml. Special attention should be given to sites with substantial variations due to rain, sewage outlets, currents, tides and other environmental conditions. Sites always clean or very polluted do not need constant monitoring. The resources are better used in the control of intermediate and variable sites that represent more critical areas. Sites with intermediate levels of pollution should be avoided by people with lower immunity.

Shellfish are able to concentrate microbes in their tissues (Kosawa *et al.,* 1991; Pagnocca *et al.,* 1989). In many countries, the standard for shellfish water is rather strict. Epidemiological studies are needed in tropical waters to establish significant fecal and non- fecal indicators for shellfish harvest waters. Most beaches studied along the Brazilian coast are near urban areas and do not pass the US-EPA shellfish harvest standard of 14 FC/100ml (*APHA, 1995*). Obviously, monitoring alone will not solve these problems. Construction of sewage treatment plants, treatment of sewer before releasing offshore and control over clandestine sewer outlets are needed to improve the present pollution problem of our urban coastal waters. These types of measures should be encouraged by community groups, and the tourist and fisheries industries, and the results monitored with microbial indicators.

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